Biology of aspidobothrian trematodes

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Biology of aspidobothrian trematodes

by

David William Fredericksen

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Zoology and Entomology
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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa
1973

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INTRODUCTION

Among the Platyhelminthes there occurs a small, interesting subclass of trematodes, the Aspidobothria Burmeister 1856. The group is comprised of nine recognized genera and at least 27 species, occurring in fresh and marine waters as parasites of both molluscs and poikilothermous vertebrates. In one case, however, a marine crustacean may serve as a host. In all known instances, aspidobothrians develop directly, without asexual stages, and maturity is usually reached in a molluscan host. They are easily recognized by their large, unarmed, ventral adhesive discs divided into many alveoli or separated into individual suckers. They lack the typical oral sucker of other trematodes and have, instead, a buccal disc at the anterior end of a retractable neck. The dorsal posterior portion of the aspidobothrian body usually protrudes beyond the ventral adhesive disc as a muscular cone containing the excretory pore(s). A simple rhabdocoelous gut is common to all members of the subclass.

There are six genera known to occur in the fresh-waters of the world, and of these, only three occur commonly in fresh-waters of North America, namely Cotylaspis insignis Leidy 1857, Cotylogaster occidentalis Nickerson 1902, and Aspidogaster conchicola v. Baer 1827. Although each of these parasites occurs in the intestines of various poikilothermous
vertebrates, all three are primarily parasites of pelecypod molluscs, and each occupies a different niche in the mussel host. *Cotylaspis insignis*, an ectoparasite, is found on the epidermis covering the foot, kidney, and in the water chambers of the mussel gill. *Cotylogaster occidentalis*, a lumen dweller, occurs in the intestines of mussels, whereas *Aspidogaster conchicola* resides in the pericardial cavity and kidney of mussels. Due to their nature, these three members of the family Aspidogastridae Poche 1907 lend themselves to interesting biological comparisons, the subject of this thesis. These three parasites have been investigated from various standpoints of their adult morphology, initial larval stage, development, and host-parasite relationships. Data gained from studies on the aforementioned aspects have many biological and phylogenetic implications concerning the nature of aspidobothrian trematodes.
HISTORICAL REVIEW OF SUBCLASS ASPIDOBOTHRIA

Aspidobothrians have long been of uncertain taxonomic status because of their unique characteristics and paucity of information regarding their biology. Monogenean and digenean trematodes fall within well defined boundaries, but such is not true of aspidobothrians. In all previous studies, development among members of the latter group has been shown to be direct as in the monogeneans. However, aspidobothrians lack a major monogenean characteristic, namely, sclerotized elements in their well-developed adhesive discs. Aspidobothrians resemble digeneans morphologically, and many authors presently consider these groups closely related. However, evidence needed to assess their precise position has been lacking and consequently the group has remained enigmatic. Their uncertain status is borne out by the fact that since their discovery they have sometimes been placed within the Monogenea, sometimes within the Digenea as well as having been given status coordinate with these two trematode groups.

Aspidogaster conchicola v. Baer 1827, was the first aspidobothrian fluke to be described, and in Dujardin's 1845 system of trematode classification, A. conchicola was included with the group now known as the distomate flukes. Diesing (1850) devised a system of classification which placed A. conchicola alone in the subtribe Rhabdocoela, but in the same tribe with
certain monogenean and digenean trematodes. Aubert (1855) studied the development of *A. conchicola* and noted that it undergoes neither metamorphosis nor alternation of generations. Aspidobothrian trematodes were first recognized as a separate group by Burmeister in 1856 when he referred to this group of worms as the Aspidobothrii, with status equal to his other two groups Malacobothrii and Pectobothrii. The latter two groups have been interpreted by Stunkard (1962, 1963) as containing Digenea and Monogenea, respectively. Van Beneden (1858) divided the trematodes into the two groups now known as Monogenea and Digenea, and, contrary to the report of Stunkard (1963), van Beneden placed *A. conchicola* with the monogenean group Polystomatidae. Taschenberg (1879) reviewed the systematics of the Trematoda and included aspidobothrians with the monogenean group Microcotylidae. Leuckart (1879) suggested that aspidobothrians are essentially sexually mature rediae and thus linked them to the Digenea. Cunningham described *Stichocotyle nephropis* in 1887 and indicated it should be included with the monogenean group Polystomatidae. Hoyle (1888), in a review of the trematodes, grouped them in accordance with van Beneden's (1858) views, and placed the aspidobothrians among the Monogenea. Hoyle (1888) was unable to assess the affinities of *Stichocotyle nephropis*. Monticelli (1892) treated Burmeister's Aspidobothrii as family Aspidobothridae in a suborder Aspidocotylea, and gave
it coordinate status with his two other suborders, Heterocotylea (Monogenea) and Malacocotylea (Digenea). Braun (1879) agreed with Monticelli's 1892 treatment of the trematodes but, according to LaRue (1957), Braun included the Aspidocotylea within the Digenea. Odhner (1902) favored placing the aspidobothrians with the Digenea on the basis of their morphology. Nickerson (1902) revised the family Aspidobothridae, but did not comment on the position of the group. Osborn (1903, 1905) acknowledged that Leuckart (1879) had likened aspidobothrians to sexually mature rediae, and to support this concept, he noted that the separate excretory bladders and pores in young *Cotylaspis insignis* Leidy 1851, resembled the condition in digenean rediae and cercariae. Poche (1907) changed the family name from Aspidobothridae to Aspidogastridae so as to conform to the rules of zoological nomenclature. Stunkard (1917) favored Monticelli's 1892 classification, but was unable to determine the affinities of the group. Ward (1918) included aspidobothrians with Digenea. Faust (1932) created the name Aspidogastrata, but Faust and Tang (1936) reviewed the position of the group and proposed re-establishing the aspidobothrians as equal in status to the Monogenea and Digenea. They gave the name Aspidogastrea to the group, a conclusion later favored by Williams (1942), Dawes (1941), Dickerman (1948), Dollfus (1958), and Llewellyn (1965). Hyman (1951) suppressed the
name proposed by Faust and Tang (1936) on the grounds that it was not the oldest available name and referred to the order as Aspidobothria with status equal to the Monogenea and Digenea. Manter (1931, 1947, 1954) treated the aspidobothrians as a family of the Digenea. LaRue (1957), however, concluded that aspidobothrians could not be considered together with the Digenea and should be regarded as a separate group, the Aspidogastrea. Stunkard (1963), in an excellent review regarding the status of the Aspidobothria, concluded that by definition the latter are basically monogenean but are related to the Digenea, and assigned them to the order Aspidobothrea under subclass Malacobothria. Price (1967), in agreement with Yamaguti (1963), regarded these trematodes as members of the order Aspidocotylea, a group distinct from Monogenea and Digenea. Recently, Rohde (1971f, 1972, 1973) has indicated that aspidobothrians appear close to the stem of the digenea and perhaps represent a prodigenean group.
MATERIALS AND METHODS

Specimens used in this study were taken from widely separated collecting areas. Of the three species studied (Cotylogaster occidentalis, Cotylaspis insignis, and Aspidogaster conchicola), C. insignis and A. conchicola were abundant in the mussels Leptodea fragilis Raf. and Anodonta grandis Say in the Ft. Madison area of the Mississippi River, Lee County, Iowa. C. occidentalis occurs in Lampsilis siliquoidea (Barnes), West Lake Okoboji, Dickinson County, Iowa, but only in small numbers. However, the parasite is abundant in mussels (Ligumia nasuta (Say)) from Douglas Lake, Cheboygan County, Michigan. Surveys of fish and mussel fauna in Lake Pepin, Mississippi River, Goodhue and Wabasha Counties, Minnesota, were conducted during the summers of 1969 and 1970. Sheepshead were the most common hosts for C. occidentalis in Lake Pepin, and specimens of this fish were easily obtained from commercial fishermen operating in the area. Seining carried out by the commercial fishermen was confined to a series of five general localities in Lake Pepin. Since weather and weekly quotas in pounds of fish were primary factors governing frequency of seining by fishermen, it was necessary to spend extended periods of time in this collecting area. Because a mobile research unit constructed for surveys in 1969 and 1970 was unsatisfactory for critical examination of living
material, a cabin was rented on Lake Pepin for the summer of 1971. Suitable gear and a flat-bottomed clam-boat (8' x 24') supplied with a 28hp motor were borrowed from a branch of the Tennessee Shell Co., stationed at Lake Pepin. With this equipment, large numbers of fish and mussels could be readily collected and quickly transported for examination. When it was established that *C. occidentalis* was limited to larger sheepshead, only fish of suitable size were selected for examination. At death, anal sphincters of fish relax and some *Cotylogaster* specimens escape, thus necessitating keeping fish alive until the intestines could be removed. Fish to be examined (usually 10 per sein-haul) were placed in a tub of water oxygenated with two battery-powered aerators and were transported by boat to the cabin for parasitological examination. After fish were weighed and measured, their intestines were removed. Examinations were made with the aid of a binocular dissecting microscope. Specimens of *Cotylogaster* were removed with a camel-hair brush, placed in saline or lake water, and maintained in a refrigerator until all hosts collected on a given day had been examined. Intestinal contents of the sheepshead were noted and all mollusc shells present were washed, dried, weighed, and later identified.

Mussels were collected by wading, diving with an Evinrude Aquanaut and scuba gear, dredging, and towing a clam-bar. To collect small mussels, a dredge was constructed for
use in shallow areas. Mussel hosts collected for parasitological examination were maintained at 45°F in a Living Stream Unit (Frigid Units Inc., Toledo, Ohio). Examinations of mussels were made soon after collection, and all shells of molluscs surveyed were identified by Dr. Henry van der Schalie, Mollusc Division, Ann Arbor, Michigan. The soft parts of mussels to be examined were removed, placed in a shallow dish, and examined first for Cotylaspis insignis. Such specimens were removed with curved microforceps (Dumont #7), and fixed un flattened by adding AFA to a petri dish containing the specimens. Mussels were next examined for A. conchicola, which, if present, were removed with a pipette and camel-hair brush, flattened with the weight of an 18mm sq coverslip and fixed with alcohol-formalin-acetic acid (AFA). Specimens fixed by this method were flattened laterally; to obtain dorsoventrally flattened individuals, small amounts of AFA were applied with a brush, and after they became somewhat rigid, a coverslip was applied. Mussels examined for C. occidentalis were opened by severing the adductor muscles with a thin-bladed knife. Developmental stages were found in the mouth, esophageal and stomach regions of L. nasuta, by gently teasing the tissues with microforceps. Developing C. occidentalis were flattened with a 10mm sq coverslip and fixed with AFA. Adult C. occidentalis were found in the intestines, most often near the heart. Intestines were
exposed by making a parasagittal incision through the viscera. Specimens were removed and fixed by gently applying small amounts of AFA with camel-hair brushes. One brush was placed near the posterior end of the worm and the second drawn anteriorly from that point so as to expand the ventral adhesive disc and to keep the retractile neck and buccal disc in an extended position. When worms became somewhat rigid, a square 22mm sq coverslip (No. 1) was placed on them and the area flooded with AFA. No additional pressure was applied.

All specimens for whole mounts were stained with Mayer's paracarmine and counterstained with fast green. Specimens to be sectioned were fixed in 10% neutral buffered formalin, saturated mercuric chloride, or 3% glutaraldehyde. Serial sections were stained with Harris's hematoxylin. Entire soft parts of mussels were sectioned for in situ studies. To achieve proper fixation, dehydration, and infiltration, each of these processes was carried on in a vacuum. Tertiary butyl alcohol was the best infiltrating agent for mussel tissue.

Microdissections were made on living adult worms to demonstrate the oogenotop; dissected material was stained with iron acetocarmine.

Karyotypes were determined by staining squash preparations with aceto-orcein or chrome-alum, and were verified by
Mr. P. T. LoVerde, Ann Arbor, Michigan.

Laurer's canal was demonstrated by a nonspecific esterase localization modified from Holt (1954).

Acetylcholinesterase localizations, according to the method of Bueding, Schiller, and Bourgeois (1967), were used to determine nervous system morphology.

Tegumental structures of *C. occidentalis* cotylocidia were demonstrated by using AgNO₃, according to Lynch (1933). Specimens of superior quality were obtained when mounted in a solution consisting of 8.5 gm PVP (polyvinylpyrrolidione) in 15 ml of 30% acetic acid. Following the distilled water wash as outlined in Lynch's procedure, specimens were placed in 15% glacial acetic acid for 10 min. A small amount of PVP was placed on a microscope slide, the cotylocidia pipetted onto the mounting medium, more PVP was added, and a coverslip gently applied. Mounted specimens were maintained indefinitely in a refrigerator. Silver-nitrate-treated specimens and living cotylocidia were studied with the aid of brightfield, darkfield, and phase-contrast optics of Leitz Labolux and AO Spencer compound microscopes. Nile blue sulfate was useful in demonstrating cilia and for enhancing visibility of various internal structures of cotylocidia. All light microscope photographs were taken with a Leitz 35mm camera using high-contrast copy film and type A Kodachrome II, or with an MP-3 Polaroid camera using Panatomic-X sheet film. Drawings were
made with the aid of a Leitz micro-slide projector.

To verify light microscope studies, cotylocidia of *C. occidentalis* were viewed with a Joel JSM-S1 scanning electron microscope (SEM), and photographed using Kodak Ektapan Sheet Film (Thick Film No. 4162). Living specimens were washed several times by pipetting them through several changes of filtered artificial spring water (Ulmer, 1970). They were then fixed for 1 min in a filtered solution of 6 pts 2% OsO₄ and 1 pt saturated HgCl₂ (Parducz, 1967). Specimens were then washed in three changes of doubly distilled water (15 min each) and pipetted onto a clean 10mm No. 1 round coverslip. With the aid of a dissecting microscope, the excess fluid was drawn off and the coverslip was placed on an aluminum weighing pan floating in liquid nitrogen. Frozen specimens were on the liquid nitrogen (in some cases overnight) and then transferred to an Edwards E12E4 freeze-dryer, and maintained at -20° to -30°C for 48 hrs. Dried specimens were coated with a thin carbon coat (200Å) and two coats (400Å each) of gold (40%)-palladium (60%). Adult worms viewed with the scanning electron microscope were washed several times in doubly distilled water prior to fixation but instead of being frozen on a coverslip, were pipetted directly onto an aluminum weighing pan floating on liquid nitrogen. All other procedures used for adult worms were the same as those used for cotylocidia.
For comparative purposes, specimens representing all genera in the subclass Aspidobothria, except *Lissimysia* and *Multicotyle*, were borrowed from the Manter Memorial Museum, Lincoln, Nebraska and the U.S. National Museum.
ADULT MORPHOLOGY

As indicated by LaRue (1957), early studies on the systematic arrangement of trematodes by such workers as Odhner (1902), Looss (1899), and Poche (1926) were based entirely on the comparative morphology of adult worms. More recent workers such as LaRue (1957) and Stunkard (1963) have added much to our understanding of trematode systematics by constructing systems of classification that include consideration of life histories and comparative developmental morphology. However, many of the trematode groupings as recognized by earlier workers are still valid, and LaRue (1957) observed with reference to Poche's 1926 study, "many of his views of relationship are now supported by data derived from studies of life histories." Since many relationships known to exist among the various taxonomic levels of digenetic trematodes were first determined on the basis of comparative adult morphology, the aspidobothrians, too, deserve continued study on that basis.

