STUDIES ON THE ORGANS OF REPRODUCTION IN THE NUDIBRANCHIATE MOLLUSKS
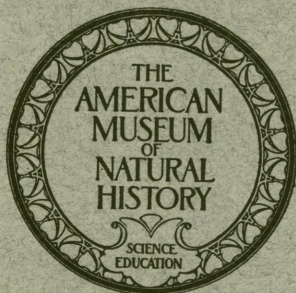
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Article VI.—STUDIES ON THE ORGANS OF REPRODUCTION IN THE NUDIBRANCHIATE MOLLUSKS, WITH SPECIAL REFERENCE TO *EMBLETONIA FUSCATA* GOULD[1]

By Leslie A. Chambers

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HISTORICAL INTRODUCTION

The group Nudibranchiata has been treated in considerable detail by the systematists of the last century and, to a lesser degree, by those of the first quarter of the twentieth century. The extensive systematic writings of Milne-Edwards, von Ihering, Vayssiere, Trinchese, Pelseneer, Hancock, Embleton, Alder, Bergh, Risbec, O'Donoghue, Eliot, Balch, and others are sufficiently well known to require no special mention. Their combined investigations, in a general way, have upheld the thesis that the Nudibranchiata represent a highly specialized group derived at some time in the past from tectibranch stock. The researches of Pelseneer (1892, 1894), Bergh (1892), and von Ihering (1876, 1877, 1892) are particularly illuminating in this connection. The existence of three natural groups of the Nudibranchiata is universally recognized:

(1) the Holohepatica, (2) the Kladohepatica, and (3) the Ascoglossa. Only in their opinions of the relative phylogenetic positions of these three groups do the systematists differ, Pelseneer regarding the Ascoglossa as derived from the Kladohepatica by specialization, Bergh and von Ihering placing the Ascoglossa in the position of a link between Tecti- branchiata on the one hand and the remaining nudibranchiate groups on
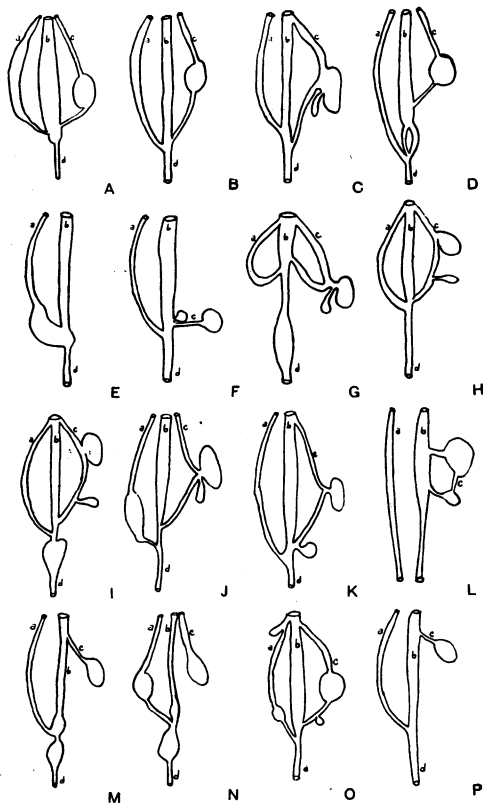
Fig. 1. Conventionalized diagrams of nudibranchiate reproductive systems adapted from figures chosen at random from the systematic literature.

A.	*Analogium*	I.	*Doriopsis*
B.	*Trevelyana*	J.	*Idalia*
C.	*Chromodoris*	K.	*Polycera*
D.	*Phlegmodoris*	L.	*Elysia*
E.	*Scyllaea*	M.	*Doto*
F.	*Bornella*	N.	*Tritonia*
G.	*Doriopsilla*	O.	*Platydoris*
H.	*Euplocamus*	P.	*Marionia*

(A–G, L, O, after Risbec, 1928; H–J, P, after Vayssiere, 1901; K, after Pohl, 1905; M, N, after Dreyer, 1911.)

a.—male duct system   *c.*—androgynous duct system
b.—female duct system   *d.*—hermaphrodite duct

the other. Numerous researches of an anatomical nature during recent years have tended to support the latter view, at least to the extent of showing the great heterogeneity of the nudibranch group as a whole, but it seems unlikely that sufficient evidence can ever be adduced from anatomical data alone to establish a satisfactory phylogeny. The great diversity of specialization encountered in almost every organ system, together with the absolute lack of paleontological links, renders discussion as to the relationships existing within the group highly speculative.

Among the three recognized divisions of the group there is a certain amount of parallel specialization when individual organ systems are considered without reference to other systems or to the organism as a whole. In no place is this tendency toward parallelism so pronounced as in those structures directly connected with the reproductive function. A survey of the systematic literature is at first misleading, giving the impression of fundamental diversity in the structures of nudibranchiate reproduction, but upon close analysis of descriptions and figures a striking similarity of structure is revealed. In figure 1, I have reduced to the lowest diagrammatic terms a series of such reproductive systems chosen at random from figures given in the works of various authors. The figures represent a wide variety of sexual ducts of genera and families including members of each of the three important groups. The descriptions and indeed the figures included in these systematic works may not be regarded as always accurate in details, but they suffice to reveal the basic similariy of the complex throughout the group Nudibranchiata. In some cases there are errors in the labelling of drawings, and in few cases is the exact relationship between the various components of the reproductive system established, but so much similarity of gross features is suggestive of fundamental affinity.

It is extremely difficult in the absence of accurate descriptions, except in a very few cases, to reduce the masses of figures and verbal data to any system. Lang (1896) recognized in the hermaphroditic Mollusca three types of organization of the genital systems, as follows:

I.—The hermaphrodite gland has a single undivided efferent duct opening through a single aperture as in *Gastropteron*, the Pteropoda, and the Cephalaspidea (*Bulla, Doridium*).

II.—The hermaphrodite gland gives rise to a hermaphrodite duct which soon divides into two parts, the vas deferens or seminal duct, and the oviduct. The former runs to the male copulatory apparatus, the latter to the female genital aperture. The male aperture and the penis lie in front of the female . . . and both lie on the right. This second type is derivable from the first if we assume that the common duct of the hermaphrodite gland bifurcated into a male and female duct, and that the

seminal furrow closed to form a canal in continuation of the male duct. Examples of this type are found especially among the Pulmonata.

III.—In all Nudibranchia and a few Tectibranchia (e.g., *Pleurobranchaea*) the hermaphrodite gland gives rise to the hermaphrodite duct, which, as in the second type, sooner or later divides into a male and female duct. These, however, do not open through distinct apertures but again unite to form a common atrium genitale or genital cloaca.

Thus Lang recognizes the fundamental similarity of the reproductive complexes of the various Nudibranchiata, at the same time indicating by his descriptive terms a considerable variety in the extent of bifurcation of the hermaphrodite duct.

Agersborg (1923) has found it convenient to add a fourth type to the above list, exemplified by *Melibe leonina* (Gould). This he describes as being "equivalent to types II and III (of Lang) plus a more complete duct system." The principal points of difference seem to lie in the complete separation of the male and female apertures, and in the bifurcation of the hermaphrodite duct into male and female components at points very near the acini of the hermaphrodite gland, each acinus having its individual pair of ductules. This type of organization has been recognized by earlier writers dealing with ascoglossan types, particularly by Trinchese (1874, 1876, 1877, etc.), Pelseneer (1894), and von Jhering (1892).

A basis for further classification of the reproductive systems of the nudibranchs has been suggested by von Ihering (1876) in the presence or absence of a secondary bifurcation of the oviduct. It was believed by this author that the diaulic condition of the complex, the oviduct being undivided throughout its course, was typical of the Kladohepatica, while a triaulic condition was characteristic of the Holohepatica and the Ascoglossa (Sacoglossa). Pelseneer (1891) pointed out that certain Ascoglossa are not triaulic, while certain of the Kladohepatica, notably *Aeolidia papillosa* (Eliot, 1910), have been found to be essentially triaulic. It should be noted that von Ihering's interpretation of the diaulic or triaulic condition rested upon the presence or absence of a third external orifice.

