FISH-CARRIED LIVER TREMATODES

OF MANITOBA MAMMALS

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The present research began in 1961 as an enquiry into the role of fish as vectors of mammalian and avian helminths. This line of research has been followed in this department for the last 33 years and has culminated in 1952 in the publication of "Zoology of Tapeworms" by Wardle and McLeod.

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INTRODUCTION

The commercial fisheries of Manitoba make up the fifth most important primary industry of the province. Thus it is necessary to know what parasites the freshwater fish are carrying and which of them are pathogenic to man or fish-eating animals. It has been a known fact for many years that fish harbour larval forms of helminths. The most common of these worms, parasitizing both man and animals, are the Dibothriocephalus tapeworms and the liver flukes Clonorchis sinensis and Opisthorchis felineus. The definitive host becomes infected by eating raw or under-cooked fish. However most people cook fish sufficiently, therefore cases of human infection in Manitoba are unlikely to occur.

Raw fish has been and still is an important part of the diet of dogs, cats, mink, fox, and other carnivores, both wild and domestic. Fur production is Manitoba's fourth most important primary industry and many of the above mentioned carnivores are important to it. In Ontario mink and fox (cultured) have been reported infected with fish-borne digenetic trematodes (Cameron, 1944 (10); Price, 1929 (27)).

The realization that Metorchis conjunctus is present in Manitoba freshwater fish and that it has caused deaths of sledge dogs in the northern portion of the province on at least two occasions (Allen and Wardle, 1934 (1); Mongeau, 1961

(26)), has prompted this research. *Catostomus commersonii*, the known secondary intermediate host of *Metorchis conjunctus* in Ontario (10), was suspected of serving as such in Manitoba. However, it was felt that species of fish other than the common sucker might also be involved. This has induced the undertaking of feeding experiments with several species of fish. Cats served as experimental animals.

Populations of liver flukes obtained experimentally showed much variation in certain characteristics used for differentiation of species of the genus *Metorchis*. An adequate differentiation of these species necessitated thus a thorough study of the individual variability of the morphological characters regarded by some authors as characteristic of species. This is why the present work consists of two parts. The first is the search for the secondary intermediate hosts of the opisthorchids in Manitoba, and the second, a study of the variability of the morphological characteristics used for separating the species of *Metorchis*.

HISTORICAL REVIEW

A. HISTORY OF THE CLASSIFICATION OF METORCHINAE

The peculiar, small liver flukes of the fish-eating birds and mammals, placed at present in the subfamily Metorchinae of the family Opithorchidae, have been known for over one hundred and fifty years. Already in 1809 a fluke, taken from the liver of a European buzzard, had been described by Rudolphi (29, pp. 212). He named it *Distomum crassiusculum*; it is now placed in the genus *Metorchis*. The first representative of Metorchinae parasitizing the liver of mammals, *Amphistomum truncatum* (now called *Pseudamphistomum truncatum*), was described by the same author ten years later, in 1819.

The realization of the immense practical importance of the fish-borne liver flukes of mammals was the result of a series of discoveries started in 1884 with Rivoltas' description of a cat liver-fluke in Italy, now called *Opisthorchis felineus*, and its identification with the Siberian liver fluke of Winogradoff, *Distomum sibiricum*, by Braun in 1894. (8)

Early in the twentieth century the human opisthorchids, *O. felineus* and *Clonorchis sinensis*, were shown to infect many millions of people in Siberia, China and Japan, and to be highly pathogenic. Seventy years ago, in 1893, M. Braun

(7) pointed out that the occurrence of opisthorchid liver flukes in fish-eating birds and mammals shows that fishes are the source of infection with these parasites. Askanazy, in 1904 (2), provided experimental proof of Braun's statement, when he showed that *Leuciscus rutilis* serves as intermediate host of *O. felineus*. At present numerous species of fish are known to serve as intermediate hosts of *O. felineus*, *Clonorchis sinensis*, and *Metorchis*.

The genus *Opisthorchis* s. str. is not represented in North America except by *Opisthorchis tonkae* (31) whose placement in this genus is questionable. However, fish-borne liver flukes of mammals belonging to the genera *Metorchis* and *Amphimerus* occur in North America. In Canada *Metorchis* has been known since 1934 to cause serious diseases among sled-dogs. (1)

The genus *Metorchis* was created by Looss in 1899. (21) He placed in it eight species, four from the genus *Distomum* Zeder, 1800, and four from the genus *Opisthorchis* Blanchard, 1895. All eight species were, prior to 1895, included in the genus *Distomum*. The four species taken from *Distomum* are

Distomum truncatum	Rudolphi, 1819
Distomum campula	Cobbold, 1876
Distomum albidum	Braun, 1893
Distomum amphileucum	Looss, 1896

Blanchard in 1895 created the genus *Opisthorchis* for certain species previously included in the genus Distomum. He chose D. felineum Rivolta, 1884, as type species and gave the following definition of Opisthorchis.

> "Fasciolidae: Body distinctly, often very greatly, elongated, anterior end attenuated, posterior end broader. Skin generally smooth, without spines. Excretory system with long sigmoid stem winding between the testicles, and with short branches. Copulatory organs absent. Testicles in posterior portion of body, the one obliquely posterior to the other, margins lobed. Ovary simple or lobate. Laurer's canal present. Receptaculum seminis prominent. Uterine coils anterior to ovary, but not extending materially over the intestinal caeca. Vitellaria moderately developed, lateral of intestinal caeca not extending anteriorly beyond the acetabulum and extending posteriorly to the ovary; vitellaria form one region. Habitat - parasitic in the gall ducts of mammals, birds and fishes." (6)

Looss (21) placed the following species of Opisthorchis into the genus Metorchis:

Opisthorchis crassiusculus	(Rudolphi, 1809)
Opisthorchis xanthosomus	(Creplin, 1846)
Opisthorchis conjunctus	(Cobbold, 1860)
Opisthorchis complexus	(Stiles and Hassall, 1894)

Looss chose Metorchis albidus (Braun, 1893) as type species of his genus Metorchis. His definition of this genus is as follows:

> "Middle-sized or small Opisthorchiinae with a relatively short, wide body, clearly tapering anteriorly. Skin mostly spinose, in some cases smooth. Digestive apparatus as in Opisthorchis; in some cases the intestinal caeca reach only the level of the testis. The latter are more often rounded than lobed; situated rather one beside the other than one behind the other. Ovary, receptaculum seminis and Laurer's canal as in

> *Opisthorchis*. Uterine coils more compact than in this last genus, and mostly transgressing the intestine laterally. The vitellaria also more compact, shifted anteriad and in all cases extending anteriorly to the ventral sucker."

Thus at the time of its establishment in 1899 the genus *Metorchis* contained the following eight species:

Metorchis crassiusculus	(Rudolphi, 1809)
Metorchis truncatus	(Rudolphi, 1819)
Metorchis xanthosomus	(Creplin, 1846)
Metorchis conjunctus	(Cobbold, 1860)
Metorchis campula	(Cobbold, 1876)
Metorchis albidus	(Braun, 1893)
Metorchis complexus	(Stiles & Hassall, 1894)
Metorchis amphileucus	(Looss, 1896)

M. crassiusculus (Rudolphi, 1809) a parasite of aquatic birds, is the oldest recorded species of this genus. Skrjabin gives the following description of this trematode:

> "Body spatulate, 1.75 - 3.75 mm. long and 1 - 1.5 mm. wide. Anterior conical portion of the body separated from the posterior by lateral constrictions. Cuticle with spines, oral sucker 0.216 - 0.284 mm., ventral sucker almost circular, 0.1 - 0.3 mm. in diameter. Oesophagus absent. Testes fill only a small portion of the posterior end of the body, which remains free of other organs. Testes lobate, posterior testis mostly five lobed. Ovary 0.275 - 0.375 mm., ovoidal, sometimes lobate; usually not covered by the uterine coils. Receptaculum seminis pear shaped, slightly larger than the ovary. Vitellaria lateral, extending from the level of the pharynx to the level of the ovary. Almost the entire uterus is situated in the anterior half of the body; only a few uterine coils penetrate into its posterior half. Eggs 0.0306 - 0.0324 x 0.016 mm. (29, p. 212)"

Since 1899 eleven species have been added to the genus Metorchis and five have been removed from it. The species added are:

M. caeruleus	Braun, 1902
M. tener	Kowalewski, 1903
M. pinguinicola	Skrjabin, 1913
M. orientalis	Tanabe, 1921
M. taiwanensis	Morishita & Tsuchimochi, 1925
M. zacharovi	Layman, 1926
M. caintaensis	Tubangui, 1928
M. revilliodi	Baer, 1931
M. felis	Hsu, 1934
M. intermedius	Heinemann, 1937

The five species that have been removed from the genus Metorchis are:

M. truncatus	(Rudolphi, 1819)
M. campula	(Cobbold, 1876)
M. amphileucus	(Looss, 1896)
M. camplexus	(Stiles & Hassall, 1894)
M. caintaensis	Tubangui, 1928

In removing the species M. truncatus from the genus Metorchis Lühe, in 1908, made it the type species for his new genus Pseudamphistomum. (23) He gave the following definition of this genus:

"Metorchinae with a short body slightly tapering anteriorly and provided with a funnel shaped or sucker shaped concavity on the posterior end of the body. Cuticle provided with small spines. The intestinal caeca reach the posterior end of the body. testes rounded, in the posterior portion of the body, side by side or slightly diagonal. Ovary round or kidney shaped, anterior to the testes and separated from these by uterine coils. The excretory pore opens in the posterior depression of the body. Parasites of the biliary ducts of mammals. Type species Pseudamphistomum truncatum (Rudolphi, 1819)."

In 1908 Lühe (23) removed the species Metorchis campulus and Metorchis amphileucus and placed them in his new genus Cyclorchis with Cyclorchis amphileucus as type. (23) He defined Cyclorchis thus:

"Opisthorchinae; body elongate and spindle shaped, skin smooth, intestinal caeca do not reach the posterior end of the body. The excretory canal S-shaped, between the testes, opening at the posterior end of the body. Testes round and one behind the other rather than one beside the other. Uterine loops not crowded, transgressing the intestinal caeca laterally. No loops anterior to the ventral sucker. Vitellaria lateral, in the middle portion of the body, comparatively short. Occurrence; in the liver of marine mammals."

Skrjabin, in 1943, removed M. caintaensis from the genus Metorchis and created for it the genus Tubangorchis, with T. caintaensis as type and sole species (29 p. 262). Skrjabin gave the following definition of this genus:

"Metorchinae of small size, 0.9 - 2.0 mm. Body width equal along the entire length of the body. Oesophagus absent. Ventral sucker shifted anteriorly, on the level of the pharynx, almost in contact with the oral sucker. Testes large and lobed. The vitellaria running along almost the entire margin of the body, reaching its posterior end, where they

> partially encircle the intestinal caeca and penetrate into the inter-caecal space reaching the posterior testes. Sex pore median between the ventral and oral suckers. Ovary and receptaculum seminis covered by uterine coils. Eggs 36 x 21 μ. Parasites of the intestine (or the liver?) of birds."

The author believes that the removal of these four species (M. truncatus, M. campula, M. amphileucus, M. caintaensis) from the genus Metorchis and the creation for them of the new genera is justified. However the creation by Skrjabin, in 1913, of the genus Parametorchis (29 p. 248) to include M. complexus is hardly warranted.

Studying the genus Metorchis, Skrjabin came to the conclusion that M. complexus (Stiles & Hassall, 1894) has to be placed in a separate genus Parametorchis. His definition of the new genus is as follows:

> "Metorchinae with an elongated anterior and rounded posterior end of the body. Cuticle provided with spines. Suckers weakly developed, of equal size. Ventral sucker near the boundary between the first and second fourths of the body. Pharynx and short oesophagus present. Intestinal caeca reach the posterior end of the body. Testes lobate, situated in the posterior half of the body, one behind the other. The uterine coils in the anterior portion of the body, surrounding the ventral sucker in the shape of a rosette. Vitellaria in the anterior half of the body, lateral to the intestinal caeca. Lobated ovary anterior to the testes. Receptaculum seminis lateral to the ovary." (29 p. 248)

Thus Skrjabin created the genus Parametorchis to include species differing from Metorchis only in possessing a rosette shaped uterus and the testes one behind the other in the sagittal plane. Since 1913 the following species have been added to the genus *Parametorchis*:

P. noveboracensis	Hung, 1926 (20)
P. intermedius	Price, 1929 (27)
P. canadensis	Price, 1929 (27)
P. manitobensis	Allen & Wardle, 1934 (1)

Morozov, in 1939, divided the genus *Parametorchis* into two subgenera: *Parametorchis* S.STR. and *Metametorchis*. This division was the result of Morozov's finding, in the liver of a weasel, of a trematode closely resembling *Parametorchis*, but differing from the type species of this genus in not possessing a rosette shaped uterus. He described his trematode as a new species *Parametorchis* (*Metametorchis*) *skrjabini*. (29 p. 205)

Baer, in 1943, apparently without the knowledge of Morozov's work, (according to Skrjabin), (29 p. 249) removed from the genus *Parametorchis* the species *P. intermedius*, *P. manitobensis*, *P. canadensis* and created for them the genus *Allometorchis* with the type species *A. intermedius* (Price, 1929). He based the segregation of these species into his new genus on the absence of a rosette shaped uterus. In other words, according to Skrjabin, he did what Morozov had done four years earlier, the difference being that Morozov had ascribed a subgeneric status to his taxon *Metametorchis* whereas Baer has regarded *Allometorchis* as a genus. Therefore Skrjabin feels that the subgeneric name Metametorchis of Morozov is equivalent to the generic name Allometorchis of Baer and has to be regarded as a senior synonym of it. According to the rules of Zoological nomenclature Morozov's name Metametorchis has priority. Metametorchis therefore becomes a genus with the type species M. skrjabini (Morozov, 1939).

As a result of the splitting of the genus Parametorchis into two genera, its six species became distributed in the following way: (29 p. 249)

Parametorchis	Skrjabin, 1913
P. complexus	(Stiles & Hassall, 1894)
P. noveboracensis	(Hung, 1926)
Metametorchis	Morozov, 1939 emend. Skjabin, 1953
M. skrjabini	Morozov, 1939
M. canadensis	(Price, 1929)
M. manitobensis	(Allen & Wardle, 1934)
M. intermedius	(Price, 1929)

Cameron, in 1944, (10) apparently unaware of the work of Morozov and Baer, referred to the four species of the genus Parametorchis as follows:

> In 1926, Hung described a fluke from the cat; this he called Parametorchis noveboracensis. In 1929, Price described P. intermedius and P. canadensis from the silver fox and the mink respectively, from material collected by Law and Kennedy in Canada, and in 1934, Allen and Wardle described P. manitobensis from the dog. All of these are separable from each other on the basis of the specimens examined at the time of the various descriptions. However, when a large

> series is available and different hosts are infected experimentally from the same source, the differences disappear and all appear to be synonyms of Cobbold's *Distomum conjunctum*.
>
> All, however, had been placed in the genus *Parametorchis*. I have been unable to find any character that justifies the placing of Cobbold's species in any genus other than *Metorchis*. This leaves the genus *Parametorchis* with a single species, *P. complexus*, characterized by the union of the vitellaria.

If what Cameron suggests is true, then the genus *Metametorchis* must also contain only one species, *M. skrjabini*, Morozov, 1939.

In referring to *P. complexus*, Cameron says:

> However, in 1913, Skrjagin made it the type of a new genus, *Parametorchis*, of which the main character was this union of the yolk glands anterior to the "rosette"-shaped uterus; the genus *Metorchis*, which also had a "rosette"-shaped uterus, had the yolk glands in two separate lateral groups. (10)

Skrjabin did create the genus *Parametorchis* on the basis of the presence of a "rosette-shaped" uterus. However, he regarded the convergence of the yolk glands only as a specific character of *P. complexus*, not a generic one of *Parametorchis*. Moreover, he included as a generic characteristic the position of the testes one behind the other (rather than one obliquely posterior to the other) (29 p. 249) a character disregarded by Cameron. When referring to the "rosette"-shaped uterus, Cameron says:

> The word 'rosette' should not be taken literally; it refers to a tubular uterus, with coils heaped up on themselves and more or less surrounding the acetabulum, contrasted with the undulating form seen

in *Opisthorchis*.

Further he points out that the "rosette"-shaped uterus is a characteristic of both *Metorchis* and *Parametorchis*. (10)

Thus we see that

1. since Cameron has shown that the "rosette"-shaped uterus is a characteristic of both *Parametorchis* and *Metorchis*;
2. and since Cameron has not regarded the arrangement of the testes as a valid generic characteristic;
3. and since Skrjabin ascribed only specific value to the convergence of the vitellaria in *P. complexus*;

the latter species should be returned to the genus *Metorchis*. If this standpoint is accepted, it also becomes obvious that *Metametorchis skrjabini* Morosov,1939, should be placed in the genus *Metorchis*, and the genera *Parametorchis* Skrjabin, 1913 and *Metametorchis* Morozov, 1939 shall be regarded as junior synonyms of *Metorchis* Looss,1899.

B. LIST OF SPECIES OF THE GENUS *METORCHIS*

After the addition of *M. skrjabini* and the return of *M. complexus* to the genus *Metorchis* this genus contains a total of 15 species. They are as follows:

1. *M. crassiusculus* (Rudolphi, 1809) Looss, 1899

   definitive hosts: *Circus*, *Buteo*, *Anas*, and other birds

2. *M. xanthosomus* (Creplin, 1846) Looss, 1899

   definitive host: *Colymbus septentrionalis*

3. M. conjunctus (Cobbold, 1860) Looss, 1899

   definitive hosts: man, dog, red fox, mink, raccoon and cat

4. M. albidus (Braun, 1893) Looss, 1899

   definitive host: cat

5. M. complexus (Stiles & Hassall, 1894) Looss, 1899

   definitive host: cat

6. M. caeruleus Braun, 1902

   definitive host: Cairina moschata

7. M. tener Kowalewski, 1903

   definitive host: Mergus merganser

8. M. pinguinicola Skrjabin, 1913

   definitive host: Spheniscus demersus (Penquin)

9. M. orientalis Tanabe, 1921

   definitive hosts: Anas boschas and Anas domesticus

10. M. taiwanensis Morishita and Tsuchimochi, 1925

    definitive host: Anas boschas

11. M. zacharovi Layman, 1926

    definitive host: Anas circia

12. M. revilliodi Baer, 1931

    definitive host: Neomys fodiens

13. M. felis Hsu, 1934

    definitive host: cat

14. M. intermedius Heinemann, 1937

    definitive host: duck

15. *M. skrjabini* Morosov, 1939

definitive host: *Putorius putorius*

Only six of the above species use mammals as definitive hosts, the remaining nine parasitize the bile ducts of birds.

C. MAMMALIAN SPECIES OF *METORCHIS*

1. *M. albidus* (Braun,1893) (7)

Body length 2.5 - 3.5 mm., width 1.0 - 1.6 mm. Body spatulate or spoon-like. Anterior half narrower and separated from the flat, wide posterior half by a constriction. Posterior end rounded and never possesses a sucker-like depression. Skin usually densely spinous. Spines of the anterior portion of the body are slightly larger than those of the posterior one. They are shed easily and are absent in some specimens. Ventral sucker 0.242 - 0.301 mm.; oral sucker 0.323 - 0.269 mm.

Ventral sucker on the boundary between the anterior and middle thirds of the body, often obscured by uterine coils. Pharynx immediately behind the oral sucker. Bifurcation of the intestine close to the pharynx. Intestinal caeca extend to the posterior end of the body, their ends converging. Testes always lobed, 0.242 - 0.65 mm. in diameter, in posterior half of body, one anterior to the other. Receptaculum seminis pyriform, to the right of the ovary, bigger than the latter. Vitellaria extend from the bifurcation of the intestine to the middle of the body or slightly behind it. Eggs 27 - 32 μ long and 13 - 16 μ wide.

2. M. conjunctus (Cobbold, 1861)

Cobbold's Latin description runs as follows: "Corpus planum, oblongum, antrorsum sensim angustatum utrinque obtusum. Collum continuum. Acetabulum, ore paulo majus, ad colli basin. Aperturae genitales supra et pone acet. Longit. 1/4 unc.; crass, 1/12 unc." (11)

Cameron gives the following description of this species:

Body length 1.0 - 6.6 mm., width 0.59 - 2.60 mm. Oral sucker 0.090 - 0.240 mm. in diameter but varies according to its state of contraction. Diameter of the acetabulum is the same as that of the oral sucker, both being equal to the length of the pharynx.

Body linguiform, greatest width at the level of the testes. Acetabulum lies near the boundary of the first and second thirds of the body length. Genital pore median, just anterior to the acetabulum. Cuticle spinous. Excretory pore terminal, bladder tubular, winding between the testes, bifurcating at the anterior margin of the anterior testis.

Intestinal caeca almost reach the posterior end. No pre-pharynx present. Two testes, placed in the third quarter of the body either tandem or slightly obliquely, roughly spherical, often lobate (up to eight lobes), never dendritic.

Ovary slightly anterior to the anterior testis, varies from spherical or oval to trilobed. Yolk glands lateral to the intestinal caeca, extending from the ovary to the boundary between the first and second sixths of the body length. Uterus anterior to the ovary, between the intestinal caeca, slightly overlapping the latter. Uterine coils closely heaped together, extending slightly anterior to the acetabulum. Eggs 22 - 32 by 11 - 18 μ. (10)

3. M. *felis* Hsu, 1934

The original description runs as follows:

The body is relatively short and broad, tapering slightly in front and rounded behind, measuring 2.4 - 5.3 mm. in length and 0.8 - 1.4 mm. in width, with the posterior one-fourths of the body expanded and rounded. It is, therefore, broadest at about the posterior one-fourths of the body. The body is translucent and reddish when fresh. The cuticle is closely set with small scale-like spines, being more prominent at the anterior part of the body. The terminal oral sucker varies in shape as shown in the table of measurements, but in most cases it is transverse, being broader than long. It measures 14 μ - 39 μ x 14 μ - 35 μ. The acetabulum is about the size of oral sucker being a little larger. It is armed with strong radiating muscles. It measures 22 - 37 in diameter, and lies at the posterior limit of the anterior third of the body. The distance between the oral sucker and the acetabulum varies from 35 μ - 125 μ.

The mouth is relatively large being situated at the center of the oral sucker. The oesophagus is very short. It immediately divides into two intestinal crura which are narrow in the anterior three-fourths of the body. Beginning from the anterior limit of the posterior one-fourths of the body where the body expands, the two intestinal crura enlarge and completely embrace the testes laterally and posteriorly.