External Morphology

Although the characteristics of external body form among aspidobothrian trematodes are well known, scanning electron microscopy opens a new dimension in perspective and reveals details of surface structure too small to be discerned with the light microscope. Halton and Lyness (1971)
published a scanning electron micrograph showing the antero-ventral portion of *Aspidogaster conchicola*, but because their specimen was adversely affected by fixation, it did not show surface details. Allison et al. (1972), in a study concerning the preparation of helminths for SEM, included *Cotylaspis insignis* as one of their subjects; however, they used no special fixation techniques and fixation artifacts are apparent in photomicrographs of their preparation. Rohde (1973) has shown several SEM views of *Lobastostoma manteri*, but his specimens appeared to have suffered from shrinkage.

Scanning electron microscope studies conducted on *Cotylogaster occidentalis*, *Cotylaspis insignis*, and *Aspidogaster conchicola* reveal additional data noted below.

*Cotylogaster occidentalis*  
(Figures 45-52)

*Cotylogaster occidentalis* is elongate and consists of a tube-like body extending anteriorly as a retractable (telescopic) neck that ends in a pentolobate and heart-shaped buccal disc (Figure 45). The buccal disc may be expanded or constricted (Figure 46). The body extends posteriorly as a dorsal cone (Figure 46) containing two osmoregulatory pores and the opening of Laurer's canal (Figures 48, 49). Attached to the body wall ventrally is a massive ventral adhesive disc extending from the base of the neck to the base of the dorsal
cone. The ventral adhesive disc is elongate, ovoid, and composed of alveoli bordered by tegumental ridges (Figure 47). Alveoli include a peripheral row encircling a median longitudinal row of transversely elongated alveoli. Posteromost alveoli are small and presumed to be newly formed. A protrusible pyriform-shaped marginal papilla occurs peripherally within each of the ridges bordering the marginal alveoli (Figures 52, 53).

Aspidogaster conchicola
(Figures 44 and 54-60)

The tube-like body of Aspidogaster conchicola extends anteriorly as a much narrowed retractile neck terminating in a circular buccal disc. When constricted, the opening of the latter is oval or slit-like (Figure 58). Posteriorly, the body extends as a muscular dorsal cone containing the two osmoregulatory pores in a recessed area, the caudal foramen (Figures 59, 60). The ventral body surface is modified forming a pyriform-shaped ventral adhesive disc (Figures 44, 54, 55) which does not extend the full length of the body posteriorly. Alveoli of the ventral adhesive disc include a peripheral row of marginal alveoli encircling two median longitudinal rows; the latter are separated by a median longitudinal ridge. For descriptive purposes, the ventral adhesive disc of A. conchicola may also be described as con-
sisting of transverse rows each including four alveoli. Anteriorly, alveoli are small, and newly formed ones occur posteriorly where the ventral adhesive disc is greatly narrowed. Alveolar formation appears to occur in a series of steps whereby single sucker-like alveoli are formed and are then displaced anteriorly as more new alveoli are added. As alveoli are displaced anteriorly, they are subdivided by ridges: first, a single median longitudinal ridge; and then, two additional longitudinal ridges, one on each side of the former. Evidence supporting the above description of alveolar formation can be observed in the ventral adhesive disc of most adult worms. In some specimens, the posteromost alveolus appears as a transversely elongated structure which may or may not possess a single, weakly developed, median ridge (Figure 55). Immediately anterior to this alveolus is a row which in some specimens has a well developed median ridge and one or two poorly developed lateral longitudinal ridges. In all specimens examined, the third most posterior row always possesses four fully formed alveoli as do all additional rows.
Cotylaspis insignis
(Figures 43 and 61-71)

*Cotylaspis insignis* is ovoid in outline when contracted or when detached from its host. When seen *in situ* (Figures 106, 107), its body appears small and tube-like with the anterior end extending as a retractile neck that ends in a circular, funnel-like disc. The latter may be constricted to form a pore-like opening (Figures 61-64) or expanded as a flat disc (Figures 62, 63). Posteriorly, the body is usually flattened and blends into the dorsum of the large, broadened ventral adhesive disc, but may extend as a dorsal cone. A ventral adhesive disc extends beyond the body on either side and consists of a peripheral row of marginal alveoli that encircles a median row of transversely elongated alveoli (Figure 43). All alveoli are approximately the same size except for the antero- and postero-most alveoli, which are small (Figures 66, 67). A protrusible pyriform-shaped papilla occurs peripherally within each of the ridges bordering the marginal alveoli (Figure 68) except for those around the posteromost alveolus (Figure 67).

Other Aspidobothria

The simple tube-like body described for *Cotylogaster occidentalis*, *Aspidogaster conchicola*, and *Cotylaspis insignis* is typical of other known aspidobothrian trematodes.
The dorsal cone, a posterior extension of the body, is also characteristic of the group. Although Hendrix and Short (1965) could observe only a single excretory pore in the dorsal cone of Cotylaspis insignis, two could be seen with the SEM. All aspidobothrians examined have a retractable neck, but it is telescopic in only two genera, namely, Cotylogaster and Lobatostoma. Similarly, species in these two genera are the only aspidobothrians with lobed buccal discs. In Cotylogaster, this disc is only slightly pentolobate and appears heart-shaped, but in Lobatostoma (Figures 111, 112) the five lobes composing its buccal disc are deeply separated. All other aspidobothrian trematodes have a simple circular buccal disc. Attachment organs vary markedly among the Aspidobothria. Stichocotyle nephropsis (Figure 108) has a single longitudinal row of ventrally situated individual suckers. Species of Taeniocotyle (Figure 109) possess a single longitudinal row of fused suckers (sometimes termed "alveoli"), and in taenio-cotylids each alveolus is supplied laterally with a marginal organ (Figure 110). The latter are present in all aspidobothrians except Stichocotyle nephropsis, and among taenio-cotylids they resemble the tube feet of an echinoderm. In the genera Cotylogaster, Cotylaspis, and Lissimysia, the ventral adhesive disc consists of a single median row of transversely elongated alveoli encircled by a peripheral row of smaller marginal ones. Marginal organs in species of these
genera are small, protrusible, pyriform-shaped, and are restricted to the ridges separating each of the marginal alveoli. In species of *Lobatostoma*, the ventral adhesive disc is similar to that of *Cotylogaster*; however, a weakly developed median longitudinal ridge appears in the anterior portion of the ventral adhesive disc. If the median longitudinal ridge in the ventral adhesive disc of *Lobatostoma* were hypothetically extended the entire length of its ventral adhesive disc, the resultant disc would resemble that found in *Multicotyle purvisi* Dawes 1941, *Aspidogaster*, and *Lophotaspis*. All these genera of aspidobothrians have a peripheral row of marginal alveoli encircling two median longitudinal rows. Marginal organs of *Aspidogaster* and *Multicotyle* as described by Rohde (1966b), resemble those described by Nickerson (1901) for *Cotylogaster*. Species of *Lophotaspis* (Figure 113) have tentacle-like marginal organs that are also found on the ridges bordering each median alveolus (Ward and Hopkins, 1932, Wharton, 1939).

On the basis of external morphology, and particularly in view of the variation that occurs in the ventral adhesive disc, aspidobothrians may be arranged in a series in accordance with the complexity of their ventral adhesive discs (Figures 108-113). In *Stichocotyle nephropsis*, the disc is actually a series of individual suckers arranged in a single longitudinal row. In species of *Taeniocotyle* such suckers
appear to have fused, and marginal organs have been added to them. In some genera (Cotylogaster, Cotylaspis, and Lissimysia), a peripheral row of marginal alveoli encircles the median longitudinal row, and in Lobatostoma the longitudinal row is partially subdivided longitudinally. Continued subdivision appears to have resulted in a condition seen in Aspidogaster, Lophotaspis, and Multicotyle.
NERVOUS SYSTEM MORPHOLOGY

Until recent times, students of trematode morphology have considered the nervous system of flukes to be poorly developed. Early workers such as Bettendorf (1897), Looss (1894, 1895), and Zailer (1914) employed methylene blue stains as well as silver nitrate impregnation techniques for demonstrating trematode nervous tissues. Techniques employing heavy metals for studies of aspidobothrians have been used with marked success recently by Rohde (1968a,b,d, 1970c, 1971c,1972), who found the nervous system of *Multicotyle purvisi* to be well developed and complex.

Bueding (1952) demonstrated biochemically the presence of a nervous system enzyme, acetylcholinesterase, in the tissues of *Schistosoma mansoni* Samborn 1907. A method for the histochemical localization of acetylcholinesterase was developed by Koelle and Friedenwald (1949), a technique later modified by Gomori (1952), Gerbertzoff (1959), and Douglas (1966). From Gomori's modification, Bueding et al. (1967) adapted a procedure for localizing acetylcholinesterase in *Schistosoma mansoni* so as to quantitatively determine the effects of drugs on its nervous system. This technique (referred to by Bueding et al. (1967) as the Koelle-Gomori technique for acetylcholinesterase) was also successfully employed by Timofeyeva (1971) for nervous system studies on
Aspidogaster conchicola. A less precise nonspecific esterase technique, introduced by Barnett and Seligman (1951), was also used by Holt and Withers (1952), and was later modified by Holt (1954). This method was used by Huehner and Etges (1972b) to demonstrate the nervous system of immature Cotylogasteroides barrowi Huehner and Etges 1972b (=Cotylaster occidentalis).

Though specialized nervous system techniques were not used in early morphological studies on aspidobothrians, authors frequently referred to sensory structures, ganglia, and nerves. Huxley (1856) was unable to find any trace of a nervous system in Aspidogaster conchicola, but predicted it would be found eventually. Voeltzkow (1888) described the nervous system of this species as consisting of a "brain" giving rise to a dorsal and a ventral pair of anteriorly directed nerves and a larger pair of posteriorly directed nerves. Nickerson (1895) found the suckers of Stichocotyle nephropsis well supplied with nerves and sensory structures. Observations on the nervous system of Cotylaspis insignis were made by Osborn (1903, 1905), and Stunkard (1917) commented on the eye spots of Cotylaspis cokeri Barker and Parsons 1914. Monticelli (1892) reconstructed the nervous system of Cotylaster michealis from sectioned material, and observed that the "brain" gives rise to four pairs of anteriorly directed and three pairs of posteriorly directed nerves.
Rohde's (1970b, 1971b, 1972) electron microscope studies have shown that in *Multicotyle purvisi* a sheath occurs around neurons, a condition not previously observed among monogenean or digenean flukes.

In the present investigation details of the nervous system of *Cotylogaster occidentalis*, *Cotylaspis insignis* and *Aspidogaster conchicola* were studied from whole mount specimens treated with the Bueding et al. (1967) modification of the Koelle-Gomori technique for acetylcholinesterase.

**Cotylogaster occidentalis**
(Figures 72-83)

The cerebral ganglia (C) (Figures 73, 82) of *Cotylogaster occidentalis* are located anterodorsal to the pharynx and are fused dorsomedially; each extends ventrolaterally around the prepharynx as a circumpharyngeal connective (CP). Ventrolateral to the prepharynx, the connectives are directed posteriorly and each divides into two posteriorly directed nerves. Cerebral ganglia and connectives give rise to six pairs of dichotomously branching nerves supplying the buccal disc and mouth region. Five pairs of nerves extend posteriorly to supply the neck, body, and ventral adhesive disc.

The expanded buccal disc (Figures 72, 73, 82) of adult *C. occidentalis* is pentolobate and heart-shaped (Fredericksen, 1972). It consists of an anterior median lobe and one pair
each of symmetrically arranged lateral and ventrolateral lobes. The anterior median lobe is supplied with a pair of anteromedian nerves (AM) extending from the anterior surface of the cerebral ganglia to the tip of the lobe where the finely branched nerve fibers anastomose. A small pair of anterodorsal nerves (AD) arise from the posterodorsal surface of the cerebral ganglia and also innervate the anterior median lobe. Each lateral lobe of the buccal disc receives an anterolateral nerve (AL) from the anterior surface of each circumpharyngeal connective. The ventrolateral lobes are each supplied with two nerves, an anteroventrolateral nerve (VL) and an anteroventral nerve (AV). The former is large and originates ventrally on the circumpharyngeal connective near the point where the latter divides posteriorly; the anteroventral nerve (AV) originates near the base of the anterolateral nerve. The mouth is supplied with a pair of oral nerves (O) originating on or near the base of the anteromedian nerves and connecting with an oral nerve ring (OR) around the mouth opening. Nerves of the buccal disc are highly branched and are interconnected. Near their distal ends, buccal disc nerves are joined by a peripheral buccal nerve ring (BR) which becomes very thin at the tip of the anterior median lobe. Peripherally, the buccal nerve ring is connected to many sensory structures.

Near the base of the oral nerves, paired pharyngeal
nerves (P) arise (Figure 82) and extend posteriorly to form a plexus around the prepharynx, pharynx, and intestine.

Among the five pairs of posterior nerves (72-75, 82, 83) three pairs are superficial and form an orthogonal network of fibers around the neck and body (excluding the ventral adhesive disc). A pair of posterodorsal nerves (PD) originate in common with the anterodorsal nerves. These nerves extend posteriorly along the neck and body, meet behind the base of the dorsal cone, and thus form a complete loop. The posterodorsal nerves receive connectives from small nerve rings around the osmoregulatory pores in the caudal foramen. Paired posterolateral nerves (PL) originate from the circumpharyngeal connectives and extend laterally along the neck, each receiving a large external genital commissure (GC) (Figure 80) from the gonopore. A nerve plexus occurs around terminal portions of male and female ducts; these plexuses appear to extend posteriorly from the genital commissure. At the base of neck, the posterolateral nerves each receive a thick commissure from the large posteroventral nerves (PV). In flattened specimens, the latter two nerves may appear fused. The posterolateral nerves meet posteriorly to form a complete loop. Paired superficial posteroventral nerves (Figure 82) take their origin on the lateral aspect of the circumpharyngeal connectives and with the anteroventral nerves of the buccal disc. Each superficial posteroventral
nerve extends posteriorly along the ventral aspect of the neck and also communicates with the external genital commissure. As the former nerve pair extends into the body musculature dorsal to the ventral adhesive disc, it becomes very faint, and in this region commissures from the posterior lateral nerves connect to the large posteroventral nerves.

The posteroventral nerves (PV) (Figures 82, 83) are the largest of the posteriorly directed nerve cords. They arise from the circumpharyngeal connectives together with the posterolateral nerves and are contained within, but not connected to, the superficial orthogonal network of the neck region. Each posteroventral nerve cord passes through the neck region; lateral to the gut and near the base of the neck, each receives a short thick commissure from the posterolateral nerve. From this point, the posteroventral nerves proceed posteriorly where they meet and thus form a large nerve loop just dorsal to the ventral adhesive disc. In the region of the ventral adhesive disc, posteroventral nerve cords receive commissures from the posterior lateral nerves and the muscular body septum. The posteroventral nerve cords communicate with the ventral adhesive disc via ventral adhesive disc connectives (VC) (Figures 77, 83) which arise, usually in pairs, throughout the length of each cord and connect with an inner superficial nerve ring (IS) (Figures 75, 83) in the ventral adhesive disc. One pair of connectives
enters this nerve ring for each marginal alveolar ridge in
the ventral adhesive disc. An enlarged pair of nerves, the
ventral adhesive disc rami (VR) (Figures 74, 83) extend from
the posterior ventral nerve cords to ganglia in the inner
superficial nerve ring at approximately the level of the
3rd transversely elongated alveolus. The inner superficial
nerve ring lies in or near the ridge separating the marginal
alveoli from the transversely elongated alveoli and is con­
nected to an outer superficial nerve ring (OS) (Figure 83)
by pairs of marginal alveolar nerves (MA) (Figures 76-83)
which extend through the marginal alveolar ridges. A single
superficial transverse alveolar nerve (ST) (Figures 78, 83)
ocurs anteriorly in the base of each transverse alveolar
ridge, and is paralleled by a weakly developed deep trans­
verse alveolar nerve (DT). Marginal papillae of the ventral
adhesive disc are each supplied with a separate papillary
nerve (PN) (Figure 83) arising from one of the two ventral
adhesive disc connectives that extend to each marginal alveolar
ridge. In addition, a papillary nerve ring (PNR) lies at the
base of each papilla, and extending into each papilla is a
pair of nerves giving rise to an inner plexus (PL) (Figures
80, 83) around the lumen of the papilla. The inner papillary
nerves (IPN) are extensions of the marginal alveolar nerves
and are best developed in the anterior portion of the ventral
adhesive disc. These nerves and their plexuses end a short
distance from the terminal pore that occurs in each marginal papilla.