Only the most careful morphological and histological investigations have revealed the true course of the bifurcated or non-bifurcated oviduct in any species of nudibranch and researches of this type are almost negligible in number. The extreme complexity and delicacy of the anterior genital mass render almost useless such anatomical data as are usually included in the systematic literature. A comparison of the reproductive system in *Aeolidia papillosa* as described by Alder and

Hancock (1845–55) with the same system as described by Eliot (1910) shows that the earlier authors, dealing only with gross dissections, made serious errors, such as calling the prostate gland "testis," but the most striking point of difference in the two descriptions lies in the failure of Alder and Hancock to observe any secondary bifurcation of the oviduct. If so important a point could be overlooked completely by such skilled workers it seems highly probable that other errors of the same type may have been made in the less careful systematic investigations that form so large a part of nudibranchiate literature. Extensive histological and anatomical investigation will be necessary before any exact degree of similarity or dissimilarity in the reproductive systems of the various nudibranchs can be established.

Histological investigations of the organs of reproduction in the Nudibranchiata date principally from the closing years of the last century. Scattered histological information is to be found in the writings of Bergh, Pelseneer, and particularly Trinchese. The anatomical papers of Alder and Embleton (1845, 1852) relative to *"Eolis"* and *"Doris"* are valuable in a general way. But the first detailed histological analysis of the entire reproductive system in a single species, so far as I know, is that ot Böhmig (1893) dealing with the taxonomically doubtful *Rhodope veranii*. In summarizing, Böhmig outlines the essential features of the system in this way:

> Der Genitalapparat der *Rhodope* besteht also—aus einer Zwitterdrüse, welche vermittels des Zwitterdrüsenganges mit dem einfachen Genitalgange (Oviduct + Vas deferens) verbunden ist. In diesen öffnet sich seitlich eine voluminose, mehrlappige Drüse, "glandula uterina" von Kölliker, "glandula dell'albume e del nidamento" Trinchese, welch wahrscheinlich den Eiern Eiweiss- und Schalensubstanz liefert. Der Genitalgang geht über in ein blasiges Organ an das sich das Atrium genitale anschliest, das auf der rechten Seite des Thieres durch den Porus genitalis nach aussen mündet.

Rhodope may not be described as a typical nudibranch, hence this picture of a monaulic system is enlightening only in so far as it shows the wide limit of variation exhibited by the group. It is surprising that some one of the systematists has not seized upon this form as a connecting link between nudibranch and tectibranch.

Bruel in 1904 published an important paper on the finer structure of the reproductive and digestive systems of *Caliphylla mediterranea*, a very typical ascoglossan form. In this study a vestigial triaulic condition is described, only the upper portion of the oviduct showing bifurcation into oviduct proper and a channel for the passage of incoming spermatozoa. There are only two external orifices, the female aperture serving the functions of both copulation and ova deposition.

The work of H. Pohl (1905) established as a certainty the existence of a true triaulic type in one of the Holohepatica, *Polycera quadrilineata*. In this form the hermaphrodite duct bifurcates a short distance beyond the ampulla; the oviduct runs into a fertilization chamber from which pass a channel for the reception of foreign spermatozoa and a channel through which the outgoing ova may pass. Both of these secondary divisions, together with the vas deferens, eventually terminate at a single external genital atrium.

Eliot (1910), in his 'Monograph of the British Nudibranchiate Mollusca,' treats in detail the anatomy of the reproductive system of *Aeolidia papillosa*. As pointed out above, this form was first supposed (by Alder and Hancock) to represent a true diaulic type. Eliot showed the existence of a secondary bifurcation in the oviduct that had not been suspected up to his time. He showed that the oviduct proper passes from the fertilization chamber through a convoluted portion of the nidamental gland, while a shorter, more direct passage leads from the same chamber to converge with the oviduct at a point near the common external orifice.

Dreyer (1911) in a brief contribution outlined the relation of the various ducts in *Doto fragilis*, *Berghia*, *Tritonia plebia*, *Coryphella rufibranchialis*, *Calma cavolinii*, and *Rizzolia peregrina*. Of these *Doto*, *Coryphella*, and *Calma* are reported to differ only in the structure of the penis and the ductus ejaculatorius. They show in their duct relationships a true diaulic condition. The arrangement in the other forms is not perfectly clear from the figures and descriptions, but in none is a secondary bifurcation of the oviduct demonstrated.

The investigation of Agersborg (1923) into the anatomy of *Melibe leonina* has been mentioned previously. Agersborg's contribution is distinctive in that he presents much of the finer anatomy of the reproductive system, but the extreme condition of complete bifurcation of the hermaphrodite duct throughout its length, which he describes, has been observed in numerous Ascoglossa since the time of von Ihering.

From the scattered information available it seems possible to classify nudibranchiate reproductive systems roughly on a basis of arrangement of the channels through which ova and spermatozoa are conducted to the exterior, and incoming spermatozoa brought into contact with the ova at the proper moment. Such a classification as follows may be regarded only as a broad generalization, but, with the exception of the one form, *Rhodope*, all reproductive duct complexes of nudibranchs heretofore described fit quite nicely into the scheme suggested.

TYPE I.—Exemplified in *Doto fragilis* as recorded by Dreyer. The hermaphrodite duct leading from the hermaphrodite gland into the anterior genital mass undergoes a bifurcation into vas deferens and oviduct. Annectant to the oviduct are a single seminal receptacle probably serving as bursa copulatrix, and a nidamental gland complex. A portion of the oviduct itself may be glandular in some cases, but in *Doto* the nidamental gland and other glandular structures seem entirely apart, emptying their products into the oviduct at the time of passage of the ova. There are two possibilities with reference to the external orifices: (1) There may be two separate openings as in *Doto fragilis*. (2) There may be a single genital atrium into which vas deferens and oviduct converge.

TYPE II.—Finds exemplification in the majority of the Holohepatica. The hermaphrodite duct first bifurcates as in Type I, but immediately the oviduct shows the secondary bifurcation already referred to in discussion of Pohl's work on *Polycera quadrilineata*. The oviduct proper passes to its external orifice either through a portion of the nidamental gland complex or directly, receiving contributions from the adjacent glands. The third channel (distinct from vas deferens and oviduct) is that provided for the incoming spermatozoa. It leads from the fertilization chamber in the oviduct to the seminal receptacles, primary and secondary, and thence through the female copulatory tube to the exterior. With this type of arrangement there might be expected three different dispositions of the external openings: (1) one in which all three channels empty into a common genital atrium; (2) one in which the vagina and oviduct converge, thus leaving two separate apertures; and (3) one in which all three ducts remain distinct, leaving three apertures. All three expectations seem justified by figures scattered throughout the literature, although (1) and (2) are by far the most frequently encountered.

TYPE III.—This type of system is figured repeatedly by authors dealing with the Ascoglossa. Risbec (1928) describes it in two new species of *Elysia*. The bifurcation of the hermaphrodite duct in this type occurs at the outlet from each individual acinus of the hermaphrodite gland. Thus each acinus has its own branch of both vas deferens and oviduct. The ductules converge anteriorly into the two channels described under Type II, and their disposition is essentially the same as in that group. Three external orifices seem to be the rule, although it would not be surprising to find the other two alternatives when more anatomical data are available.

TYPE IV.—Finds its type in *Aeolidia papillosa* as described by Eliot (1910). It is essentially triaulic in the sense that it shows a secondary bifurcation of the oviduct along a part of its course. The chief point of difference between Types II and IV is in the relation of the third channel to the seminal receptacle. In II the duct passes from the fertilization chamber directly into the secondary and primary seminal receptacles and then into the vagina or copulatory pouch. In Type IV there is but a single receptacle, and it is not entered directly by the third channel. The receptacle is rather a unit in itself, connected with the vagina by a short duct. The third duct passes from the fertilization chamber to the vagina at a point anterior to the receptacle. In *Aeolidia papillosa* the vagina subsequently converges with the oviduct, and, together with the sperm duct (penis), they empty into a common atrium. It is conceivable, however, that other species may be found in which two or three external orifices exist. Indeed the researches outlined below show *Embletonia fuscata* as having a duct arrangement of this type while showing two contiguous but separate terminal pores. Risbec (1928) has recently figured a number of reproductive systems of Type IV, all of them occurring in Aeolidia-form species.

The four types of duct system described above are figured diagram-
matically in figure 2. Only the barest outlines are employed, since only
in their basic arrangements are the diverse species similar. The hundreds
of figures that have appeared in the systematic writings as well as in the
more accurate anatomical researches demonstrate in their great variety
the many possibilities of specializations and recombinations based on the
same fundamental duct relationships.