The two transversely elongated and irregularly lobate testes occupy the central portion of the expanded posterior one-fourths of the body, one behind the other. The anterior testis measures 31μ - 69μ x 20μ - 52μ. The posterior testis measures 29μ - 67μ x 22μ - 27μ. Each testis leads into a very minute vas deferens running anteriorly. The two vasa deferentia unite behind the acetabulum to form a convoluted vesicula seminalis lying on the left side of the acetabulum. The common genital aperture opens directly in front of the acetabulum. The ovary is a prominent rounded organ situated along the median line at the anterior limit of the posterior one-fourths of the body, immediately in front of the anterior testis. It measures 11μ - 27μ in diameter. The oval-shaped receptaculum seminalis lies on the left side of the ovary. It measures 7μ - 23μ x 3μ - 12μ. The shell gland is situated in front of the ovary. The vitellaria which consist of small follicles are confined to the narrow lateral fields from in front of acetabulum to the posterior limit of the anterior three-fourths of the body. The paired transverse vitelline ducts lie immediately in front of the ovary, leading to the ootype. The uterus is distended with eggs and thrown into close irregular loops. It occupies the greater part of the middle portion of the worm. It extends in front of the acetabulum to the middle in between the oral sucker and the acetabulum. It opens out through the common genital aperture in front of the acetabulum. The elliptical, operculate eggs from the uterus measure 12μ x 6μ. The excretory system could not be made out in detail. (19)

4. M. complexus (Stiles & Hassall, 1894) Looss, 1899

Length 5 - 7 mm. (can stretch to 10 mm.); breadth 1.5 - 2 mm.; body linguiform, not so separated into anterior and posterior portion as in D. albidum; anterior end rather pointed, posterior end rounded, but does not possess any muscular ridge. Integument covered with spines. Oral sucker terminal or by contraction may appear subterminal, of about the same size as acetabulum (0.33 to 0.39); acetabulum in median line about on boundary between first and second fourths of body and very much obscured by uterus. Oral sucker followed by pharynx, eosophagus very short; intestinal caeca extend to posterior end of body. Testicles lobate (three to eight lobes) in posterior half of body, one (right or left) in front of the other (left or right), separated by sigmoid end of excretory system. Ovary trilobate, lateral of ovary is situated a pyriform receptabulum seminis; shell-gland same as in D. albidum; vitellogene glands confined to anterior half of body, cross the intestines and meet in the median line, thus encircling the uterus. Genital opening anterior to acetabulum; penis not seen. Eggs average 0.024 by 0.012 mm. Color of fresh worm greenish; of preserved specimens whitish to pinkish, with dark brown spot (uterus)" (30).

5. M. skrjabini (Morozov, 1939)

Body length 3.5 mm., maximum body width 0.559 mm. Oral sucker 0.203 mm. in diameter, ventral sucker 0.214 mm. Vitellaria lateral, starting at a distance of 0.374 mm. from the anterior end and extending down to the middle of the body. In their anterior portion the vitellaria are in contact with each other. Their posterior portions are connected by vitelline ducts. The uterus is simple, not "rosette" shaped, and anterior to the vitelline ducts. The round ovary is 0.209 mm. in diameter. Testes entire, ovoid, the anterior 0.428 mm. long and 0.374 mm. wide, posterior 0.374 mm. long and 0.289 mm. wide. Eggs oval, 26 - 29 μ by 13 - 16 μ. (29 p. 241)

6. M. revilliodi Baer, 1931

Body length 1.1 - 1.7 mm.; width 0.82 - 1.0 mm. Ellipsoidal in shape with a sudden widening behind the level of the ventral sucker. Oral sucker 144 - 198 μ in diameter, ventral sucker 137 - 198 μ in diameter. Pharynx always longer than wide 65 - 90 by 54 - 70 μ. Oesophagus present but not easily seen in contracted specimens. Intestinal caeca almost in contact with each other behind the testes. Testes relatively large, never clearly lobed, situated obliquely to each other or else side by side. Cirrus pouch absent. Ovary smaller than the testes, more or less spherical, sometimes ovoidal, anterior to the testes and usually near the mid-line of the body. Only in contracted specimens is it situated on the level of the testes. Receptaculum seminis large, spherical or elongated. Vitellaria lateral, extending from the level of the intestinal bifurcation almost to the level of the ovary. The uterus, very long, lies in the space between the vitellaria, the bifurcation of the intestine and the ovary. 2 to 3 uterine loops are anterior to the ventral sucker. Eggs 26 - 30 μ by 12 - 13 μ, very numerous. The excretory pore visible only in sections, situated on the ventral surface at the level of the testes. (4)

In North America three species of the genus Metorchis have been reported: M. conjunctus (Cobbold, 1861) several times from both Canada (1, 9, 10, 11, 26, 27) and the United States (14, 20, 25, 27); M. complexus (Stiles & Hassall, 1894) twice, once from Baltimore, Md., and Washington, D.C. (30); and once from Rochester, Minn. (28); this last was probably M. conjunctus; and M. albidus (Braun, 1893) from Alaska. (17)

THE GEOGRAPHICAL DISTRIBUTION OF METORCHIS AND AMPHIMERUS pseudofelineus IN NORTH AMERICA

A. GENUS METORCHIS

The first record of a Metorchis in the liver of a North American mammal is probably that of Cobbold who, in 1860, has described a trematode from the biliary ducts of the American red fox, Canis fulvus, in the London Zoo, London, England. He named this trematode Distomum conjunctum. (11) This species of Distomum is one of the oldest placed by Looss (1899) into his genus Metorchis. It must be made clear here that Cobbold's finding only suggests the presence of this parasite in North America. It is possible that the infection was acquired in England.

Stiles and Hassall in 1894 have found a new species of trematode in the bile ducts of two cats; 18 specimens in a cat from Baltimore, Md., U.S.A., and two in a cat from Washington, D.C., U.S.A. They named it Distomum complexum. (30) In 1899 it was placed in the genus Metorchis by Looss (21) and became thus Metorchis complexus (Stiles and Hassall, 1894), Looss, 1899. Later, in 1913, it was chosen by Skrjabin as type species of his new genus Parametorchis (29 p. 248) and became Parametorchis complexus (Stiles and Hassall, 1894) Skrjabin, 1913. Skrjabin's genus Parametorchis was shown by Cameron in 1944 to have been erected on the basis of a non-existent character, the "rosette"-

shaped uterus. (10) It became thus obvious that Stiles and Hassall's species should never have been removed from the genus Metorchis. It shows all the characteristics of this genus; however the vitellaria converge anteriorly to the uterus. This trait, if proven to be constant, could be regarded as a specific character. Its constancy, however, was never proven, and it is possible that Stiles and Hassall have described as a new species an individual variant or an aberration of Metorchis conjunctus. (Fig. 1, Points 1 and 2)

Hung, in 1926, (20) has examined three specimens of a trematode from the gall bladder of a cat from Ithaca, New York, and described these as a new species Parametorchis noveboracensis. (20) He placed it in the genus Parametorchis because of its apparently "rosette"-shaped uterus. However, P. noveboracensis, according to Cameron (10) has to be regarded as a junior synonym of M. conjunctus (Cobbold, 1860). (Fig. 1, Point 3)

Price, in 1929, (27) has described two species of liver flukes of fur-bearing animals, which he placed in the genus Parametorchis. One of them, from the gall bladder of a silver fox Vulpes fulva, from Wisconsin, he named P. intermedius, believing it to be an intermediate form between P. complexus (Stiles and Hassall, 1894), and P. noveboracensis (Hung, 1926). The other, from the gall bladder of a mink Mustela vison, from Ontario, Canada, he named P. canadensis. According to

Figure 1: Distribution of *Metorchis* and *Amphimerus pseudofelineus* in North America

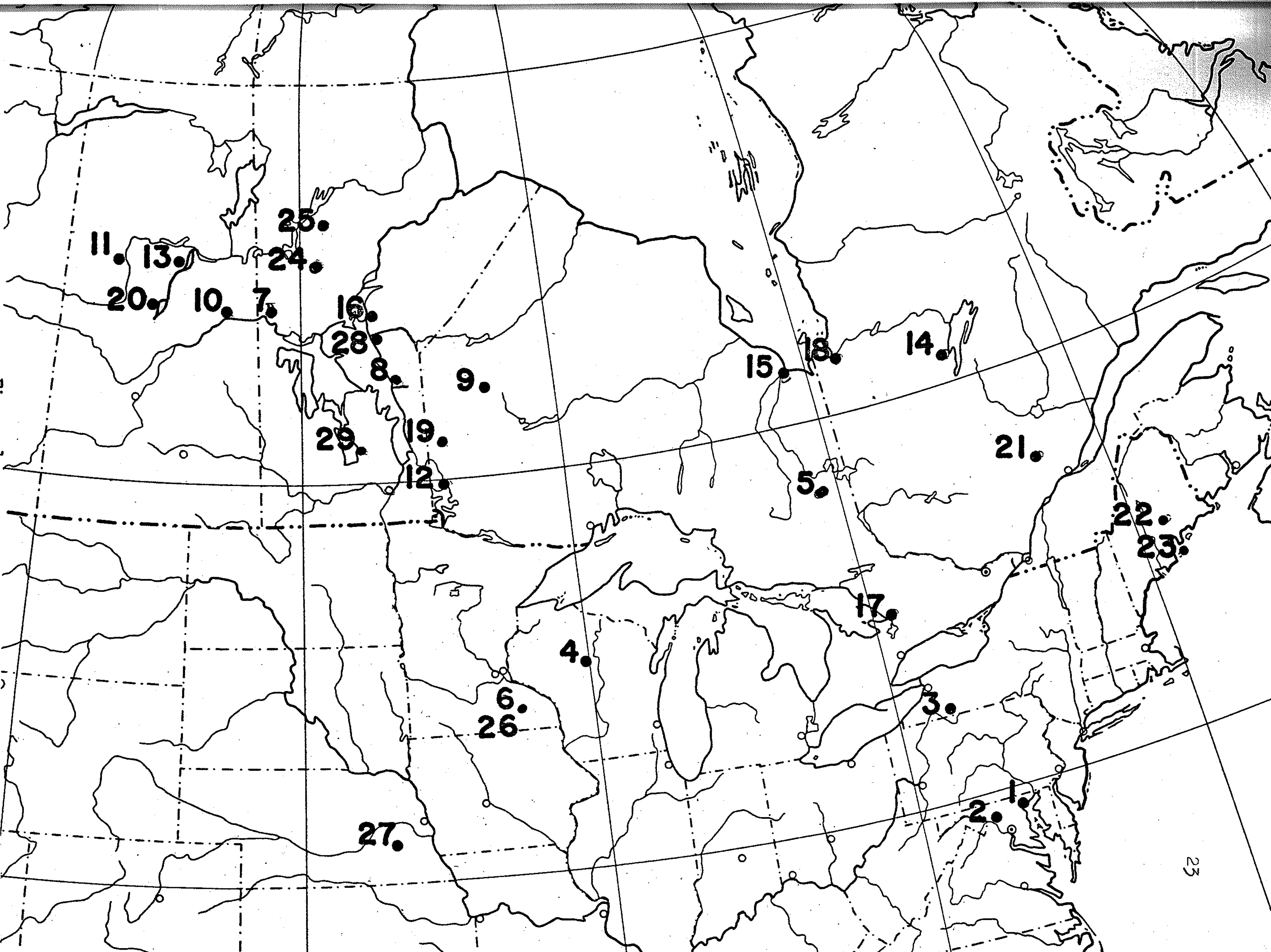


Figure 1

Cameron (10) both P. intermedius and P. canadensis should be regarded as junior synonyms of M. conjunctus (Cobbold, 1860). (Fig. 1, Points 4 and 5)

Essex and Bollman, in 1930, have found two species of trematode in the gall bladder of a cat. (14) The cat was believed to have been infected by eating trout from a creek near Rochester, Minnesota. They identified one of the trematodes as Metorchis complexus (Stiles and Hassall, 1894). The description of the specimens and the photograph of one of them convince the author that Essex and Bollman were dealing with M. conjunctus (Cobbold, 1860). The other trematode they identified as Opisthorchis pseudofelineus; it will be discussed later in the section on Amphimerus pseudofelineus. (Fig. 1, Point 6)

Allen and Wardle, in 1934, found numerous liver flukes in a sled dog from Cormorant Lake, 46 miles north east of The Pas, Manitoba, Canada. (1) They named it Parametorchis manitobensis. In his paper of 1944 (10) Cameron correctly regarded it as a junior synonym of M. conjunctus (Cobbold, 1860). (Fig. 1, Point 7)

Freeman and Ackert, in 1937, found flukes in the bile ducts of a husky dog imported from Alaska. (17) They identified these as Metorchis albidus (Braun, 1893), a species hitherto known only from Europe. Cameron (10) has pointed out that these trematoes may have been identified correctly, but that they are probably *M. conjunctus* (Cobbold, 1860). (Alaska not included on map)

Cameron, in 1940, published a list of localities in Canada where *Metorchis conjunctus* had been found in dogs. (9) The list is as follows:

Berens, Manitoba	See Fig. 1, Point 8
Cat Lake, Ontario	" " " 9
Cumberland House, Saskatchewan	" " " 10
Ile a la Cross, Sask.	" " " 11
Kenora, Ontario	" " " 12
Lac la Ronge, Sask.	" " " 13
Lake Mistassini, Quebec	" " " 14
Moosonee, James Bay, Ont.	" " " 15
Norway House, Manitoba	" " " 16
Pine River, Ontario	" " " 17
Rupert House, James Bay, Quebec	" " " 18
Trout Lake, Ontario	" " " 19
Waskesiu Lake, Sask.	" " " 20

In a later paper (1944) Cameron stated that the Lake Edward area (Quebec County) is a focus of infection with *M. conjunctus* and that both mink and fox from this area are heavily infected. (10)(Fig. 1, Point 21)

Meyer, in 1949, (25) published his finding of 12 specimens of *Metorchis conjunctus* in a dog from Bangor, Maine,

U. S. A. and of numerous Metorchis conjunctus in two raccoons from Swan Island, Maine, U. S. A. (Fig. 1, Points 22 and 23)

Heavy infections with M. conjunctus in sled dogs from both South Indian Lake and Nelson House, Manitoba, were reported by Mongeau in 1961 (26). He noted that the infected dogs were also suffering from canine hepatitis. (Fig. 1, Points 24 and 25)

In the present paper, the author reports the presence of Metorchis conjunctus in cats fed Catostomus commersonii and Perca flavescens from the Black River area on Lake Winnipeg, Manitoba. (Fig. 2, Point 6)

B. AMPHIMERUS PSEUDOFELINEUS (WARD 1901)

Amphimerus pseudofelineus was found by Ward in 1895 in the bile ducts of cats and a coyote from Lincoln, Nebraska. He first identified this trematode as Distomum felineum (Rivolta,1884) (32). Later, in 1901, after reading the classical paper of Looss (36), he realized that this trematode was a new species and named it Opisthorchis pseudofelineus. In 1911 Barker has created for this species a new genus Amphimerus. (6) (Fig. 1, Point 27)

Essex and Bollman in 1930 reported the finding of two species of trematodes in the gall bladder of a cat from Rochester, Minnesota. (14) One they identified as Metorchis complexus, already mentioned, the other as Opisthorchis

Figure 2: Distribution of *Metorchis* and *Amphimerus pseudofelineus* in Manitoba.

Legend

1. South Indian Lake
2. Nelson House
3. Norway House
4. Cormorant Lake
5. The Pas District
6. Black River
7. Berens River
8. Oak Point

▲ *Metorchis conjunctus* found in fish

■ *Amphimerus pseudofelineus* found in fish

All other points are findings of *M. conjunctus* in carnivores

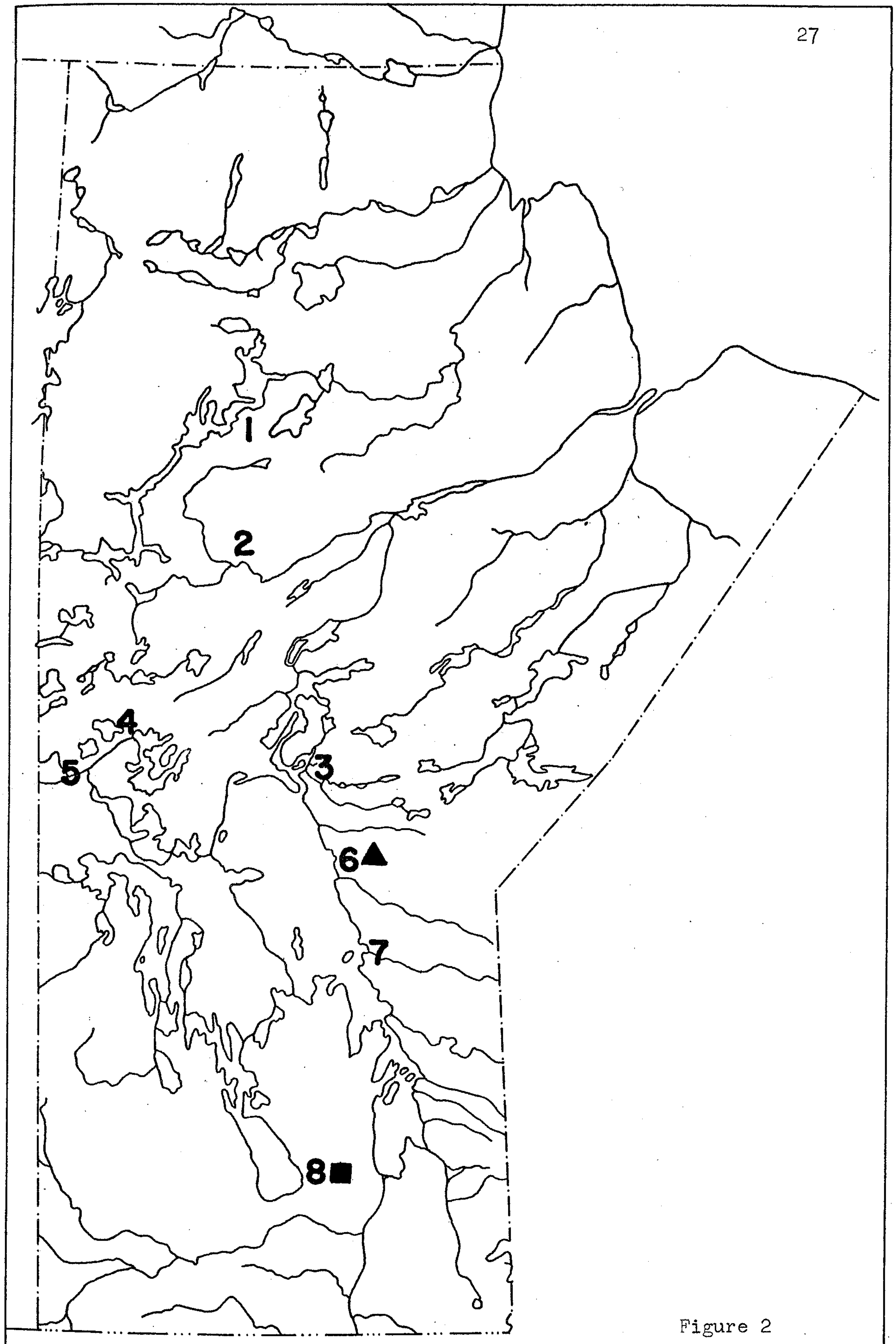


Figure 2

pseudofelineus (Ward, 1901). This last is of course *Amphimerus pseudofelineus* (Ward 1901), Barker, 1911. (Fig. 1, Point 26)

In the fall of 1962, the author found 36 specimens of *Amphimerus pseudofelineus* in an experimental cat fed *Catastomus commersonii* from Oak Point on Lake Manitoba, Manitoba, Canada. (15) (Fig. 2, point 8) This finding was published in 1963. (15).

Representatives of the genus *Metorchis* are known to occur in North America between latitudes 58° and 39°N. and longitudes 68° and 110°W. They are found in the drainage systems of the Hudson Bay, the St. Lawrence and the Mississippi Rivers and are thus not restricted to any particular drainage system. This fact, as well as the finding of *M. albidus* in Alaska by Freeman and Ackert in 1937, (17) shows that *Metorchis* is widely distributed in North America. It is certainly not restricted to the above area, but it is impossible to say how wide its actual distribution is.

TABLE I summarizes the distribution of *Metorchis* and *Amphimerus pseudofelineus* in North America.

TABLE I

DISTRIBUTION OF METORCHIS conjunctus AND AMPHIMERUS pseudofelineus

IN NORTH AMERICA

Map No.	Locality	Date	Author	Original Name	Definitive Host	Remarks
0	North America	1860	Cobbold (11)	Distomum conjunctum	North American Red Fox (Canis fulvus)	Found in London Zoo, London, England.
1	Baltimore, Md.	1894	Stiles & Hassall (30)	Distomum complexum	Cat	
2	Washington, D.C.	1894	" "	"	"	
3	Ithaca, New York	1926	Hung (20)	Parametorchis noveboracensis	Cat	Cameron showed this to be M. conjunctus (1944) (10)
4	Wisconsin	1927	Price (27)	Parametorchis intermedius	Silver Fox	Cameron showed this to be M. conjunctus (1944) (10)
5	Kirkland, Ontario	1929	Price (27)	Parametorchis canadensis	Mink	Cameron showed this to be M. conjunctus (1944) (10)
6	Bear Creek, near Rochester, Minnesota	1930	Essex & Bollman (14)	Metorchis complexus	Cat	Figure shows this to be obviously M. conjunctus
7	The Pas, district in Manitoba	1934	Allen & Wardle (1)	Parametorchis manitobensis	Dog	Cameron showed this to be M. conjunctus (10)
0	Alaska	1937	Freeman & Ackert (17)	Metorchis albidus	Dog	Probably this is M. conjunctus

TABLE I (continued)

Map No.	Locality	Date	Author	Original Name	Definitive Host	Remarks
8	Berens, Manitoba	1940	Cameron, Parnell & Lyster (9)	*Metorchis conjunctus*	Dog	
9	Cat Lake, Ontario	1940	"	"	"	
10	Cumberland House, Sask.	1940	"	"	"	
11	Ile a la Cross, Sask.	1940	"	"	"	
12	Kenora, Ontario	1940	"	"	"	
13	Lac la Ronge, Sask.	1940	"	"	"	
14	Lake Mistassini, Que.	1940	"	"	"	
15	Moosonee, James Bay, Ontario	1940	"	"	"	
16	Norway House, Man.	1940	"	"	"	
17	Pine River, Ontario	1940	"	"	"	
18	Rupert House, Quebec	1940	"	"	"	
19	Trout Lake, Ontario	1940	"	"	"	
20	Waskesiu Lake, Sask.	1940	"	"	"	
21	Lake Edward, Quebec County, Quebec	1944	Cameron (10)	*Metorchis conjunctus*	Cat	
22	Bangor, Maine, U.S.A.	1949	Meyer (25)	*Metorchis conjunctus*	Dog	

TABLE I (continued)

Map No.	Locality	Date	Author	Original Name	Definitive Host	Remarks
23	Swan Island, Maine, U.S.A.	1949	Meyer (25)	*Metorchis conjunctus*	Raccoon	
24	Nelson House, Manitoba	1961	Mongeau (26)	*Metorchis conjunctus*	Dog	
25	South Indian Lake, Man.	1961	Mongeau (26)	*Metorchis conjunctus*	Dog	
26	Bear Creek near Rochester, Minn., U.S.A.	1930	Essex & Bollman (14)	*Opisthorchis pseudofelineus*	Cat	
27	Lincoln, Nebraska	1895	Ward (32 & 36)	*Opisthorchis pseudofelineus*	Cat, Coyote	Became *Amphimerus pseudofelineus* (6)
28	Black River, Manitoba	Present Paper	Author	*Metorchis conjunctus*	Cat	From experimentally infected cats
29	Oak Point, Manitoba	1963	Author (15)	*Amphimerus pseudofelineus*	Cat	From experimentally infected cats

MATERIALS AND TECHNIQUES

Cats, obtained from a biological supplier, were dewormed with piperazine citrate, and their feces checked repeatedly for the presence of trematode eggs, using the Teleman sedimentation technique. The cats were known never to have eaten fish prior to the author's experiments.

Technique of Stool Examination

Sedimentation (Teleman) technique.

1. 10-15 mls. of feces were thoroughly shaken up in approximately 100 mls. of tepid tap water.
2. The suspension was strained through two layers of wet gauze into a beaker.
3. The strained fluid was placed in centrifuge tubes and centrifuged for 30 seconds at approximately 1500 r.p.m.
4. The supernatant was poured off and more tap water added. The sediment re-suspended, centrifuged, and the supernatant discarded. The washing was repeated four times, or until the supernatant became clear.
5. 10 mls. of 10% formaldehyde were added and mixed thoroughly with the sediment. The suspension was allowed to stand for two or three minutes; 3 mls. of ether were added and the test tube shaken vigorously.
6. The suspension was centrifuged for two minutes at 1500 r.p.m.