Nerves of the ventral adhesive disc are connected to tegumental sensory structures by numerous fine branches to be discussed later.

Aspidogaster conchicola
(Figures 84-92)

Timofeyeva (1971) described the nervous system of Aspidogaster conchicola, but did not mention certain aspects of this system which are necessary for comparative purposes. For this reason, and to standardize terminology, a redescriptions of this species' nervous system is given below.

The cerebral ganglia (C) (Figure 90) are fused medially and lie dorsal to the prepharynx. Circumpharyngeal connectives (CP) extend ventrally from the ganglia around the prepharynx and then turn posteriad, passing through the neck region as a single large posterior nerve trunk (PT). Five pairs of anteriorly directed nerves supply the buccal disc, and four pairs of posteriorly directed nerves occur in the neck region. One pair of these nerves, the posterior nerve trunk, divides into two nerves, and thus a total of five nerves supply the body and ventral adhesive disc.

The circular buccal disc (Figures 84, 85, 90, 92) of A. conchicola is not lobate and is innervated in part by a single pair of anterior median nerves (AM). These arise from
the anterior surface of the cerebral ganglia and each receives a connective from each member of a pair of antero-dorsal nerves (AD). The latter nerves arise just posterior to the anteromedian nerves, in common with the paired posterodorsal nerves (PD). In some specimens, branches of a small pair of superficial dorsolateral nerves (DL) that arise from lateral commissures in the neck region, extend anteriorly on each side and may also enter the dorsolateral aspect of the buccal disc. Laterally on the circumphtaryngeal connectives, anterolateral nerves (AL) arise, and at the ventralmost extremity of each connective an anteroventralateral nerve (VL) originates. Near the same point, an anteroventral nerve trunk (AT) extends ventrally on each side and each branches into an anteroventral nerve (AV) and a superficial posteroverentral nerve (SP). An oral nerve ring (OR) around the mouth receives connectives from the anterolateral nerves and from the anteroventralateral nerves. A peripheral buccal nerve ring (BR) connects all branches of nerves supplying the buccal disc. There does not appear to be an anteromedial anastomosis of nerve fibers in the buccal nerve ring of A. conchicola.

Posteriorly, a small pair of pharyngeal nerves (P) extends from the median surface of the circumphtaryngeal connectives and innervates the prepharynx, pharynx, and intestine. Individual connectives extend from the pharynx to the pos-
terior nerve trunk. Paired posterodorsal nerves (PD) (Figures 84-85, 90-92) extend posteriorly from their common origin with the anterodorsal nerves; each receives two pairs of cervical commissures, including an anterior pair (CC₁) and a large 'Y' shaped pair (CC₂) (Figures 85, 90, 92) where the neck expands into the body. Posterodorsal nerves continue posteriad, receive connectives from a nerve ring (OP) encircling each osmoregulatory pore, and ultimately join one another behind the dorsal cone to form a complete loop. The posteroventral nerve trunks (PT) extend to the base of the neck where each divides into a pair of posterolateral nerves (PL) and a pair of large posteroventral nerves (PV). The latter two nerves are connected on each side by the two cervical commissures. Posterolateral nerves continue along the body wall dorsal to the ventral adhesive disc and receive commissures from both posterodorsal and posteroventral nerves. The latter nerves extend along the length of the ventral adhesive disc to form a complete nerve loop.

The ventral adhesive disc of *A. conchicola* appears to be divided into four longitudinal rows of alveoli including a peripheral row and two longitudinal median rows. Ventral adhesive disc connectives (VC) (Figures 87-88, 91) arise singly throughout the length of the posteroventral nerve cord, one for each marginal alveolus. Each connective usually gives rise to three branches, one extending to the inner super-
ficial nerve ring (IS), one to the marginal papilla, and one to a marginal alveolar nerve. From the posteroventral nerves, the inner superficial nerve ring receives a ventral adhesive disc ramus (VR) (Figures 88, 91) on each side at approximately the level of the fourth or fifth transverse row of alveoli. Ganglia appear in the inner superficial nerve ring where rami of the ventral adhesive disc connect. An outer superficial nerve ring (OS) (Figure 91) occurs peripherally in the ventral adhesive disc, and in some specimens an additional but poorly developed outer nerve ring may occur. Pairs of marginal alveolar nerves (MA) lie in each marginal alveolar ridge. These nerves connect the inner and outer superficial nerve rings, and extend across the midregion as superficial transverse alveolar nerves (STA) in the transverse alveolar ridges. At each point where the marginal alveolar nerves intercept the outer superficial nerve ring, a small papillary nerve ring (PNR) (Figures 88, 91) appears. From the latter, a small plexus (IP) extends into the central portion of the marginal papilla. The outer superficial nerve ring usually receives a number of connectives from the posterolateral nerves. A single, very slender, deep transverse alveolar nerve (DT) (Figure 91) paralleling each pair of superficial transverse alveolar nerves, joins the posterior lateral nerves.

Superficial nerves of the ventral adhesive disc and
buccal disc supply numerous tegumental sensory structures to be discussed later.

*Cotylaspis insignis* (Figures 93-99)

Osborn (1903, 1905) described the nervous system of *Cotylaspis insignis* as consisting of a transverse commissure crossing above the pharynx and giving rise to single pairs of anterior and posterior nerves. He also described eyespots in the form of "hollow cups".

Cerebral ganglia (C) (Figure 97) of *C. insignis* are enlarged, fused medially, lie dorsal to the prepharynx, and extend ventrolaterally around it as circumpharyngeal connectives (CP). The circular nonlobate buccal disc is supplied with four paired nerves arising on the cerebral ganglia and circumpharyngeal connectives. In the neck region, three pairs of nerves are directed posteriorly, one of which, the posterior nerve trunk (PT), divides; thus a total of four paired nerves supply the body and ventral adhesive disc.

Anteromedially, the buccal disc (Figures 97, 99) is supplied with two pairs of nerves, one pair of large anteromedian nerves (AM) and a pair of smaller anterodorsal nerves (AD). The former arise on the anterior aspect of the cerebral ganglia; the latter arise in common with the posterodorsal nerves (PD). Ventrolateral to each eyespot (E), a large nerve
trunk arises and divides into two nerves thus forming a pair of anterolateral nerves (AL) and a pair of anteroventrolateral nerves (VL). An oral nerve ring (OR) around the mouth is connected to each anteromedian nerve as well as to a median branch of each anteroventrolateral nerve. Occasionally, branches from a small pair of superficial dorsolateral nerves (DL) that arise from lateral commissures in the neck region, extend anteriorly on each side and may also enter the lateral aspect of the buccal disc. A small pair of anteroventral nerves (AV) originates in common with the superficial posteroverentral nerves at or near the base of the anteroventrolateral nerves. All nerves of the buccal disc are provided with many fine branches and are connected peripherally by a buccal nerve ring (BR).

The pharynx is supplied by a pair of pharyngeal nerves (P) (Figure 97) that extend from near the base of the anteroventrolateral nerves to a pair of ganglia located posteriorly on each side of the pharynx. Surrounding the lumen of the pharynx is a particularly well developed nerve plexus (Figure 97). The prepharynx is extremely short and its nerve supply appears to be a plexus extending inward from the oral nerve ring.

A pair of superficial posteroverentral nerves (SP) originates in common with the anteroventral nerves. In the neck region, the former receive several commissures from the
posterior nerve trunk. A genital nerve ring (GR) (Figure 94) surrounding the gonopore receives connectives from the superficial posteroventral nerves. Dorsally, paired posterodorsal nerves (PD) (Figures 93, 99) extend from their origins with the anterodorsal nerves, and as they pass through the neck region, each receives laterally a large pair of cervical commissures (Figures 93, 99). The first cervical commissure \( (CC_1) \) occurs at the level where the posterolateral nerves separate from the posterior nerve trunk, and the second cervical commissure \( (CC_2) \) occurs at the base of the neck and continues ventrally connecting the posterolateral and posteroventral nerves. Posterodorsal (PD) nerves receive connectives from a nerve ring around each of two osmoregulatory pores and then meet, to form a complete nerve loop. Each posterior nerve trunk (PT) divides into two nerves, a posterolateral nerve (PL) and a posteroventral nerve (PV), and the resultant pairs each form complete nerve loops. The posterolateral nerves follow the lateral margins of the body, and are connected by commissures with the outer superficial nerve ring of the ventral adhesive disc. At the base of the neck, each posteroventral nerve receives an enlarged extension of the second cervical commissure (Figures 93, 99). In the region of the ventral adhesive disc, each posteroventral nerve sends a large ventral adhesive disc ramus (VR) (Figures 95, 98) to a ganglion located in the inner superficial
nerve ring (IS). Throughout the course of the posteroventral nerves, ventral adhesive disc connectives (VC) (Figure 98) arise and extend to the inner superficial nerve ring, marginal papilla, and marginal alveolar nerves (MA) (Figure 98). In each marginal alveolar ridge, paired marginal alveolar nerves connect the inner superficial nerve ring with an outer superficial nerve ring (OS), and extend medially through the transverse alveolar ridges as superficial transverse alveolar nerves (ST) (Figure 98). A deep transverse alveolar nerve (DTA) parallels the superficial pair. At the base of each marginal papilla, a papillary nerve ring (PNR) (Figure 98) is connected to a plexus (IP) around the central lumen of the papilla.

Nerves associated with the gonads could not be discerned; however, the terminal genital ducts are each provided with a plexus that extends posteriorly from the nerve ring surrounding the gonopore.

Superficial nerves of the ventral adhesive disc and the buccal disc nerves are connected to numerous tegumental sensory structures to be discussed later (Figure 95).

Most authors accept the planuloid-acoeloid theory for the evolution of the Bilateria, and agree that Platyhelminthes evolved from a very simple acoeloid type of turbellarian that lacked a gut, (Hyman, 1951). As presented by Hyman (1951), this theory also suggests that the evolution of the platy-
helminth nervous system involved a change from an original superficial position as in certain Acoela, followed later by a more internal position, with the "brain" developing as a plexus around a superficial statoblast, the latter also assuming a deeper position. The resultant nervous system was thus an internal orthogonal network. In certain Rhabdocoela too, an internal orthogonal network is present. According to Rohde (1968a), Ax (1964) has postulated that the most primitive Platyhelminthes are not the Acoela, but those forms with an intestine such as the Rhabdocoela. According to Bullock and Horridge (1965), the acoel turbellarians have the most primitive platyhelminth nervous systems. These free-living flatworms have three to six pairs of longitudinal nerve cords (usually five); monogeneans and digeneans usually have three or less pairs of longitudinal nerve cords (Bullock and Horridge, 1965). Rohde (1971f, 1972) has postulated that the aspidobothrians are among the most primitive trematodes, indicated in part by their nervous systems. Thus, to clarify their phylogenetic position, Rohde (1968a) has indicated that much work is needed on the comparative anatomy of platyhelminth nervous systems.

A comparative summary of similarities and differences existing among the nervous systems of *Cotylogaster occidentalis*, *Aspidogaster conchicola*, *Cotylaspis insignis*,
and Multicotyle purvisi is presented below. Synonyms of descriptive terms used in this study are given in Appendix A.

In all aspidobothrians studied, the cerebral ganglia are enlarged structures that lie dorsal to the prepharynx, are fused medially, and each extends ventrally around the prepharynx as a circumphtaryngeal connective. Small commissures joining these connectives ventrally have led Rohde (1968a, 1972) to liken the "brain" of Multicotyle purvisi to an enlarged commissure, the "cervical commissure" Rohde (1968a, 1972). Cerebral ganglia and connectives give rise to anteriorly as well as posteriorly directed nerves, and for purposes of discussion, these remaining elements of the nervous system may be divided into three portions, namely; posterior nerves supplying the body proper, nerves of the ventral adhesive disc, and anterior nerves supplying the buccal disc.

**Posterior nerves to the body proper**

The posteriorly directed nerves that supply the body usually form complete nerve loops, and all are joined by a great many commissures. A pair of pharyngeal nerves is common to all aspidobothrians studied thus far; they arise from the circumphtaryngeal connectives, innervate the pharynx, and form a well developed plexus around the length of the sac-
like intestine. Two posteriorly directed nerve pairs arise in common with anteriorly directed nerves, namely, the posterodorsal and superficial posteroventral nerves. The posterodorsal nerves receive connectives from nerve rings that occur around the osmoregulatory pores, and demonstrate little variation among species examined. The superficial posteroventral nerves, not seen in M. purvisi by Rohde (1968a), arise in common with the anteroventral nerves. The former are weakly developed, connect to the genital nerve ring or commissure, and enter the body region dorsal to the ventral adhesive disc. In the posterior portion of the body, they are less prominent than the posteroventral nerves.

In C. insignis and A. conchicola, a pair of dorsolateral nerves originates among several cervical commissures, extends anteriorly, and in some specimens reaches the dorsolateral aspect of the buccal disc. Although this nerve pair was not observed in C. occidentalis, Rohde (1968a, 1972) described a similar pair as being well developed in M. purvisi and connected to the cerebral ganglia. Such a connection of the dorsolateral nerves, however, was not observed in C. insignis or A. conchicola. According to Rohde (1968a, 1972), a pair of "dorsolateralis posterior nerves" extends posteriorly on each side along the lateral aspect of the body parallel to the posterodorsal nerve pair. Because of discrepancies to be discussed below, these posterior extensions of the dorso-
lateral nerve pair in *M. purvisi*, as described by Rohde, are considered to be posterolateral nerves. The circumpharyngeal connectives extend as posterior nerve trunks through part of the neck region of *A. conchicola* and *C. insignis* before dividing into posterolateral and posteroventral nerve pairs. In *C. occidentalis*, the circumpharyngeal connectives branch into these two nerve pairs immediately at the base of the prepharynx. In the latter species as well as in *A. conchicola* and *C. insignis*, an enlarged portion of a cervical commissure at the base of the neck connects posterolateral and posteroventral nerves. In *A. conchicola* and *C. insignis*, this connection is formed by cervical commissure "CC₂" (Figures 90, 99). Except for this connection, cervical commissures in adult *C. occidentalis* are not enlarged. In both *C. insignis* and *A. conchicola*, well developed cervical commissures (CC₁ and CC₂) appear at the point where the posterior nerve trunk divides, as well as at the point where posterolateral and posteroventral nerves are connected. Rohde (1968a, 1970a) did not describe these commissures in *M. purvisi* and although they are not apparent in adult *C. occidentalis*, they do appear in older juveniles of this species and will be described later. According to Rohde (1968a), each posterior nerve trunk in *M. purvisi* gives rise to a dorsal ramus at the anterior level of the
pharynx; this ramus rejoins the posterior nerve trunk where the latter divides into the posteroventral nerve and what Rohde calls the "ramus ventralis anterior". At this point, the dorsal ramus, according to Rohde (1968a), again separates from the posteroventral nerve, rejoins it after a short distance, and after separating from the posteroventral nerve a third time, the two nerves fuse at the level of the ovary. Rohde (1968a) based his description on reconstructions made from sectioned material, and may not have properly interpreted the branching of the posterior nerve trunk. In a drawing by Rohde (1968a, 1972), an enlarged portion of a cervical commissure appears near where the dorsal ramus first rejoins the posterior nerve trunk; where the dorsal ramus rejoins a second time, the connection itself appears to be made by an enlarged cervical commissure. The latter is located at the point where cervical commissure "CC₂" occurs in C. insignis and A. conchicola.