It seems likely that all four of the types described may be referred
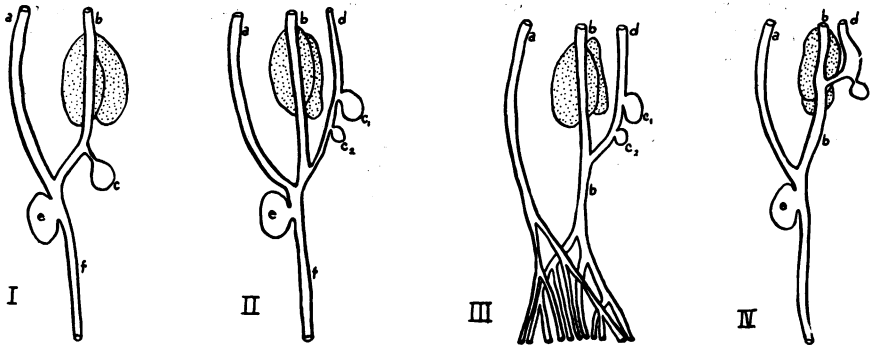to a single primitive arrangement so far as function is concerned. The

Fig. 2. Diagrammatic representation of the arrangement of male, female,
hermaphrodite, and androgynous ducts in the four types of nudibranchiate reproduc-
tive system.

Type I is of general but not universal occurrence among the Kladohepatica (*Doto, Tritonia, Calma, Coryphella, Pleurophyllidia*, etc.)

Type II occurs in practically all Holohepatica.
Type III occurs in some Aeolidiadae (*Aeolidia, Embletonia*).
Type IV is found only among the Ascoglossa (*Elysia, Limapontia*).

a—male duct	d—vagina (androgynous)
b—oviduct	e—ampulla
c—sperminal receptacle (androgynous)	f—hermaphrodite duct

lack of paleontological links and the irregular distribution of the types
throughout the order Nudibranchiata render impossible the choice of
one or another as the most primitive type. A glance at the diagrams
(Fig. 2) will suffice to show that any one might have been derived from
any other. It may be significant that Type I occurs in such forms as
Tritonia which Pelseneer regarded as very near the root of the nudi-
branch phylogenetic tree. On the other hand Type III, found in the
Ascoglossa, was regarded by Pelseneer as a derivative of Type I which he
supposed prevalent among the Aeolidiomorpha, while Bergh and von
Ihering, holding the Ascoglossa to be the primitive stock, naturally

assumed that Type I was derived from Type III. The inconclusiveness of such discussion has already been mentioned. As pointed out by Eliot, it is probable that of the original phylogenetic tree we know only the tips of the topmost branches. The connecting links have not been found nor, in all probability, do they exist in living forms.

However, the obvious similarity in arrangement of the duct complex suggests a similarity in function. With spermatozoa and ova produced side by side or in adjacent acini of an hermaphrodite gland, with an hermaphrodite duct for more or less of the distance to the exterior, but with the universal bifurcation into vas deferens and oviduct implying a selective separation of eggs and spermatozoa, there are seen to be physiological problems involved the solution of which in one species would probably shed much light on the functioning in the whole group. The suggestions as to the path followed by the germ cells in *Polycera* (Pohl), *Caliphylla* (Bruel), *Aeolidia* (Eliot), and others seem logically deduced and instructive, but nowhere is the question raised as to why such a course is followed, or what is the selective mechanism by which spermatozoa and ova are separated.

The histological and anatomical researches recorded on the following pages were undertaken with precisely these questions in mind. The selection of material was fortuitous, but fortunate because of the abundance of the species studied most (*Embletonia fuscata*), and because of the ease with which it could be prepared for histological study.

Briefly, the investigation follows two courses:

1.—A description of the anatomy and histology of a typical nudibranch reproductive system.

2.—An interpretation, based on anatomical as well as on physiological evidence, of the functional significance of the component parts of the system.

MATERIALS AND METHODS OF STUDY

On November 11, 1928, several hundred specimens of *Embletonia fuscata* Gould were scraped from the piles of the bridge across Barnegat Bay, New Jersey, near Beach Haven. They were feeding on the very abundant masses of the bryozoan *Amathea dichotoma* and some hydroids of several different species. Large numbers of the kidney-shaped egg-masses were taken at the same time, the eggs being in various stages of development from the second or third cleavage up to the fully formed veliger almost ready to escape from the spawn jelly. The nudibranchs showed a certain amount of copulatory activity even after capture. Upon return to the collecting ground two weeks later it was found that the colony had completely and mysteriously disappeared. Such "mi-

grations" are recorded repeatedly throughout the literature in connection with a variety of species and will be considered later.

On July 11, 1929, a second lot of the same species was taken by Mr. Vincent Gregg of the Princeton Biological Laboratory near Seaside Heights, New Jersey, under conditions comparable with those which obtained in the first case. This second catch was less numerous than the first.

In both cases the spawning and copulatory activity assured a variety of stages in the reproductive cycle. This, coupled with their abundance and with the ease of cutting serial sections incident upon their extremely small size and lack of hard parts, made the organism especially favorable for histological study of the reproductive organs.

Representatives of some forty other species of Holohepatica and Kladohepatica were available for comparison. Thirty-five of these were taken in the vicinity of Mt. Desert Island, Maine, during the months of June, July, August, and September, 1929. This list will be published later, with descriptions of five new species. Three additional species were available from Barnegat Bay, of which one (*Polycerella emertoni*) was especially abundant. The collections of the Princeton Biological Museum furnished several alcoholic specimens, chiefly of European species.

The method of investigation involved simple fixation in toto, serial sectioning, and subsequent wax reconstruction. *Embletonia* is entirely too small to allow of gross dissection with profit.

A variety of fixatives were employed, among them Bouin's picro-formol-acetic mixture, Flemming's strong mixture, formalin, seventy per cent alcohol, Perenyi's fluid, chrom-aceto-formol mixture, and Duboscq and Brasil's modification of Bouin's fluid. A modification of the latter by the addition of 0.5 per cent of urea crystals was quite successful. The best fixative for all purposes was undoubtedly that of Bouin.

Sections were cut in thicknesses ranging from five to fifteen micra.

In staining, the iron hematoxylin of Heidenhain and the Delafield's hematoxylin-eosin combination gave excellent results. Great difficulty was encountered in obtaining full expansion of the soft-bodied nudibranchs. Various narcotics were used before fixation but none of them with more than mediocre results.

At this point I wish to express appreciation to Prof. Ulric Dahlgren for his assistance and guidance during the course of the investigation; to Prof. E. G. Conklin for many valuable suggestions; and to Mr. Vincent Gregg for his technical assistance.

TAXONOMIC STATUS AND GENERAL MORPHOLOGY OF *EMBLETONIA*
FUSCATA GOULD

The genus *Embletonia* was first described in 1844 by Alder and Hancock under the name *Pterochilus*. The original description reads:

Body oblong, nearly linear, tapering behind. Head anterior, terminal, having a flat extended lobe on each side forming a kind of veil above the mouth. Jaws corneous. Tentacula two, linear, dorsal with the eyes behind them. Branchiae papillary, elongated, arranged down the sides of the back. Anus on the right side behind the generative organs.

Later it was found that *Pterochilus* was already the name of a hymenopteran genus, so the same authors in 1845 adopted the name *Embletonia*. They give no details of the internal anatomy except the structure of the radula. A single statement disposes of the reproductive complex:

The reproductive organs are bulky; the testis (figured) being short and thick and of a tubular form.

The "testis" referred to is either the basal portion of the penis or the prostate gland.

These authors regarded *Embletonia* as related to *"Eolis"* and to *Calliopaea* of d'Orbigny. The species described is *Embletonia pallida*.

Bergh ('Beitrage zur Kenntnis der Aeolidiaden,' 1886) points out that the morphology of the genus is not well known, but he adds little to our knowledge, his acquaintance with the group apparently being limited to three specimens of *E. fuscata* presented to him by Prof. A. E. Verrill and taken at Woods Hole in July, 1881. He lists six species which may be ascribed to the genus but expresses considerable doubt that any real specific differences exist. Rather he is inclined to ascribe all to a single species.