7. The supernatant was poured off and the sediment examined for the presence of trematode eggs.

Cat Feeding Procedure

The experimental cats were kept in separate cages and fed fish flesh from which the skin and bones had been removed. Each cat was fed only one species of fish. The species of fish fed to cats were as follows:

1. *Catostomus commersonii* Lacépède (Common sucker)
2. *Carpiodes cyprinus* Le Sueur (Quillback sucker)
3. *Moxostoma aureolum* Le Sueur (Redhorse sucker)
4. *Catostomus catostomus* Forster (Sturgeon or Northern sucker)
5. *Perca flavescens* Mitchill (Yellow perch)

The cats were sacrificed not less than 30 days after the last feeding and the gall bladder and bile ducts, as well as the small intestine were examined for the presence of fish-borne helminths.

Liver Examination

The gall bladder was cut open, and if trematodes were not present in it, the bile was examined microscopically for the presence of trematode eggs.

The liver immersed in physiological saline was cut into half-inch slices in such a way that the bile ducts were cut through transversely. Each section was squeezed gently with the fingers in an attempt to force the trematodes out of the bile ducts. The worms were collected from the saline with the aid of a wide mouthed pipette.

Examination of the Small Intestine

The small intestine was cut into sections, usually three, and each examined separately. The sections were opened by cutting the intestinal wall lengthwise. They were then washed in physiological saline and the washings examined with a magnifying glass. Following this, each section was placed in fresh physiological saline and the internal surface of the intestinal wall was scraped gently with the edge of a microscopic slide, or the blunt edge of a scalpel. The physiological saline with the scrapings was examined with a dissecting microscope at a magnification of 30.

Killing and Fixing the Specimens

The liver flukes were killed by placing them in distilled water. 2.5% $MgCl_2$ was also tried for this purpose, but distilled water proved to be better, as the worms died more quickly in it (approximately in 10 minutes) and showed less tendency to contract. Immediately following death the worms were fixed in 10% formalin.

Though all the internal organs of the trematode could be seen clearly without staining, many specimens from each population were stained and mounted permanently for a detailed study of the internal organs and for photography.

Staining and Mounting

1. Specimens fixed with 10% formalin were washed in running water 6 - 8 hours.
2. The washed specimens were placed in Gower's carmine for 18 - 24 hours. This overstained the specimens.
3. The specimens were then destained with acid alcohol (about 2 to 4% concentrated HCl in 70% ethanol). The destaining was watched through dissecting microscope.
4. The worms were dehydrated, then cleared with terpineol and xylene. The series of reagents used and the timing were as follows: 50%, 80%, 90%, 95% and again 95%, 15 minutes each.

absolute alcohol	30 mins.
absolute alcohol, second change	30 mins.
terpineol	60 mins.
50 - 50 mixture of xylene and absolute alcohol	5-10 mins.
pure xylene, two changes	3-5 mins. each

The mounting medium used was Permount.

Microscopic Examination of the Trematodes

All measurements were made on unstained specimens placed in 10% formalin under a coverslip resting on thin pieces of glass. Structures were examined with a microscope using a X10 eye piece and a X3 objective.

Photographic Procedures

Two techniques were used.

1. Negative printing

   The prints were obtained by placing stained and mounted specimens of *M. conjunctus* and *A. pseudofelineus* into the film holder of a 35 mm photographic enlarger or in the slide holder of a bioscope and projecting the image on F_3 Kodabromide medium contrast photographic paper. In some cases F_4 high contrast paper was used. The prints were developed with Ilford Id 36 developer. (Plates I, II, III, IV, V and VI)

2. Microphotography

   The eggs of *M. conjunctus* were photographed using a Zeiss microscope in conjunction with Zeiss Universal camera.

RESULTS

A. RESULTS OF THE FEEDING EXPERIMENTS

Feeding experiments were carried out using fishes from Lake Winnipeg and Lake Manitoba. It was decided that, since common sucker is known to be the host of Metorchis conjunctus, all Catostomidae that could be found in the above lakes should be tested, and so should yellow perch, a common game and market fish of both areas.

On Lake Winnipeg fishes from three areas were tested:

1. Black River: 3,580 gms. of Catostomus commersonii (Lacepede) from this locality were fed to four cats. All four became infected with Metorchis conjunctus.

3,784 gms. of Perca flavescens (Mitchill) were fed to three cats. Only one became infected with opisthorchids; two others became infected with Diphyllobothrium latum. The cat which became infected with opisthorchids was found to harbour only one specimen of M. conjunctus. This probably means that perch is an accidental host as the cat in question had eaten 1550 gms. of perch flesh.

2. Tamarch Island: 3,385 gms. of quillback sucker Carpiodes cyprinus (Le Sueur), caught in this locality, were fed to three cats. None became infected. This is a fairly substantial amount of fish for a test and it may be concluded that Carpiodes cyprinus probably does not serve as an inter-

mediate host for opisthorchids in this area.

3. Kinowow Bay: 3,370 gms. of sturgeon sucker Catostomus catostomus (Forster) from this locality were fed to two cats. Neither became infected. Again a substantial amount of fish has been tested, thus it is probable that Catostomus catostomus does not serve as an intermediate host for opisthorchids in this area.

2,722 gms. of northern redhorse sucker, Moxostoma aureolum (Le Sueur), also from this locality were fed to two cats; neither became infected. Thus it is unlikely that this fish harbours opisthorchid metacercaria in this area.

On Lake Manitoba only one station, Oak Point, was studied. Though only 700 gms. of Catostomus commersonii from this area were fed to one cat, it became infected with Amphimerus pseudofelineus. This is the first finding of this parasite in Canada and the first report of its secondary intermediate host for the world.

400 gms. of Perca flavescens from the same locality were fed to one cat which did not become infected. However, this cannot be considered a negative finding as not enough fish was fed.

The results of all the feeding experiments are given in TABLE II.

TABLE II

RESULTS OF FEEDING EXPERIMENTS CONDUCTED DURING THE SUMMERS OF 1961 and 1962

Cat No.	Species of Fish	Amount Fed Gms	Source of Fish	Results
1	*Catostomus commersonii*	1040	Black River	47 *Metorchis conjunctus*
2	*Catostomus commersonii*	604	Black River	16 *Metorchis conjunctus*
3	*Carpiodes cyprinus*	1105	Tamarch Island	
4	*Carpiodes cyprinus*	1140	Tamarch Island	
5	*Carpiodes cyprinus*	1140	Tamarch Island	
6	*Perca flavescens*	1550	Black River	1 *Metorchis conjunctus*
7	*Perca flavescens*	1154	Black River	1 *Diphyllobothrium latum*
8	*Catostomus catostomus*	1677	Kinowow Bay	
9	*Catostomus catostomus*	1693	Kinowow Bay	
10	*Moxostoma aureolum*	1675	Kinowow Bay	
11	*Moxostoma aureolum*	1642	Kinowow Bay	
12	*Perca flavescens*	1080	Black River	2 *Diphyllobothrium latum*
13	*Catostomus commersonii*	950	Black River	293 *Metorchis conjunctus*
14	*Catostomus commersonii*	950	Black River	238 *Metorchis conjunctus*
15	*Perca flavescens*	400	Oak Point on Lake Manitoba	
16	*Catostomus commersonii*	700	Oak Point on Lake Manitoba	36 *Amphimerus pseudofelineus*

B. THE FINDING OF AMPHIMERUS *pseudofelineus*

The finding of this parasite in the liver of an experimental cat fed *C. commersonii* from Oak Point was unexpected and very interesting.

36 specimens were found. They varied in length from two-thirds of an inch to almost a full inch, their width from about one-tenth to one-eighth of an inch. They are thus much larger and more elongate than *Metorchis conjunctus*. (PLATE I, 4) Their well developed vitellaria were divided into anterior and posterior regions by a break at the level of the ovary. Anteriorly the vitellaria do not reach the ventral sucker, whereas their posterior ends extend to the level of the posterior testis (PLATE I, 1, 2, 3). These characters compelled the author to place these opisthorchids into the genus *Amphimerus* Barker, 1911 (6, pps. 535-536). Measurements of 21 undamaged specimens from the experimental cat are summarized in TABLE III.

Historical Review

This species was found by Ward in cats and a coyote in Lincoln, Nebraska, U. S. A., and reported in 1895 as *Distoma felineum* (32). He noted the differences in the morphology of vitellaria of the European and American forms, but thought that they "do not warrant the creation of a new species for this form. At most, if shown to be constant and constantly

TABLE III

MEASUREMENTS (IN MM) OF FORMALIN FIXED SPECIMENS OF *AMPHIMERUS pseudofelineus* FROM THE EXPERIMENTALLY INFECTED CAT

	$M \pm \delta_m$	$\pm\delta$	C.V.(%)	Observed Range
Max. length	20.50 ± 0.45	2.01	9.8	16.2 - 23.8
Max. width	2.70 ± 0.03	0.14	5.2	1.7 - 3.2
Width at vent. sucker	1.47 ± 0.08	0.35	23.6	1.22 - 2.10
Width at ovary	2.54 ± 0.01	0.33	13.0	1.68 - 3.32
Length of oral sucker	0.38 ± 0.01	0.06	16.3	0.31 - 0.51
Width of oral sucker	0.46 ± 0.01	0.06	13.0	0.29 - 0.55
Pharynx length	0.24 ± 0.01	0.04	16.0	0.13 - 0.29
Pharynx width	0.26 ± 0.005	0.03	10.0	0.21 - 0.29
Oesophagus length	0.22 ± 0.01	0.05	22.7	0.17 - 0.40
Oesophagus width	0.13 ± 0.01	0.04	27.3	0.06 - 0.19
Diam. of ventr. sucker	0.29 ± 0.01	0.03	8.7	0.25 - 0.34
Distance from ventr. sucker to ant. end	4.47 ± 0.11	0.50	11.2	3.57 - 5.29

unlike the European form, they would entitle the American form to rank as a variety." (32 p. 154) This view he has maintained in a subsequent publication of the same year (33) and in a German note of 1895 (34). In a paper of 1898 (35) he again refers to the American cat fluke as "Distoma felineum" but states that "the two forms (European and American, Auct.) are possibly not the same species, but are certainly so closely related that this species may also well be a parasite of man under favourable circumstances (l.c., p. 307). In December 1899 there appeared the classical paper of Looss (21) on the revision of the genus Distomum in which he stressed the constancy of the morphological characters of the vitellaria within species and their importance for the identification of species (l.c., p. 552). Under the influence of this paper, Ward, in a footnote to a paper on Microphallus published in 1901 (36 p. 180) recognized the American "Distoma felineum" as a separate species and proposed for it the name Opisthorchis pseudofelineus.

In 1907 Barker, working in Ward's laboratory, thoroughly studied the variation of the vitellaria in Opisthorchis pseudofelineus and in related species (5) and in 1911, in a revision of the genus Opisthorchis (6), he has created the genus Amphimerus, which differs from Opisthorchis s. str. in possessing vitellaria divided into two distinct regions by a break in the line of acini near the ovary; the antovarial region of the vitellaria does not extend cephalad beyond the acetabulum; the postovarial portion extends caudad to or beyond the posterior testis. Thus characters, believed by Ward in 1895 to be insufficient for the establishment of a new species, were in 1911 recognized as being sufficient for the creation of a new genus.

Discussion

Four species of Amphimerus have been found in mammalian hosts: A. lancea (Diesing, 1850) Barker, 1911 from dolphins in Brasilian and Indian waters; A. noverca from dogs, cats, and pigs in India, a species reported as a human parasite from the same country; A. pricei (Foster, 1939) Skrjabin and Petrov, 1950 from the woolly opossum, Philander laniger pallidus from Panama R. P. (16, pp. 191-192, PLATE III, Figs. 18 and 19; and 29, pp. 165-166 and p. 167, Fig. 58); and A. pseudofelineus (Ward, 1901) Barker, 1911 from cats and a coyote from Lincoln, Nebraska, U. S. A. Our specimens differ widely from A. lancea of dolphins, which is smaller (up to half inch long), has a large ventral sucker and a pointed posterior body end. They also differ from A. noverca which is also smaller than our specimens, relatively much wider, has a larger pharynx, and a very small ventral sucker situated just behind the pharynx. Amphimerus pricei differs from our opisthorchids in possessing vitellaria which extend posteriad behind the posterior testis. On the other hand the comparison of the author's measurements with Barker's data for Amphimerus pseudofelineus (6 p. 544) show that these are almost identical. (TABLE IV) The smaller variability in our material depends probably on its character: we have examined a population from an experimentally infected cat, in which all worms were probably derived from only one infected fish. One of our specimens (Pl. I, 1) bears a striking resemblance to Barker's figure of Amphimerus pseudofelineus (6, Pl. XVII, 4). There can be no doubt that our opisthorchids have to be identified as Amphimerus pseudofelineus (Ward, 1901) Barker, 1911. (PLATE I, 1, 2, 3)

The finding of A. pseudofelineus in a cat fed the flesh of Catostomus from Lake Manitoba extends the known area of distribution of this opisthorchid into central Canada. It indicates, however, nothing as to the natural mammalian hosts of A. pseudofelineus in this region. A search for these hosts and the examination of the entire life cycle of this trematode are problems well worthy of further research.

TABLE IV

A COMPARISON OF THE AUTHOR'S MEASUREMENTS OF *AMPHIMERUS pseudofelineus* WITH THOSE OF BARKER (6 p. 525). MEAN VALUES AND OBSERVED LIMITS OF VARIATION (in mm.)

Dimensions	Barker's Data	Author's Data
Length of body	16(5 - 22)	20.50(16.20 - 23.80)
Breadth of body across acet.	1.25(0.75 - 1.5)	1.47(1.22 - 2.10)
Breadth of body across ovary	2.0 (1.0 - 3.0)	2.54(1.68 - 3.32)
Oral sucker length	0.31(0.19 - 0.45)	0.38(0.32 - 0.50)
Oral sucker width	0.38(0.24 - 0.54)	0.46(0.29 - 0.55)
Pharynx length	0.23(0.12 - 0.26)	0.24(0.13 - 0.29)
Pharynx width	0.21(0.14 - 0.28)	0.26(0.21 - 0.29)
Oesophagus length	0.24(0.04 - 0.38)	0.22(0.17 - 0.40)
Acetabulum length	0.26(0.14 - 0.31)	0.29(0.25 - 0.34)
Acetabulum width	0.24(0.17 - 0.32)	0.29(0.25 - 0.34)

C. VARIABILITY OF *METORCHIS*

The mammalian species of the genus *Metorchis* have been described on the basis of examination of only a few specimens. The consistency of characters used for the separation of these species has never been examined in populations sufficiently large to determine whether they are constant enough to be regarded as specifically significant. In the present paper the variability of these characters has been examined in two large population of *Metorchis conjunctus*.

Variation in Body Shape

The variation in body shape was studied, as this character is used by many authors when separating species of the genus *Metorchis*.

Three basic body shapes were found. These are shown in Figure 3. See also PLATE IV, 18-26.

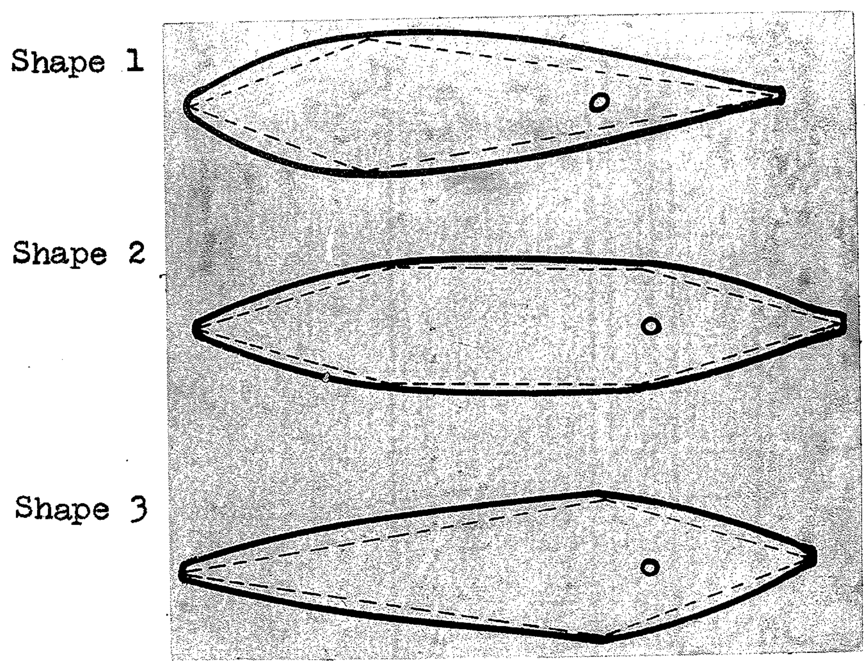


Figure 3: The Three Body Shapes of *Metorchis conjunctus*

Shape 1: Greatest body width in the posterior portion of the body

Shape 2: Anterior and posterior portions of equal width

Shape 3: Greatest body width in the anterior portion of the body

TABLE V

COMPARISON OF THE BODY SHAPE VARIATION IN POPULATIONS 1 and 2

Body Shape	Population 1 $\% \pm m_{\%}$	Population 2 $\% \pm m_{\%}$
Shape 1	59 ± 4.91	44 ± 4.96
Shape 2	37 ± 4.83	49 ± 4.99
Shape 3	4 ± 1.96	7 ± 2.55

Population 1 contains 238 specimens; population 2 contains 293 specimens. 100 specimens were examined from each population.

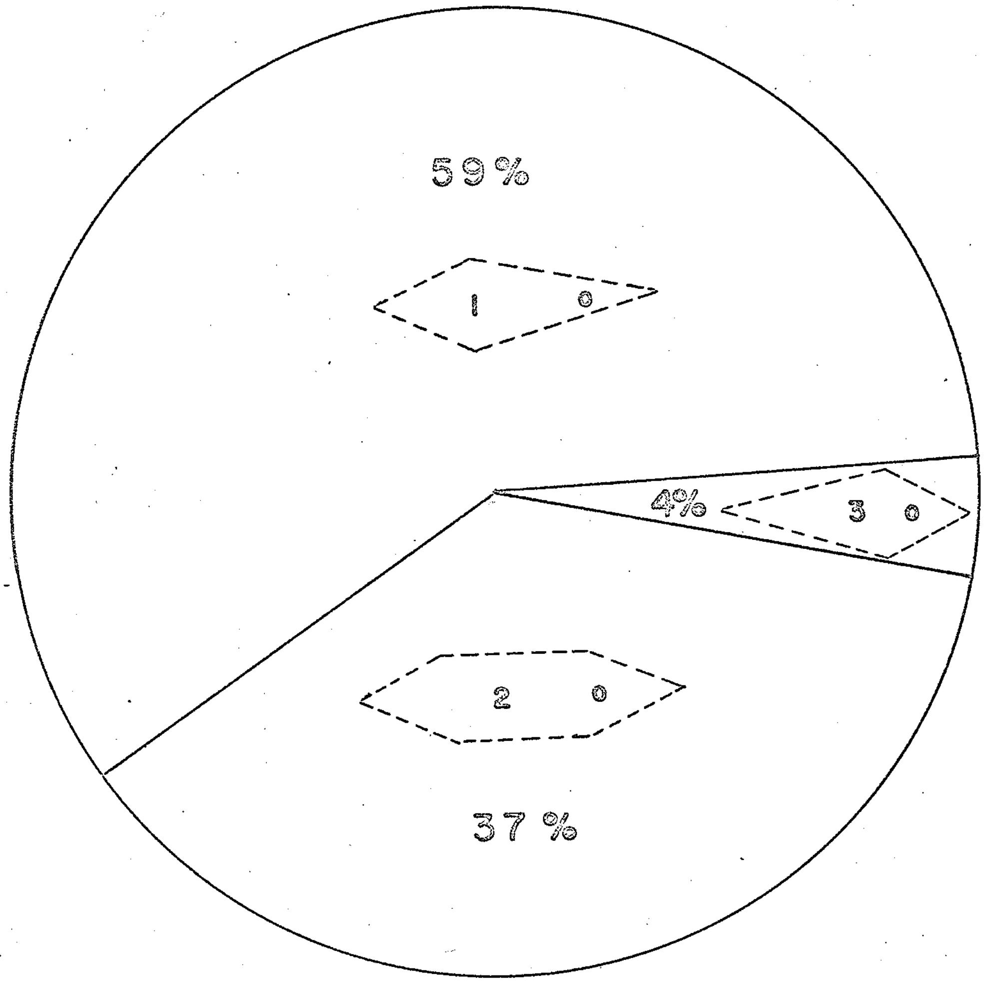


Figure 4: Variation in Body Shape of Population 1

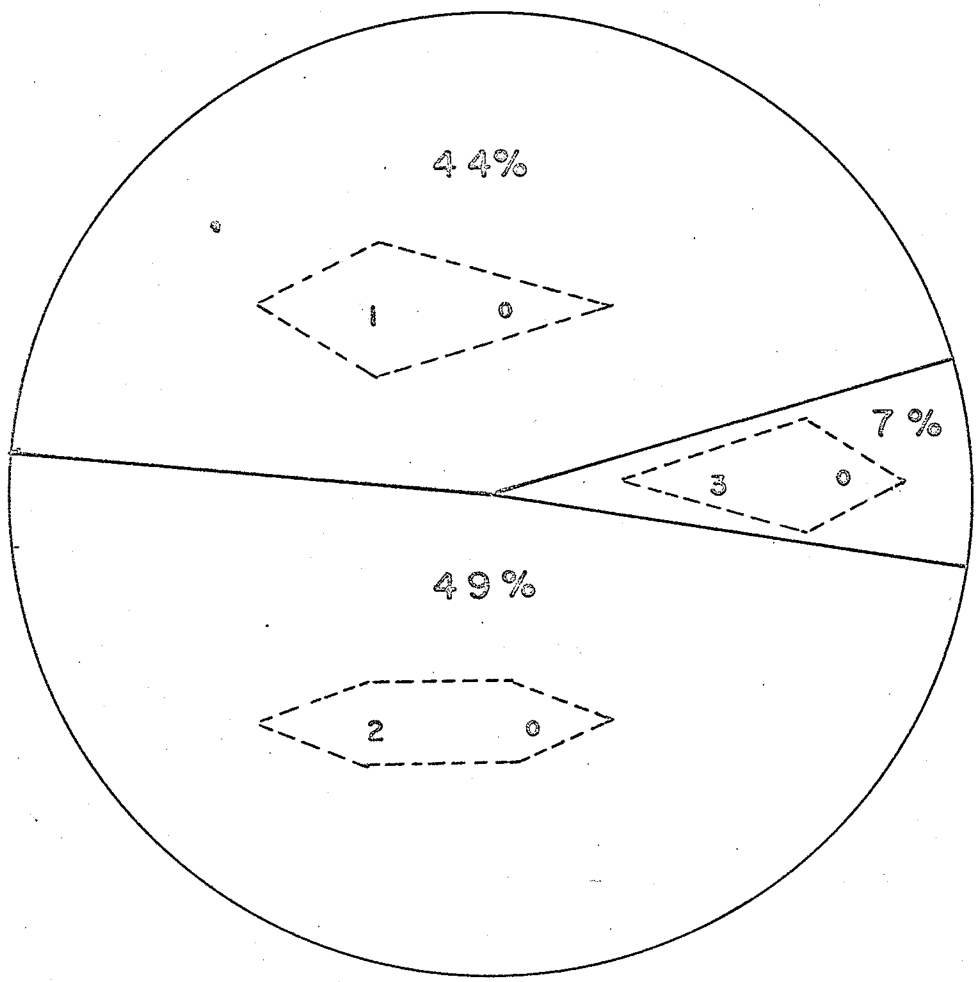


Figure 5: Variation in Body Shape of Population 2

Variation in Body Size

Body size is often used for the separation of species of Metorchis. Therefore the variation in body size was examined here.