Rohde's drawing of M. purvisi does not extend beyond this point, and in C. insignis and A. conchicola this is the area where the superficial dorsolateral nerve originates from the cervical commissures; it does not extend posteriorly beyond this point. The dorsal rami of the posterior nerve trunks as described by Rohde (1968a) are probably posterolateral nerves, and each of the second two connections made by the rami with the posterior nerve trunks are probably enlarged cervical
commissures \((CC_1 \text{ and } CC_2)\). The dorsolateral nerve, as described by Rohde (1968a), probably originates from cervical commissures as it does in \textit{A. conchicola} and \textit{C. insignis}, but whether it connects to the cerebral ganglia anteriorly is a matter of conjecture. It is doubtful that this nerve extends posteriorly beyond the cervical commissures, and what Rohde (1968a) refers to as the "dorsolateralis posterior nerve" is interpreted to be the posterolateral nerve.

According to Rohde (1968a), each of the posterior ventral nerve trunks in \textit{Multicotyle purvisi} branches into an anterior ventral ramus supplying the anterior part of the ventral adhesive disc, and a posterior ventral ramus that continues as the posteroventral nerve. In \textit{A. conchicola}, \textit{C. insignis}, and \textit{C. occidentalis}, a long ventral adhesive disc connective supplying the anteromost alveoli appears to be the equivalent of the anterior ventral ramus in \textit{M. purvisi}. In all aspidobothrians studied thus far, the posteroventral nerves receive commissures from the posterolateral nerves; these are connected ventrally by commissures that serve as deep transverse alveolar nerves. Occasionally, branches from the posterolateral nerves bypass the posteroventral nerves and connect with the outer superficial nerve ring of the ventral adhesive disc. Connections of the posteroventral nerve pair with nerves of the ventral adhesive disc vary greatly even within a given specimen, but the basic pattern appears to be similar among all species.
that have been studied. Ventral adhesive disc connectives arise from the posteroventral nerve cords and join both inner and outer superficial nerve rings as well as the marginal alveolar nerves and marginal papillae. Paired, well-developed ventral adhesive disc rami connecting the inner superficial nerve ring with the posteroventral nerve cord in *A. conchicola*, *C. insignis*, and *C. occidentalis* were not observed in *M. purvisi* by Rohde (1968a).

**Nerves of the ventral adhesive disc**

In those aspidobothrian trematodes studied to date, the position of nerves in the ventral adhesive disc coincides with ridges in this region of the body. An outer superficial nerve ring encircles the ventral adhesive disc and may or may not have associated with it an accessory outer superficial nerve ring. An inner superficial nerve ring occurs in or near the ridge separating the outer row of marginal alveoli from the medially located alveoli. This nerve is usually better developed than the outer superficial nerve ring, and receives most of the connectives from the posteroventral nerve cords, including the ventral adhesive disc rami. Occasionally this nerve may also be accompanied by an accessory nerve. Only in *M. purvisi* and in *A. conchicola* where there is a median longitudinal ridge in the ventral adhesive disc, and an accompanying median longitudinal nerve. In *M. purvisi*,
Rohde (1968a) has indicated the presence of a pair of very slender nerves in the median longitudinal ridge. Nerves also occur in each transverse ridge of the ventral adhesive disc of all species of aspidobothrians that have been studied. Pairs of marginal alveolar nerves occur in the ridges bordering the marginal alveoli; these nerves connect the inner and outer superficial nerve rings, and extend across the central portion of the ventral adhesive disc as transverse alveolar nerves. In C. occidentalis, there is but a single transverse alveolar nerve in each transverse alveolar ridge; all other species studied possess a pair of nerves in each transverse alveolar ridge.

Each genus has a specific pattern of ventral adhesive disc nerves whose distribution parallels that of the alveolar ridges.

Nerves of the buccal disc

Nerves of the buccal disc are best developed and most complex in C. occidentalis; they are the least complex in C. insignis. Buccal disc nerves of A. conchicola and M. purvisi appear to be similar. From the cerebral ganglia and circumpharyngeal connectives, buccal disc nerves arise and branch dichotomously to the periphery of the disc where they are connected by a buccal nerve ring. Rohde (1968a) described an anterior terminal commissure occurring in the dorsal portion.
of the buccal disc of *M. purvisi* and this commissure appears to correspond with the buccal nerve ring occurring in the buccal discs of *C. occidentalis*, *A. conchicola*, and *C. insignis*. Two pairs of buccal disc nerves are anterior extensions of nerves that also occur posteriorly, namely, anterodorsal nerves and anteroventral nerves. In studies on *A. conchicola* by Timofeyeva (1971), and *M. purvisi* by Rohde (1968a), the anterodorsal nerves were not observed to reach the buccal nerve ring. In this study, all nerves of the buccal disc except the dorsolateralis (present sometimes), extend to the buccal nerve ring. The anteromedian nerves appear similar in all species studied thus far, except in *C. occidentalis* where these nerves anastomose peripherally in the anterior lobe of the buccal disc. In *C. insignis*, the anterolateral and anteroventrolateral nerves arise from a common trunk, whereas in *C. occidentalis*, *A. conchicola*, and *M. purvisi* they arise separately. Although the oral nerve ring in *C. occidentalis* is supplied by separate nerves from the circumpharyngeal connectives, the oral nerve ring in other species simply connects to superficial nerve branches occurring in the buccal disc.

The phylogenetic significance of differences occurring in nervous systems among *Multicotyle purvisi*, *Cotylogaster occidentalis*, *Aspidogaster conchicola*, and *Cotylaspis insignis* are difficult to assess. The nervous systems of adult
M. purvisi and A. conchicola are very similar. The buccal disc of C. insignis, in contrast to that of other species, has fewer nerves originating independently from the cerebral ganglia and circumpharyngeal connectives. The nerve network occurring in the ventral adhesive disc of C. insignis seems intermediate between the condition found in C. occidentalis and that seen in M. purvisi and A. conchicola. C. occidentalis has the simplest arrangement of ventral adhesive disc nerves, but the most complex arrangement of buccal disc nerves.

Thus, nerves appear to parallel changes in external morphology; consequently, comparative developmental studies of the nervous system will be necessary before the significance of minor differences in adult nervous systems can be properly assessed.

In general, aspidobothrian nervous systems seems intermediate between those of the Acoela and Rhabdocoela. Thus far, aspidobothrians have been found to possess four to five longitudinal nerve cords; like the acoels, five are usually present. According to Bullock and Horridge (1965), longitudinal nerve cords are much reduced among the rhabdocoels; however, they possess some interesting well developed commissures that might be compared to the cervical commissures that are apparent in aspidobothrians.
SENSORY STRUCTURES

Aubert (1855) was probably the first to observe sensory structures in aspidobothrians. He described "small marginal secreting bodies" corresponding in size and location to what Stafford (1896) described as being sensory structures; these are now known to be uniciliate structures, presumably sensory. Certain "skin glands" were described by Voeltzkow (1888) and Stafford (1896) in the tegument of A. conchicola. Halton and Lyness (1971) confirmed the presence of these glands and noted that there were no ultrastructural differences between those associated with the marginal papillae and those gland cells located elsewhere in the tegument. Osborn (1903, 1905) described cuticular sensory structures occurring in the tegument of Cotylaspis insignis, and noted they were sometimes associated with tegumental elevations; in sectioned material some appeared to have a solid core. Nickerson (1895) observed that many sensory structures are associated with the ventral suckers of Stichocotyle nephropsis. At the ultrastructural level, Halton and Lyness (1971), described two morphologically distinct types of sensory receptors occurring in the tegument of Aspidogaster conchicola; namely, the so-called marginal organs or papillae, and numerous uniciliate sensory structures. The latter occur in large numbers around the forebody and along the margin of the ventral adhesive disc.
According to Halton and Lyness (1971), the uniciliate type sensory structures in the tegument of *A. conchicola* are similar to those in the tegument of digeneans, but certain of their ultrastructural characteristics show similarities to cestode and monogenean sensory structures. Rohde (1966a) described up to ten classes of sensory structures found in or below the tegument of *Multicotyle purvisi*, and Rohde (1973) listed six of these as occurring commonly in the tegument of *M. purvisi*. Rohde (1973) published a scanning electron micrograph of a uniciliate sensory structure in the tegument of *Lobatostoma manteri* and estimated that at least 8440 such "papillae" occur in the tegument of this aspidobothrian trematode.

In the present study, acetylcholinesterase localizations revealed the presence of numerous structures associated with the ventral adhesive disc and buccal disc and these were presumed to be sensory. SEM studies of *Cotylogaster occidentalis*, *Aspidogaster conchicola*, and *Cotylaspis insignis*, confirmed the presence of these structures and showed them to be of two types; uniciliate and dome-shaped. New and additional information concerning these presumed sensory structures is given below.
Uniciliate sensory structures (0.8μ to 1.0μ in diameter) (Figure 33) are most numerous on the surfaces and margins of buccal and ventral adhesive discs of \textit{C. occidentalis}. The cilia of many are retracted, and each may thus appear as a white dot in the tegument (Figure 35). An average of 40 uniciliate type sensory structures occur in each marginal alveolus and as many as 200 occur in each transverse alveolus. In a large specimen from \textit{Aplodinotus grunniens} approximately 29,100 uniciliate sensory structures were associated with the ventral adhesive disc. In addition, approximately 12,000 dome-shaped sensory (Figure 37) structures may also be present in the ventral adhesive disc. Although sensory structures may seem to be unusually numerous, they occupy less than 0.3% of the surface area on the ventral adhesive disc. The buccal disc, particularly its peripheral margin, possesses large numbers of uniciliate sensory structures, numbering approximately 1500 in one specimen. Relatively few sensory structures could be counted on the dorsal body surface and neck. In these regions, approximately 1000 were estimated by counting representative areas and multiplying by the total surface area. Thus, a total of approximately 43,600 sensory structures are observable in well developed \textit{C. occidentalis} with the SEM.

Counts of sensory structures, as indicated by acetyl-
cholinesterase localizations, (Figure 78-80) show approximately 50 in each marginal alveolus and approximately 300 in each transverse alveolus. It is thus possible for a well developed worm to contain over 43,000 sensory structures in the ventral adhesive disc alone. Certain of the localizations that are apparent with the light microscope may be subtegumental sensory structures or developing ones, and thus not visible with the SEM.

In some specimens a retractable papilla, without visible pores or sensory structures, occurs in the anteromost median aspect of the buccal disc (Figure 51). Its function is not known, and examinations of sectioned material show only muscular tissue in the area.

Aspidogaster conchicola
(Figures 56-57)

Uniciliate sensory structures (Figure 56) on the ventral adhesive disc of A. conchicola number approximately 2500, but dome-shaped sensory structures, characteristic of this area in C. occidentalis, were not seen except around the outer margin of the ventral adhesive disc and in the buccal disc. Loci of presumed ganglia were apparent (Figure 57). Approximately 1200 uniciliate sensory structures were counted on the margin of the ventral adhesive disc. Both uniciliate and dome-shaped sensory structures (approximately 1000) appear on the
surfaces of the buccal disc (Figure 58). Not more than 500 sensory structures could be counted on the dorsum of \textit{A. conchicola}. Consequently, a total of approximately 5200 sensory structures may be seen with the SEM.

\textit{Cotylaspis insignis} (Figures 61, 69-71)

In \textit{C. insignis}, an average of 30 uniciliate and 10 dome-shaped sensory structures are visible in each alveolus of the ventral adhesive disc (2400 total). Approximately 800 uniciliate sensory structures also occur around the periphery of the disc and an additional 300 of these are evenly distributed over the ventral neck region; approximately 40 of these are concentrated near the gonopore. An estimated 1000 uniciliate sensory structures occur on the surfaces of the buccal disc (Figure 65). Many pore-like apertures 0.1\textmu to 0.2\textmu in diameter occur around the buccal disc and some appear to contain retracted or broken cilia. The tegument of the dorsal body surface is reticulate in appearance and contains relatively few visible sensory structures. A maximum of 200 could be counted dorsally on one specimen. Thus, a total of approximately 4700 sensory structures may be seen with the SEM.

When viewed under the SEM, external sensory structures of the types observed in \textit{C. insignis}, \textit{C. occidentalis}, and \textit{A. conchicola} appear identical. As indicated by Rohde (1966a,
1968b, 1971b, 1972, 1973), aspidobothrian trematodes are well supplied with sensory structures; however, as noted by Halton and Lyness (1971), more ultrastructural studies are needed before the many types of sensory structures described by Rohde (1966a, 1972) for *M. purvisi* can be confirmed.

**Chromosome studies**

Taxonomic affinities of trematodes may be shown by development of their excretory systems and by the nature of their larval development (La Rue, 1957). In addition, Britt (1947) suggested that the number of chromosomes present in a given species may provide insight into relationships between taxa, as did Walton (1959), in an extensive compilation of chromosome numbers of helminth parasites. According to Britt (1947), evolutionary changes in chromosome numbers of trematodes have probably occurred by aneuploidy (the gradual addition or loss of chromatin material). Among the Monogenea, haploid chromosome numbers range from four to six and among digeneans, haploid chromosome numbers range from 6 to 14, the majority varying from 9 to 11 (Walton, 1959). Previous studies on chromosomes of aspidobothrians include those by Brinkmann (1957), who reported *Taeniocotyle elegans* as possessing a haploid number of six or seven chromosomes, and Rohde (1973), who listed *Lobatostoma manteri* as having a haploid number of seven chromosomes.
In the present study, squash preparations stained with acetocarmine were made on two species, namely, *Cotylogaster occidentalis* and *Cotylaspis insignis*. The haploid number of the former is six and of the latter, 11. These determinations were made by Mr. Phil LoVerde, and indicate that on the basis of chromosome numbers, aspidobothrians appear to be more closely related to digeneans than to monogeneans. *C. insignis* has considerably more chromosomes than other aspidobothrians studied to date, which, according to the theory proposed by Britt (1947), indicates that *C. insignis* is widely separated from other aspidobothrian trematodes and perhaps is more highly evolved than is indicated by its morphology.
THE COTYLOCIDIUM LARVA

According to Price (1967), a comparison of larval stages is one of the most reliable means of establishing affinities among organisms. Although larvae of many aspidobothrians have been described, few have been studied in detail. In this section, a detailed description of the newly hatched Cotylogaster occidentalis larva is given, as well as observations on the newly hatched Cotylaspis insignis larva.

Wootton (1966) proposed the term cotylocidium to set apart the larval stage of aspidobothrian trematodes from the oncomiracidium of monogeneans and the miracidium of digeneans. All known cotylocidia are elongate, cylindrical, possess a well-developed posterior sucker or posteroventral disc (Rohde, 1972), buccal disc and cavity, mouth, prepharynx, pharynx, and rhabdocoelous gut. Dorsal, paired excretory bladders open posteriorly via separate pores. Eyespots may or may not be present. When apparent, cilia are limited to tufts appearing near the midregion and behind the posterior sucker. Ciliated cotylocidia are rapid swimmers, and some are capable of inchworm-like movements, similar to those observed in nonciliate types.