Following Bergh's classification scheme, *Embletonia* is a genus of the family Nudibranchiata Kladohepatica and of the subfamily Aeolidiadae, and is synonymous with the *Calliopaea* of d'Orbigny. Bergh adds the following to the generic description of Alder and Hancock:

Margo masticatorius mandibularum vix denticulatus; dentes linguales cuspide paulum prominento, utrinque denticulati. Penis inermis.

The specific description given by Bergh follows very closely the description of *E. pallida* given by Meyer and Moebius (1865), and those of *E. fuscata* and *E. remigata* given by Gould (1870). Gould also describes a *Calliopaea* (?) *fuscata* which can be no more than a variety of the species. Bergh, in dealing with this vexing problem of specific validity concludes that:

This *Calliopaea fuscata* (of Gould) is without doubt the *Embletonia pallida* of the English authors (Alder and Hancock) and with the same identity we regard the *E.*

fuscata of Gould. Also the *E. remigata* of the same author is likely only a variety of a widely distributed northern species.

The great variety observed in the specimens of *E. fuscata* examined here gives credence to this notion that the genus may be represented by a single species of widespread distribution.

In Bergh's treatise the head is described as comparatively large, half-moon-shaped with prolonged angles. The rhinophores are relatively long, simple, and almost cylindrical. The branchial papillae occur in irregular groups or singly, in two lateral rows with four or five tufts or single cerata in each row. The cerata are club-shaped and show a large internal gland. The anus is strongly marginal and lies between the second and third groups of papillae. The foot is slightly less wide than the body and its angles are rounded.

No adequate description of the central nervous system in the species or genus exists, but Bergh notes an arrangement that is typical for the Aeolidiadae.

Bergh describes the follicles of the hermaphrodite gland as quite large and says that the anterior genital mass appears rounded. He continues:

Samenstrang stark, nicht lang, mehrere Biegungen machend; der Penis ziemlich stark, die kegelformige Glans innerhalb des dünnen Präputiums zeigend, durch die ganze Lange der Glans konnte der Samenleiter verfolgt werden. Die Samenblase gross, von ovalem Umrisse von reifem Samen strotzend; der vaginale Gang ziemlich kurz.

Apart from these rather confusing fragments the anatomy of *Embletonia*, even in its gross aspects, is unknown. It is not the purpose of this investigation to offer a description of the anatomy of the species, aside from the reproductive system, except in so far as is necessary to establish the true topographical relations of the various components of the generative complex.

GENERAL TOPOGRAPHY OF THE REPRODUCTIVE SYSTEM OF
EMBLETONIA FUSCATA

In its superficial features the reproductive system of *Embletonia fuscata* Gould resembles that of *Aeolidia papillosa* as described by Eliot (1910). In the classification given in the first section of this paper such a duct complex falls under Type IV. The finer details of anatomy will be seen to bear out this preliminary grouping.

The genital complex with its accessory gland structures constitutes at least two-thirds of the total bulk of the animal. In a specimen of three millimeters length, two and one-half millimeters are occupied by re-

productive elements. The brain, buccal bulb, digestive tube, and circulatory system take up scarcely one-tenth as much space in the body. The buccal bulb and central nervous system are anterior to the nidamental and prostate glands, the heart lies dorsal to the medial portion of the complex, while the digestive tube is surrounded by the hermaphrodite duct, its branches, and the follicles of the hermaphrodite gland. The liver of course is concentrated in the cerata.

The entire reproductive system falls roughly into two divisions: the posterior hermaphrodite gland with its duct, and the anterior genital mass composed of a seminal ampulla or vesicle, vas deferens, oviduct, vagina, seminal receptacle, prostate gland, penis, and nidamental glands. The two divisions are bridged by the hermaphrodite duct. The posterior mass constitutes two-thirds of the entire system in volume but not in complexity.

The posterior division consists of six or seven ovoid follicles lying on the ventral floor of the body cavity. They are arranged in order of descending size from anterior to posterior, the most posterior being very small. These are the male acini in which the spermatozoa are produced. A staggered arrangement of the acini, the first lying to the left, the second to the right, and so on, tends to form a central groove along which the digestive tube passes. The hermaphrodite duct lies dorsal to the gut and with its lateral branches completes a meshlike covering for that tube. The lateral branches pass somewhat posteriorly from the posterior portion of each male acinus to form a connection with the hermaphrodite duct.

Each male acinus bears from three to eight smaller ovigerous follicles. These are invariably arranged in the area immediately surrounding the attachment of the hermaphrodite ductule. In a few cases the most posterior male follicles bear no female acini. These are interpreted to be immature.

As the hermaphrodite duct passes forward into the anterior genital mass it expands into an ampulla, thin-walled but muscular, which on sectioning is always found to contain spermatozoa except at the precise moment of spawning. From the ampulla (see Fig. 3) the duct passes into the more complex portion of the anterior mass. It divides shortly into an oviduct and vas deferens.

The vas deferens passes immediately into a series of glandular convolutions, the prostate gland, from which it proceeds through a short muscular duct to the penis. Through this latter structure the spermatozoa are forced during copulation. A unique mechanism for the evagination of the penis exists in this species, the essential features of which are

the muscular bulb at the base of the penis,,and the peculiar structure of the intromittent organ itself. A full description is given later.

From the point of bifurcation of the hermaphrodite duct the oviduct passes forward into a slight expansion, the fertilization chamber. At this point a secondary bifurcation occurs (Fig. 3), a very short thin tube leading into the vagina, while the oviduct proper becomes the
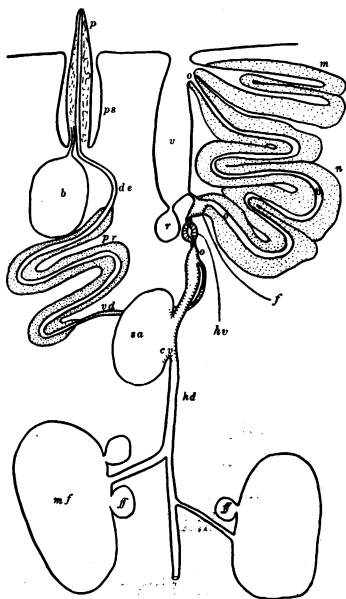

Fig. 3.   Diagrammatic representation of the reproductive system in *Embletonia fuscata* Gould.

p—penis
ps—penis sheath
b—penial bulb
de—ductus ejaculatorius
pr—prostate gland
vd—vas deferens
o—oviduct
v—vagina
m—mucous gland

n—nidamental gland complex
f—fertilization chamber
r—sperminal receptacle
hv—hermaphrodite valve
sa—sperminal ampulla
cv—ciliary valve
hd—hermaphrodite duct
ff—female follicle of hermaphrodite gland
mf—male follicle

lumen of the much convoluted and voluminous nidamental gland complex converging finally with the vagina near its external orifice. The vagina itself is a glandular organ whose lumen is about the same size and shape as the evaginated penis. At its inner end occurs a receptacle which is in such a position that it could conceivably serve as a bursa copulatorix.

The nidamental gland complex is massive and consists of three folds of the glandular oviduct plus a series of storage pouches which empty into the oviduct at various points.

The external genital apertures are contiguous but distinct, the penis lying just anterior to the opening into the vagina.

Thus in general topography the reproductive structures resemble those described by Eliot for *Aeolidia*. The single major point of difference is the appearance of the penial bulb at the base of the penis. Figure 3 gives in diagrammatic manner the arrangement of the various components of the reproductive system in *Embletonia*.

ANATOMY OF THE REPRODUCTIVE ORGANS IN *EMBLETONIA*
HERMAPHRODITE GLAND

The most obvious anatomical feature of a section through the posterior two-thirds of the body of *Embletonia fuscata* is the large ovoid follicle of the hermaphrodite gland with its adjoining ovigerous acini. There are six to eight such follicles in each individual, the general arrangement of which has been described above.

Serial sections reveal that the follicles (sperm producing acini) are in the shape of ovoids somewhat laterally compressed by contact with the body wall on the one hand and with adjacent follicles on the other. The female acini (ova producing) are clustered in a restricted area about the opening of the hermaphrodite ductule into the male lumen, being attached to the larger sperm producing follicle by a short pedicle. Thus, during the later stages of oögenesis and spermiogenesis at least, there is a complete separation of the two types of germ cells. At maturity both are released into the common lumen and discharged through the hermaphrodite duct. A very similar disposition is reported by Eliot (1910) for the kladohepatic form *Aeolidia papillosa* and for the holohepatic *Doris tuberculata* in which the female acini are described as "around and above" the male follicles. In the Holohepatica such an arrangement is somewhat obscured by the initimate relation of hermaphrodite gland and hepato-pancreas.