Population 1 contained 238 specimens, of which 200 specimens were examined, and population 2 contained 293 specimens, of which 160 were measured.

TABLE VI

COMPARISON OF THE BODY LENGTHS OF POPULATION 1 AND POPULATION 2. MEASUREMENTS IN MM

Population	$M \pm \delta_m$	δ	C.V. %	Range of Variation
1	6.90 ± 0.04	± 0.65	± 9.4	3.5 - 8.0
2	5.39 ± 0.05	± 0.68	± 12.6	3.0 - 7.5

TABEL VII

COMPARISON OF THE BODY WIDTHS OF POPULATION 1 AND POPULATION 2. MEASUREMENTS IN MM

Population	$M \pm \delta_m$	δ	C.V. %	Range of Variation
1	1.72 ± 0.02	± 0.29	± 16.86	0.75 - 2.5
2	1.42 ± 0.03	± 0.32	± 22.5	0.75 - 2.75

The variation in body length for populations 1 and 2 are given by Figs. 6 and 7 respectively, and the variation in body width by Figs. 8 and 9 respectively.

Figure 6: Variation in Body Length of Population 1

Mean	6.9 mm.
Standard deviation	± 0.65 mm.
Standard error	± 0.04 mm.
Coefficient of Variation	± 9.4%

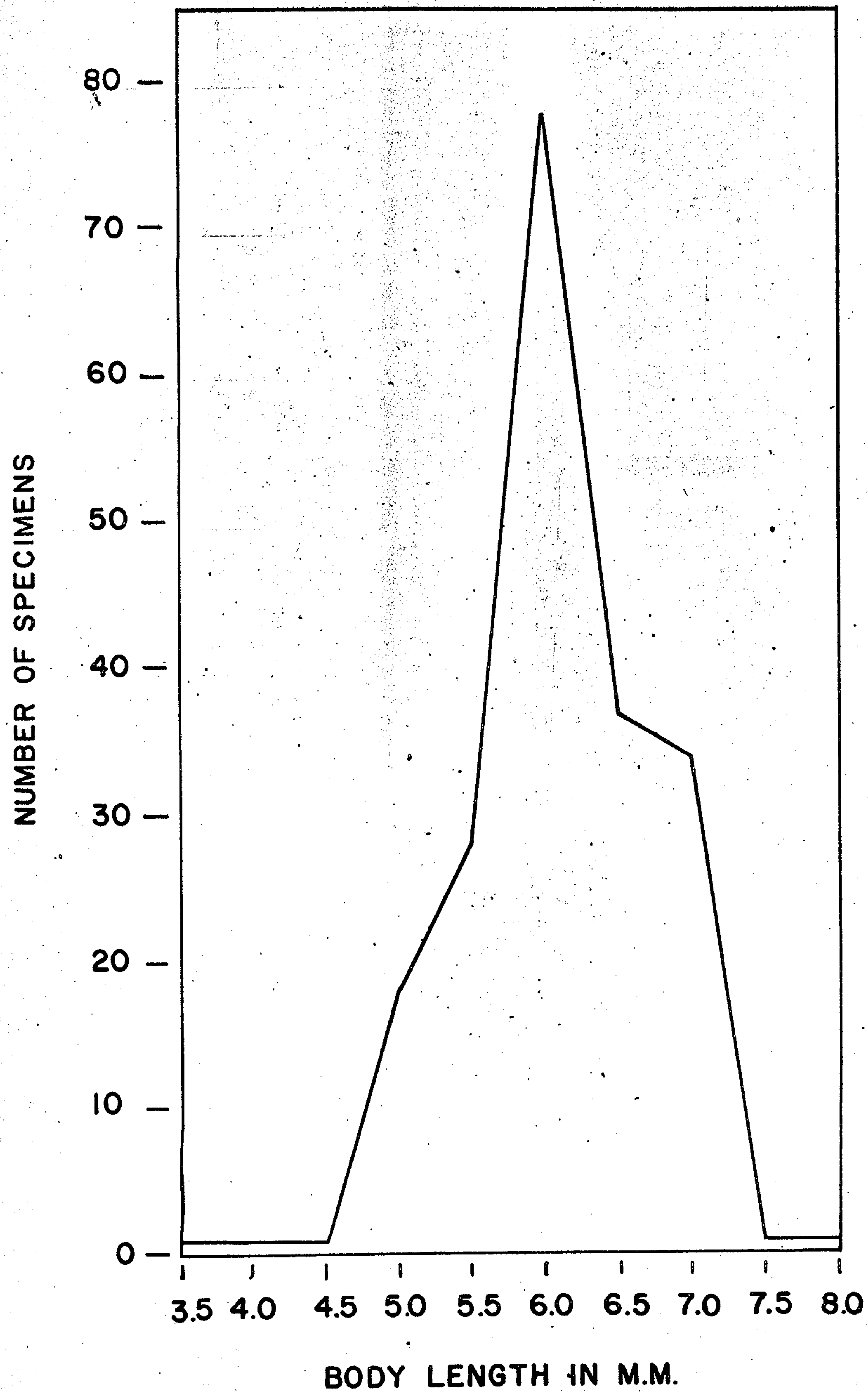
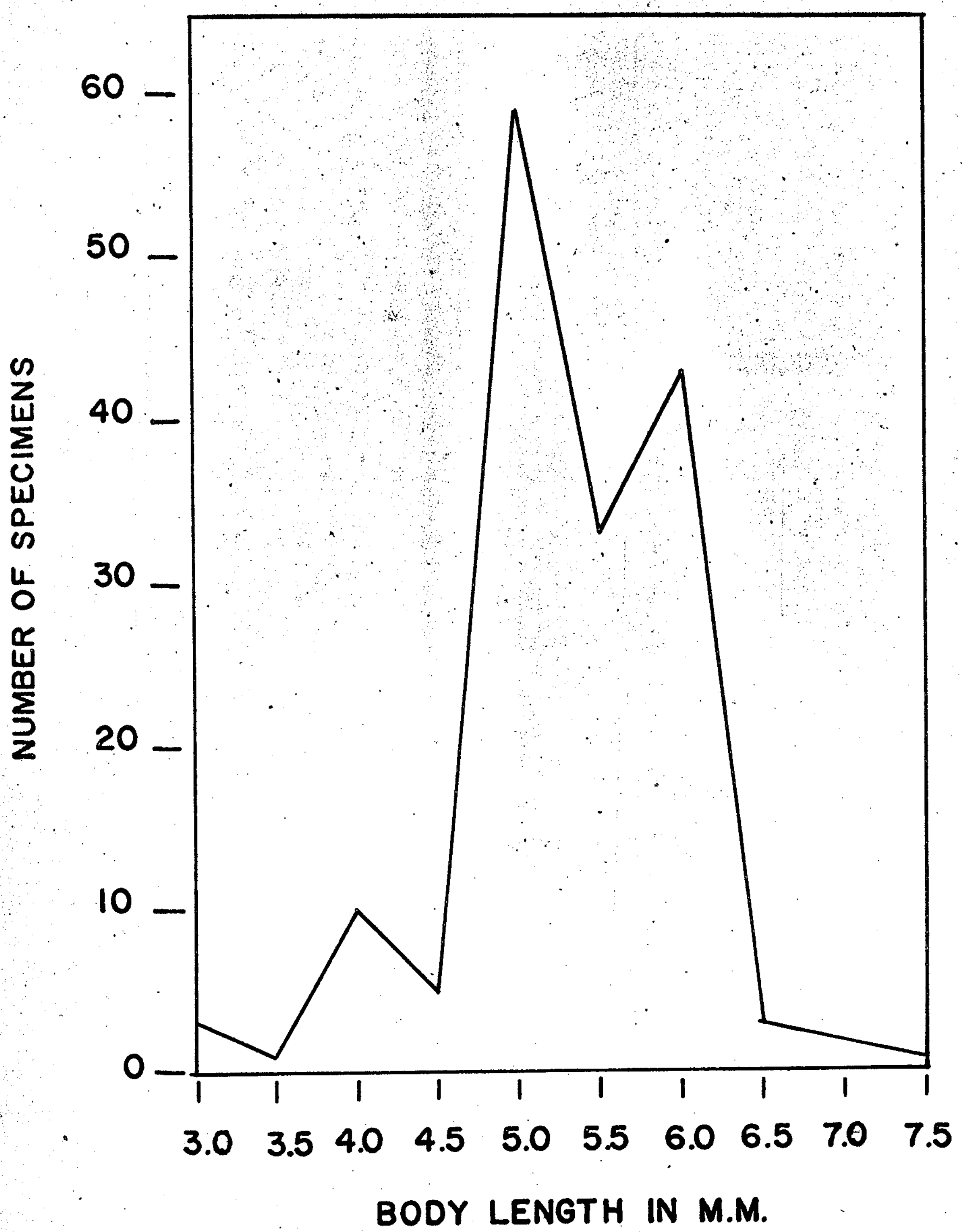

80
70
60
50
40
30
20
10
0
NUMBER OF SPECIMENS
3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
BODY LENGTH IN M.M.

Figure 6

Figure 7: Variation in Body Length of Population 2

Mean	5.39 mm.
Standard deviation	± 0.68 mm.
Standard error	± 0.05 mm.
Coefficient of Variation	± 12.6%



NUMBER OF SPECIMENS
60
50
40
30
20
10
0
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
BODY LENGTH IN M.M.

Figure 7

Figure 8: Variation in Body Width of Population 1

Mean	1.72 mm.
Standard deviation	± 0.29 mm.
Standard error	± 0.02 mm.
Coefficient of Variation	± 16.86%

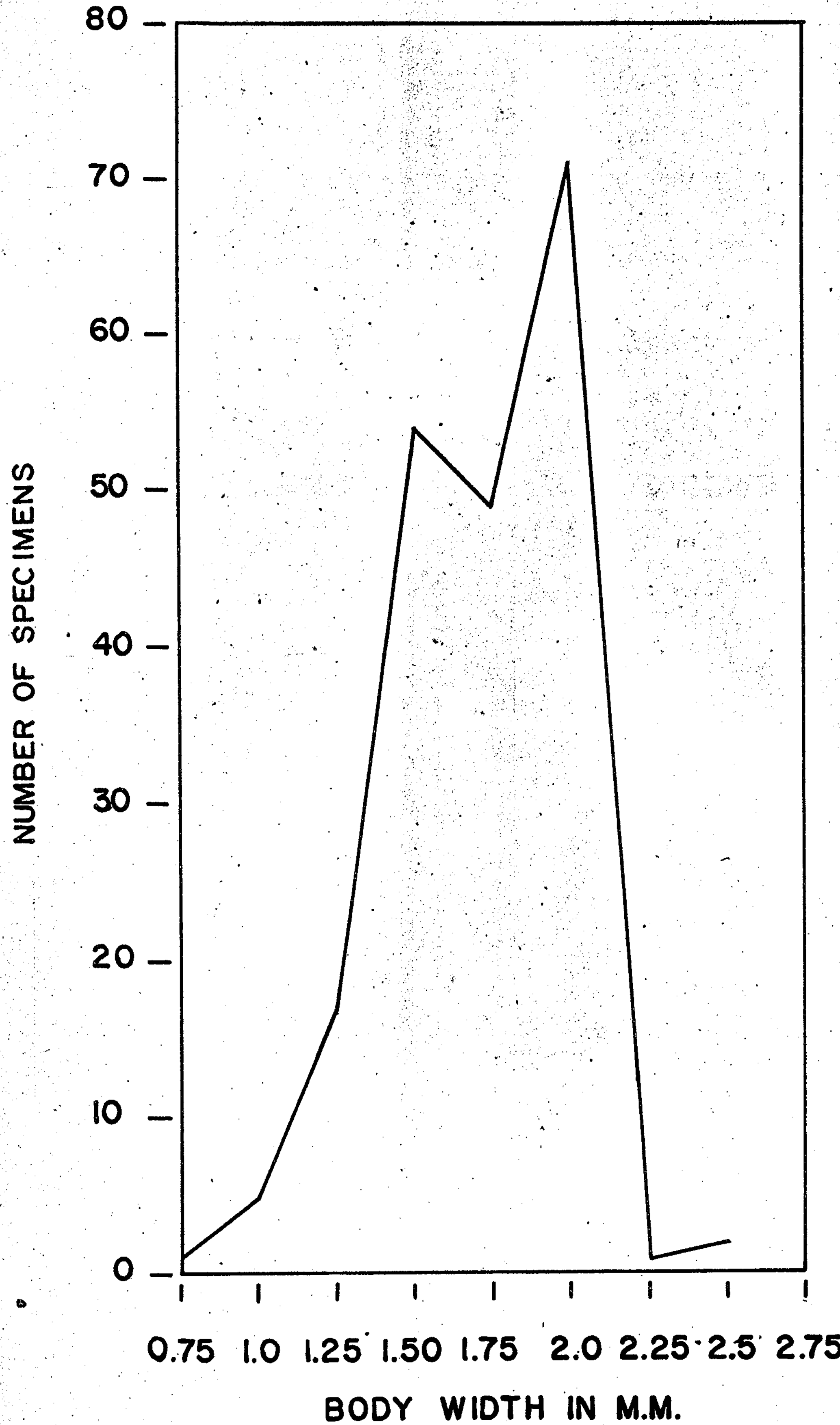

80
70
60
50
40
30
20
10
0
NUMBER OF SPECIMENS
0.75 1.0 1.25 1.50 1.75 2.0 2.25 2.5 2.75
BODY WIDTH IN M.M.

Figure 8

Figure 9: Variation in Body Width of Population 2

Mean	1.42 mm.
Standard deviation	± 0.32 mm.
Standard error	± 0.03 mm.
Coefficient of Variation	± 22.5%

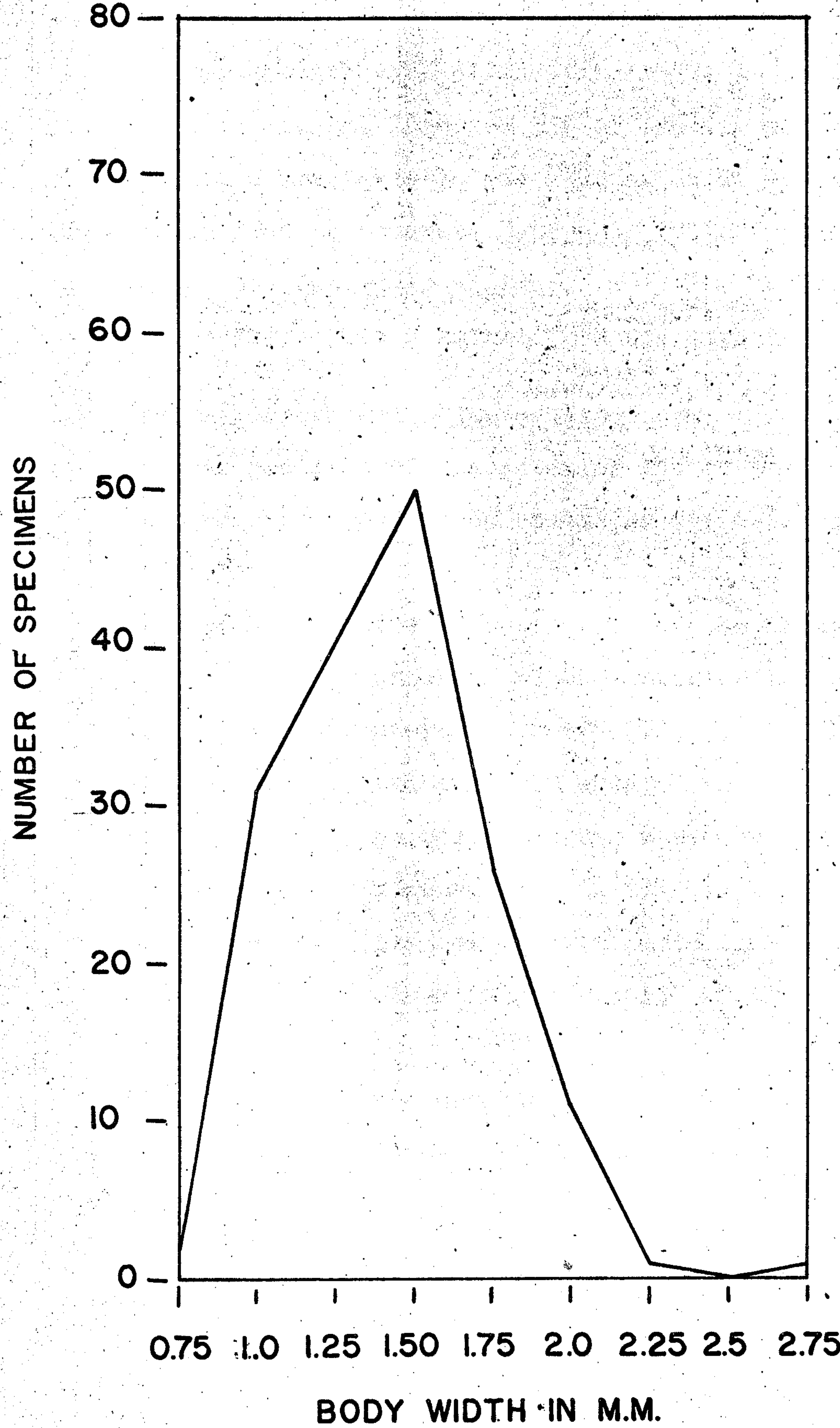

80
70
60
50
40
30
20
10
0
NUMBER OF SPECIMENS
0.75
1.0
1.25
1.50
1.75
2.0
2.25
2.5
2.75
BODY WIDTH IN M.M.

Figure 9

Anterior Extension of the Vitellaria in Relation to the Anterior Tip of the Uterus

This characteristic was studied as it was used by Cameron in 1944 to separate *Metorchis felis* from *Metorchis conjunctus* and *Metorchis albidus*. (10) The anterior extension of the vitellaria was examined from two aspects.

1. Position of Anterior Tip of Vitellaria

   Four position of the anterior tip of the vitellaria were observed. These positions are designated as follows:

   a) ahead - the anterior tips of the vitellaria are anterior to the anterior tip of the uterus. (PLATE III, 15, 16, 17)

   b) behind - the anterior ends of the vitellaria are posterior to the anterior tip of the uterus. (PLATE III, 9, 10, 11)

   c) mixed - one row of vitellaria is anterior, the other is posterior. (PLATE III, 12, 13, 14)

   d) even - the anterior tips of the vitellaria are level with the anterior end of the uterus.

TABLE VIII

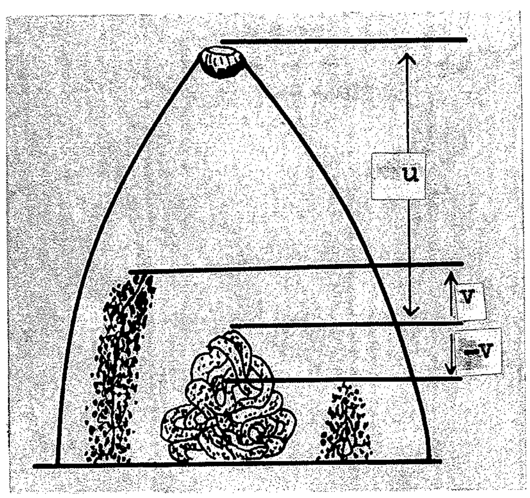
THE POSITIONS OF THE ANTERIOR TIPS OF THE VITELLARIA OF POPULATIONS 1 AND 2

Anterior extension of vitellaria	Population 1 $\% \pm m_2$	Population 2 $\% \pm m_2$
ahead	88 ± 4.14	97 ± 1.71
mixed	7 ± 2.55	2 ± 1.40
even	3 ± 1.71	1 ± 0.99
behind	2 ± 1.40	

Population 1, 238 specimens; population 2, 293 specimens. 100 specimens were examined from each population. See Figs. 11 and 12.

2. <u>Variation in the Anterior Extension of the Vitellaria</u>

The anterior extension is expressed as a percentage of the distance between the anterior tip of the uterus and the anterior tip of the body.



u = distance from anterior tip of uterus to anterior tip of body.

v = distance from anterior tip of uterus to anterior tip of vitellaria.

Figure 10: Explanation of the Measurement of the Anterior Extension of the Vitellaria

$$\text{Anterior extension of vitellaria} = \frac{100v}{u}$$

v is negative when the anterior ends of the vitellaria are posterior to the anterior tip of the uterus.

Population 1, 238 specimens; 200 specimens examined.

Population 2, 293 specimens; 160 specimens examined.