Detailed descriptions for cotylocidia of Aspidogaster conchicola have been given by v. Baer (1827), Voeltzkow (1888), Faust (1922), and Williams (1942), of Aspidogaster indica by
Rai (1964), of *Multicotyle purvisi* by Rohde (1968c,d, 1970a,c,d,e,g, 1971a,b,e, 1972), and of *Lobatostoma manteri* by Rohde (1973). Nickerson (1902) and Wootton (1966), have given brief accounts of *Cotylogaster occidentalis* cotylocidia.

**Cotylocidium of *Cotylogaster occidentalis* (Figures 1-24)**

Nickerson (1902) described the cotylocidium of *C. occidentalis* from sheepshead, based on studies of embryos contained in sections of gravid worms. He noted the presence of a mouth, mouth cavity, prepharynx, pharynx, unbranched intestine, posterior sucker, and rudimentary excretory bladders. Cilia were described as occurring in tufts, forming an incomplete band at the level of the pharynx and also as occurring posteriorly. Nickerson found no cilia on the ventral surface of the cotylocidium, and though he did not comment on the number of tufts in the anteriorly located band, eight are clearly shown in his drawing. He described the tegument as a thin homogeneous modified epithelium lacking cell outlines, with thickened, ciliated, crater-like depressions, and containing nuclei only in ridges around ciliary tufts. Nickerson presumed cotylocidia to have powers of penetration because he observed large cells containing acid-fast granules and having long processes leading to the surface, similar to the penetration glands of miracidia. He further predicted
that *C. occidentalis* probably has two hosts.

Wootton (1966) added to Nickerson's 1902 data with observations of living material and silver-nitrate-treated specimens obtained from mussels (*Ligumia nasuta* and *Anodonta grandis*). He described cotylocidia as active swimmers, somewhat larger (73μ x 172μ) than those observed by Nickerson (68μ x 98μ). According to Wootton, the cotylocidium intestine is characterized by a thin-walled ventral portion and a sphincter-like constriction separating it from a larger thick-walled dorsal portion. Wootton reported the flame cell pattern as 2(2+2), and described "ciliated epidermal cells" occurring in two transverse bands, an anterior band consisting of four dorsal and four ventral circular patches of cilia, and a posterior band with four ciliary patches.

Rohde (1972) cited Wootton (1966) as disagreeing with Nickerson (1902) concerning the number of flame cells; Nickerson (1902), however, gave no account of flame cells.

Nickerson (1902) examined cotylocidia from worms in fish, and Wootton (1966) based his studies on specimens from mussels. In the present study, specimens were taken from the following pelecypods: *Lampsilis siliquoidea*, West Lake Okoboji, Iowa, *Ligumia nasuta* and *Anodonta grandis*, Douglas Lake, Michigan, and fish: *Aplodinotus grunniens*, Lake Pepin, Mississippi River, Minnesota.
Cotylocidia of C. occidentalis are cylindrical, elongate (100µ to 185µ x 35µ to 55µ), (living cotylocidia may extend up to 250µ in length or contract to become 100µ in diameter) with a buccal disc forming a subterminal opening and cavity. A minute mouth at the base of the cavity is followed by a short prepharynx, pharynx, and a rhabdocoelous gut extending to the level of the osmoregulatory bladders. A well developed posterior sucker (20µ to 40µ in diameter) is apparent (Figures 3, 19).

Silver nitrate localizations were useful in demonstrating circular ciliated areas of the tegument (Figures 4, 5, 9, 15). Cilia are restricted to fourteen circular tufts or patches, each having a crenulated margin 10µ in diameter (Figures 9, 15). Scanning electron microscope (SEM) observations show each patch as containing approximately 400 cilia (Figure 22). The latter average 18µ in length, each possessing an enlarged base 0.02µ in diameter. Circular patches of cilia appear in two areas on the cotylocidium as follows: a ventrally incomplete ring of eight encircling the body approximately 50µ from the anterior end (Figures 4, 9), and a group of six evenly spaced in a circle around the base of a conical, muscular elevation posterodorsal to the posterior sucker (Figures 5, 15, 20, 21). Cilia are absent on the ventral surface of the cotylocidium (Figures 9, 10). In addition to ciliated areas, other tegumental structures were indicated with
silver nitrate localizations and confirmed by SEM studies.

The tegument of the *C. occidentalis* cotylocidium appears fuzzy when observed with the SEM, which is probably due to the presence of a "glycocalyx" (Bennett, 1963) covering the larva.

Osmoregulatory pores appear in the form of signet rings (4μ diameter) on the dorsal surface, anterior to the postero-dorsal group of cilia (Figures 15, 17). Observations with the SEM show that each osmoregulatory pore is recessed in a tegumental elevation, consisting of a circular ridge with a groove at its anterior margin (Figure 23), hence, its signet ring appearance with the light microscope.

Openings of cephalic glands (Figure 8) are apparent lateral to the mouth, and SEM studies show them to be large papillae protruding from slightly raised pores (Figures 12, 14).

Presumed sensory structures may be seen as raised dots (Figures 4-9), some outlined by a peripheral ring (Figures 17, 18). With the light microscope 46-54 of the former type were counted in the anterior third, and ten to fifteen in the posterior two-thirds of the cotylocidium. That type characterized as being ringed, number approximately twenty and occur only in the posterior two-thirds of the cotylocidium. SEM observations showed that structures appearing as raised dots in silver-nitrate-treated material are rounded elevations.
(0.8μ x 1.5μ) in the tegument, each bearing a cilium 1μ to 3μ long (Figures 10-14). Those dots outlined by a ring are dome-like structures 0.8μ - 0.1μ in diameter, each surrounded by a circular tegumental thickening 2.0μ to 2.5μ (Figures 19, 23). Uniciliate sensory structures are most numerous in the cephalic region; greatest concentrations occur in the immediate region of the buccal opening, and among those specimens examined, a consistent pattern of sensory structures was apparent. Two sensory structures are found laterally on the dorsal surface of the buccal cavity (Figures 13-14). Occurring around the mouth in eight groups of three, are 24 cilia bearing sensory structures. In three of four well preserved specimens examined, each group of sensory structures usually consisted of a structure bearing a cilium approximately 2μ - 3μ long, the others with cilia each 1μ - 1.5μ in length. In addition to the above, 20-25 additional cilia-bearing sensory structures were observed scattered over the anterior region. Comparatively few visible sensory structures are found on the posterior two-thirds of the cotylocidium. Surrounding the posterior sucker are six evenly spaced cilia bearing sensory structures (Figure 19). At the level of the excretory pores and in the midregion, ten to twelve dome-type sensory structures encircle the cotylocidium (Figure 19). Additional dome-type structures could be seen near the anterior group of ciliary tufts, but because of
the long cilia their total number could not be discerned. An estimated total of 80-90 sensory structures occur externally in the tegument of *C. occidentalis* cotylocidia.

The osmoregulatory system (Figure 24) is characterized by a 2(2+2) flame cell pattern as described by Wootton (1966). A pair of osmoregulatory ducts empties into completely separate dorsal osmoregulatory bladders (18μ in diameter) (Figures 1, 2, 16, 24), each containing a large irregular concretion (Figures 2, 24).

Acetylcholinesterase localizations (Figure 3) in the cotylocidium indicate a large anterior concentration of nervous tissue, with processes extending posteriorly on each side and joining another concentration around the posterior sucker.

In living cotylocidia, at least 3 pairs of cephalic gland cells with processes leading to the previously described anterior openings, can be distinguished (Figure 24). Anteriorly, two are located on each side of the pharynx, and in the midregion one cell appears on each side of the gut.

The cotylocidium develops within a nonoperculate egg (35μ - 45μ x 110μ - 140μ) which is usually retained in the uterus until mature. Hatched cotylocidia may be observed regularly in the adult uterus. Eggs liberated into filtered lake water and containing fully developed cotylocidia hatch within minutes of being extruded. Prior to hatching, secre-
tions appear to be extruded from openings of the cephalic
glands, and shortly afterward the egg ruptures anterolaterally.
Eggs not containing mature cotylocidia either failed to hatch
or give rise to erratic-swimming, short lived specimens. Dif­
f erences were apparent in numbers of viable eggs produced
by worms from various hosts examined, as shown in Table 1.
Temperature appears to affect the longevity of cotylocidia.
The latter, when maintained in filtered lake water from the
time of hatching remained alive for an average of 56 hrs.
at 4°C, 72 hrs. at 10°C, and 48 hrs. at room temp. Specimens
allowed to hatch in saline at room temperature lived for a
maximum of 24 hrs.; in distilled water they died within
minutes.

Cotylocidia from both mussel and fish hosts of various
ages (2, 4, 6, 12, 24, and 48 hrs.) were exposed to snails
(Pleurocera acuta and Goniobasis livescens), mussels
(Lampsilis ventricosa, Lampsilis siliquoidea, Anodonta
grandis and Carunculina sp.) and young sheepshead (Aplodinotus
grunniens). Examinations of exposed molluscs and fish at
1, 2, 7, 14 and 30 days were negative.

Cotylocidium of Cotylaspis insignis (Figures 25, 26)

Detailed accounts of Cotylaspis insignis cotylocidia are
lacking. Osborn (1905), described a "young individual" of
C. insignis from the renal epidermis of Anodonta grandis;
Table 1. Cotylocidium production by gravid *C. occidentalis* from fish and mussel hosts

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Number of gravid adults recovered</th>
<th>Number of gravid adults producing cotylocidia</th>
<th>Average Number of eggs extruded</th>
<th>Average Number cotylocidia produced by each worm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lampsilis siliquoidea</em></td>
<td>29</td>
<td>13 (45%)</td>
<td>13 (50)a</td>
<td>3 (20%) b</td>
</tr>
<tr>
<td><em>Ligumia nasuta</em></td>
<td>35</td>
<td>24 (69%)</td>
<td>42 (125)</td>
<td>12 (28%)</td>
</tr>
<tr>
<td><em>Aplodinotus grunniens</em></td>
<td>100</td>
<td>82 (82%)</td>
<td>56 (142)</td>
<td>23 (50%)</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>164</td>
<td>119 (72%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Maximum in parentheses.

*b* Percentage of average number of eggs hatched in parentheses.
he published three figures of this specimen. Neither measurements nor magnifications were given, but he likened it to a particular developmental stage of *Aspidogaster conchicola* (25μ x 100μ), described and figured by Voeltzkow (1888). Osborn (1903, 1905) described his specimen as having a mouth funnel, pharynx leading into an unbranched intestine, separate osmoregulatory bladders opening independently, and ventral sucker without apparent subdivisions or sensory structures. A "single lumpy concretionary mass" was observed in each osmoregulatory bladder, and according to Osborn, behavior of the "young individual" paralleled that of the adult. Eggs of *C. insignis* as measured in stained whole mounts by Hendrix and Short (1965) averaged 87μ x 159μ in contrast to Osborn's measurements of unfixed eggs (100μ to 160μ x 220μ x 360μ). Osborn (1905) recorded a maximum of 13 eggs in the adult uterus; Hendrix and Short (1965) observed a maximum of seven (average three); both reported eggs as yellow and operculate. Osborn observed their curved outline and noted that some eggs were bioperculate. Tandon (1949) incubated eggs of *Lissemysia ovata*, an Indian species that is similar to *C. insignis*, but was unable to obtain hatched embryos. Osborn (1905) maintained *C. insignis* eggs in an aquarium but none hatched, and he concluded that a secondary host is probably needed to supply the hatching stimulus.

In the present study, gravid *Cotylaspis insignis* (30
specimens) contained one to six (average three) yellow, crescent-shaped eggs measuring 68\(\mu\) to 90\(\mu\) x 160\(\mu\) to 190\(\mu\) (average 76\(\mu\) x 175\(\mu\)). Living cotylocidia of *C. insignis* (three studied) lack cilia, measure 85\(\mu\) x 180\(\mu\), contracting to 125\(\mu\), and when extended, are nearly 300\(\mu\) long (Figure 25). Buccal disc and posterior sucker were used by the cotylocidium to effect leech-like movements, and one specimen was observed floating upside down with the posterior sucker adhering to the water surface. The posterior sucker (60\(\mu\) to 140\(\mu\)), possesses approximately 20 evenly spaced peripheral structures presumed to be sensory (Figure 25). The posterior sucker is capable of being shifted to a terminal position from which the animal can extend 360° in any direction. The buccal disc is muscular and forms a subterminal opening and cavity; a pore-like mouth lies at the base of the cavity. The prepharynx is short, the pharynx is well developed and protrusible, and a rhabdocoelous gut extends just anterior to the level of the osmoregulatory bladders. The latter are separate, open dorsally near the posterior end; each contains a single concretion (Figure 26). Eyespots are present, lateral to the pharynx.

The presence or absence of cilia is an obvious morphological variable among cotylocidia of different species. Those species whose cotylocidia are known to possess cilia include *Cotylogaster occidentalis*, *Multicotyle purvisi*, and
Lophotaspis vallei. C. occidentalis cotylocidia have the largest number of ciliary tufts among those forms that have been studied; six occur posteriorly (Wootton, 1966, reported four) and eight occur anterior to the midregion, for a total of 14. According to Rohde (1972), M. purvisi cotylocidia have six posteriorly located tufts and four that are located just posterior to the midregion, for a total of ten. Manter (1932) described the cotylocidia of L. vallei as having a single posterior tuft of cilia, and two tufts posterior to the midregion, for a total of three, and according to Manter (1932) each tuft is approximately 20μ in diameter. Ciliary tufts in M. purvisi described by Rohde (1972), and those in C. occidentalis (this study) measure approximately 10μ in diameter. Swimming movements in cotylocidia of M. purvisi according to Rohde (1972), and those of C. occidentalis (this study), are helical and clockwise. Manter (1932) described L. vallei cotylocidia as being rapid swimmers and that they were also capable of inchworm-like movements, such as are seen in cotylocidia of Cotylaspis insignis. Those aspidobothrian species whose cotylocidia are aciliate include Cotylaspis insignis, Aspidogaster conchicola, Aspidogaster indica, and Lobatostoma manteri. Except for Lobatostoma manteri cotylocidia of the above species demonstrate leech-like movements and can float upside down while adhering to the water surface. According to
Rohde (1973) cotylocidia of _L. manteri_ hatch after the eggs are ingested by a snail, a phenomenon also observed by Huehner and Etges (1972a) when they experimentally infected snails with _A. conchicola_.

Ciliation of larval stages in trematodes is considered a primitive condition (Hyman, 1951; Llewellyn, 1965). According to Hyman (1951), acoel and rhabdocoel turbellarians typically have a ciliated epidermis both as larvae and adults. Llewellyn (1965) is of the opinion that in monogeneans cilia were retained only by the larval stage of these parasites for the purpose of invading new hosts. According to Stunkard (1963) miracidia of the digenean trematode _Haplometra cylindracea_ have been observed by various workers as being ciliated at the time of hatching as well as shedding their cilia prior to hatching. From this observation Stunkard (1963) suggests that aciliate miracidia are derived from ciliated ones. Aspidobothrians with ciliated larvae might for these reasons be regarded as more primitive than those without ciliated cotylocidia. Furthermore, if reduction of cilia among aspidobothrian cotylocidia is another example of specialization in more highly evolved species, then of those forms which have been studied, _Cotylogaster occidentalis_ might be considered the most primitive.