Arrangements for the isolation of sperm and ova producing areas of the gland seem to have become increasingly efficient with the progressive evolution of the reproductive system in the euthyneurous Gastropoda. Only in rare cases are both germ-cell products produced from the same acinus in the Nudibranchiata, and in those cases there is an obvious differentiation of certain areas, usually proximal to the hermaphrodite gland, for the production of spermatozoa, and of other areas, usually distal, for the development of ova. In the latter cases there is evidently but one cycle in the life of a follicle, the mature sperm being released before the ova are ready for fertilization. Thus the probability of self fertilization is removed.

Trinchese, in *Janus cristatus,* Bergh in *Rizzolia, Trevelyana* and numerous other genera, and Dreyer in *Doto, Calma* and others, describe a relation of male and female acini essentially similar to that found in *Embletonia.* In *Coryphella landsburgii* Pelseneer (1896) finds no isolation of male and female acini but finds sperm production localized in the proximal portion of each acinus, with ova in the distal portion. He reports that similar conditions obtain in all the Elysioidea (*Cyerce, Hermaea, Elysia, Limapontia*). Bruel (1904) finds that in *Caliphylla* ova production, in its later stages, is exclusively peripheral, while sperm production is restricted to the central portion of the lumen.

The Ascoglossa (Elysioidea, etc.) show a rather different type of arrangement in this respect from that which seems general in the other nudibranchiate groups. In these forms there is usually found a larger number of individual follicles. Agersborg (1923) in his researches on *Melibe* found both types of germ cells produced in a few acini but also observed that the majority of the follicles were either exclusively male or exclusively female. In any phylogenetic consideration this anomaly in disposition of the germ producing areas adds to the doubtful position of a group concerning which much discussion has already been aroused.

Accepting fully the pulmonate-tectibranch-nudibranch course of phylogeny it is interesting to note the general progression from the lower group to the higher in respect to this isolation of germ producing areas. Bonnevie (1916) describes for *Cuvierina columnella* a condition which seems typical for the Pulmonata. The acini of the hermaphrodite gland are elongated and sperm production is not strongly restricted to any particular area. However, the developmental sequence is such that the mature spermatozoa are released into the "Zwittergang" before development of the ova is complete. In this type of acinus only one cycle of germ-cell production is to be expected.

Mazzarelli (1891) describes the disposition of the hermaphrodite gland in a number of tectibranchs. In the acini of the Aplysiidae, male and female products are often present at the same time, much as in the Pulmonata. In *Pleurobranchaea*, however, there are separate male and female acini. This author notes that while "the formation of spermatozoa and of ova in separate acini is abnormal in the tectibranchs, it is seen to be generally in effect among the nudibranchs."

Pelseneer (in Lankester's 'Treatise on Zoölogy,' 1906) summarizes this apparent evolutionary series in the Euthyneura:

In the most simple arrangement the two kinds of products are developed side by side as may be seen in *Valvata* and the majority of the Tectibranchiata and Pulmonata.

In the more specialized condition there are male and female acini, the latter opening into the spermatogenous sacs in *Oncidiopsis*, the Pleurobranchidae, and the majority of the Nudibranchiata with the exception of the Elysiomorpha.

A cross-section of a spermatogenous follicle in *Embletonia* reveals that the acinus is made up of a very thin outer covering of connective tissue with more or less infrequent muscle fibers surrounding the contained mass of loosely arranged oögonia, spermatogonia, spermatocytes, spermatids, and maturing spermatozoa. Although the individual follicles vary greatly in size the extreme dimensions are somewhat as follows:

Lateral axis...................................... 675 micra
Dorsoventral axis............................... 725 micra
Longitudinal axis.............................. 875 micra

Approximately the ventral third of the follicular wall is in intimate relationship with the lining of the body cavity; in most cases the two are fused into a single layer of fibrous connective tissue. Such an attachment to the body wall doubtless serves to maintain the proper orientation of the follicles and allows them to keep a constant relative position during the frequent contractions and expansions of the soft-bodied animals.

It is in the area of contact of follicular epithelium and ventral body wall that the production of spermatozoa takes place. The contact area may be described as roughly circular. From the center of the circle a wave of spermatogenesis passes peripherally around the walls of the acinus. Thus in any one follicle there may be present at this central point a group of mature spermatozoa, with successively earlier stages of spermatids and spermatocytes arranged in concentric rings about them. The dorsal two-thirds of each acinus is free from indications of differentiation although the apparently equipotential mother-cells are present in a loose layer adherent to the acinal wall. The determining factor in the differentiation of these so-called "mother-cells" into spermatozoa or ova seems to be related to their position in the follicle.

No attempt has been made to follow in detail the course of spermatogenesis but it appears to offer no deviation from what is already known concerning germ-cell genesis in the Gastropoda. In the process of spinning out the tail of the spermatozoan a cytoplasmic body is sloughed off into the lumen of the acinus. This body, which frequently appears nucleated due to the presence of the so-called "chromatoid body," passes from the acinus along with the spermatozoa and its subsequent presence in various portions of the duct system, together with other

bodies released with the sperm mass such as undifferentiated mother-cells and spermatogonia, gives some evidence as to the direction of ciliary currents and to other details of mechanics in the system.   This evidence will be noted in a later consideration of these ducts.

The extreme dorsal portion of the follicular wall is free from germ cells or undifferentiated mother-cells, particularly in a small area surrounding the orifice into the hermaphrodite ductule.   There is no semblance of a valve at this point, and indeed the behavior of the follicle during debouchement of its contents obviates the necessity for one.   By a concerted contraction of the follicular wall and of the body wall the ripened and loosened spermatozoa are forced in a mass into the opening of the hermaphrodite duct where a series of peristaltic movements completes their transportation to the ampulla.   Plate XXIX, figure 2, shows a portion of an acinus fixed at the moment of emptying its contents into the hermaphrodite duct.   It will be seen that a number of detached mother-cells and other bodies are following in the wake of the germ cells.

At an early stage in the development of the spermatozoa certain cells begin to increase in size.   These cells occur in a ratio of about one in thirty undifferentiated cells in the area given over to sperm production. While the cytoplasm of the gonia eventually undergoes changes in staining property from basic to acid, these cells retain their basic property throughout.   They are invariably in intimate contact with the follicular wall and by extreme vacuolation they eventually form a layer separating completely the maturing spermatozoa from the follicular wall. (Pl. XXVIII, figs. 1 and 3.)   As soon as the vacuolation of these cells has become visible there is to be noted a clustering of the spermatids about them.   The vacuoles increase in size until in the final stages before release of the spermatozoa the cells consist of a single huge vacuole surrounded by a thin layer of cytopalsm, and with the nucleus pressed into any available space near the periphery.   When the moment of release of the spermatozoa occurs the contractions of follicular wall and body-wall burst the thinly covered vacuoles and their contents are passed into the lumen along with the spermatozoa that were once attached to them. Thus nourishment is evidently provided for the germ cells until the time when they may be ejected from the ampulla.

In very young specimens of *Embletonia* the follicles of the hermaphrodite gland contain only undifferentiated germ mother-cells.   In slightly older stages some differentiation begins to appear in the region of spermatogenesis, but only at a late stage is any indication of oögenesis

apparent. Certain cells in the area indicated above, the dorsal third of the follicle, undergo appropriate divisions, increase in size, and begin to push out toward the periphery of the lumen. Their continued growth results in the production of a projection which soon becomes the oögenous acinus, and which remains attached to the male acinus by a narrow pedicle filled with oögonia.

The female acinus is thus covered by the same thin connective tissue and muscular retaining sheath as the male follicle. A cross-section of the acinus at a time just preceding release of the eggs shows a very simple arrangement (Pl. XXVIII, fig. 4). There are usually not more than four mature ova together with an assortment of immature oöcytes and nurse cells in each acinus. The entire group of oöcytes is in the form of a sphere with the more mature cells centrally located while the smaller cells are compressed into the remaining irregular spaces. The majority of the latter naturally fall into close relationship with the thin wall of the acinus. There is no certain means of distinguishing between developing ova in the earlier stages and the nurse cells, if any exist. All the cells continue to give a deep basophilic staining reaction until the deutoplasm begins to appear. Gradually the reaction becomes more acid until the mature ova stain brilliantly with ordinary acid dyes.