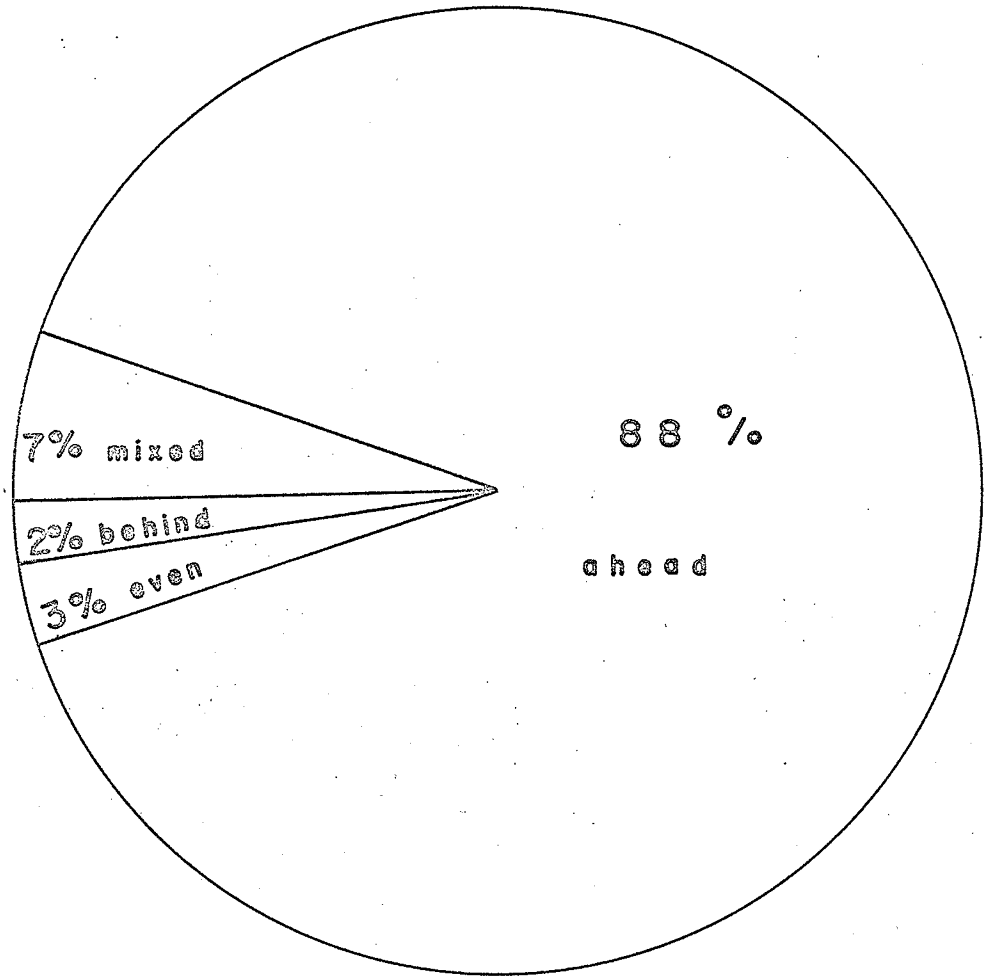


Figure 11: Position of the Anterior Tip of the Vitellaria in Population 1

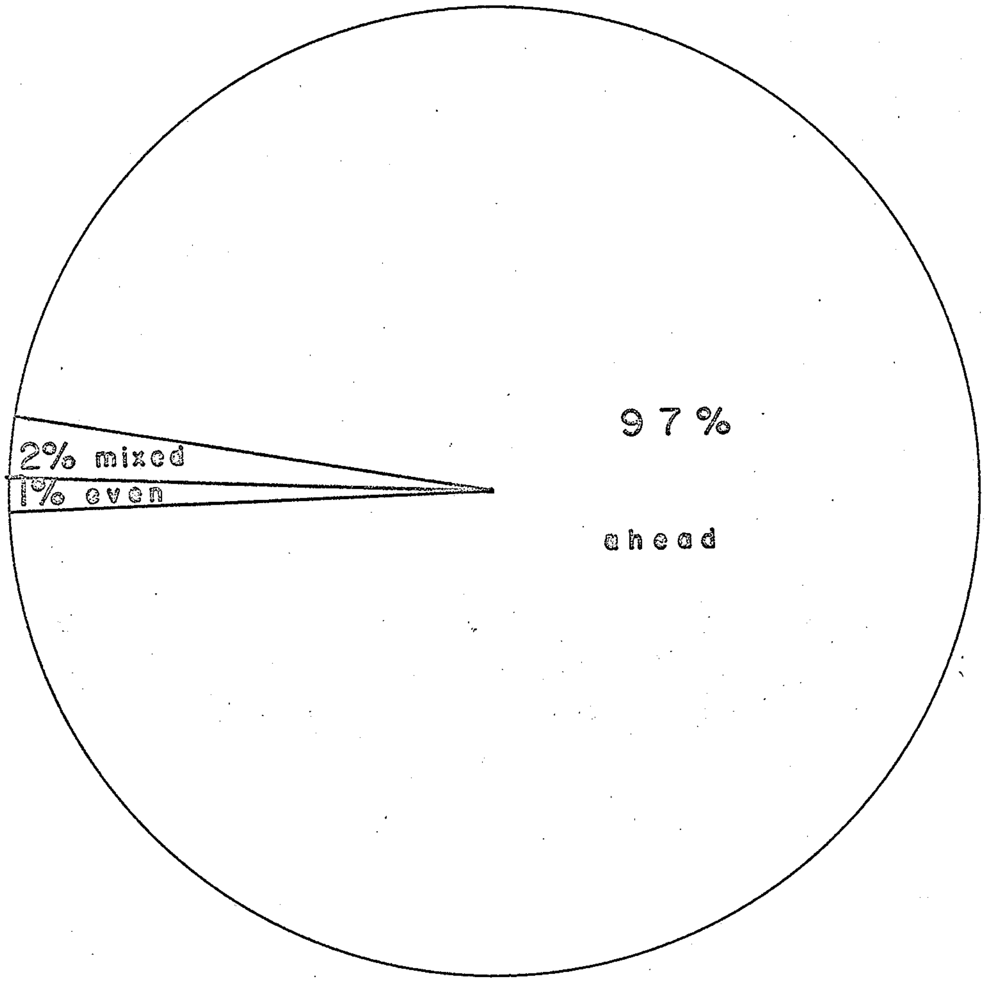


Figure 12: Position of the Anterior Tip of the Vitellaria in Population 2

Figure 13: Variation in the Anterior Extension of the Vitellaria in Relation to the Anterior Tips of the Uterus in Population 1

Mean	20.35
Standard deviation	± 12.40
Standard error	± 0.87
Coefficient of Variation	± 60.9%

Figure 13

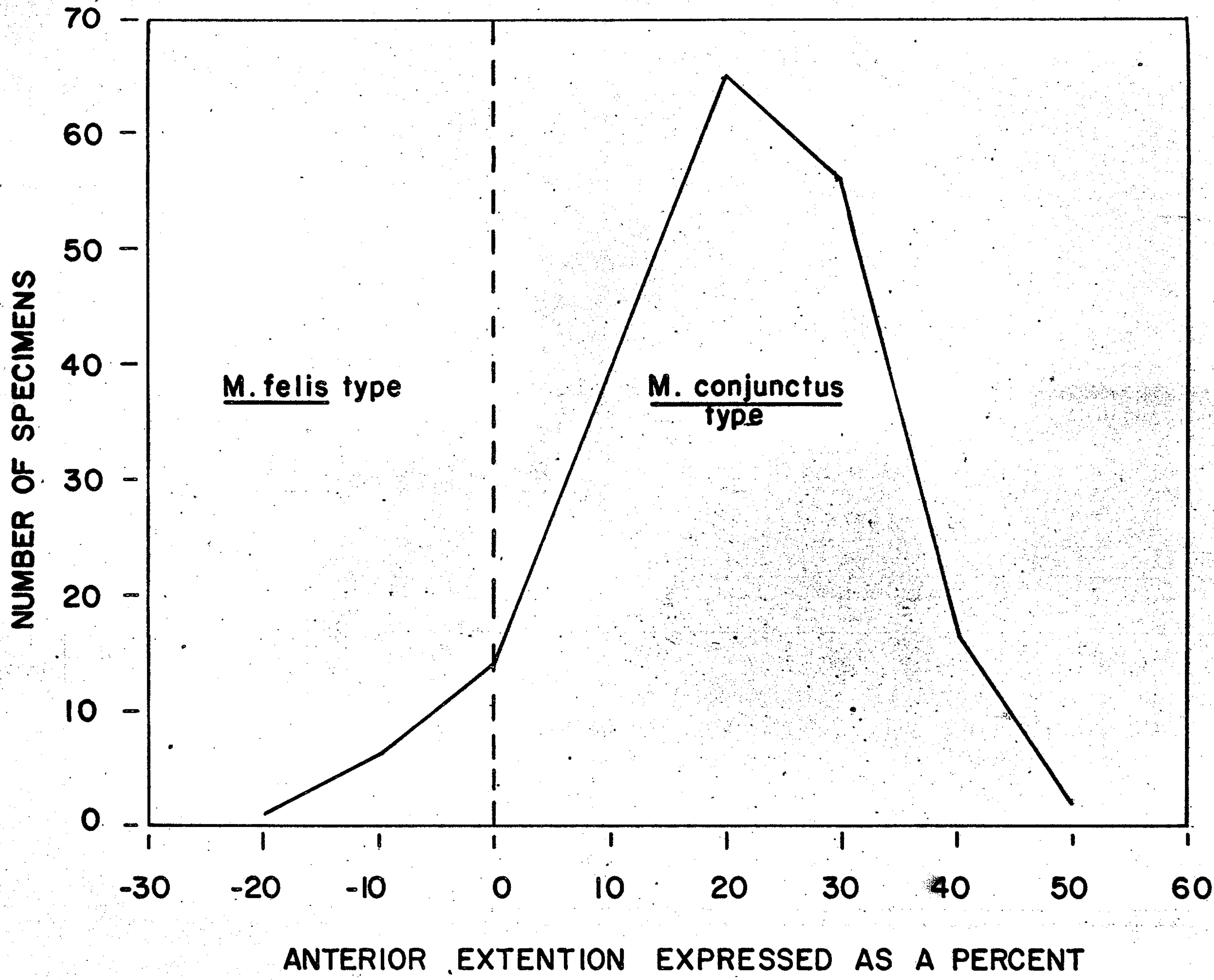

NUMBER OF SPECIMENS
0
10
20
30
40
50
60
70
-30
-20
-10
0
10
20
30
40
50
60
ANTERIOR EXTENTION EXPRESSED AS A PERCENT
M. felis type
M. conjunctus type

Figure 14: Variation in the Anterior Extension of the Vitellaria in Relation to the Anterior Tip of the Uterus in Population 2

Mean	21.1
Standard deviation	± 12.4
Standard error	± 0.97
Coefficient of Variation	± 58.8%

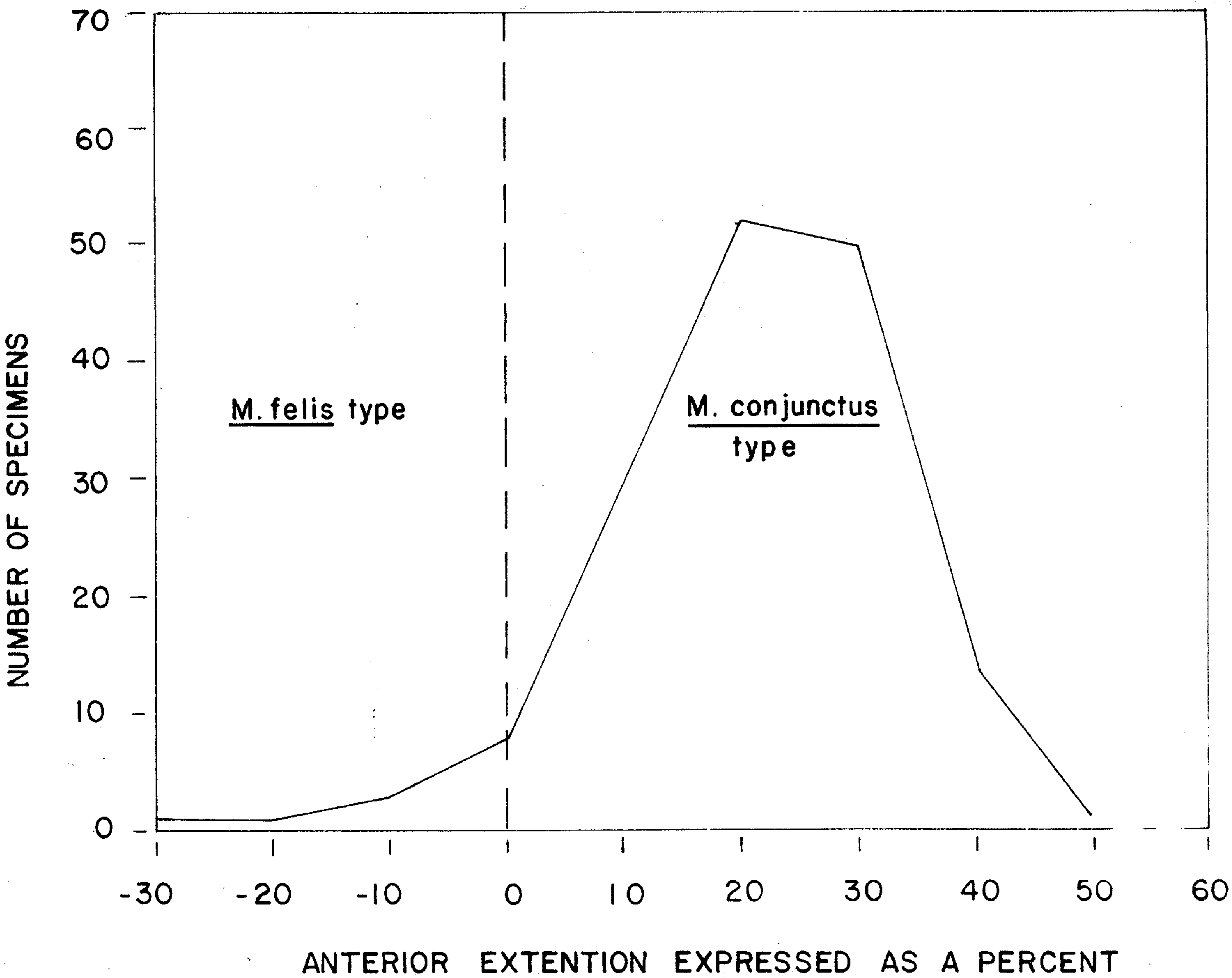

NUMBER OF SPECIMENS
70
60
50
40
30
20
10
0
M. felis type
M. conjunctus type
-30
-20
-10
0
10
20
30
40
50
60
ANTERIOR EXTENTION EXPRESSED AS A PERCENT

Figure 14

Convergence of the Vitellaria Anterior to the Uterine Coils

This character was given serious consideration as it was regarded as a species characteristic of *Metorchis complexus* (Stiles & Hassall) (30) differentiating it from *Metorchis albidus* (Braun).

TABLE IX

COMPARISON OF THE CONVERGENCE OF THE ANTERIOR TIPS OF THE VITELLARIA IN POPULATIONS 1 AND 2

Convergence	Population 1 % $\pm$ m$_{\%}$	Population 2 % $\pm$ m$_{\%}$
Vitellaria converging (Fig. 15)	2 $\pm$ 1.40	1 $\pm$ 0.99
Vitellaria mixed (Fig. 16)	1 $\pm$ 0.99	1 $\pm$ 0.99
Vitellaria not converging (Fig. 17)	97 $\pm$ 1.71	98 $\pm$ 1.40

Population 1, 238 specimens; population 2, 293 specimens. 100 specimens were examined from each population.

Figure 15: Both vitellaria are converging

Figure 16: Mixed, i.e. one row of vitellaria converges and one does not

Figure 17: Both vitellaria are not converging

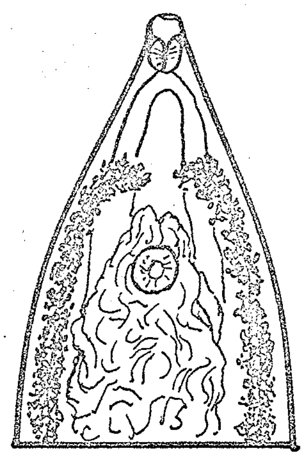


Figure 15

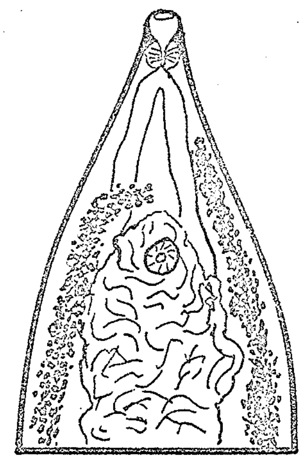


Figure 16

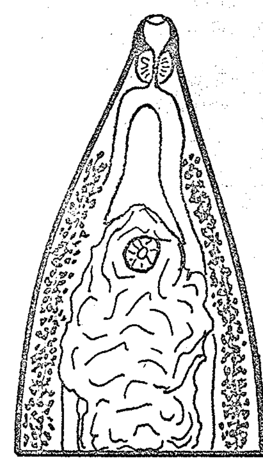


Figure 17

Lobation of the Ovary

This particular characteristic was examined because it was chosen by Stiles and Hassall (30) as a specific character when in 1894 they have described Distomum complexum, as a new species distinct from Metorchis albidus (Braun, 1893).

TABLE X

COMPARISON OF OVARY LOBATION IN POPULATIONS 1 AND 2

Lobation	Population 1 % ± $m_{\%}$	Population 2 % ± $m_{\%}$
Entire	39 ± 4.88	56 ± 4.96
2 lobes	3 ± 1.71	3 ± 1.71
3 lobes	46 ± 4.98	40 ± 4.90
4 lobes	12 ± 1.72	1 ± 0.99

Population 1 contains 238 specimens; population 2 contains 293 specimens. 100 specimens were examined from each population. See Figs. 18 and 19.

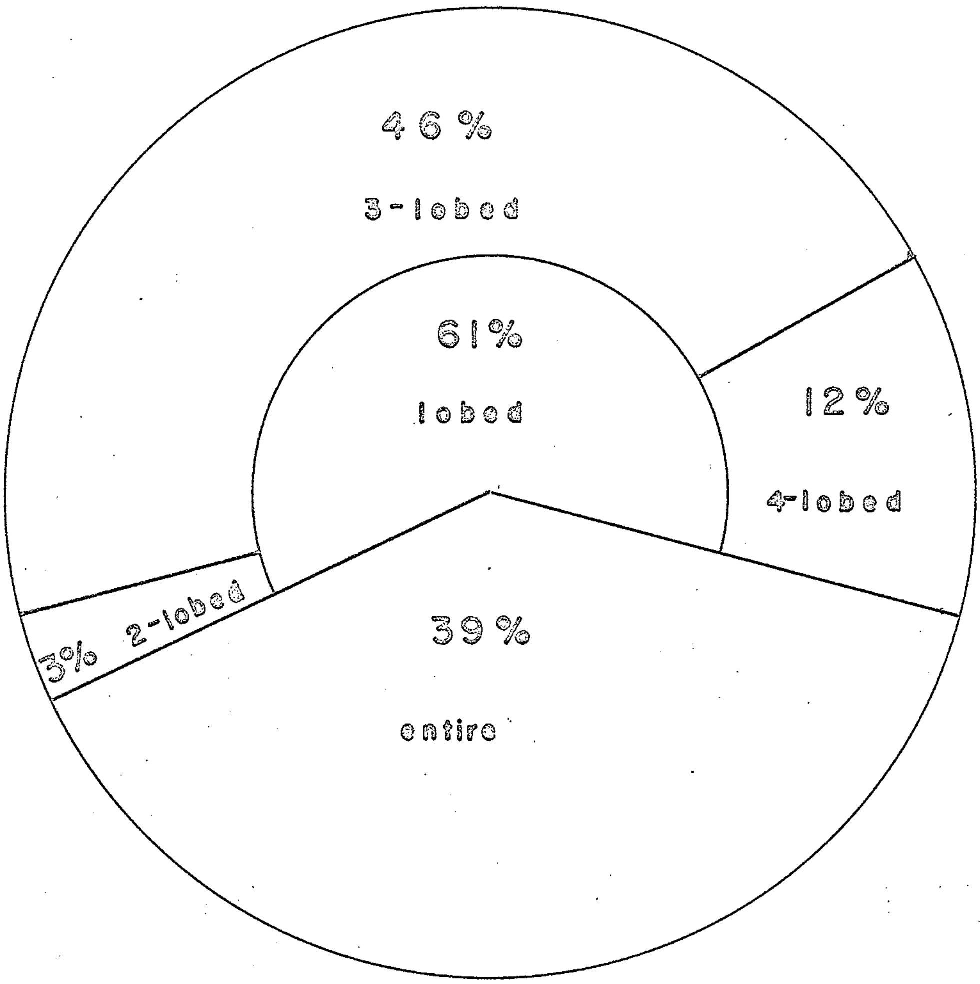


Figure 18: Variation of Ovary Lobation in Population 1

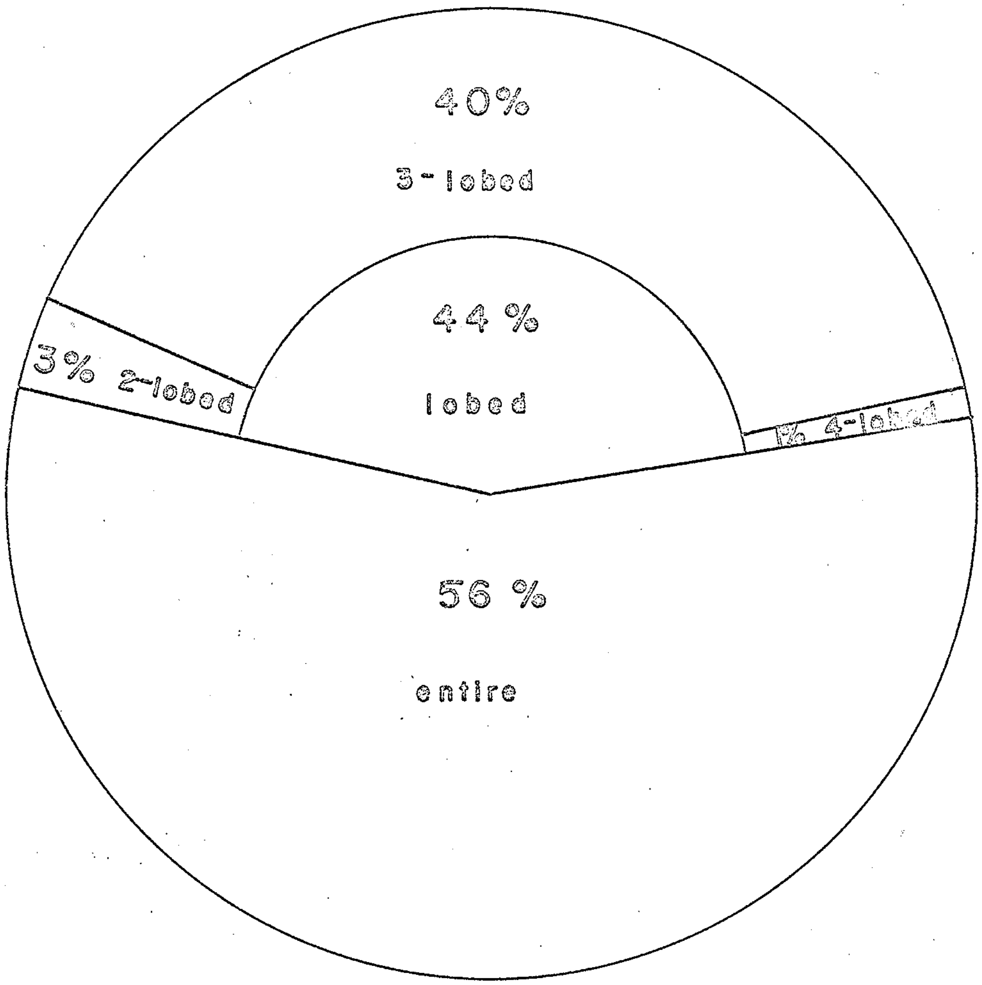


Figure 19: Variation of Ovary Lobation in Population 2

Variation in the Egg Size of <u>Metorchis</u> <u>conjunctus</u>

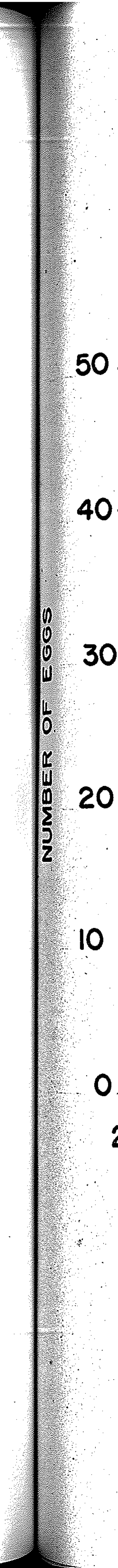
The variation in egg size was studied because this size is widely used for the differentiation of trematode species. One hundred were measured, all of which came from the same individual. The egg length ranged from 22 to 32 and the width from 14 to 19 .

The variation curves of egg length and egg width are represented in Figs. 21 and 22. Fig. 20 is a photomicrograph taken at a magnification of 3000.

Figure 20: Photo Micrograph of <u>Metorchis</u> <u>conjunctus</u>
Eggs x 3000

Figure 21: Variation in the Length of the Eggs of *Metorchis conjunctus*. Based on 100 Eggs, from one worm.