Types of sensory structures described for _C. occidentalis_ cotylocidia are very similar to those seen in adult _C._
occidentalis. Rohde (1966a, 1972) counted 111 and 115 "ring-shaped papillae" distributed in the teguments of two M. purvisi cotylocidia. According to Rohde (1972), papillate sensory structures are approximately symmetrical in arrangement dorsally, but their number and arrangement is quite variable. Rohde (1972) also described several "paired dark bodies" that correspond in size and location to dome-shaped structures presumed to be sensory. Rohde (1972) published a series of three micrographs from ultrastructural studies of sensory structures of cotylocidia; however, the plane of sections and the low magnification make interpretation difficult. Further ultrastructural studies are needed before the true nature of tegumental sensory structures in cotylocidia can be determined, because in the case of the dome-shaped structures it is possible that these are openings of gland cells, and are not sensory as supposed.

The glycocalyx covering, thought to be present of C. occidentalis cotylocidia was described by Rohde (1972) for M. purvisi cotylocidia. According to Rohde (1972), M. purvisi cotylocidia are covered with "microfilia," a covering that in other parasites is regarded as a glycocalyx by Bennett (1963), Wright and Lumsden (1968), Lumsden et al., (1970), and Halton and Lyness (1971).

Concretions found in the osmoregulatory bladders of C. occidentalis and C. insignis cotylocidia were observed in
young juveniles of *C. insignis* by Osborn (1903, 1905). Nickerson (1895), in studies on juveniles of *Stichocotyle nephropsis*, noted that gas is formed when concretions present in the excretory system of *S. nephropsis* come in contact with acids such as picrosulphuric. He thus interpreted these concretions to be a carbonate. Concretions (corpuscles) have been reported as occurring in the osmoregulatory system of many adult aspidobothrians, and Pearson (1972) has observed that the presence of excretory corpuscles in the osmoregulatory bladders of digenean cercariae tends to accompany primitive two-host life cycles. The significance of Pearson's 1972 observation when applied to aspidobothrians cannot be assessed at this time. As noted in Table 1, the fish host (*Aplodinotus grunniens*) appears to be more favorable than either of the mussel hosts (*Lampsilis siliquoidea* and *Ligumia nasuta*) for the production of large numbers of eggs and cotylocidia in *C. occidentalis*. Additional discussion on this topic will be given in the section on host-parasite relationships.

Behavior of certain larval as well as adult aspidobothrians, such as *Cotylaspis insignis*, appears to be rather complex and resembles that of a certain rhabdocoeel. According to Bullock and Horridge (1965), Honjô (1937) observed that a small temnocephalan rhabdocoeel *Caridinicola*, cannot perform leech-like movements without a brain. Extirpation
experiments on aspidobothrians may reveal interesting information concerning their dependence upon a "brain" to coordinate complex behavior.
Details of postembryonic development among aspidobothrian trematodes are poorly known, even though one or more developmental stages have been described for all genera except Lissimysia. Polyembryony has not been demonstrated in aspidobothrians, and in all accounts of development the cotylocidium simply loses its cilia, if present, and grows to adulthood. The most conspicuous aspects of this transformation, other than general size increase, include growth and differentiation of the posterior sucker into the massive adult ventral adhesive disc, and development of reproductive structures.

Early studies on trematodes were concerned primarily with adult morphology, and, until the significance of presence or absence of asexual generations in life cycles became important in understanding their taxonomic affinities (van Beneden, 1858), no concerted efforts to elucidate aspidobothrian development were made. Aubert (1855), in describing several developmental stages (herein referred to as juveniles) of Aspidogaster conchicola v. Baer 1827, concluded that it metamorphoses directly without asexual stages, a suggestion which had been made earlier by Dujardin (1845). Burmeister (1856) consequently gave aspidobothrians status equal
Voeltzkow (1888) described a reasonably complete series of developing juvenile *Aspidogaster conchicola*. He observed a general increase in body size of the juvenile prior to differentiation of its posterior sucker into alveoli, a characteristic also observed in *Aspidogaster conchicola* by Williams (1942), by Rohde (1968c, 1970c,g, 1971a, 1972) in *Multicotyle purvisi*, and in *Lobatostoma manteri* by Rohde (1973). In *A. conchicola*, the posterior sucker of early juveniles ultimately differentiates into four longitudinal rows of alveoli, but how this occurs remains unclear. Voeltzkow (1888) observed that in the same species the posterior sucker first differentiated into a series of transversely elongated alveoli, followed by the appearance of a single median and 2 lateral longitudinal ridges. When three longitudinal ridges are present, the ventral adhesive disc of *A. conchicola* had reached its adult condition. SEM data given earlier in this study appear to support Voeltzkow's 1888 observation. Rai (1964) described several juveniles in a similar species (*Aspidogaster indica*), but in his youngest specimens (53 days) the ventral adhesive disc was already adult-like. Williams (1942) outlined the development of *A. conchicola* as consisting of four stages during which a deep sac-like posterior sucker evaginates to become the ventral adhesive disc. Williams observed that marginal
(lateral) alveoli appear first. In *Cotylogaster occidentalis* according to Wootton (1966), and in *Multicotyle purvisi*, as observed by Rohde (1968c, 1970c,g, 1971a, 1972), alveoli first appear in the anterior margin of the posterior sucker. Rohde (1970a) observed that the four longitudinal rows of alveoli in juvenile *M. pursivi* differentiate simultaneously, and concluded that growth of the ventral adhesive disc is by apposition and stretching. A posterior growth zone has been identified as the point of alveolar formation in many aspidobothrians, namely: *Stichocotyle nephropsis* by Cunningham (1887) and Nickerson (1895), *Taeniocotyle elegans* by Jägerskiöld (1899), Brinkmann (1957), and Burt (1968), in *Multicotyle purvisi* by Rohde (1968c, 1971a, 1972), and in *Lobatostoma manteri* by Rohde (1973).

**Development in *Cotylogaster occidentalis***

Development in *Cotylogaster occidentalis* is a gradual growth process whereby the cotylocidium loses its cilia, passes through a growing juvenile stage, and becomes an adult. Juvenile worms first appear in the mouth and esophagus of the host mussel, and as development continues they migrate through the stomach into the intestine where they attain sexual maturity. Gravid worms are most often found near the heart in the distal portion of the intestine.

A representative series of *C. occidentalis* juveniles
(Figures 27-41) was taken from naturally infected mussels (*Ligumia nasuta*). Measurements of these living juveniles and their locations within the host are presented in Table 2.

In the following section, specimens I through VI are described individually.

**Specimen I**  
(Figures 31-33)

Juveniles recovered from the mouth of *Ligumia nasuta* are morphologically little different from cotylocidia, except for their larger size and lack of cilia. Posterodorsal to the posterior sucker, a raised area (Figure 32) is present that corresponds in location to the posterior cilia-bearing cone of the cotylocidium and the dorsal cone of older specimens. Sensory structures, identical to those present on cotylocidia, appear on the bodies of juveniles. At this stage, sensory structures include a type bearing an external cilium, numbering approximately 40 on the posterior sucker (Figures 32, 33). Osmoregulatory bladders are elongated, each containing a single irregular concretion; bladders open dorsoterminal via separate pores. Acetylcholinesterase localizations show weakly developed nerve cords running anteriorly from a cerebral ganglia that is located dorsal to the pharynx. Dorsal and lateral posterior nerve cords are weakly developed and cross commissures are difficult to discern. Posterior
Table 2. Measurements (in microns unless otherwise stated) of living juvenile *Cotylogaster occidentalis* and their locations within the host

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>Total Length $^a$</th>
<th>Posterior Sucker Length $^b$</th>
<th>Gut Length</th>
<th>Pharynx</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>450</td>
<td>100 (22%)</td>
<td>180</td>
<td>35 x 50</td>
<td>mouth</td>
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<tr>
<td>II</td>
<td>650</td>
<td>150 (25%)</td>
<td>250</td>
<td>45 x 51</td>
<td>esophagus</td>
</tr>
<tr>
<td>III</td>
<td>750</td>
<td>200 (30%)</td>
<td>470</td>
<td>70 x 75</td>
<td>esophagus</td>
</tr>
<tr>
<td>IV</td>
<td>950</td>
<td>315 (33%)</td>
<td>630</td>
<td>78 x 80</td>
<td>stomach</td>
</tr>
<tr>
<td>V</td>
<td>1.2mm</td>
<td>650 (54%) expanded</td>
<td>780</td>
<td>75 x 80</td>
<td>stomach</td>
</tr>
<tr>
<td>VI</td>
<td>1.3mm</td>
<td>775 (60%)</td>
<td>900</td>
<td>80 x 90</td>
<td>stomach</td>
</tr>
</tbody>
</table>

$^a$Total length includes neck and buccal disc.

$^b$Percent of total body length in parentheses.
ventral nerve cords are apparent and each connects with a nerve ring in the posterior sucker.

**Specimen II**

In this specimen the only apparent change is size and location of the juvenile within the host. Alveoli are not apparent in the posterior sucker, and rudiments of the reproductive system cannot be discerned. Nervous and osmoregulatory systems remain unchanged.

**Specimen III**
(Figures 21, 34, 38)

The posterior sucker of this juvenile is pyriform in outline, lacks alveoli, and 70 cilium-bearing sensory structures were counted on its outer surface. Internally, a crater-like depression, presumed to be the zone of differentiation (Figure 34), occurs in the floor of the posterior sucker. Primordial elements of terminal genitalia and the developing gonads are present. A gonopore, outlined by darkly stained cells, appears midway between the buccal disc and posterior sucker. Extending posteriorly from the gonopore, primordial terminal genitalia (Figure 38) extend as a solid mass of darkly stained cells. This mass of cells is constricted a short distance from the gonopore, continues posteriorly, and then branches into a pair of cords that can be traced for a short distance before they disappear. Gonadal
primordia appear as a single mass (Figure 38) of darkly stained cells posteroventral to, and contiguous with, the intestine. Osmoregulatory and nervous systems show little change.

Specimen IV
(Figures 28, 39)

Marginal and transverse alveoli appear in what may now be termed the ventral adhesive disc. In fixed or frozen specimens the anterior portion of the ventral adhesive disc is expanded; however, in living specimens it may be constricted (Figure 39) giving it a sucker-like appearance. Alveoli are well developed anteriorly and become less prominent posteriorly to a point where there is a depression from which alveoli appear to arise in anteriorly directed crescent-shaped waves (Figure 28). Alveoli are not visible posterior to the depression. Pores of the marginal papillae are present, and at least 300 uniciliate sensory structures are visible on the ventral adhesive disc. The buccal disc is well supplied with sensory structures of both types, as found in the cotylocidium. Genital ducts are connected to the gonadal primordium (100µ x 190µ), and the latter is no longer contiguous with the intestine (Figure 39). Developing terminal male genitalia consist of an enlarged prostatic sac (250µ long) not yet supplied with prostatic cells, seminal vesicle
(60μ long), and a vas deferens. Male and female ducts appear fused at the point of their association with the primordial gonadal mass. Cross commissures of the nervous system are more apparent, and the terminal nerve ring in the buccal disc is visible. Nerves supplying the alveoli of the ventral adhesive disc are extremely faint.

Specimen V
(Figures 29, 40)

Increased growth of the ventral adhesive disc is apparent (Figure 29). When constricted, it still appears sucker-like. Little change has occurred in the genital ducts. They have elongated, and the gonadal primordium shows two groups of darkly stained cells within its substance, each approximately 50μ in diameter (Figure 40).

Specimen VI
(Figures 30, 35-37, 41)

The ventral adhesive disc is fully differentiated into alveoli, consisting of a peripheral ring of marginal ones surrounding a median row of transversely elongated alveoli (Figures 30, 36, 37, 41). Terminal genitalia have increased in size, each containing a lumen. Male and female ducts are slightly coiled, and are connected to their respective gonads (Figure 41). The pyriform ovary (70μx90μ) is connected to an anteriorly reflected oviduct which in turn gives rise to the
uterus. Laurer's canal is present, but does not open to the outside (Figure 35). Elements of the vitelline follicles, vitelline ducts, and Mehlis' gland do not appear. The nervous system is similar to that of adult _Cotylaspis insignis_. Nerves supplying the alveoli of the ventral adhesive disc can be traced anteriorly, but are extremely faint posteriorly. Osmoregulatory pores are separate (Figure 35).

**Young Adult**
(Figure 42)

Young adult specimens of _C. occidentalis_ occur in the intestines of mussels and demonstrate typical adult morphology as described by Fredericksen (1972).

_Cotylogaster occidentalis_ appears to be unique in that reproductive development begins before alveoli appear in the posterior sucker or primordial ventral adhesive disc. According to Voeltzkow (1888), the embryonic reproductive system of _Aspidogaster conchicola_ becomes evident in juveniles 1mm long, after alveoli have appeared. According to Voeltzkow (1888), the first elements of the reproductive system to appear are solid masses of cells; one ectodermal mass extends from the gonopore posteriorly between the gut and body septum, and joins a concentration of mesodermal cells lying ventral to the body septum and near the posterior extremity of the intestine. Voeltzkow (1888) considered the vas deferens,
cirrus, cirrus sac, uterus, and metraterm to arise from ectoderm, and the ovary and testis from mesoderm. He also described a dorsal group of ectodermal cells radiating inward to form a "receptaculum vitelli", now known to be Laurer's canal. Unlike other aspidobothrians, the Laurer's canal in A. conchicola never opens to the outside. Yolk glands in A. conchicola develop from the ovary as a cord adjacent to the excretory duct (Voeltzkow, 1888). In studies on the same species, Stafford (1896) reported that Laurer's canal did not originate dorsally from ectoderm growing inward, but that instead it developed outwardly from rudiments of the gonads. Steinberg (1931) also refuted Voeltzkow's idea that the Laurer's canal develops from ectoderm, and described rudiments of the metraterm, cirrus, and cirrus sac developing as a hollow tube instead of as a solid mass. According to Rohde (1971d, 1972), elements of the reproductive system in M. purvisi are visible after alveoli are well differentiated and first appear as a thickening in the terminal portion of the intestinal wall, which later gives rise to both genital organs and ducts. The latter are at first solid, and eventually divide into male and female portions that later separate from the intestine, and as these ducts mature they become hollow (Rohde, 1972).

In C. occidentalis, development of reproductive structures appears to parallel that described for A. conchicola by
Voeltzkow (1888), as emended by Stafford (1896, 1898), and Steinberg (1931). In both species, elements of the reproductive system appear simultaneously in two separate locations - near the gonopore, and near the posterior extremity of the intestine. As in *A. conchicola*, the terminal genitalia of *C. occidentalis* are apparently formed by an inward reflection of ectoderm, and this later joins mesodermally derived gonadal elements. In *C. occidentalis*, the genital ducts become hollow after they differentiate.

In *C. occidentalis*, differentiation of the juvenile posterior sucker into alveoli apparently takes place first anteriorly, on its inner surface. What is interpreted to be a zone of differentiation (that which is responsible for initiating alveolar formation), may be seen on the floor of the posterior sucker in a young juvenile (Figure 34). As differentiation continues, the zone of alveolar differentiation assumes a more posterior location in the developing ventral adhesive disc, and as this occurs, the disc appears to evaginate, revealing the newly formed alveoli. The latter appear to be differentiated in a wave-like procession of transverse rows (Figure 28). As alveoli appear, marginal papillae appear also, and uniciliate sensory structures increase in number on the surfaces of the former. The completely evaginated ventral adhesive disc continues to grow even after sexual maturity is reached, and its continued growth appears
to occur posteriorly as is indicated by the lack of maturity among posteromost alveoli. Once differentiated, alveoli themselves do not give rise to additional ones, because in all specimens examined from juveniles through the large adults, numbers of anteromost marginal alveoli (those anterior to the first transverse alveolar ridge) remain constant. Thus, C. occidentalis like other aspidobothrians, appears to have a posterior growth zone.

