OBSERVATIONS ON RHABDOCOELES OF ALBEMARLE COUNTY, VIRGINIA

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Among the earlier writers on Turbellaria in the United States, and on the Rhabdocoeles in particular, were Leidy (1848, 1852), Silliman (1885), and Woodworth (1896, 1897). Among the writers since 1900, von Graff (1911) has given descriptions and notes on some seventy-five species, many of which are new and peculiar to this country. Child (1901-1903) has contributed valuable information on *Stenostomum*. Linton (1910), Patterson (1912) and Ball (1916) have done extensive work on a new Rhabdocoel commensal with *Modiolus plicatus*. Stringer (1909, 1913) published accounts of some of the mid-western Turbellaria, and Higley (1918) added descriptive accounts of several new species from the same region. Kepner (1911, 1913, 1925) has made detailed studies on the morphology and physiology of some of the simpler Rhabdocoeles which occur in abundance in the waters near the University of Virginia.

The present paper is merely a tabulated account of several genera and species studied in collections made during the past two years, with special reference to three forms which are new to this community.

List of Genera and Species Observed in the Vicinity of University, Va.

1. *Polycystis goettei* Bresslau (1906)
2. *Gyratrix hermaphroditus* Ehr. (1831)
3. *Dalyellia*, at least four unidentified species
4. a. *Stenostomum leucops* Ant. Duges (1828)
   b. *Stenostomum*, at least three other species, not identified
5. *Microstomum caudatum* Leidy (1852)
6. a. *Macrostomum appendiculatum* O. Fabricus (1826)
   b. *Macrostomum sensitivum* Silliman (1885)
7. a. *Prorhynchus applanatus* Kennel (1888)
   b. *Prorhynchus stagnalis* Schultze (1851)
8. *Rhynchomesostoma rostratum* Müller (1774)
9. *Opistomum pallidum* O Schmidt (1882)
10. *Phaenocora agassizi* Von Graff (1911)

*11. *Catenula* sp?
*12. *Bothrioplana* sp?
*13. *Otomesostoma auditum* Pless (1874)
*14. *Fuhrmannia* sp?
*15. *Rhycoscolex* sp?

Observations on a Few Definite Species

These observations on Rhabdocoelida were taken at random—more or less as each species was observed in collections, and found to be of partic-

* These specimens have been found since this paper was written in 1928. Accounts of these species given in separate publications.

ular interest. Since vegetative individuals of *Stenostomum leucops*, *Microstomum caudatum* and *Prorhynchus applanatus* were being studied by others in the laboratory, I confined my studies to sexual individuals of these forms. *Prorhynchus applanatus* was studied with special reference to the general development of the sexual organs, and to the development and rearing of the embryos. I made more extensive studies upon *Rhynchomesostoma rostratum* and *Opistomum pallidum*, the results of which appear in other papers, and upon *Phaenocora agassizi*.

1. Stenostomum. On October 26th two *Stenostoma* were discovered to have developing ovaries in the mesenchyme near the anterior left margin of the enteron. These animals were identified as *Stenostomum leucops* but were very much smaller than the normal vegetative members of this species. They were observed from day to day.

The egg in each individual grew until it almost filled the diameter of the body; and, in this process of growth, gradually forced the left wall of the enteron against the right wall until both appeared as a single thread. This connecting thread never was broken through. The animals refused food but remained healthy and active although they gradually decreased in size until they became almost microscopic. During this entire period (October 26-November 6) they gave no evidence of fission.

On November 6th, these two eggs were found outside their respective parents' bodies. These eggs were in the four-celled stage and were floating in a gelatinous sphere. They were seen to go through several subsequent stages of cleavage, and then to disintegrate. This disintegration probably was caused by unfavorable environmental conditions.

The parents were present in the depression slide and were in apparently good health on November 7th. Both of them contained a huge vacuole where the egg had been, and the enteron was arching about this space. One individual was lost in manipulation; but the other was still present two days after, thus establishing the fact that the mother does not die upon laying the egg. This is contrary to von Graff's statement (1913) that the mother is killed by the eruption of the egg through the body wall.

Several species of Stenostomum have been observed in different collections but were not identified. One species was of special interest in that it consisted of a long chain of six nearly complete zooids. Within two days it broke into two individuals, each consisting of four completely developed feeding mouths; and, within each complete zooid thus formed was the beginning of a secondary zooid. No other specimens of this character were observed, and this individual was not conclusively identified.

2. Microstomum. The only observations made on Microstomum were on those individuals showing sexual development. These were found in great abundance from October 3 through November 17. The males appeared first in great numbers, some of which were isolated. Three male indivi-

duals were kept from October 3 to October 24 in a dish. The male gonads disintegrated during that time; but the individuals gave no signs of developing female gonads. From these observations, together with the fact that many males were taken from a specific culture, it is inferred that the sexes in Microstomum are either separate, or that the animals are protogenous.