Mean	27.32 μ
Standard deviation	$\pm$ 1.66 μ
Standard error	$\pm$ 0.17 μ
Coefficient of Variation	$\pm$ 6.1%



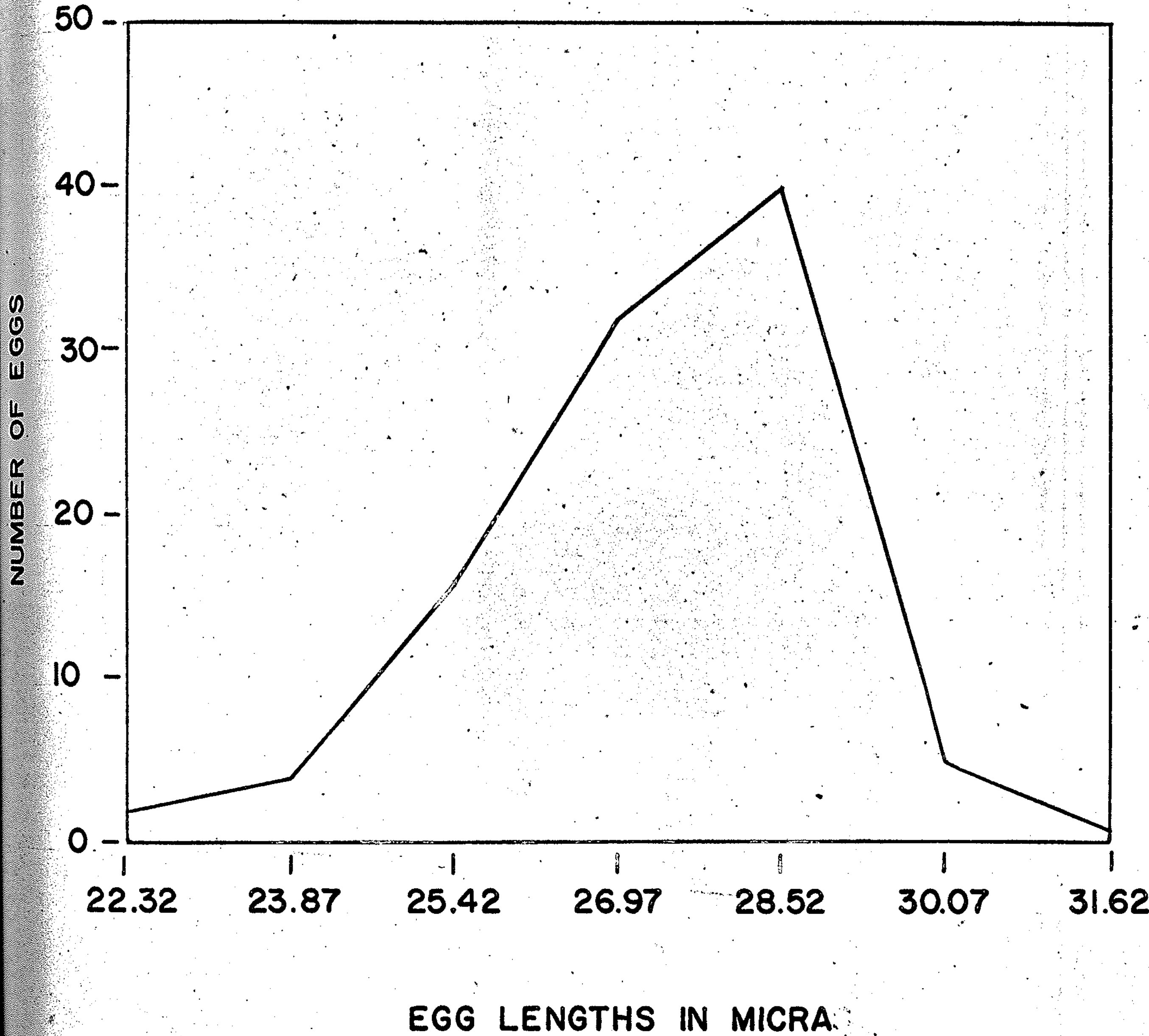

NUMBER OF EGGS
50
40
30
20
10
0
22.32
23.87
25.42
26.97
28.52
30.07
31.62
EGG LENGTHS IN MICRA

Figure 21

Figure 22: Variation in the Width of *Metorchis conjunctus* Eggs. Based on 100 Eggs, from one worm

Mean		15.50 μ
Standard deviation	±	0.68 μ
Standard error	±	0.07 μ
Coefficient of Variation	±	4.40%

NUMBER OF EGGS

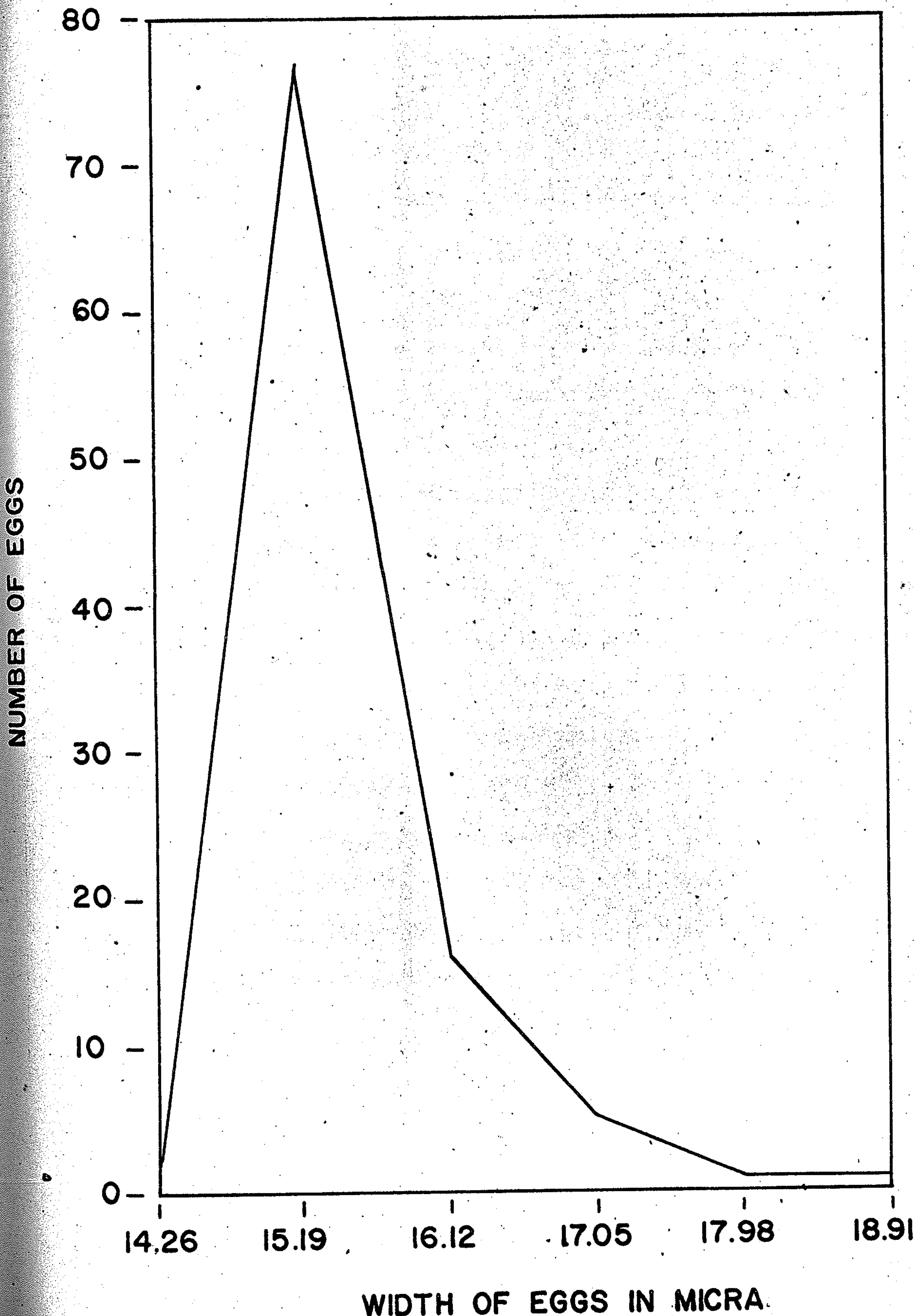

80
70
60
50
40
30
20
10
0
NUMBER OF EGGS
14.26
15.19
16.12
17.05
17.98
18.91
WIDTH OF EGGS IN MICRA

Figure 22

Lobation of the Testes

Cameron used this character in 1944 (10) when separating Metorchis conjunctus (Cobbold, 1860) from Metorchis albidus (Braun, 1893) and Metorchis felis Hsu, 1934. He used the following terms to describe the lobation of the testes: "entire", meaning the testes are not lobed; "slightly lobed", meaning the testes are lobed, but the lobes are not sharply defined; and "deeply lobed", meaning the testes are lobed and the lobes are sharply defined.

Examples of all three categories were present in each of the two populations studied. (See Figs. 23, 24, 25, 26) Also observed, however, were animals with one testis lobed and the other entire (TABLE XII) and animals with one testis deeply lobed, and the other slightly lobed. These cases are of course combinations, in one individual, of the different categories. (See Figs. 27 and 28)

The variation in the number of lobes per testis was also studied (TABLE XI). This lobation was found to be quite variable, the number of lobes of the anterior testes varying from 0 - 4 and that of the posterior testes from 0 - 6. (See Figs. 23, 24, 25, 26)

TABLE XI

VARIATION IN LOBATION OF THE INDIVIDUAL TESTES

Lobation	Anterior Testes		Posterior Testes	
	Population 1 % ± m%	Population 2 % ± m%	Population 1 % ± m%	Population 2 % ± m%
Entire	42 ± 4.93	66 ± 4.74	31 ± 4.63	50 ± 5.00
2 lobes	16 ± 3.67	10 ± 3.00	6 ± 2.37	5 ± 2.18
3 lobes	15 ± 3.56	11 ± 3.13	14 ± 3.47	10 ± 3.00
4 lobes	27 ± 4.44	13 ± 3.36	20 ± 4.00	14 ± 3.47
5 lobes	- -	- -	28 ± 4.49	20 ± 4.00
6 lobes	- -	- -	1 ± 0.99	1 ± 0.99

TABLE XII

VARIATION IN THE TESTES COMBINATIONS OF THE INDIVIDUAL WORMS

Testes Combination	Population 1 % ± m%	Population 2 % ± m%
Both testes lobed	52 ± 4.99	28 ± 4.49
Anterior lobed posterior entire	7 ± 2.55	6 ± 2.37
Anterior entire posterior lobed	19 ± 3.92	23 ± 4.21
Both testes entire	22 ± 4.14	43 ± 4.95

Population 1, 238 specimens; population 2, 293 specimens. 100 specimens were examined from each population.