Llewellyn (1965) has suggested that development of a posterior sucker in trematodes, particularly monogeneans, resulted from the necessity for stabilizing the body while feeding upon active prey. This organ of attachment is present in most trematodes and occurs in all known aspidobothrian cotylocidia. In the latter trematodes, the posterior sucker develops into a remarkable ventral adhesive disc of tremendous size. The ability of the ventral adhesive disc to secure the organism is not apparent except in ectoparasitic species. Adult obligate endoparasitic aspidobothrians have never been described as being capable of adhering tightly to a substratum, except by their oral discs. In C. occidentalis when attachment occurs, it appears to be effected primarily by the buccal disc. These observations tend to indicate that the ventral adhesive disc may serve a purpose other than attachment in most adult aspidobothrians. It is supplied with numerous sensory structures and the adult ventral adhesive
disc must be considered, in part, sensory. Many gland cells are present in the ventral adhesive disc; certain of these open at the marginal papillae, whereas others, according to Halton and Lyness (1971), open over the general surface of the ventral adhesive disc in _A. conchicola_. In sheepshead, the villi under the ventral adhesive disc of _C. occidentalis_ in situ, are partially eroded (Figure 100). Rohde (1972) has noted that with age the gut decreases in size and apparent importance in the trematode _M. purvisi_. In light of these observations, it seems possible that the ventral adhesive disc may have become an organ of extracellular digestion in some aspidobothrians.

**Host-Parasite Relationships**

Information concerning host-parasite relationships of aspidobothrian trematodes is scanty, and data as to whether aspidobothrians are primitively parasites of molluscs or vertebrates are inconclusive. Furthermore, little is known concerning taxonomic relationships among families, genera, and species of the Aspidobothria.

Because digenean trematodes, with rare exceptions, begin their development in molluscs, they have been considered primitively parasites of molluscs by Chandler (1923), Stunkard (1946), LaRue (1957), and Wright (1960). Among those aspidobothrians whose life cycles are known, development begins in a
mollusc as in the Digenea. Thus, Stunkard (1967), Dollfus (1958), and Rohde (1972) have concluded that aspidobothrians are more closely related to the Digenea than to the Monogenea, and Rohde (1972) has further concluded that aspidobothrians probably represent what he terms the "Prodigenea." Except for Lobastoma manteri, aspidobothrians whose life cycles are known are fresh-water species and may not represent the primitive condition, because published host records indicate that marine aspidobothrians have closer ties with their vertebrate hosts than do fresh-water species. Indeed, Rohde (1973) has found that in at least one locality, the marine aspidobothrian Lobastoma manteri requires a vertebrate (fish) host to complete its life cycle. According to Linton (1940) and Odhner (1898, 1910), Stichocotyle nephropsis adults occur in the bile ducts of Raja sp., an elasmobranch fish, whereas juveniles have been reported only from lobsters (Nephrops norvegicus L. and Homarus americanus Edwards) by Cunningham (1884, 1887), MacDonald (1877), Nickerson (1895), and Linton (1940). Taeniocotyle elegans (Olsson, 1869), Stunkard, 1962, occurs regularly in the bile ducts of the elasmobranch Chimera monstrosa L. as reported by Olsson (1869), Jägerskiöld (1899), and Brinkmann (1957). The location of the above aspidobothrians in a specific region of the vertebrate hosts suggests that a vertebrate is obligatory in the life cycle. Consequently, an understanding of relationships of aspido-
bothrians to other trematodes and to one another requires data concerning their respective hosts.

**Cotylogaster occidentalis**
(Figures 100-101)

Species of the genus *Cotylogaster* are known from both fresh and marine waters, and the only known fresh-water species, *Cotylogaster occidentalis*, has been reported from the digestive tracts of pelecypod (Figure 101) and gastropod molluscs as well as from sheepshead (Figure 100). In mussels, *C. occidentalis* occurs in a restricted area of the digestive tract (Kelly, 1926; Fredericksen, 1972). In sheepshead, *C. occidentalis* occurs in a specific posterior region of the intestine separated from the rest of the tract by a sphincter (Dickerman, 1948; Fredericksen, 1972). According to Dickerman (1948), young sheepshead of Lake Erie are never infected, but when he fed *C. occidentalis* from *Goniobasis* sp. to them, worms remained alive in the intestines for at least 5 days. Thus, a vertebrate may be important in the life cycle of *C. occidentalis*.

In the present study (Table 1), adult worms were recovered from the intestines of naturally infected mussels (*Lampsilis siliquoidea, Lampsilis ventricosa, and Ligumia nasuta*), and from sheepshead (*Aplodinotus grunniens*).

In mussels, juvenile stages of *C. occidentalis* (to be
discussed later) were recovered from the mouth, esophagus, and stomach of *Ligumia nasuta*. The smallest juveniles were located near the mouth, and the largest were found in the stomach; sexually mature worms were found only in the intestines of these mussels. Gravid adults were recovered from the distal portion of the intestine either proximal to, or in that region of the intestine passing through the pericardium of the mussel. When in situ, *C. occidentalis* appear as pinkish areas within the host's intestine and when several are present, all are usually close to one another. Eggs and ctylocidium may sometimes occur in the mussel's intestine. Differences in percentages of infection were noted in mussels of different species and in mussels from different localities. Surveys of *Lampsilis siliquoidea* in West Lake Okoboji, Iowa, for example, revealed 25% to be infected with *C. occidentalis* (Table 3). Less than 3% of Lake Pepin lampsilids were infected with this species; in Douglas Lake, Michigan, 90% of *Ligumia nasuta* and only 15% of *Anodonta grandis* were infected (Table 3).

In sheepshead, where *C. occidentalis* is invariably found in the posterior region of the digestive tract, specimens are not always securely attached. Many worms can be found crawling among the villi, or may be lodged in the intestinal mucus. When attached, the neck of the worm is extended deep among the intestinal villi, and here the buccal disc is closely
Table 3. Frequency of aspidogastrid infections in pelecypod and gastropod molluscs of Lake Pepin, Mississippi River; Ft. Madison, Mississippi River; and Douglas Lake, Michigan (1971-1972)

<table>
<thead>
<tr>
<th>Hosts and Localities</th>
<th>Number Examined</th>
<th>Number Infected with Cotylogaster</th>
<th>Number Infected with Aspidogaster</th>
<th>Number Infected with Cotylaspis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pelecypoda-Lake Pepin:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblema costata Rafinesque</td>
<td>103</td>
<td>-</td>
<td>84</td>
<td>-</td>
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<tr>
<td>Anodonta corpulenta Cooper</td>
<td>12</td>
<td>-</td>
<td>8</td>
<td>-</td>
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<td>Anodonta grandis Say</td>
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<td>-</td>
</tr>
<tr>
<td>Anodonta imbicilllis Say</td>
<td>23</td>
<td>-</td>
<td>18</td>
<td>-</td>
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<td>Carunculina parva Barnes</td>
<td>37</td>
<td>-</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Cyclonaias tuberculata (Rafinesque)</td>
<td>14</td>
<td>-</td>
<td>16</td>
<td>-</td>
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<tr>
<td>Elliptio complanatus (Dillwyn)</td>
<td>31</td>
<td>-</td>
<td>28</td>
<td>-</td>
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<td>Fusconaia undata (Rafinesque)</td>
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<td>12</td>
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<td>16</td>
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</tr>
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<td>Truncilla truncata Rafinesque</td>
<td>12</td>
<td>-</td>
<td>2</td>
<td>-</td>
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<td><strong>Gastropoda-Lake Pepin:</strong></td>
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<td>Pleurocera sp.</td>
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<tr>
<td>Viviparus sp.</td>
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<td>-</td>
<td>-</td>
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<td>Hosts and Localities</td>
<td>Number Examined</td>
<td>Number Infected with Cotylogaster</td>
<td>Number Infected with Aspidogaster</td>
<td>Number Infected with Cotylaspis</td>
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<tr>
<td><em>Ambelena costata</em> Rafinesque</td>
<td>83</td>
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<tr>
<td><em>Anodonta corpulenta</em> Cooper</td>
<td>26</td>
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<tr>
<td><em>Anodonta grandis</em> Say</td>
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<td><strong>Pelecypoda-Douglas Lake:</strong></td>
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</tr>
<tr>
<td><em>Ligumia nasuta</em></td>
<td>29</td>
<td>27</td>
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<td>13</td>
<td>2</td>
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</table>
applied to the gut wall. During feeding, the pharynx protrudes (Figure 100). The ventral adhesive disc of the worm extends over the tops of the intestinal villi, which are usually inflamed; in sectioned specimens in situ, villi under the ventral adhesive disc appear partially eroded (Figure 100).

Sheepshead of different weight categories show marked differences in percentage of fish infected (Table 4). In Lake Pepin, 25.7% of the sheepshead examined harbored *C. occidentalis*, but specimens weighing 0.91 kg or less were never infected. Larger fish (1.33 kg to 1.77 kg) were 65% infected, as were 85% of those weighing over 1.78 kg. Efforts to experimentally infect sheepshead with cotylocidia were negative, and apparently the presence of *C. occidentalis* only in larger fish may be explained by food habits of larger individuals. Crushed mollusc shells were frequently seen in their intestines, whereas intestines of younger fish usually contained aquatic insects, small crustaceans, and small fish, but never mollusc shells. Infected sheepshead contained an average of 5.4 grams of mollusc shells per fish, and an average of six worms per infected host. Among the genera of mollusc shells present in the intestinal contents of the sheepshead, known pelecypod hosts of *C. occidentalis* were frequently represented (Table 5). The ability of larger sheepshead to ingest and crush mollusc shells is associated
Table 4. *Cotylogaster occidentalis* infections and presence of mollusc shells in relation to size (weight) of sheepshead collected in 1971 from Lake Pepin

<table>
<thead>
<tr>
<th>Weight of fish in kilograms</th>
<th>Number examined</th>
<th>Number and percentage infected</th>
<th>Average number of worms per host</th>
<th>Average number of grams of mollusc shells per infected host</th>
</tr>
</thead>
<tbody>
<tr>
<td>to 0.45</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.46 - 0.91</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.92 - 1.32</td>
<td>49</td>
<td>9 (20%)</td>
<td>4.7</td>
<td>2.8</td>
</tr>
<tr>
<td>1.33 - 1.77</td>
<td>20</td>
<td>13 (65%)</td>
<td>7.1</td>
<td>4.0</td>
</tr>
<tr>
<td>1.78 - 2.23</td>
<td>3</td>
<td>3 (100%)</td>
<td>4.3</td>
<td>8.3</td>
</tr>
<tr>
<td>2.24 - 2.68</td>
<td>6</td>
<td>5 (83%)</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>2.69 - 3.14</td>
<td>3</td>
<td>2 (66%)</td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td>3.15 -</td>
<td>5</td>
<td>5 (100%)</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Totals 144</td>
<td>37 (25.7%)</td>
<td>5.6</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Mollusc shells (genera) occurring in the gut contents of Lake Pepin sheepshead, their frequency, and relation to presence of Cotylogaster occidentalis

<table>
<thead>
<tr>
<th>Shell Genera</th>
<th>Total number recovered from all gut contents</th>
<th>Frequency of occurrence in fish examined</th>
<th>Average number specimens per host</th>
<th>Average number worms present when mollusc shells present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anodonta</td>
<td>29</td>
<td>13</td>
<td>2.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Carunculina</td>
<td>21</td>
<td>5</td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td>Lampsilis</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Elliptio</td>
<td>3</td>
<td>4</td>
<td>1.2</td>
<td>14.5</td>
</tr>
<tr>
<td>Lasmigona</td>
<td>3</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Truncilla</td>
<td>3</td>
<td>2</td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>Pleurocera</td>
<td>61</td>
<td>5</td>
<td>14.8</td>
<td>11.5</td>
</tr>
<tr>
<td>Vivipara</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>13.5</td>
</tr>
</tbody>
</table>
with the development of certain modified anterior visceral arches to form large crushing plates, each of which bears numerous bead-like pharyngeal teeth. Thus, it seems reasonable to assume that sheepshead become infected with *C. occidentalis* by ingesting infected molluscs.

Because no aspidobothrian has yet been demonstrated to grow in a vertebrate host (Rohde, 1972), it was of interest to find that pronounced size differences occur between *C. occidentalis* from mussels and those from sheepshead. Measurements of gravid worms from *Lampsilis siliquoidea* range from 3.5 to 6.0mm (mean 4.9mm), whereas worms from sheepshead range from 3.1 to 18.1mm (mean 9.8mm) (Fredericksen, 1972). *C. occidentalis* recovered from *Ligumia nasuta* and *Lampsilis ventricosa* lie within the size range given for worms from *L. siliquoidea*. In addition to size, differences also appear in the number of gravid *C. occidentalis* recovered from fish versus those from mussels. In the former, 82% were gravid; in the latter, only 40% were gravid, indicating that although the vertebrate host is not necessary for *C. occidentalis* to complete its life cycle, it does seem that increased reproductive capacity of *C. occidentalis* is associated with its further development in a vertebrate host.
Aspidogaster conchicola
(Figure 103)

Aspidogaster conchicola is a cosmopolitan parasite occurring most often in the pericardial and renal cavities of mussels (Voeltzkow, 1888; Kelly, 1899; Stunkard, 1917; Hendrix and Short, 1965; Stromberg, 1971). A. conchicola has also been reported by Faust (1922), Michelson (1970), and Huehner and Etges (1971) from snails of the genus Viviparus, and in Goniobasis livescens by Huehner and Etges (1971); it is rarely found in vertebrate hosts. Michelson (1970) showed that in Viviparus malleatus, A. conchicola becomes encysted within the digestive gland and the cyst is entirely of host origin. Reports by Kelly (1899) and Pauley and Becker (1968) that A. conchicola is sometimes encysted within the mantle and visceral mass of heavily infected mussels, led Rohde (1972) to liken the phenomenon of tissue encystment in this aspidobothrian to the occurrence of certain digenean life cycle stages in tissues of molluscs. Only few reports are known of A. conchicola from vertebrates. Kofoid (1899) reported the species in a fish, Cyprinus carpio L., and in a turtle, Moxostoma macrolepidotum (Le Sueur). Babero and Lee (1961) listed a single dead parasite resembling A. conchicola from the intestinal contents of the nutria, Myocastor coypus (Molina).

In the present study, A. conchicola was found in the
pericardial cavity and sometimes the renal cavity of pelecypod molluscs (Table 1). All Lake Pepin and Ft. Madison reports represent new locality records, but not new host reports. In situ sections of *A. conchicola* in *Anodonta grandis* show the parasite unattached in the pericardial cavity (Figure 103).

It is significant that *A. conchicola* was never found in the intestinal tract of Lake Pepin sheepshead, although several of the commonly recorded mussel hosts for this parasite were frequently found in the intestines of these fish. Therefore, *Aspidogaster conchicola* does not appear to be a parasite of vertebrates, and demonstrates little host-specificity among invertebrate hosts.

*Cotylaspis insignis*  
(Figures 103-107)

Species of the genus *Cotylaspis* have been reported from fresh-water mussels, fish, and turtles. *Cotylaspis insignis* is known only as an ectoparasite on the foot, epithelium covering the kidneys, and gills of North American molluscs (Leidy, 1857; Kelly, 1899; Stunkard, 1917; Hendrix and Short, 1965; Hendrix, 1968; Stromberg, 1971). Kelly (1899) recorded one instance where a single *C. insignis* was found in the pericardial cavity of a heavily infected mussel. The greatest numbers of *C. insignis* have been found on the foot and on
kidney epithelium, with relatively few having been reported from the branchial channels of gills (Kelly, 1899; Stromberg, 1971). Host-specificity in *C. insignis* apparently varies among localities. Survey studies of Iowa, Illinois, and Pennsylvania by Kelly (1899) listed 20 species of mussels infected with *C. insignis*; the greatest numbers of infected mussels occur in Illinois and Iowa. In Kelly's 1899 survey, more mussels were infected with *A. conchicola* than with *C. insignis*. Stromberg (1971) obtained essentially the same results in a survey study of Ohio mussels; he found nine species parasitized by *C. insignis* and 23 by *A. conchicola*. Hendrix and Short (1965) surveyed the mussels of Florida, and found that *C. insignis* occurs in more species of mussels than does *A. conchicola*.