No careful attempt was made to follow the process of oögenesis but in general it was seen to follow the course described by Smallwood (1904) in *Haminea*. The ova are released from the acinus into the lumen of the spermatogenous follicle while in the metaphase of the first reduction division. The metaphase spindle occupies a central position in the ovum (Pl. XXVIII, fig. 2).

The mechanism of release of the ova from their acini seems essentially similar to the same phenomenon in the case of the mature spermatozoa. There is a contraction of the muscular sheath together with compression from adjacent structures, causing the large ova to distend the lumen of the pedicle and forcing them into the lumen of the male follicle. In one or two cases this expelling force was seen to be so violent as to extrude the entire contents of the acinus, but in the majority of individuals in which the process was occurring at the moment of fixation only the mature ova were forced out. Plate XXVIII, figure 1, shows an ovum in the lumen of the male acinus while the female follicle in which it was developed appears in longitudinal section to the left.

Protandry is easily demonstrated by these histological studies. In addition there is abundant evidence that *Embletonia* at least is polycyclic in its protandry. In the first place the younger individuals always

show the formation of spermatozoa some time before any differentiation is observable in the oögenic areas. In the second place many individuals show spermatozoa being discharged into the hermaphrodite duct before the ova are fully ripened. In the third place, at the time of expulsion of the ova into the male lumen there is a fresh "crop" of spermatozoa in process of maturation (see Pl. XXVIII, fig. 1). In the fourth place, very old acini show large spaces in the central portion of the spermatogenous area which are devoid of germ cells in any stage of development. This indicates a depletion of these areas by the expulsion of one or more previous generations of ripe spermatozoa.

     All this evidence is further supported by certain other observations not directly connected with the hermaphrodite glands. The seminal receptacle is frequently filled with spermatozoa, indicating that copulation has taken place even while both spermatozoa and ova are in process of formation in the gland follicles. Since copulation has been observed to be reciprocal, the inference is that one generation of spermatozoa has already been used up in the mating process.

     This evidence justifies the conclusion that not only are spermatozoa released first in the reproductive cycle but that there may be two or more such cycles in the life of an individual. This is contrary to the conclusions of Hecht (1895) in regard to *Aeolidia papillosa* based solely upon observation of individuals in captivity. I have been unable to corroborate his observation that death occurs in this species immediately after spawning takes place. Indeed several specimens of *Aeolidia* were allowed to spawn in aquaria at the Mount Desert Island Biological Laboratory, during the summer of 1929, without any noticeable ill effects. An individual of *Coryphella* species, kept in captivity at Princeton, deposited three ribbons of spawn at intervals of two to three weeks in the middle of the winter, and in the end died only because of the impossibility of providing suitable food.

HERMAPHRODITE DUCT

     The hermaphrodite ductule, into which the germ cells are forced for transportation toward the anterior genital mass, is a tube of narrow lumen capable of great distension. It is composed of the same types of muscular and connective tissue cells that make up the follicular outer covering.

     The hermaphrodite duct proper shows a different and unique structure although it retains the single cell thickness and is likewise made up of specialized connective tissue cells. The lumen, when the duct is

empty, does not exceed 50–75 micra in diameter. One can readily visualize its degree of elasticity when it is remembered that the ova which must pass through it average 250 micra and are capable of only slight compression. An increase of about four times in diameter occurs during the passage of the ova.

The individual cells that make up this highly elastic and contractile structure are of a type that suggests a dual function, contractility, and secretion. The cells are basically like those of the follicular wall. The basal portion is occupied by muscle fibrillae while an area of highly vacuolated cytoplasm without any fibrillar structure is situated in the half of the cell adjoining the lumen of the duct. In this cytoplasmic region the nucleus is located, often crowded into a very small space by the excessive vacuolation (see Fig. 4).

As the ova pass along the hermaphrodite duct there is probably a peristaltic wave of contraction of the duct wall passing from posterior
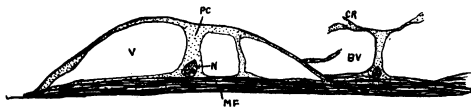

Fig. 4.   Diagram of muscle-gland cell from wall of the hermaphrodite gland.

v—vacuole
n—nucleus
pc—protoplasmic column
bv—burst vacuole (after passage of ova)
cr—remnant of vacuolar wall

to anterior. It has been observed that during the deposition of ova there is a constant rhythmic contraction and expansion of the body walls of the nudibranch. This motion undoubtedly aids in forcing the relatively large ova through the small duct system.

As the ova pass along the duct the larger vacuoles of the muscle cells may break through the very thin protoplasmic membrane emptying their colorless, unstainable contents into the lumen. One can only speculate as to the function of this excreted substance; it may be nutritive or it may serve simply as a lubricant to facilitate the passage of the ova. The breaking of the vacuoles leaves a peculiar appearance at the surface of the secretory-contractile cells. The basal fibrillae remain of course, and rising from this elongated portion of the cell are unsupported columns of cytoplasm, somewhat granular, and containing the nucleus. These columns represent the cytoplasmic remnant of the evacuated secretory portion of the cell. Remnants of the vacuolar membrane adhering to the cytoplasmic columns simulate cilia (see Fig. 4) and in-

deed may be mistaken for cilia unless very careful examination is made. There are no true ciliated structures in the hermaphrodite duct except where it enters the ampulla in the posterior portion of the anterior genital mass.

AMPULLA OR SEMINAL VESICLE

The ampulla is a pouch-like, muscular expansion of the hermaphrodite duct in which spermatozoa are stored but which is never occupied by ova. It is of such a nature that a complete contraction of its walls produces the effect of a straight tube continuous with the incoming and outgoing hermaphrodite duct. Contraction is usually complete at the time ova are passing through the duct; thus no opportunity is offered for self fertilization at this point since the stored spermatozoa are disposed of previous to the appearance of the ova.

The walls of the ampulla are composed of cells identical with those found in the hermaphrodite duct. Thus there is the suggestion not only of · contractility and elasticity, but also of a secretory function. In its most extreme degree of distension the ampulla is spherical in shape with a diameter of about 500 micra.

Several types of bodies frequently are present in the ampulla along with the mass of spermatozoa. These may be either nucleated or non-nucleated. Some are the sloughed off cytoplasmic masses resulting from spermiogenesis, others the mother cells loosened from the walls of the hermaphrodite follicle during its contraction, while others may be spermatocytes or even oöcytes from the same source. Remnants of these bodies frequently pass out with the spermatozoa through the valve at the bifurcation of the hermaphrodite duct, through the vas deferens and penis and into the seminal receptacle of another individual. They are never carried with the ova beyond the valve, for reasons which will be apparent after consideration of that structure.

The entrance of the hermaphrodite duct into the ampulla (Pl. XXXI, fig. 4) is guarded by what I have chosen to term a ciliary valve. The cells of the duct wall at this point are heavily ciliated and the effective stroke of the cilia is toward the lumen of the ampulla. The current thus set up seems sufficient to prevent any back passage of the spermatozoa. The cells bearing the cilia are of a simple cuboidal type.

The anterior continuation of the hermaphrodite duct is lined completely with heavily ciliated cuboidal epithelium resting on elastic connective tissue which is a continuation of that of the more posterior portions of the duct. The effective stroke of the cilia in this case is away

from the lumen of the ampulla. As a consequence spermatozoa and accompanying protoplasmic bodies are carried, in an apparently passive state, toward the bifurcation of the duct. This continuation of the hermaphrodite duct is of about the same diameter as the more posterior portions, i.e., about 50 micra.

At no point in the hermaphrodite duct or in the ampulla is there any indication of independent motility on the part of the spermatozoa. If such motility were existent one would expect an orientation of spermatozoa with respect to the direction of the movement through the duct. No such orientation was observed, the male germ cells invariably being propelled along in jumbled masses.