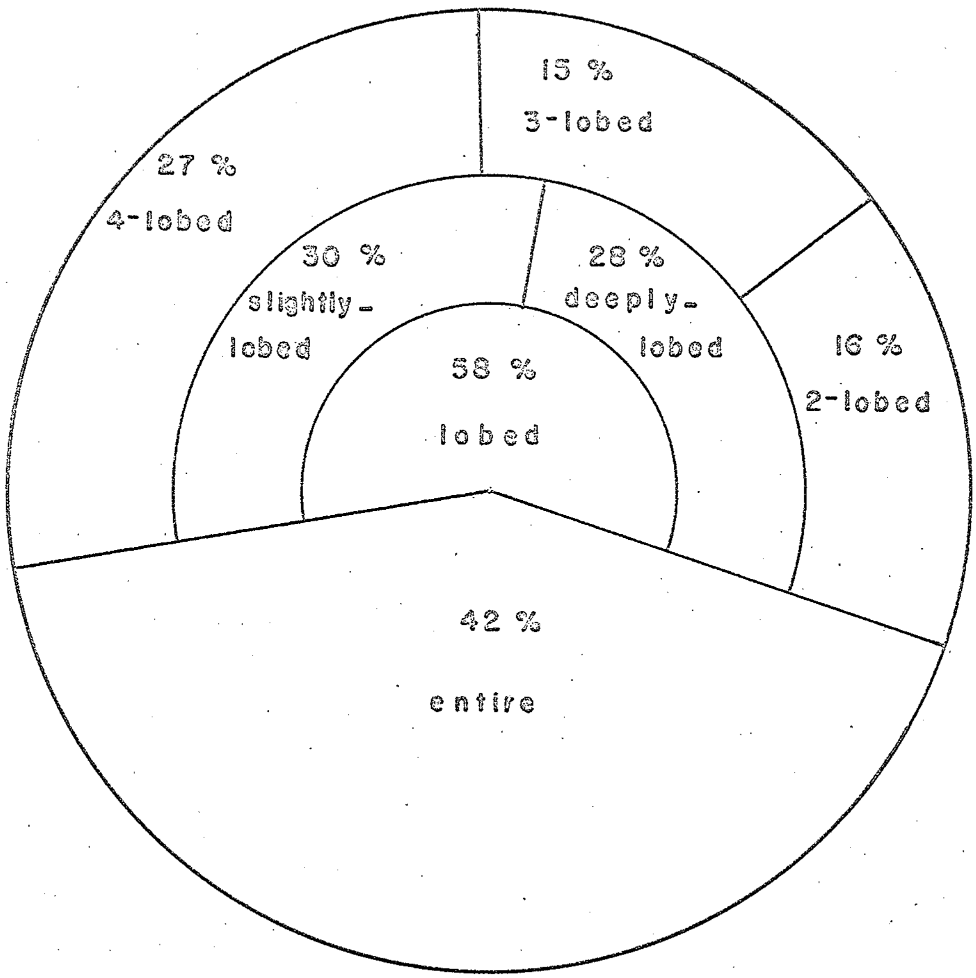


Figure 23: Breakdown of Anterior Testes Lobation of Population 1

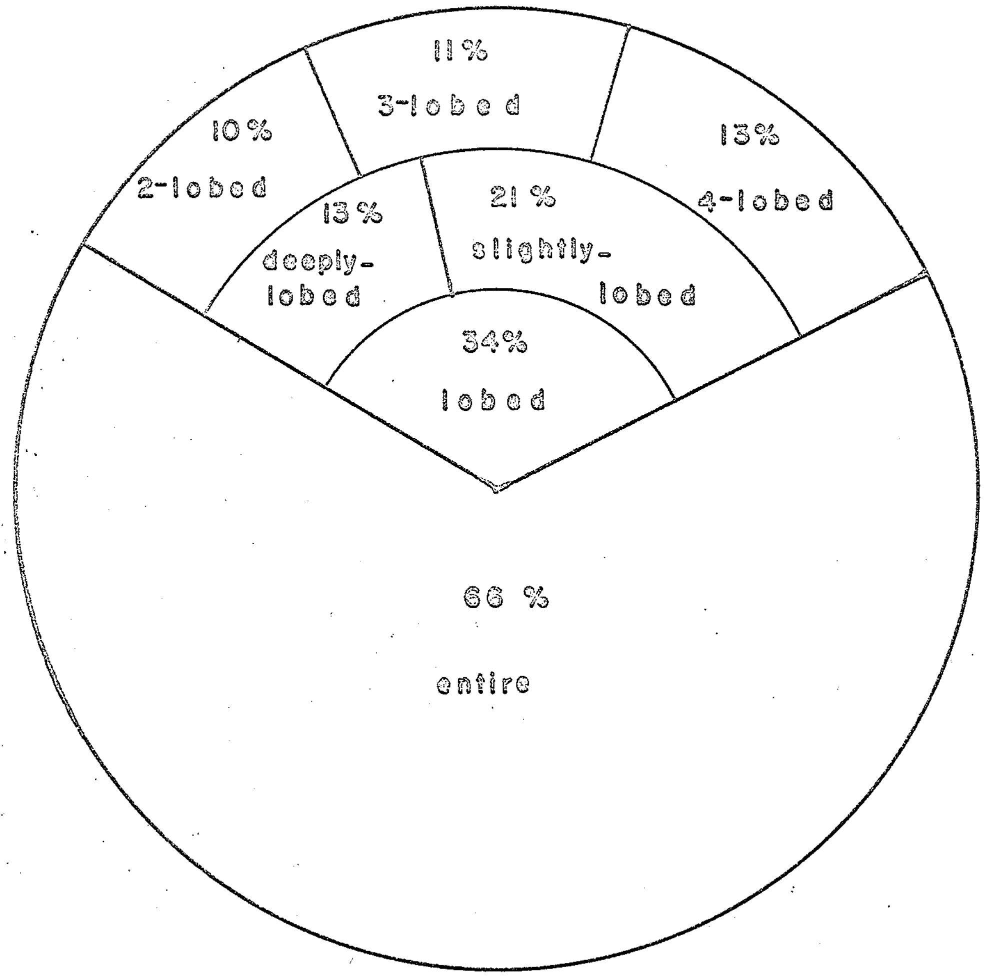


Figure 24: Breakdown of Anterior Testes Lobation of Population 2

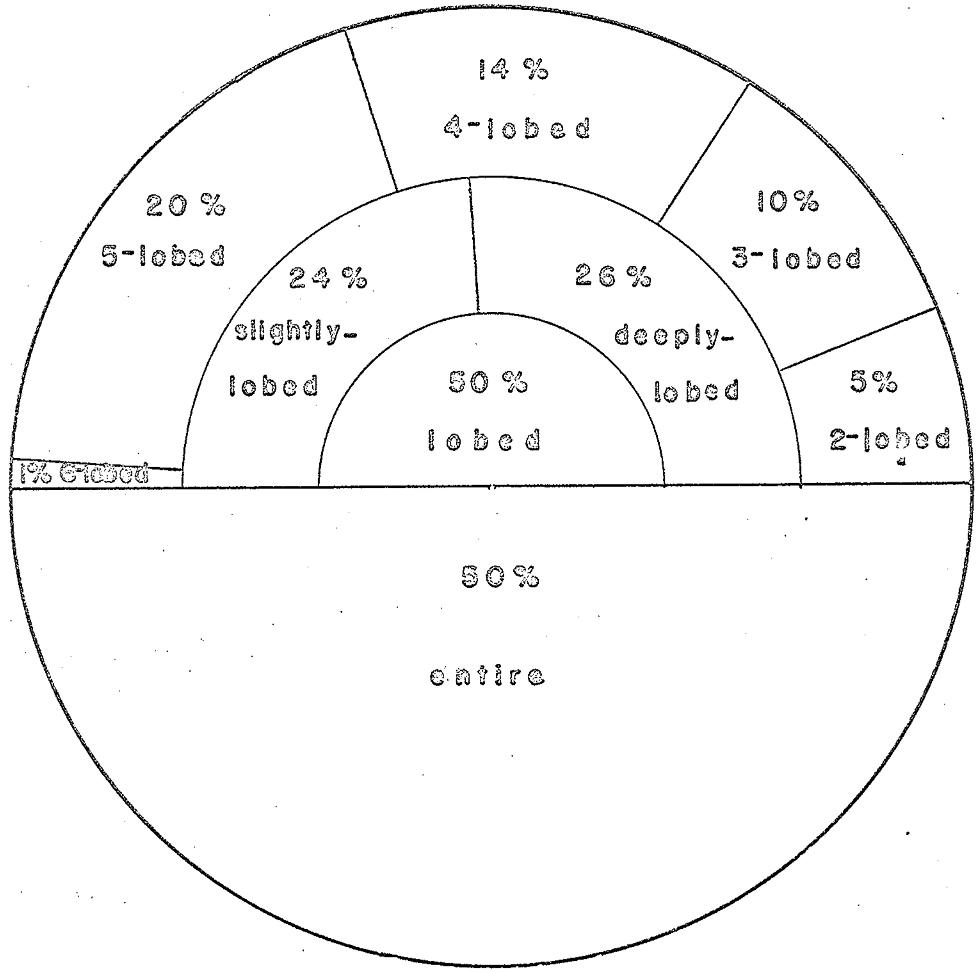


Figure 25: Breakdown of Posterior Testes Lobation of Population 1

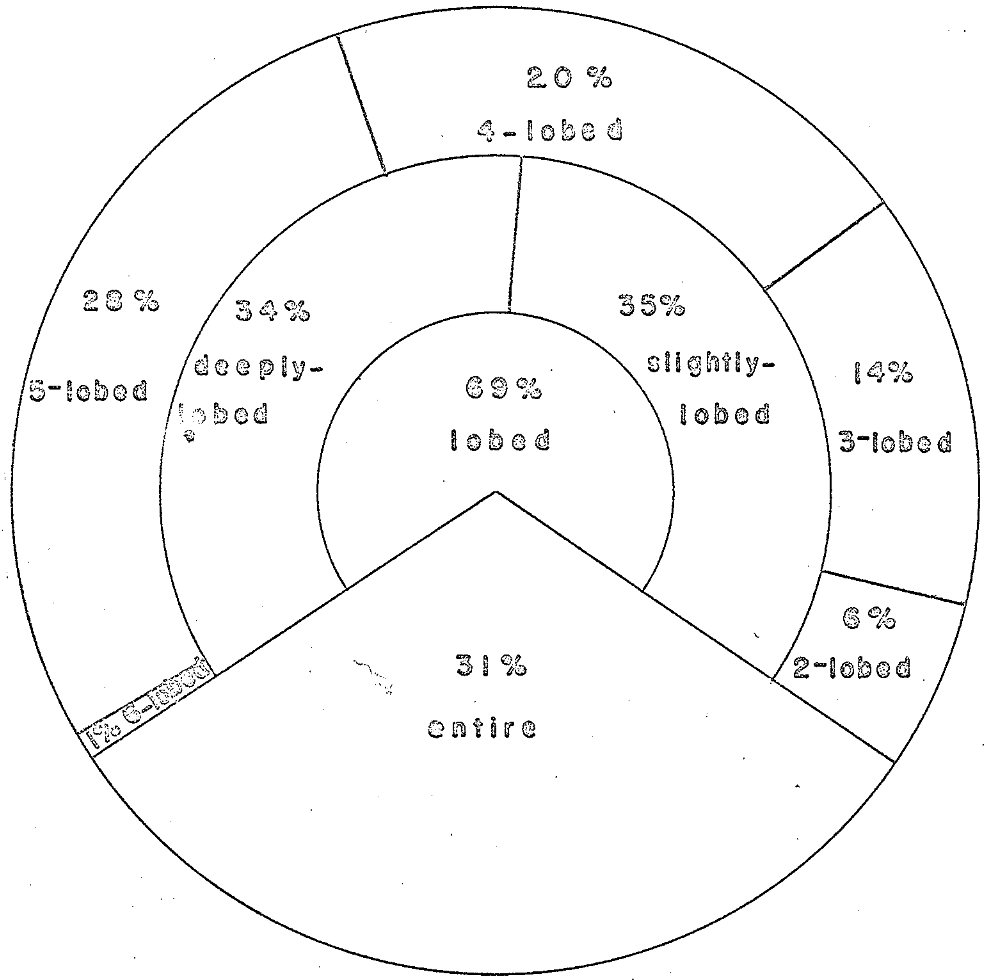


Figure 26: Breakdown of Posterior Testes Lobation of Population 2

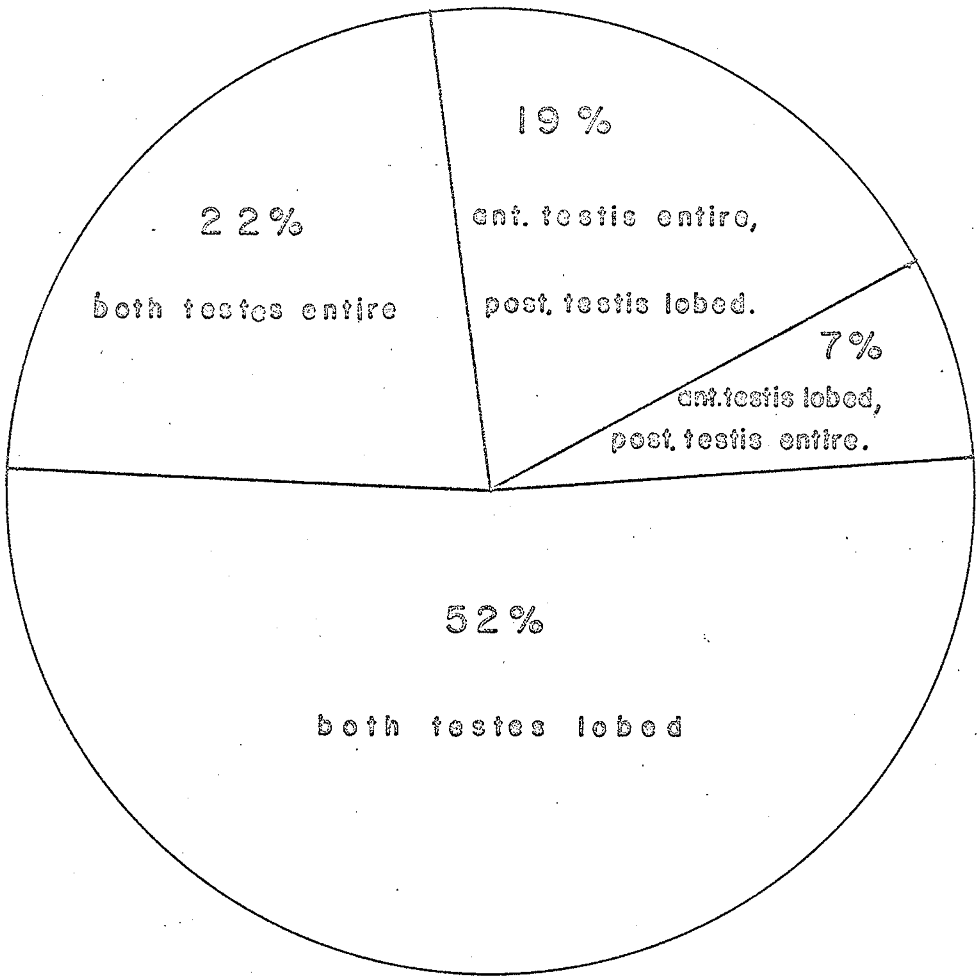


Figure 27: Variation in Testes Combinations of Individual Worms in Population 1

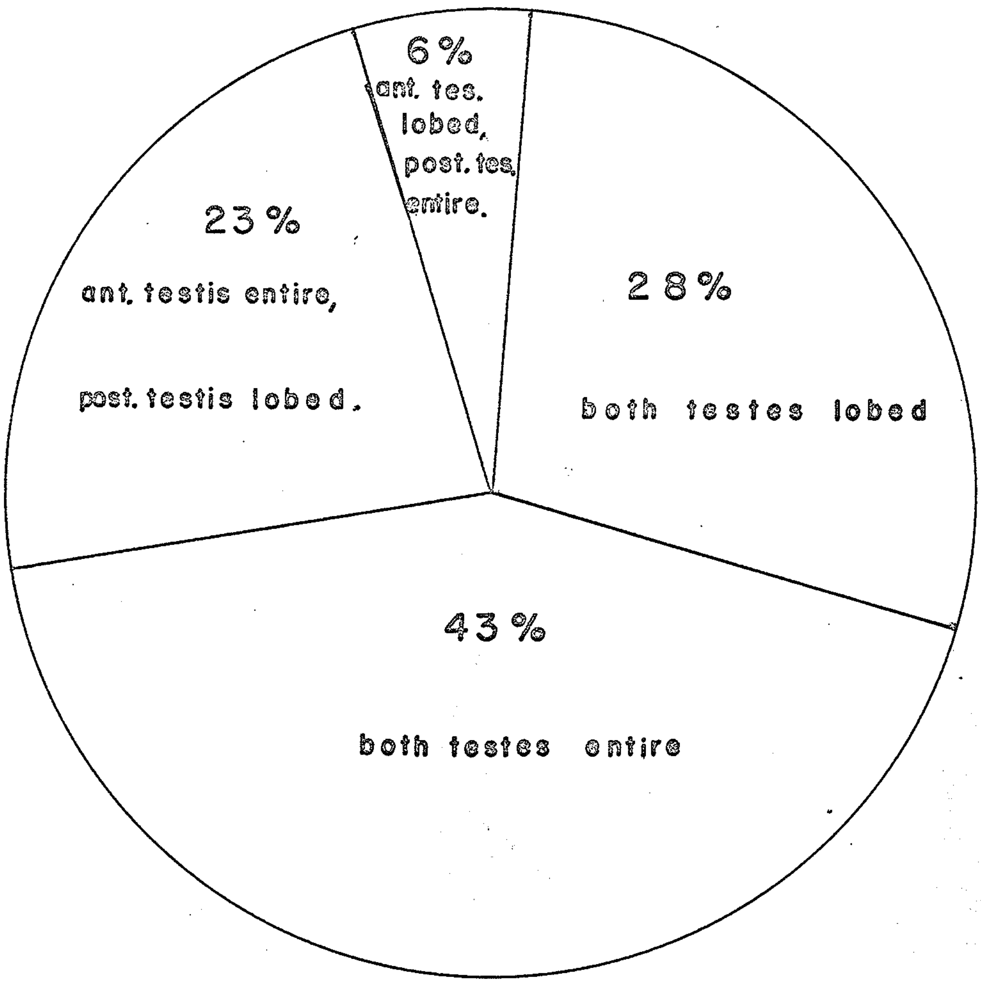


Figure 28: Variation in Testes Combinations of Individual Worms in Population 2

Position of the Testes

The testes were found to be arranged in two different ways in the posterior portion of the body. They were either positioned obliquely to the body axis or in the saggital plane, one behind the other. This last characteristic was emphasized by Skrjabin (1913) (29 p. 248) who regarded it as a generic character of the genus Parametorchis. (See Pl. 5)

TABLE XIII
COMPARISON OF TESTES ARRANGEMENTS IN POPULATIONS 1 AND 2

Population	Testes arranged obliquely % $\pm m_E$	Testes in saggital plane one behind the other % $\pm m_E$
1	64 $\pm$ 1.95	36 $\pm$ 1.95
2	39 $\pm$ 4.88	61 $\pm$ 4.88

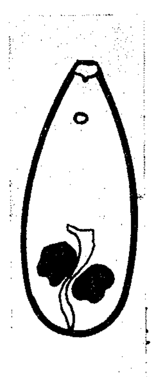


Figure 29

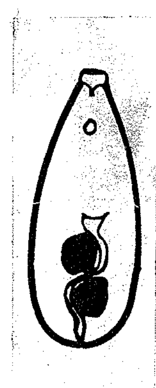


Figure 30

Figure 29: Testes Arranged Obliquely

Figure 30: Testes Arranged in Saggital Plane One Behind the Other

Population 1 contains 238 specimens; population 2 contains 293 specimens. 100 specimens were examined from each population.

DISCUSSION

In the past it was a common practice to describe new species of parasites on the basis of the examination of only a few specimens. Often specific value was ascribed to morphological characters differing but slightly from those of the known species of the genus. In many cases differentiation of species has been based on highly variable characters and, as a result, individual variants have been described as separate species. Mayr (24) points out that the under-estimation of intrapopulation variation, the individual variation, is the reason for the existence of more than half the synonyms. He stresses that, "A careful study of the phenomena of individual variation in general, and specifically in the group with which the taxonomist is concerned, is an indispensable prerequisite to all sound taxonomic work." (24)

The five species of _Metorchis_ parasitizing mammals are separated from one another on the basis of one or more of the following characteristics: (TABLE XIV)

1. Degree of lobation of the ovary
2. Relation of the anterior end of the vitellaria to the anterior tip of the uterus
3. Convergence of the anterior tips of the vitellaria
4. Size of the eggs
5. Degree of lobation of the testes

TABLE XIV

CHARACTERS USED BY DIFFERENT AUTHORS TO SEPARATE THE MAMMALIAN SPECIES OF *METORCHIS*

Author	Species	1 Degree of ovary lobation	2 Rel. of ant. end of the vit. to ant. tip of the uterus	3 Converg. of the ant. tips of the vitellaria	4 Egg Size in mm.	5 Degree of testes lobation	6 Arr. of the testes	7 Body Size in mm.	8 Body Shape
Stiles & Hassall 1894	*M. complexus*	trilobed		converge	0.024x0.012			5-6	
	M. albidus	entire		do not converge	0.027-0.032 X 0.013-0.016			2.5-3.5	
Hsu; Du, & Chow 1938	*M. felis*		vitellaria do not exceed the anterior tip of the uterus		0.023-0.030 X 0.013-0.016	lobation more pronounced than in *M. albidus*		3.3-5.8 X 1.2-1.72	Posterior portion narrower than that of *M. albidus*
	M. albidus		vitellaria exceed the ant. tip of the uterus		0.027-0.032 X 0.013-0.016			2.15-2.60 X 1.20-1.55	
Cameron 1944	*M. conjunctus*		" " "			testes entire or slightly-lobed			Linguiform
	M. albidus		" " "			testes slightly-lobed			Spatulate
	M. felis		vitellaria do not exceed the anterior tip of the uterus			testes deeply-lobed			Linguiform

6. Arrangement of the testes

7. Body size

8. Body shape

These five species are M. albidus, M. felis, M. complexus, M. skrjabini and M. conjunctus. Cameron in 1944 pointed out that three of them, M. conjunctus, M. albidus and M. felis, can be separated from each other on the basis of three of the characters listed above: 2. relation of the anterior end of the vitellaria to the anterior tip of the uterus; 5. body shape; 6. degree of lobation of the testes. He commented on the morphological peculiarities as follows:

> M. albidus: Europe. Body spatulate, testes slightly lobed, yolk glands extend anterior to uterine coils. M. felis: China. Body linguiform, testes deeply lobed, yolk glands do not extend anterior to the uterine coils. M. conjunctus: North America. Body linguiform, testes entire or slightly lobed, yolk glands extend anterior to the uterine coils. (10)

Hsu, Du, and Chow in 1938 compared M. felis with M. albidus and pointed out that these species can be separated on the basis of four of the aforementioned characters: 2. relation of the anterior end of the vitellaria to the anterior tip of the uterus; 5. body shape; 8. body size. They say that

> After a comparison of the European and Chinese Metorchis specimens, we found that, in general, all essential morphological characteristics were the same. However, a careful study revealed certain definite differences. The European specimens are comparatively smaller than the Chinese specimens. The posterior

> portion, where the two testes are situated, is more spacious and broader in the European specimens than in the Chinese specimens. The anterior margin of the vitelline gland of the European specimens usually extends beyond the anterior margin of the uterine convolutions, whereas in the Chinese specimens the anterior margin of both the vitelline gland and the uterine convolutions are usually at the same level. The lobulation of the testes in the European specimens are comparatively less distinct than in the Chinese specimens."

And further

> "We are therefore of the opinion that the _Metorchis_ species from cats and dogs in China do not belong to the European species, _Metorchis albidus_, and therefore it should be regarded as _Metorchis felis_, named by Y. C. Hsu in 1934. On the other hand we should like to point out that _Metorchis albidus_ and _Metorchis felis_ are certainly very closely related. Their specific differentiations are not very sharply marked as in the case with other species of trematodes, and it is possible that investigators may in the future find in certain specimens an overlapping of their specific variations. This might be especially true in regard to the anterior extension of the vitelline gland beyond the anterior margin of the uterine convolutions" (18).

Stiles and Hassall in 1894 also considered four of the characters listed above to be of specific significance when they described _Metorchis complexus_ as a species distinct from _Metorchis albidus_. The characters they dealt with are as follows: (30)

1. Body size

   M. complexus 5 - 6 mm. in length (preserved specimen)

   M. albidus 2.5 - 3.5 mm. in length

2. Convergence of the anterior tips of the vitellaria

   M. complexus   vitellaria converge

   M. albidus   vitellaria do not converge

3. Degree of lobation of the ovary

   M. complexus   ovary trilobed

   M. albidus   ovary entire

4. Size of the eggs

   M. complexus   0.024 by 0.012 mm.

   M. albidus   0.027 - 0.032 mm. by 0.013 - 0.016 mm.

When the author first examined specimens of Metorchis from the liver of an experimental cat, he believed that he had found individuals belonging to four species of Metorchis: M. felis, M. albidus, M. conplexus and M. conjunctus. However, further examination revealed specimens that had some characteristics of one species and some of another, being thus intermediate between two species. Within a single population many such forms were present, all with different combinations of characters believed to be peculiar to different species. Thus it became obvious that a detailed examination of the variability of morphological characteristics used to separate the mammalian species of Metorchis was necessary to determine whether or not these characters are of specific significance. This study should be based on as many large populations as possible.

In this work two such populations of Metorchis *conjunctus*, from the livers of experimental cats were examined. One consisted of 238 worms, the other of 293. Characteristics listed previously were studied and all were found to be quite variable. So many specimens with different combinations of these characters were present that the separation of the worms into groups on the basis of them was impossible (TABLE XV). Yet these characters have been used to separate the mammalian species of *Metorchis* from each other; each species being described as possessing a definite combination of these characters.

The examination of the two populations has shown that some of the characters varied continuously. e.g. the relation of the anterior end of the vitellaria to the anterior tip of the uterus (Figs. 13, 14). Specimens were found with the anterior ends of the vitellaria ahead, level with, or behind the anterior tip of the uterus (PLATE III, 9-11, 15-17). Moreover in some individuals one side of the body had the anterior end of the vitellaria ahead of the tip of the uterus while the other side had it behind the tip (PLATE III, 12, 13, 14). This fact, as well as the unimodal variation of the anterior extension of the vitellaria shows that this character can be used for specific differentiation only when studied statistically in sufficiently large populations and not in a few individuals.

Another character that varied continuously was the degree of testes lobation. The posterior testes varied from entire to six-lobed (Figs. 25, 26) and the anterior from

TABLE XV

RAW DATA SHOWING SOME OF THE MANY CHARACTER COMBINATIONS ENCOUNTERED IN THE EXAMINATION OF THE METORCHIS POPULATIONS

NO.	Vitellaria: Relationship to ant. end of uterus: ahead	behind	even	converge	dont converge	Ovary: entire	lobed	no. of lobes	BODY SHAPE: 1	2	3	Testes: Position: st. line	oblique	Lobation: Anterior: entire	sl. lobed	deeply lobed	Posterior: entire	sl. lobed	deeply lobed
1	I			I		I				I		I		I					I
2	I				I		I	4	I				I			I			I
3	I				I	I			I				I		I			I	
4	I				I		I	3		I		I		I			I		
5	I				I	I				I		I		I				I	
6			I		I		I	4			I	I				I	I		
7	I				I	I			I			I				I			I
8	I				I		I	3		I			I			I			I
9	I				I	I			I				I			I			I
10	I				I	I			I			I		I				I	
11	I				I	I				I			I	I				I	
12	I				I	I				I		I			I			I	
13	I				I		I	3			I	I			I			I	
14	I				I		I	4	I				I	I				I	
15	I				I	I			I			I				I			I
16	I				I		I	3	I				I			I			I
17	I				I		I	3	I				I	I			I		
18	I				I		I	3		I		I			I			I	
19	I				I	I			I				I		I		I		
20	"mixed"				I	I				I		I		I				I	
21	I				I		I	3	I				I		I		I		
22		I					I	3	I				I	I			I		
23	I				I		I	3	I				I		I			I	

entire to four-lobed (TABLE XI; Figs. 23, 24). The testes also ranged from entire, through slightly-lobed to deeply-lobed (PLATE VI, 31-34). There is no definite line of distinction between slightly-lobed and deeply-lobed testes.

The excretory duct divides the posterior portion of the fluke into right and left halves (30) with one testes in each. Specimens were found in which one testis was lobed and the other was entire (Figs. 27, 28).

Thus, as in the case of the anterior extention of the vitellaria, one side of the individual had a characteristic of one species, the other side that of another species. This fact, together with the continuous variation of the testes lobation, makes it clear that this lobation cannot be regarded as a character suitable for species differentiation.

The body size of the flukes in the two populations were found to vary over a range wide enough to include the body sizes of all the mammalian species of Metorchis (Figs. 6, 7, 8, 9). All the worms in the populations studied were sexually mature and approximately of the same age. Thus it is obvious that the body size is valueless for the differentiation of species (Cameron, 1944). (10)

It is known that in Opisthorchis felineus the body size, the lobation of, and the size of the testes, depend on the size of the population; individuals belonging to small populations are larger and have both absolutely and relatively larger and more deeply-lobed testes. On the other hand worms from larger populations are smaller and possess smaller testes with less pronounced lobation (22). In the populations studied here the same seemed to be true for body size and testes lobation.

The convergence of the anterior ends of the vitellaria did not show much variation. Only in a small percentage of each population did the anterior tips of the vitellaria converge (TABLE IX). Specimens were found in which the anterior end of one row of vitellaria was bent medially while that of the other was not (PLATE II, 7). Such variants, plus the presence of individuals both with non-converging and with converging vitellaria (PLATE II, 5, 6, 8) in both populations led the author to believe that the convergence of the vitellaria is in this case a character of an individual variant and not characteristic of a species.

The range of variation in the size of the eggs was found to be wide enough to include the egg sizes of all other mammalian species of the genus Metorchis (Figs. 21, 22). Thus it is obvious that this character cannot be used to separate these species unless large numbers of eggs are measured and the mean values are found to differ significantly.

The arrangement of the testes, that is whether they are placed obliquely or in the saggital plane of the body (PLATE V, 27-30), was considered by Skrjabin (1913) (29 p. 248) to be a character of generic significance. He created the genus *Parametorchis* which he said differed from *Metorchis* as it had the saggital plane arrangement of the testes whereas *Metorchis* had the oblique one.

The author examined this character in both populations and found that the two types of arrangements were well represented in each (TABLE XIII). There is, however, no definite line of distinction between these two types of testes arrangement. Presumably in the saggital plane arrangement ("straight line") the line joining the centers of the two testes is parallel to the longitudinal axis of the body and in the oblique arrangement this line intersects the longitudinal axis (Fig. 31).

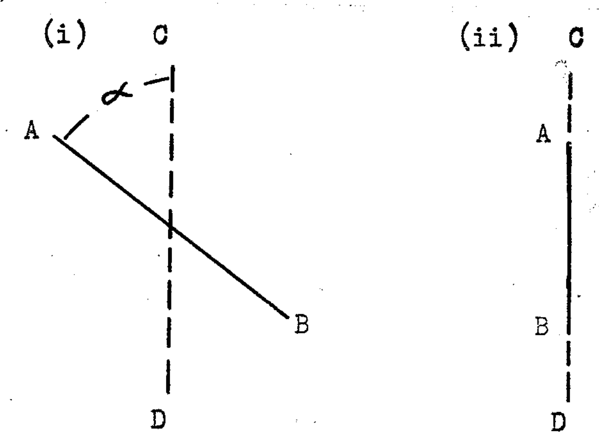


Figure 31: Explanation of Testes Arrangement
(i) Oblique arrangement
(ii) Saggital Plane arrangement

AB - Straight line joining the center of the testes

CD - Longitudinal axis of the body

α - Angle made by the intersection of AB and CD

The angle α in diagram (Fig. 31, i) is extremely variable. When it is small it is hard to tell whether the testes are arranged obliquely or in the saggital plane. An arbitrary line of distinction must be drawn between the two to make the distinction between "straight" and obliquely arrangement of the testes objective. This has never been done. However since the angle α is extremely variable (0° - 80°) and since it is highly unlikely that the two populations examined by the author consisted of more than one species, it is quite probable that such a line would have been of little taxonomic significance even if it would have been drawn.

The size of the angle α may depend to a degree on the state of contraction of the posterior portion of the animal. As the length of this portion increases the width decreases and the testes are forced towards the longitudinal axis. The result is a decrease in the size of the angle α. When the width increases and the length decreases the testes move away from the longitudinal axis and the angle α increases.

Stiles and Hassall (1894) stated that M. complexus has a trilobed ovary and that the ovary of M. albidus is entire. (30) The author examined the lobation of the ovary in the populations studied here and found specimens with entire, two-, three-, and four-lobed ovaries (Figs. 21, 22). This wide variation indicates that the lobation of the ovary is not a species characteristic but a highly individually variable character.

Though the body shape is extremely plastic in Metorchis, shapes characteristic of species have been described. Braun applied the term "spatulate" to M. albidus (7) and Cameron referred to M. conjunctus and M. felis as "linguiform" (10). Three basic body shapes were found in the populations studied here (Figs. 3, 4, 5). Shape 1. corresponds to "spatulate", Shape 2. to "linguiform" and Shape 3, though similar to "spatulate", differs from it by having the greatest width in the anterior portion of the body. When one compares the three shapes it becomes immediately apparent that they are really phases in a wave of body contraction; shape 2. being an intermediate phase between 1. and 3. (PLATE IV, 18-26). The shape of Metorchis, like that of many other trematodes, is to some extent dependent upon the state of contraction of the animal. It is probable that all the representatives of this genus can assume any of the shapes discussed. Therefore extreme caution must be exercised when using body shape to differentiate the species of this genus.

To establish the validity of a species described on the basis of only a few specimens it is necessary to examine the variability of the characters used for species differentiation in large populations of the organism in question. However most of the mammalian species have been described on the basis of only a few specimens, large series of paratypes have never been studied. The task of obtaining large populations of topotypes from all the type localities could hardly be tackled. As long as this is not done it can be suspected that species of *Metorchis* separated from each other on the basis of variable characters are not valid species but individual variants of *Metorchis conjunctus* (Cobbold, 1860). Further progress in the taxonomic study of the genus *Metorchis* can only be achieved on the basis of the examination of large populations and not of a few individuals.

PLATES

PLATE I

1 - 3 Paper negatives of *Amphimerus pseudofelineus*. 1 and 2 - Adults. 3 - Subadults.

4. Contact negative prints of *Amphimerus pseudofelineus* and *Metorchis conjunctus*. Note the differences in size and shape. Scale is in millimeters and centimeters.

Plate I

PLATE II

5 - 8 Paper negatives of the anterior portion of M. conjunctus x25. 5 and 6 - The anterior ends of the vitellaria converge. 7 - One row of vitellaria bends medially but the other does not. 8 - The anterior tips of the vitellaria do not converge.

Plate II

PLATE III

9 - 17 Paper negatives of the anterior portion of <u>Metorchis conjunctus</u> x55. 9, 10 and 11 - The anterior ends of the vitellaria are behind the anterior tip of the uterus. 12, 13 and 14 - The anterior end of one row of vitellaria is ahead while that of the other is behind the anterior tip of the uterus. 15, 16 and 17 - The anterior ends of the vitellaria are ahead of the anterior tip of the uterus.

Plate III

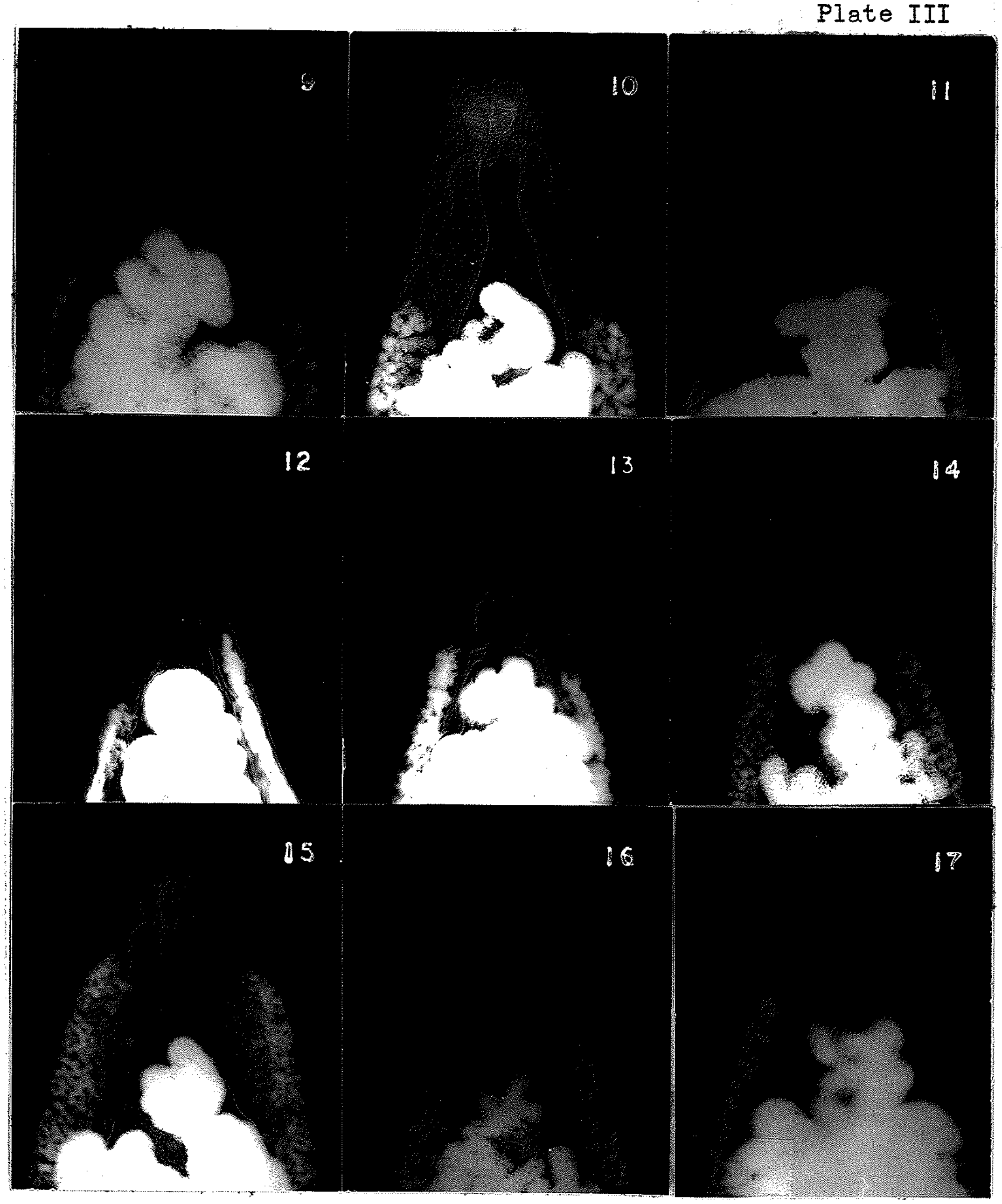


PLATE IV

18 - 26 Paper negatives of <u>Metorchis</u> <u>conjunctus</u> x10. 18, 19 and 20 - are examples of Shape 1. i.e. the greatest body width is in the posterior portion of the worm. 21, 22 and 23 - are examples of Shape 2. i.e. the anterior and posterior portions are of equal width. 24, 25 and 26 - are examples of Shape 3. i.e. the greatest body width is in the anterior portion of the body.