Although *Cotylaspis insignis* was not found in Lake Pepin mussels, 100% of three mussel species (*Anodonta corpulenta*, *Anodonta grandis*, and *Leptodea fragilis*) collected in the Ft. Madison area were infected (Table 1). In these mussels, *C. insignis* attaches to the base of the foot near the posterior adductor muscle, to the epithelium covering the glandular nephridium, and to the water channels within the gills (Figures 102-107). Contrary to the findings of previous workers, greater numbers of *C. insignis* were found in the water channels of the gills than in other locations. In some cases when only a few occurred on the foot, as many as
65 were recovered from the water channels of each gill. Thus it would seem that location within the host may also vary among localities.

Scanning electron microscope observations of *C. insignis* in situ, show its position on the epithelium of the glandular nephridium and demonstrate the nature of the worms' attachment (Figures 104, 105). Imprints of the buccal and adhesive discs as well as the pharynx are apparent (Figures 62, 105). In the water channels of the gills, *C. insignis* usually attaches by its ventral adhesive disc (Figure 107) to interlamellar connectives. In situ sections of *C. insignis* in *Anodonta grandis* indicate the extensive distribution of this parasite on the soft parts of mussels.

As was concluded by Osborn (1903, 1905), *Cotylaspis insignis* must be considered an ectoparasite of pelecypod molluscs, and although other species in this genus occur in vertebrates *C. insignis* has apparently not adapted to life within them.

Information based on host-parasite relationships of *Cotylaspis insignis*, *Aspidogaster conchicola*, and *Cotylogaster occidentalis* suggests that *C. insignis* and *A. conchicola* are parasites only of molluscs, and that the former is only associated with pelecypods, whereas the latter is also found in gastropods as well as pelecypods. Furthermore, *C. insignis* may demonstrate more host specificity than does *A. conchicola*. 
depending upon the geographical locality. Both *C. insignis* and *A. conchicola* seem to be less host-specific than *Cotylogaster occidentalis*. The latter aspidogastrid appears to include a vertebrate host (sheepshead) in its life cycle, and comparative data between *C. occidentalis* from mussels and *C. occidentalis* from sheepshead suggest that this vertebrate host is of benefit to the species in terms of increased powers of reproduction. The fact that sheepshead are swimming vertebrates may also benefit *C. occidentalis* in terms of dispersal.
PHYLOGENY

Stunkard (1917), in discussing the aspidobothrians states that "Whether the Aspidogastridae are primitive forms or secondarily degenerate is yet undecided. The simple archaic character of the intestine, the eye-spots, the direct development, and the ectoparasitic habit as it occurs in the family, together with the parasitic infections of molluscs by adult forms, strongly suggest a very primitive and ancient group. It is probable that complete evidence concerning the structure and life-history of this family would go a long way toward solving the problem of whether the invertebrate or vertebrate is the original host of and the attendant problem of the origin of double hosts."

LaRue (1957) concluded "There remains the possibility that more information on the life history of this family may show, as suggested by Stunkard (1946), that these trematodes belong with the Digenea, and that the life cycle is either primitive or secondarily simplified. To date no such evidence has been presented."

In 1963 Stunkard stated that "the affinities of the aspidogastrids are clearly with the Digenea, although present information is insufficient to determine whether their life cycle is primitive or secondarily simplified. They constitute an aberrant, isolated group of parasites of
molluscs and of lower vertebrates which feed on such molluscs and infect both marine and fresh-water hosts in all parts of the world. It appears that aspidogastrids and digenetic forms have descended from a common turbellarian-like ancestor which initially became parasitic in mollusks, and that the aspidogastrids never acquired asexual methods of reproduction and become mature in their molluscan hosts or in vertebrates that feed on those hosts, whereas members of the Digenea developed polyembryonic asexual reproduction in the mollusk and, with the acquisition of vertebrate hosts, sexual maturity was more and more deferred to worms in the definitive hosts."

Rohde has proposed in (1971f) in light of his extensive work on Multicotyle purvisi, that "the Aspidogastrea are archaic forms, poorly adapted to parasitism and close to the Digenea. All these are characteristics which would be expected of the hypothetical ancestors of the present Digenea, i.e., the Prodigenea. It appears that the Aspidogastrea are close to the root of the Digenea, i.e., surviving through modified Prodigenea, in other words: living fossils." I am in agreement with the statements of Stunkard (1963) and LaRue (1957), and in my opinion, the conclusions of Rohde (1971f) are premature. Among those data upon which Rohde (1971f) based his conclusions was the observation that no aspidobothrian has ever been demonstrated to grow in its vertebrate host, although he (1973) has shown that a verte-
brate host is necessary for *Lobatostoma manteri* to complete its life cycle, at least in the locality in which it was studied. Data from this thesis suggest that a vertebrate host enhances growth and reproductive powers of *Cotylogaster occidentalis*. Studies by Brinkmann (1957), and Burt (1968) on *Taeniocotyle elegans* as well as those by Odhner (1910) on *Stichocotyle nephropsis* have revealed that juvenile as well as adult parasites of both species occur in a very specific location (the bile ducts) in their vertebrate hosts; it is therefore likely that these species grow in their vertebrate hosts. Furthermore, both *Taeniocotyle* and *Stichocotyle* appear morphologically simple and perhaps primitive, but among the aspidobothria, these genera are among the least known. Detailed studies of their morphology and life histories will be necessary before meaningful conclusions as to the origin of the Aspidobothria can be properly assessed.
SUMMARY AND CONCLUSIONS

1. Adult aspidobothrian trematodes (Cotylogaster occidentalis, Aspidogaster conchicola, and Cotylaspis insignis) have been studied with reference to certain aspects of their adult morphology, cotylocidium larvae, development, host-parasite relationships, and phylogeny.

2. Scanning electron microscope (SEM) studies of adult C. occidentalis, A. conchicola and C. insignis indicate that each has a simple tube-like body extending anteriorly as a retractile neck (telescopic in C. occidentalis), and terminating posteriorly as a muscular dorsal cone containing osmoregulatory pores in its summit. In C. occidentalis the cone also contains the opening of Laurer's canal. Growth of the ventral adhesive disc in adult A. conchicola is characterized by the development of individual sucker-like alveoli appearing posteriorly in the disc; these gradually become subdivided by the addition of three longitudinal ridges. In accordance with the complexity of their ventral adhesive discs, aspidobothrians may be arranged in a series, with Stichocotyle and Taeniocotyle representing simple types; Cotylogaster, Cotylaspis, Lissimysia, and Lobatostoma are considered to be of intermediate complexity, whereas Aspidogaster, Lophotaspis, and Multicotyle are complex.

3. Morphology of the nervous systems was studied, based on acetylcholinesterase localizations (Bueding et al., 1967)
in whole mounts of *C. occidentalis*, *C. insignis*, and *A. conchicola*. A standardized terminology is proposed for the nerves. In each species, a well developed orthogonal network is apparent, composed of four or five longitudinal nerve cords with numerous transverse commissures. In numbers of nerve cords, aspidobothrians resemble acoel turbellarians; however, enlarged cervical commissures of aspidobothrians also occur in certain rhabdocoels. The ventral adhesive disc of each aspidobothrian species studied is richly supplied with nerves and several pairs of dichotomously branched nerves appear in the buccal disc. Characteristic marginal organs occur peripherally in the ventral adhesive disc and each is supplied with nerves and with a plexus encircling the lumen. As seen under SEM, differences between species in number and distribution of nerves are related to body form and surface structure.

4. Externally, uniciliate structures seen with both light microscope and SEM occur in large numbers, especially on the ventral adhesive and buccal discs. Innervations of these structures, together with acetylcholinesterase localizations in them suggest that these uniciliate structures are truly sensory in function. Dome-shaped structures, also presumed to be sensory, occur externally. As many as 43,600 externally visible sensory structures may occur in the tegument of *C. occidentalis*, 5200 in *A. conchicola*, and 4700 in *C.*
insignis. Comparable numbers of sensory structures have not been observed in other trematode groups. The complex behavior of certain aspidobothrians, such as C. insignis is likely to be facilitated by large numbers of such sensory structures.

5. The range of chromosome numbers in aspidobothrian species resembles that of the Digenea. C. occidentalis has a haploid number of six chromosomes; C. insignis, 11; in this respect the latter appears widely separated from other members of the subclass.

6. The cotylocidium larva of C. occidentalis is provided with 14 tufts of cilia; eight tufts occur in a band encircling the body anterior to the mid-region, and six occur around the base of a posterodorsally located muscular cone. This large number of ciliated tufts on C. occidentalis cotylocidia is indicative of a primitive type aspidobothrian larva. Each of two osmoregulatory bladders opening posterodorsally contains a large concretion. C. insignis cotylocidia are aciliate and resemble those of other aciliate aspidobothrian species.

7. Previously unknown developmental stages of C. occidentalis were recovered from naturally infected mussels. Development of C. occidentalis is a gradual growth process whereby cotylocidia lose their cilia and undergo juvenile stages in the mouth, esophagus, and stomach of the host mussel, and adulthood is attained in the mussel's intestine.
Development of the reproductive system begins before alveoli appear in the posterior sucker (primordial ventral adhesive disc). Alveoli first appear anteriorly on the inner surface of the juvenile posterior sucker. A presumed zone of differentiation appears posteriorly on the floor of the sucker and as alveoli are differentiated in a wave-like procession of transverse rows, the ventral adhesive disc evaginates. The ventral adhesive disc continues to grow after sexual maturity is reached. Primordia of the reproductive system appear in two separate locations; near the gonopore, and near the posterior extremity of the intestine.

8. A vertebrate host appears to enhance growth and stimulate increased reproductive capacity to *Cotylogaster occidentalis*. The mean size of gravid worms from mussels was 4.9mm whereas a mean size of 9.8mm was recorded from adults from sheepshead fish. Among those worms recovered from mussels (*Lampsilis siliquoidea*) 40% were gravid; 80% of those recovered from sheepshead were gravid. More cotylocidia hatched from eggs of worms found in fish than from eggs of worms found in mussels. Efforts to infect sheepshead directly with *C. occidentalis* cotylocidia were negative, and infection of sheepshead with this parasite appear to be related to ingestion of molluscs by this fish.

9. Data indicate that genera of aspidobothrians known only from fresh-water (*Aspidogaster, Cotylaspis, and Multi-
cotyle, Lissimysia) are limited to molluscan hosts and tend to be more specialized than those genera limited to marine waters (Lobatostoma, Taenicotyle, and Stichocotyle) or occurring both in fresh and marine waters (Cotylogaster and Lophotaspis); the latter either may use or require a vertebrate host and tend to be less specialized.
LITERATURE CITED


Dawes, B. 1941. On Multicotyle purvisi, n.g., n.sp., an aspidogastrid trematode from the river turtle, Siebenrockiella crassicollis, in Malaya. Parasitology 33: 300-305.


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APPENDIX
<table>
<thead>
<tr>
<th>This Study</th>
<th>Rohde (1968) Multicotyle purvisi</th>
<th>Timofeyeva (1971) Aspidogaster conchicola</th>
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<tbody>
<tr>
<td>Cerebral ganglia</td>
<td>Cerebral ganglia</td>
<td>Cerebral ganglia</td>
</tr>
<tr>
<td>Circumpharyngeal connectives</td>
<td>Descending portion of cerebral ganglia</td>
<td>Descending portion and posteriorly directed portions of cerebral ganglia (above 2 constitute the peripharyngeal ring)</td>
</tr>
<tr>
<td>Anteromedian nerve</td>
<td>Nervus medialis anterior</td>
<td>Anterior medial nerve</td>
</tr>
<tr>
<td>Anterodorsal nerve</td>
<td>Nervus dorsalis anterior</td>
<td>Anterior dorsal nerve</td>
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<tr>
<td>Anterolateral nerve</td>
<td>Nervus lateralis anterior</td>
<td>Anterior lateral nerves</td>
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<tr>
<td>Anteroventrolateral nerve</td>
<td>Nervus mediodorsalis with Ramus lateralis, Ramus intermedius, and Ramus medialis</td>
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<td>Buccal nerve ring</td>
<td>Anterior terminal commissure (in part)</td>
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<td>Pharyngeal nerve</td>
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<td>Pharyngeal nerve</td>
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<td><strong>This Study</strong></td>
<td><strong>Rohde (1968)</strong></td>
<td><strong>Timofeyeva (1971)</strong></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>--------------------------------------</td>
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<td><strong>Superficial posteroventral nerve</strong></td>
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<td>Nervus dorsalis posterior</td>
<td>Posterodorsal</td>
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<td>Ramus dorsalis</td>
<td>Posterior lateral</td>
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<td><strong>Posteroventral nerve</strong></td>
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<td>Posterior ventral nerve</td>
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<tr>
<td><strong>Ventral adhesive disc connectives</strong></td>
<td>Connections between extra and interplantar commissures in turn connected to nervus ventralis post</td>
<td>Small branches</td>
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<td>Nervus intra plantaris intermedius major and minor</td>
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<td>Ramus ventralis posterior</td>
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<td><strong>Marginal alveolar nerves</strong></td>
<td>Commissura intraplantaris</td>
<td>Annular nerve of transverse cells</td>
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*Note: The table compares the nervous system structures for different species as discussed by Rohde (1968) and Timofeyeva (1971).*
<table>
<thead>
<tr>
<th>This Study</th>
<th>Rohde (1968) Multicotyle purvisi</th>
<th>Timofeyeva (1971) Aspidogaster conchicola</th>
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<tr>
<td>Longitudinal nerve of disc</td>
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PLATES
Abbreviations

A  Accessory outer superficial nerve ring
AD  Anterodorsal nerve
AL  Anterolateral nerve
AM  Anteromedian nerve
AV  Anteroventral nerve
B  Body
BD  Buccal disc
BR  Buccal nerve ring
C  Cerebral ganglia
CC\textsubscript{1}  Cervical commissure No. 1
CC\textsubscript{2}  Cervical Commissure No. 2
CL  Cilium
CO  Concretion
CP  Circumpharyngeal connective
D  Dorsal cone
DL  Dorsal lateral nerve
DS  Dome-shaped sensory structure
DT  Deep transverse alveolar nerve
E  Eye spot
F  Flame cell
GC  Genital commissure
GM  Gonadal mass
I  Intestine
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<td>Inner papillary nerves</td>
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<tr>
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<td>Laurer's canal</td>
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<td>MG</td>
<td>Mehlis' gland</td>
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<td>MO</td>
<td>Mouth</td>
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<td>MP</td>
<td>Marginal papilla or organ</td>
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<td>NR</td>
<td>Nerve ring</td>
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<td>Seminal vesicle</td>
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<td>Tuft of cilia</td>
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<td>Ventral adhesive disc ramus</td>
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Legend

BD Buccal disc
GD Genital duct
I Intestine
LC Laurer's canal
M Metraterm
MG Mehlis' gland
MO Mouth
O Ovary
OB Osmoregulatory bladder
PH Pharynx
PG Gonadal primordial
PS Posterior sucker
S Prostatic sac
SV Seminal vesicle
TE Testis
TG Terminal genitalia
U Uterus
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VT Vitelline follicles
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