Gamble, in the "Cambridge Natural History," Vol II, p. 44, states, "the two latter (Microstomum and Stenostomum) are of distinct sexes, contrary to the usual hermaphroditic condition of Flatworms." Kepner, in "Animals Looking into the Future" indicates that Microstomum is not only hermaphroditic, but also protandrous. From the observations on Microstomum made in this laboratory and stated above, I am inclined to agree with the opinion of Gamble.

In Microstomum the presence of the gametes does not inhibit fission although it retards the rate of fission considerably. One individual containing testes and penis, isolated October 10, had formed a zooid by October 14. As soon as the sexual organs degenerated zooid formation was accelerated.

No new observations were made as to the morphology of the reproductive organs other than the fact that the testes may occur singly as well as in pairs.

3. **Prorhynchus applanatus** Kennel (1888). Specimens of this species appear in practically every collection made from ponds near this laboratory, few appearing in the fall, but many during January and February, and later on in the cool spring months. A few specimens which were sexually mature were isolated in a covered dish. One large specimen (A) had fully developed testes, four lobes on one side and five on the other. A second large animal (B) was very opaque because of the presence of seven or eight eggs which were shortly deposited in the dish. One other specimen (C) was quite small and transparent, evidently a young individual.

The eggs of (B) were closely watched during their subsequent development; and it was discovered that some of the eggs contained two embryos, while some contained only one. This is evidently a real case of polyembryony somewhat similar to that described for *Graffilla gemellipara* Linton. This conclusion is based upon the anatomy of the vitello-germarium and upon observation of the egg itself.

One of the twins that developed was isolated successfully and reared to sexual maturity. On March 30 it was observed to be developing an ovary in the mid-ventral region. The genital opening could be clearly distinguished in the central region of the ovary. Two days later white dots appeared in the ovary which gradually disappeared.

Likewise, the young animal (C) taken in the original collection was seen to develop an ovary first, then testes; but the ovary never developed any eggs. These facts would lead to the conclusion that *Prorhynchus applanatus* is protogenous were it not for the fact that the large male animal (A) taken

from the original collection has retained its testes to the present, and has now within the last week (April 21) developed an ovary containing one egg. The testes are still lobed. It is evident that no definite conclusion can be reached as to the exact nature of sexuality in *Prorhynchus applanatus* with the observations at hand, and much of the interest remains to be discovered in regard to this point.

Prorhynchus applanatus exhibits no signs of fission, though it readily repairs injury to itself for specimens can be cut at least three-eighths of the way across their bodies with no apparent harm being done. They are ravenous eaters, readily swallowing huge pieces of tadpole brain or of liver larger than themselves. They can also subsist for days without food whatever, other than that which they can extract from the pond water in their dishes.

4. **Rhynchomesostoma rostratum** Müller (1774). The first specimens were found in this laboratory the last of January, 1927, in an aquarium. The collection had been made the previous September, and had remained perfectly balanced with the addition of tap water at intervals. The first few specimens found were rather small (1 mm. in length) and quite transparent.

For a short time they disappeared from the culture, then were found in abundance again; this time much larger than before. Several contained eggs, some as many as three. This occurred several times; and from this fact the conclusion was reached that they appear in cycles, and are large or small according to the varying amounts of food present. During the fall and winter of 1928 specimens were found in abundance in collections made at an old reservoir which is stagnant and full of Elodea and other water plants. The individuals taken from these fresh collections often measure over 3 mm. in length, each one usually containing from one to three eggs. The eggs are ellipsoidal and stalked, the stalk being cemented to the substratum, and the embryo emerging from the egg membrane at the pole opposite the stalk. At this pole the egg opens by means of an operculum.

Interesting observations have been made upon the feeding habits of *Rhynchomesostoma rostratum*, and upon its specialized rhabdites. An account of these observations will be given in a later paper.

5. **Opistomum pallidum** O. Schmidt (1882). Miss Stringer in Ward and Whipple's "Fresh Water Biology" (1918) states that this genus of the Dalyellidae is not found in this country. No later reference to this animal has been found. Several individuals of the species were found in each of two collection made in the spring of 1927 from a stagnant, temporary pool formed by an overflow of the "Golf Stream." This is a small, clear stream which flows through a shallow depression and then into the Gymnasium Reflection Pool. A single specimen has been found in a collection taken from the pool itself, in April 1926. By means of macerations, sections, and

observations on the living animals, these specimens have been conclusively identified as belonging to the genus and species *Opistomum pallidum* O. Schmidt.

6. **Phaenocora agassizi** Von Graff (1911). Von Graff ('11) records *Phaenocora agassizi* among the Rhabdocoeles of America. He found them "in a few examples in the bottom of a ditch at the east wide waters of Rochester at the end of Ericson Street." In the pools and streams around the University of Virginia they occurred in the greatest abundance in the spring of 1927, and in the fall of the same year. Only one specimen was found in a collection, and only three have been found in collections this spring (1928). These three have no eggs. The temperature has been very low, with only a warm day now and then, indicating that *P. agassizi* appears in the warm waters, probably without reference to the length of day and the amount of sunlight.