HERMAPHRODITE VALVE

The mechanism by which the germ cells are selectively separated, ova being passed into the oviduct and spermatozoa along with other small bodies into the vas deferens, is unique but simple in its operation. In Plate XXIX, figure 3, a portion of the valve is shown, while text figure 5 indicates the manner in which it operates.

The continuation of the hermaphrodite duct into the anterior genital mass has already been described as to the structure of its walls. It is very short and, as in other portions of the duct, is highly contractile and elastic. At the point of bifurcation one of the two branches passes ventrally at a sharp angle. This is the vas deferens, a duct of small lumen, obviously incapable of sufficient distension to allow the passage of the large ova, but identical in structure with the upper portion of the hermaphrodite duct. The cilia in this branch as well as in the original duct continue to direct their effective strokes away from the ampulla and toward the exterior. Any small passive object such as a spermatozoan is quickly sucked into this small orifice by the agency of the ciliary currents.

The other branch, the principal channel from the ampulla, proceeds forward a short distance, about 0.2 mm. or less, until it reaches a peculiar valvular arrangement (Pl. XXIX, fig. 3). The valve consists essentially of a heavily muscular sphincter completely closing the oviduct. Beyond the sphincter the duct opens into a spherical chamber scarcely large enough to contain a single ovum at a time. The wall of this spherical chamber is formed by a large number of high columnar epithelial cells of a secretory nature. From the chamber the oviduct passes through a second sphincter identical with the first, and into the glandular enlargement that serves as a fertilization chamber.

Study of a large number of individuals in different stages of the reproductive cycle showed that this double valve has two functions:

1.—The first spincter permits the passage of ova into the oviduct while forming an effective barrier to spermatozoa.

2.—The second sphincter acts as a barrier to incoming foreign spermatozoa, preventing their passage into the hermaphrodite duct or vas deferens.

As an ovum passes from its follicle it is carried up the hermaphrodite duct to the point of bifurcation. Because of the extreme dilation of the duct the opening of the vas deferens is effectively closed to the ovum. As a consequence the ovum must pass through the first sphincter. Either
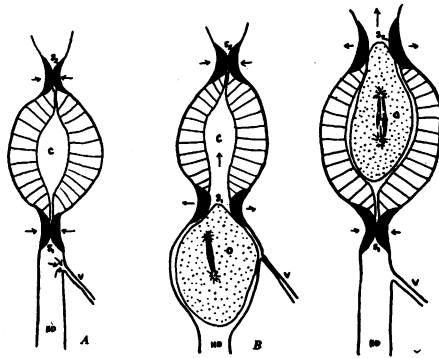

Fig. 5. Diagrams illustrating the action of the hermaphrodite valve in selectively separating ova and spermatozoa.

In A, both sphincters (s_1 and s_2) are closed; the cilia cause spermatozoa from the hermaphrodite duct (hd) to pass into the vas deferens (v).

In B, an ovum is passing from the hermaphrodite duct through the opened sphincter (s_1) and into the central chamber (c) of the valve. Distension of the hermaphrodite duct causes closure of the vas deferens.

In C, the ovum has passed into the inner chamber, the first sphincter has closed, while the second sphincter (s_2) has opened, allowing passage of the egg into the fertilization chamber.

the dilation of the tube is sufficient to open the sphincter or it is opened by some automatic coördinating mechanism not apparent anatomically, and the ovum is admitted to the spherical chamber. Various stages in this passage have been seen in a few individuals, although the deposition of the spawn ribbon is a rapid process and only by the rarest good fortune is fixation obtained at the proper moment. The opening of the second sphincter allows the passage of the ovum into the fertilization chamber where it meets the incoming spermatozoa from the vagina.

Outgoing spermatozoa and the small passive bodies accompanying them, which by some chance are carried past the cilia leading into the vas deferens, reach the first sphincter, but their size is not great enough

to force open the valve. As a consequence they cannot pass into the oviduct. Eventually they are caught in the ciliary current leading into the vas deferens and are transported toward the penis.

So far as can be determined, no similar valvular mechanism has been described in any hermaphroditic gastropod. Dreyer (1911) makes mention of some such structure in *Doto fragilis* but offers no description and ventures no conclusion as to its function. In discussing the ascending portion of the hermaphrodite duct he writes:

This . . . . tube divides far forward into two parts: the one part opens into the prostatic portion of the vas deferens, while the other opens into the oviduct by means of a small aperture, situated on a funnel shaped papilla pointing backwards into the oviduct. One can only guess at the possible function of this curious ciliated funnel; it may perhaps serve to suck back into the vas deferens any spermatozoa which may have entered the oviduct with the ova, and so help to prevent possible self fertilization.

The structure so described is obviously unlike the one described above for *Embletonia*, but the identical positions of the two with relation to the bifurcation of the hermaphrodite duct make it seem highly probable that there is a similarity in function.

Very careful anatomical investigation of this junction of vas deferens and oviduct in a large number of species will be necessary before it can be said that this method of separating ova and spermatozoa is of general occurrence. However, it is probable that some such mechanism must exist in every species; otherwise spermatozoa and ova would pass indiscriminately through both vas deferens and oviduct, a condition which never has been observed in any of the hundreds of specimens of some forty species cursorily examined in this investigation, and which never has been recorded in the literature.

VAS DEFERENS AND PROSTATE GLAND

For a short distance after its separation from the oviduct the vas deferens is a simple tube lined with flat epithelial cells rather heavily ciliated. The duct is in two layers, the outer of which consists of circularly arranged muscle fibers. Contraction of these fibers in a wave progressing anteriorly, together with the action of the cilia, is responsible for the propulsion of the spermatozoa into the glandular portion of the sperm duct.

At a point dorsal to the anterior genital mass the vas expands into a glandular, convoluted passage which is the source of the prostatic secretion. In cross-section (Pl. XXXI, fig. 3) the prostate gland shows a relatively wide lumen, its diameter depending upon the degree of disten-

sion of the glandular cells, that is, upon the stage of the secretory cycle. The outer covering of the duct consists of a thin layer of connective tissue. Directly based upon this membranous sheath is the layer of prostatic secretory cells.

The secretion process in these cells is of a type midway between holocrine and merocrine. At the onset of the secretory cycle the nucleus is centrally located, the cytoplasm is rather finely granular, and no semblance of vacuolation is present. Except in the very earliest stages the strongly basophilic reaction of the secretion granules gives the cytoplasm a decidedly blue color with hematoxylin. In successively more advanced stages the granulation of the cytoplasm becomes more dense, particularly in the region nearest the lumen of the duct. The nucleus is crowded to the basal portion of the cell but maintains its spherical shape and large size throughout the cycle. The nucleolus increases gradually in size and staining reaction. Eventually a slight vacuolation appears in the more distal region of the cytoplasm. This vacuolation increases until the vacuoles actually burst, emptying their slightly basic contents into the lumen of the gland (Pl. XXXI, fig. 3). At the time of excretion of the secretory product the distal half of the cell is completely disrupted, but the proximal half with its granular cytoplasm and the nucleus is relatively undisturbed and ready after a brief period of repair to undergo a repetition of the cycle.

The prostatic secretion thus released into the lumen of the vas deferens becomes mixed with the spermatozoa in their passage toward the penis. As the secretion plus germ cells passes from the glandular portion of the vas and into the ductus ejaculatorius it is a dense, basophilic mass with the spermatozoa in no semblance of orientation. The mass indeed behaves almost as a unit and in this respect suggests the so-called spermatophore of certain pulmonates. Not until sometime after deposition in the sperminal receptacle of another individual does the mass disintegrate, allowing the then motile spermatozoa to move freely.

After a series of convolutions, usually three, the glandular portion of the vas deferens passes into the ductus ejaculatorius which is continuous to the tip of the penis. This duct contains a very small lumen, scarcely more than 25 micra in diameter, and is lined with a simple non-ciliated, cuboidal epithelium. Surrounding this is the same type of circular muscle layer seen in the most posterior portion of the vas deferens, but here the muscular coat is extremely dense and evidently capable of powerful contraction. Upon this layer of muscle evidently

falls the function of ejecting the sperm mass forcibly into the bursa copulatorix of a second individual at the time of copulation.

In its general topography the vas deferens is essentially similar to that described by Eliot for *Aeolidia*, but that author did not recognize the presence of the prostatic region. He notes that the aeolids in general have been found to be without a prostate gland (as an appended accessory to the vas) but notes that "the upper part (of the vas) is often thick and spongy compared with the thinner and more muscular part near the exit." In *Aeolidia* two muscular layers are described for the ductus ejaculatorius and indeed for the entire length of the vas deferens, but I have been unable to distinguish more than one in *Embletonia*.