Plate IV

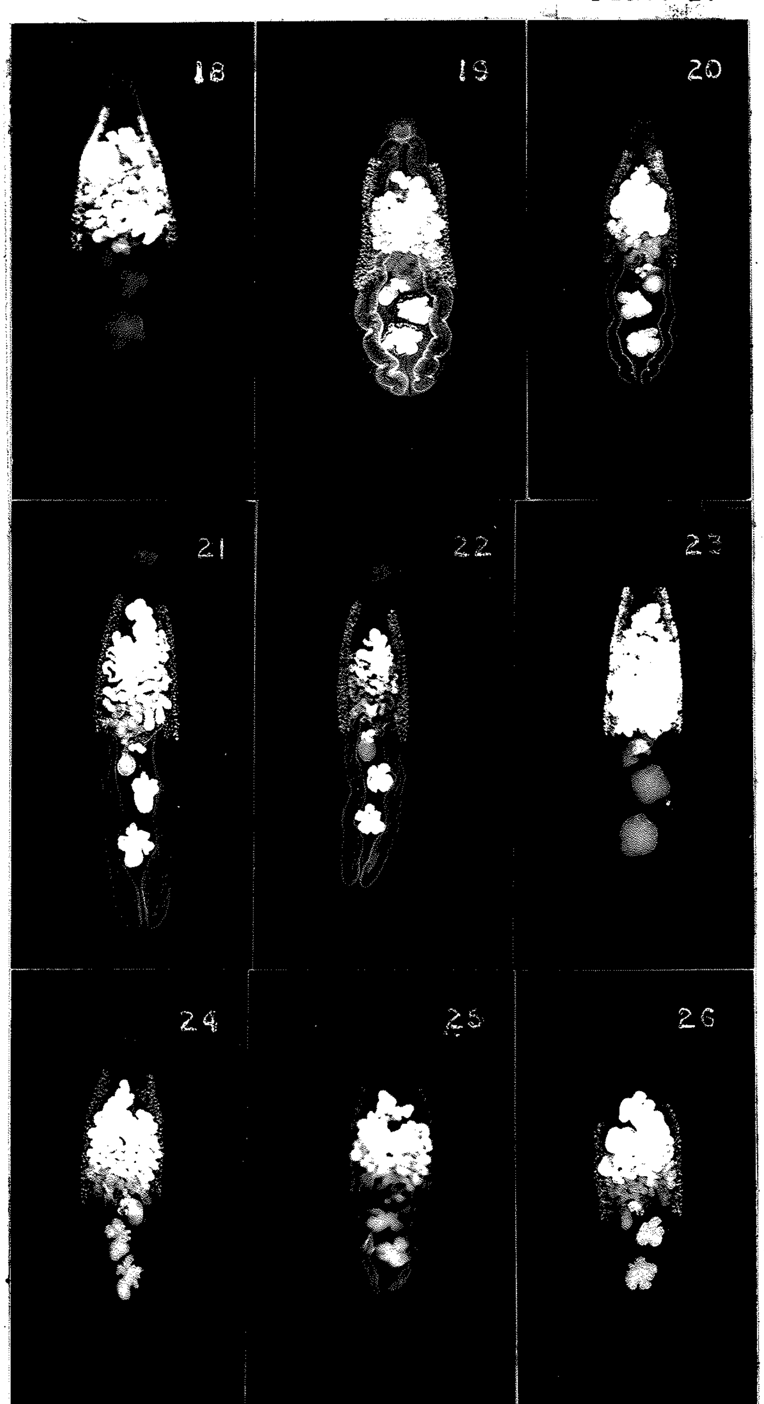


PLATE V

27 - 30 Paper negatives of the posterior portion of *M. conjunctus* x55. 27 and 28 - Testes are arranged obliquely. 29 and 30 - Testes are arranged in the sagittal plane one behind the other (i.e. in a "straight" line).

Plate V

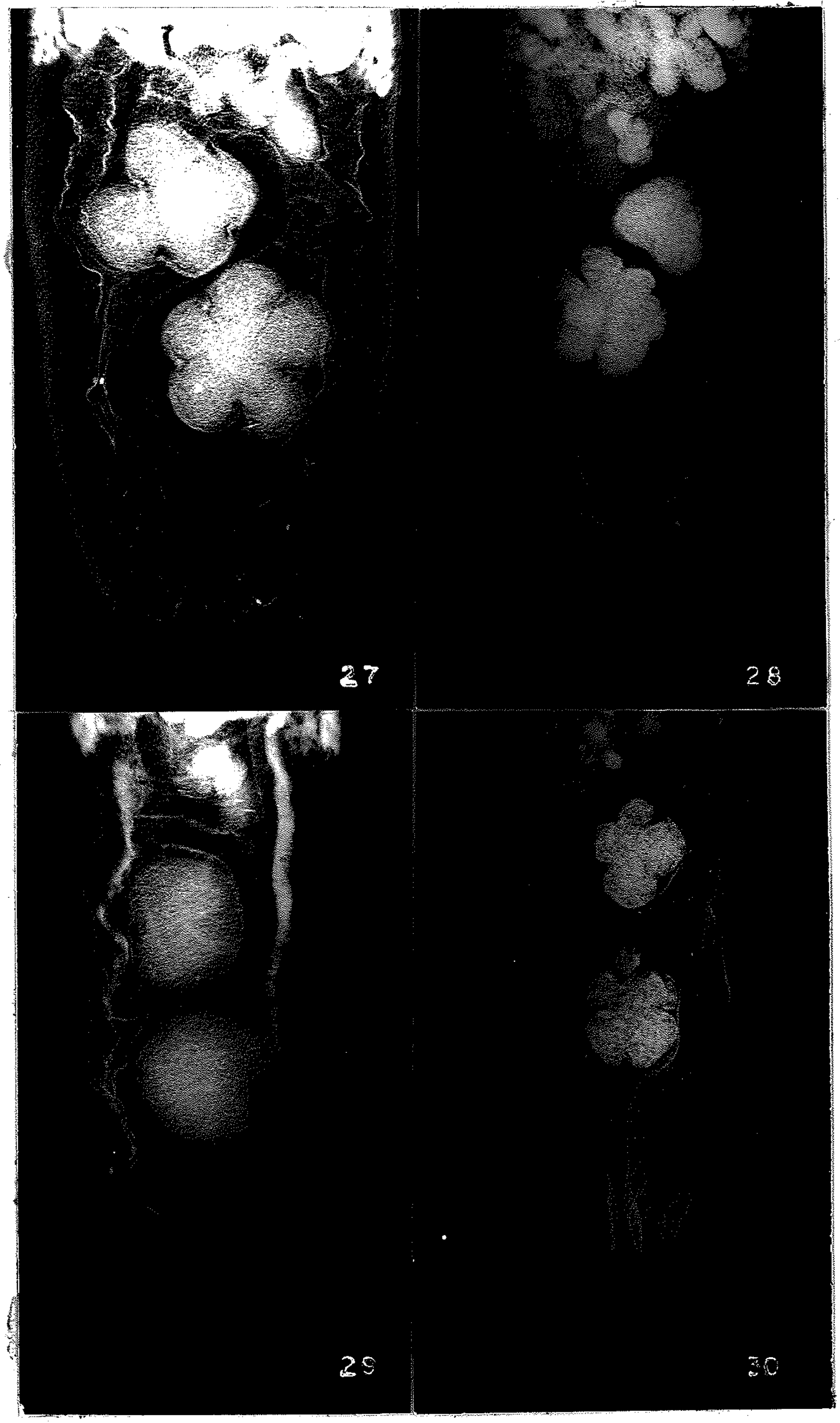
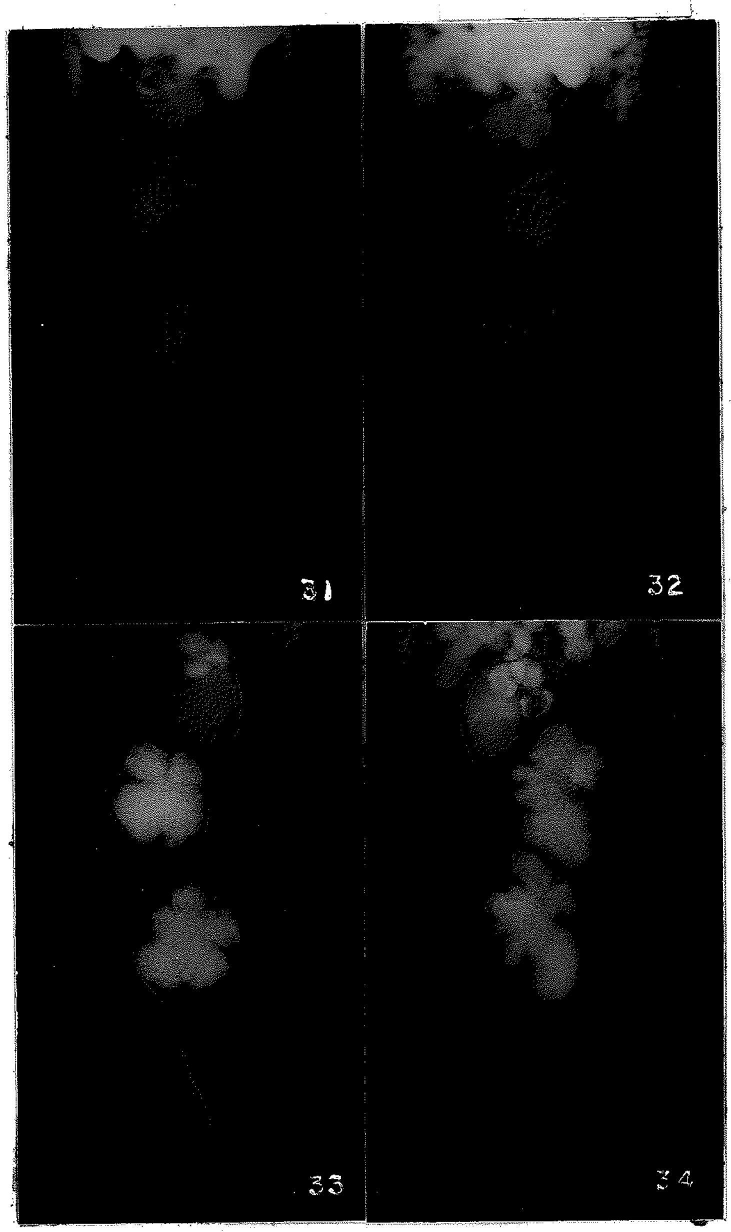


PLATE VI

31 - 34 Paper negatives of the posterior portions of *Metorchis conjunctus* x55. 31 - Testes entire; 32 - Testes slightly-lobed; 33 - Testes lobed; 34 - Testes are deeply-lobed.

Plate VI



SUMMARY

Two species of liver fluke were found in experimental cats fed fish flesh from two locations in Manitoba.

Amphimerus pseudofelineus: 36 specimens of this worm were taken from the liver of a cat fed *Catostomus commersonii* from Oak Point on Lake Manitoba. This is the first record of this parasite for Canada and the first record of its secondary intermediate host for the world.

Metorchis conjunctus: *Catostomus commersonii* and *Perca flavescens* from Black River on Lake Winnipeg were fed to experimental cats. Five cats became infected, one fed *P. flavescens* and four fed *C. commersonii*. This is the first record of *P. flavescens* serving as an intermediate host of this parasite and it is also the first finding of *Metorchis conjunctus* at Black River.

The variability of several characters used for the differentiation of the mammalian species of *Metorchis* was examined. All characters were found to be very variable. It is suggested that *M. felis*, *M. albidus*, *M. complexus* and *M. skrjabini* which are all separated from each other on the basis of these characters, are junior synonyms of *Metorchis conjunctus* (Cobbold, 1860).

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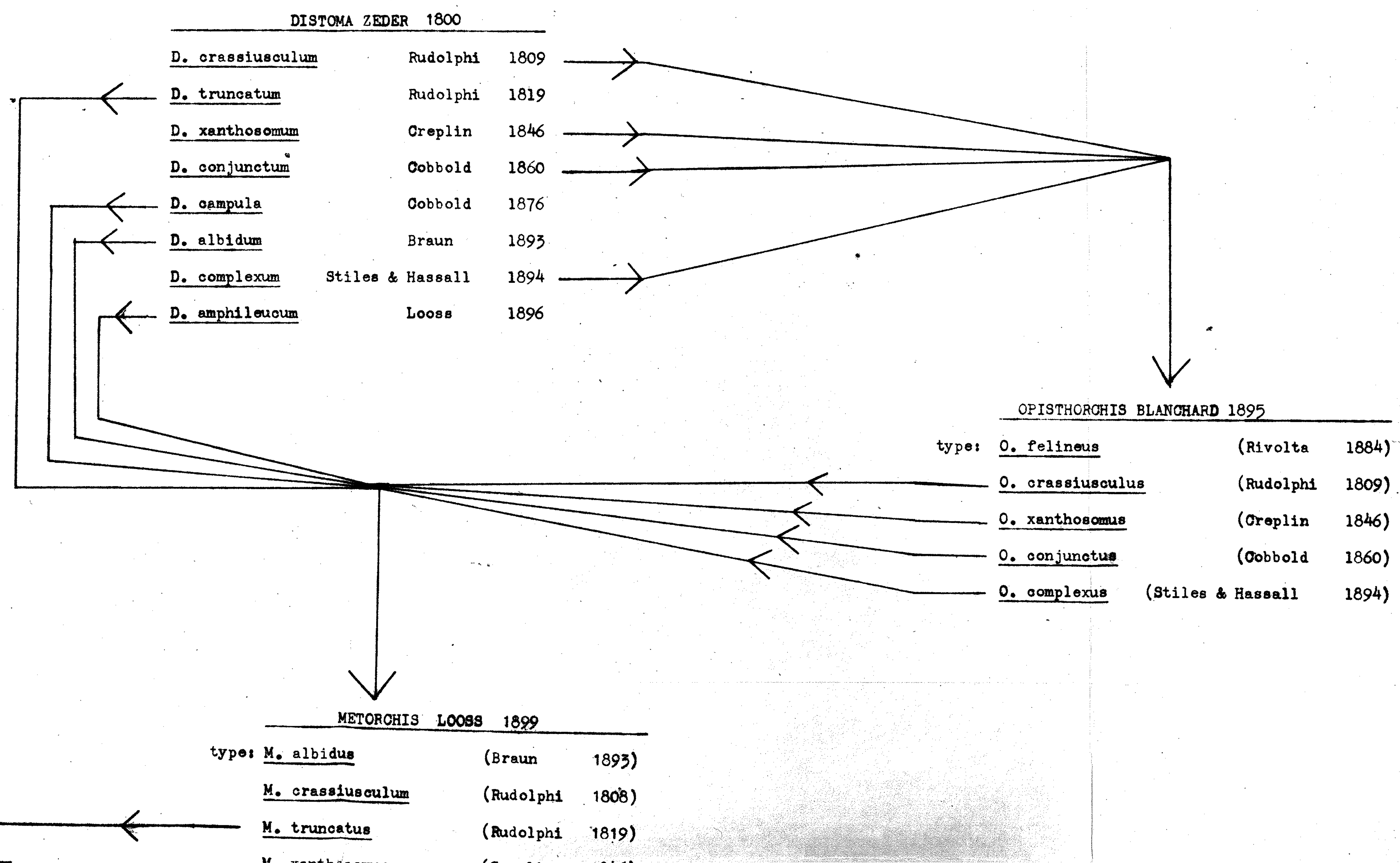
HISTORY OF THE GENUS METOR



RY OF THE GENUS METORCHIS

DISTOMA ZEDER 1800

D. crassiusculum	Rudolphi	1809
D. truncatum	Rudolphi	1819
D. xanthosomum	Creplin	1846
D. conjunctum	Cobbold	1860
D. campula	Cobbold	1876
D. albidum	Braun	1893
D. complexum	Stiles & Hassall	1894
D. amphileucum	Looss	1896

OPISTHORCHIS BLANCHARD 1895

type:	O. felineus	(Rivolta	1884)
	O. crassiusculus	(Rudolphi	1809)
	O. xanthosomus	(Creplin	1846)
	O. conjunctus	(Cobbold	1860)
	O. complexus	(Stiles & Hassall	1894)

METORCHIS LOOSS 1899

type:	M. albidus	(Braun	1893)
	M. crassiusculum	(Rudolphi	1808)
	M. truncatus	(Rudolphi	1819)
	M. xanthosomus		

M. conjunctus (Cobbold 1860)
M. campulus (Cobbold 1876)
M. complexus (Stiles & Hassall, 1894)
M. amphileucum (Looss 1896)

SKRJABIN'S ERROR 1913. THE "ROSETTE"-SHAPED UTERUS IS A NON-EXISTANT CHARACTER.

CYCLORCHIS LUEHE 1908

type : C. amphileucus (Looss 1896)
C. campulus (Cobbold 1876)

species added to Metorchis after 1899:

M. caeruleus Braun 1902
M. tener Kowalewski 1903
M. pinguinicola Skrjabin 1913
M. orientalis Tanabe 1921
M. taiwanensis Morishita & Tsuchimocki 1925
M. zackarovi Layman 1926
M. caintaensis Tubangui 1928
M. revilliodi Baer 1931
M. felis Hsü 1934
M. intermedius Heinemann 1937

TUBANGORCHIS SKRJABIN 1943

type : T. caintaensis (Tubangui 1928)

PARAMETORCHIS SKRJABIN 1913

type: P. complexus (Stiles & Hassall 1894
P. noveboracensis Hung 1926
P. canadensis Price 1929
P. intermedius Price 1929
P. manitobensis Allen & Wardle 1934

Parametorchis was divided into two subgenera by Morozov in 1939.

These taxa shown to
by Skrjabin in 1950.
was retained as it ha

PARAMETORCHIS S. STR. MOROZOV 1939

P. (Parametorchis) complexus (Stiles & Hassall 1894)
P. (Parametorchis) noveboracensis (Hung 1926)
P. (Parametorchis) canadensis (Price 1929)
P. (Parametorchis) intermedius (Price 1929)
P. (Parametorchis) manitobensis (Allen & Wardle 1934)

METAMETORCHIS MOROZOV 1939

P. (Metametorchis) skrjabini (Morozov 1939)

1819)

(Creplin 1846)

M. conjunctus (Cobbold 1860)

M. campulus (Cobbold 1876)

M. complexus (Stiles & Hassall 1894)

M. amphileucum (Looss 1896)

species added to Metorchis after 1899:

M. caeruleus	Braun	1902
M. tener	Kowalewski	1903
M. pinguinicola	Skrjabin	1913
M. orientalis	Tanabe	1921
M. taiwanensis	Morishita & Tsuchimocki	1925
M. zackarovi	Layman	1926
M. caintaensis	Tubangui	1928
M. revilliodi	Baer	1931
M. felis	Hsü	1934
M. intermedius	Heinmann	1937

SKRJABIN'S ERROR 1913. THE "ROSETTE"-SHAPED UTERUS IS A NON-EXISTANT CHARACTER.

PARAMETORCHIS SKRJABIN 1913

type: P. complexus	(Stiles & Hassall	1894)
P. noveboracensis	Hung	1926
P. canadensis	Price	1929
P. intermedius	Price	1929
P. manitobensis	Allen & Wardle	1934

Parametorchis was divided into two subgenera by Morozov in 1939.

These taxa shown to be synonyms by Skrjabin in 1950. Metametorchis was retained as it had priority.

PARAMETORCHIS S. STR. MOROZOV 1939

P. (Parametorchis) complexus	(Stiles & Hassall	1894)
P. (Parametorchis) noveboracensis	(Hung	1926)
P. (Parametorchis) canadensis	(Price	1929)
P. (Parametorchis) intermedius	(Price	1929)
P. (Parametorchis) "manitobensis	(Allen & Wardle	

METAMETORCHIS MOROZOV 1939

P. (Metametorchis) skrjabini (Morozov 1939)

ALLOMETORCHIS BAER 1943

type: A. intermedius	(Price	1929
A. canadensis	(Price	1929
A. manitobensis	(Allen & Wardle	1934

P. (Parametorchis) canadensis (Price 1929)

P. (Parametorchis) intermedius (Price 1929)

P. (Parametorchis) manitobensis (Allen & Wardle 1934)

Metametorchis given generic rank (Skrjabin 1950)

The subgenus Parametorchis is no longer necessary and is discontinued.

PARAMETORCHIS SKRJABIN 1913

P. complexus	(Stiles & Hassall 1894)
P. noveboracensis	(Hung 1926)

METAMETORCHIS (MOROZOV 1939) SKRJABIN 1950

M. skrjabini	(Morozov 1939)
M. intermedius	(Price 1929)
M. canadensis	(Price 1929)
M. manitobensis	(Allen & Wardle 1934)

The author proposes that this species be returned to the genus Metorchis making Parametorchis a junior synonym of the latter

All shown by Cameron , in 1944 , to be junior synonyms of M. conjunctus (Cobbold 1860)

P. (Parametorchis) canadensis (Price 1929)

P. (Parametorchis) intermedius (Price 1929)

P. (Parametorchis) manitobensis (Allen & Wardle 1934)

... intermedius (Price 192

A. canadensis (Price 192

A. manitobensis (Allen & Wardle 193

Metametorchis given generic rank (Skrjabin 1950)

The subgenus Parametorchis is no longer necessary and is discontinued.

PARAMETORCHIS SKRJABIN 1913

P. complexus (Stiles & Hassall 1894)

P. noveboracensis (Hung 1926)

METAMETORCHIS (MOROZOV 1939) SKRJABIN 1950

M. skrjabini (Morozov 1939)

M. intermedius (Price 1929)

M. canadensis (Price 1929)

M. manitobensis (Allen & Wardle 1934)

The author proposes that this species be returned to the genus Metorchis making Parametorchis a junior synonym of the latter

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