Phaenocora agassizi is of particular interest because of the presence of rhabdites, since all other species of this genus appear to lack them (von Graff '11). These rhabdites occur in the skin as shiny, roundish, oval bodies, 1 to 2 microns in diameter; and, in the anterior end, as needle-shaped bodies, singly, or in pockets which arise from the botryodal glands fastened to the side of the pharynx. They seem, primarily, to be prehensile in nature. Members of this species are very voracious, and are capable of ingesting pieces of brain and liver much larger than themselves by means of a pumping motion of the pharynx followed by a pulling motion of the whole body. Though apparently full of vegetable food, such as diatoms and desmids, when first taken from a collection, they seem to live better in the laboratory on a regular diet of fish brain and liver. The isolated animals are kept in stender dishes having a mud film in the bottom, and two-thirds full of the original pond water. This water is replenished frequently with ordinary tap water, as also are the larger collections kept in aquaria.

Data were kept on several individuals collected at the same time, and apparently in the same stage of development, though this could only be a matter of conjecture. The following is a table giving a short comparison of the animals on different diets.

Animal	Dates	Food	Length of life in days
Phen I	10–1 to 11–12	*Brain-liver	43
Phen II	10–1 to 11–7	Brain	38
Phen III	10–1 to 10–20	vegetable	20

* Changed from brain to liver October 20 because of an apparent lack of appetite for brain. Metabolism accelerated, five eggs laid from October 20 to November 12.

These animals, as indicated by the table, finally reached a period in which they displayed a lack of appetite and died. This was not true in the case of animals found in the spring of 1927. Though no accurate observations are available, they were kept indefinitely from about April 15 to June 1, and were then discarded. These facts may possibly be correlated with the evident occurrence of summer and winter eggs. Eggs deposited on April 16, 1927, developed. By April 22 the embryos emerged from the shells and lived until May 24 when they were accidently killed. Eggs laid in the winter of 1928 in the laboratory developed until independent movement of the embryo was clearly distinguishable, then the embryos disintegrated. The fact that eggs laid in the spring and early fall will develop completely in six days. (see data below), while those laid in the late fall and winter fail to develop completely and die, leads to the inference that Phaenocora lays both summer and winter eggs. Gamble, in the "Cambridge Natural History" (Vol II) states that Mesostoma lays two types of eggs, thin-shelled spring eggs, and thick-shelled summer eggs which lie dormant over the winter and develop rapidly in the spring. It seems highly probable, therefore, that Phaenocora does also. Many individuals of this species have been observed, and frequent egg laying is quite usual, but never has any sign of fission occurred.

It is interesting to note that no matter how white or yellowish and opaque a specimen may be when first taken from a collection, within two or three days if kept isolated in a dish it will become practically transparent, and very green in patches due to the presence of Zoochlorellae. A few green specimens are found also in nature. When the individuals become green the rhabdites show up very clearly and appear quite black.

An experiment was carried out with regards to the life history of Phaenocora. The parent Phaenocora (No. 1 of the above table) laid the egg October 1. On October 3, the egg had been eaten by a Microstomum and was found in its enteron. A few days later the Microstomum was dead and the egg lay in the dish. By October 7, a newly hatched Phaenocora appeared. The time necessary for development corresponds to the observations on one egg in the spring of 1927, which indicates that development takes place in six days.

The animal was isolated in a dish where it gradually developed, being fed frequently on fish-brain. On November 12, it contained an egg. On November 14, this egg had been laid, and a new one was developing. It then showed a lack of appetite, and its food was changed from the fish-brain to liver. The second egg was laid and the animal gradually became less active until November 30 when it finally died.

The eggs clearly showed signs of development and by November 23 the developing embryos were revolving within the shells. These had already exceeded the time necessary for complete development as shown in the

earlier eggs, and these eggs never developed any further. The fact that they developed at all, however, seems to indicate that Phaenocora is either capable of self-fertilization or of parthenogenetic development. It probably is a case of self-fertilization as Gamble states in the "Cambridge Natural History," that some of the flatworms (Mesostoma) are capable of fertilizing their own eggs.

Summary

1. Contrary to the statement of von Graff (13), the eruption of the egg from the body of Stenostomum does not kill the mother immediately.

2. From observations on Microstomum it seems probable that the sexes in this species are separate, and that the presence of gametes retards, but does not inhibit fission.

3. In *Prorhynchus applanatus* we have a case of polyembryony. It appears that the animals may be either protandrous or protogenous.

4. *Rhynchomesostoma rostratum* appears in cycles, and the size of the members of each cycle varies according to the food conditions prevalent at the time of their appearance.

5. *Opistonmum pallidum* (O. Schmidt) occurs in the ponds and streams around the University of Virginia.

6. *Phaenocora agassizi* (von Graff) is capable of either self-fertilization or of parthenogenetic development of its eggs. It apparently produces summer and winter eggs.

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