PENIS AND MECHANISM FOR ITS EVAGINATION

In most of the Aeolids the penis is described as a "muscular conical organ, of considerable size," situated in an invagination of the outer body wall, which depression serves as a praeputium. That this is the situation in *Aeolidia* as well as in *Doto, Calma,* and other genera is pointed out by Eliot (1910), and by Dreyer (1911). In other genera the penis may take a variety of forms: it may be of a corkscrew shape; it may be blunt or sharply pointed; it may be barbed or armed with chitinous projections; or it may consist only of an evertible sac in the body wall, the contents of which are emptied into the seminal receptacle of another individual. The diversity in penial structure has often been used as a feature in specific and generic diagnoses. For this reason it is not to be expected that the functional arrangement found in *Embletonia* is of universal or even of general occurrence among the Nudibranchs.

The penis of *Embletonia* is situated in an invagination of the body wall slightly anterior to the opening into vagina and oviduct. The depression is deep enough and of such shape that the penis when relaxed fits into it very nicely, an external view of the animal at such a time giving the impression of two contiguous openings into the body at a point slightly anterior to and below the first cluster of branchiae. Upon erection of the penis, preceding the copulatory act, the elongated, sharp-pointed, muscular organ may be seen projecting from its praeputial sheath to a length almost equal to the maximum breadth of the body. There is, in this species, no eversion of the penial sheath during the sexual act, erection consisting entirely in an increase in length of the intromittent organ.

The penis sheath, which is continuous on the one hand with the external epithelium of the body wall, and on the other with the outer

epithelial covering of the penis itself, consists of a layer of cuboidal to flat epithelial cells bearing, in the portion adjacent to the exterior, a large number of fine cilia. This ciliation is but an extension, and to some degree an exaggeration, of the same type of structure covering the entire body of the nudibranch. In the inner two-thirds of the sheath the epithelial lining is simple, flat and non-ciliated. The remainder of the praeputial wall is composed of loosely arranged connective tissue cells.

Sections of the penis were obtained at all stages of contraction and extension with the result that the mechanism of erection was well shown. In spite of its tapering to a sharp point a cross-section at any level shows essentially the same histological features. Plate XXX, figure 1, shows such a section taken from the middle portion of a fully erected penis. In Plate XXX, figure 2, a section taken from the basal portion of a penis is shown, while figures 3 and 5, of Plate XXX, show longitudinal sections made at the time of complete retraction within the penis sheath.

In cross-section the penis shows six distinct layers of tissue. The two layers forming the central canal are identical with the two layers of the ductus ejaculatorius described above: i.e., there is a lining of simple cuboidal epithelial cells and an outer sheath of circularly arranged muscle fibers. In order of their occurrence passing from the central canal toward the surface, there follows a relatively thick spongy layer composed of connective tissue cells loosely arranged so as to leave innumerable spaces or sinuses, a thin layer of longitudinal muscle fibers, a thicker layer of circular muscle fibers, and a final layer of flat epithelial cells identically like that which lines the inner two-thirds of the sheath and, in fact, a continuation of that layer.

At the base of the penis, where the ductus ejaculatorius enters, sealing the spongy connective tissue layer of the penis off from the body cavity, there is a large muscular bulb. Upon cursory examination this ovoid body gives every evidence of being a gland accessory to the vas deferens, emptying its contents into that duct near its entrance into the intromittent organ. Indeed it would not be surprising if the structures of analogous form and position which have been described as prostate glands should later be shown not to be the source of the prostatic fluid. It should be noted that the occurrence of such a bulb or "prostate gland" is very rare among the aeolids; only in *Chlamylla* is it recorded. Among the Holohepatica, on the other hand, the superficial appearance of a bulbous structure adjoining the vas deferens is quite general. In this latter group the structure is truly glandular, and it empties into the vas, if the majority of descriptions are to be accepted as accurate.

In *Embletonia* the penial bulb does not empty its contents into the seminal duct.   Plate XXX, figure 3, shows the relation between penis and bulb.   It also indicates the position of the fine duct leading from the bulb into the base of the penis.   This duct does not enter the vas deferens or central canal of the penis but instead expands into a funnel which empties the fluid from the bulb into the sinuses of the column of spongy tissue.   In Plate XXX, figure 2, the cross-section of the basal portion of the penis shows the relationship existing between duct and canal. The duct is bound to the canal and is loosely joined to the outer layers of the penis by connective tissue fibers.   Slightly distal to the plane of this section the duct terminates.

In section the penial bulb shows a two-layered construction.   The inner layer is composed of granular, acidophilic gland-cells of a low cuboidal type.   Around these is a heavy muscular sheath the thickness of which varies with the degree of contraction or expansion of the bulb. The lumen in all cases is filled with a somewhat acid fluid which is also present in the spongy tissue of the penis at the time of erection.   The volume of the bulb is greatest when the intromittent organ is completely withdrawn into its sheath, and least when that organ is most completely elongated.   Thus there is obviously a direct functional relationship to erection.

The mechanism of erection of the penis is stimulated to activity by some phenomenon associated with the proximity of a second individual. The muscular wall of the bulb is caused to contract, forcing the secreted contents of the lumen into the spaces or sinuses of the spongy column in the penis.   The forces set up in this organ, being of an hydraulic type, are obviously exerted in all directions.   However, the effective closure of the base of the penis by the bulb and ductus ejaculatorius makes inoperative the inwardly directed forces.   The heavy layer of circular muscle negatives lateral forces, thus leaving only one direction in which expansion can take place.   During the period of extension the longitudinal muscle fibers are relaxed and extended greatly.   After the act of union the muscular sheath of the penial bulb relaxes its pressure and the tension already set up in the longitudinal muscles of the penis causes the retraction of that organ and forces the fluid from the spongy mass into the flaccid bulb.

The circular muscle-coat surrounding the internal canal is obviously homologous in function with the same structure in the ductus ejaculatorius.   Its contractions probably assist in propelling the sperm mass toward the exterior.

So far as is known, no other similar mechanism has been described. It is presented here not as a structure whose functional principle may be of general occurrence among nudibranchs or elsewhere, but as a unique specialization of *Embletonia*.

OVIDUCT AND ITS ACCESSORY GLANDS

It is apparent from a consideration of the deposited spawn ribbon that the oviducal passage must be the site of at least five operations, each of which would seem to call for a separate functional region. First of all the union of outgoing ovum with incoming spermatozoan must be accomplished. Then in succession must follow the deposition of a layer of albumen about the fertilized egg, the building of the thin but tough "shell" coat, the formation of the gelatinous layer of mucus about each individual ovum, and finally the formation of the spawn ribbon of the same type of gelatinous material. Previous descriptions of the various glandular regions, whether appended to the oviduct or a part of it, are generally inadequate. Each reference to the nidamental gland complex in the various species of nudibranchs contains an attempt to reduce the maze of convolutions and lobes into regions distinguishable by their coloration as "whitish" or "yellowish," or by actual or imagined differences in cutting and staining qualities. Such attempts have left the state of our knowledge almost exactly as it was a century ago.

In *Embletonia*, as in other species examined, the reason for this inadequacy of description became apparent after a few efforts at sectioning. All the specimens were taken during the spawning season when the glandular lumena were filled with secretion and when the gland cells were in a state of extreme secretory activity. The secreted material is of such a nature that fixation renders it and the cells containing it extremely friable. Only in the most favorable instances were perfect series of the glandular complex obtainable and in these the finer details of cell structure were generally so obscured that research of a cytological nature was impossible. Upon gross dissection under water the mucous-secreting portions of the gland swell into an amorphous mass. The only recourse seems to lie in the possibility that more favorable material may be secured at a time other than the spawning season. Unfortunately there are very few instances on record of the capture of nudibranchs at any time other than the breeding periods. The complete disappearance of whole colonies immediately after spawning and the failure to bring individuals up in the dredge during the winter months are puzzling phenomena that have caused much speculation as to the possible life history.

With the capture of several specimens of *Polycerella emertoni* in Barnegat Bay during the winter of 1929–1930 it was thought that some light might be obtained on the structure of the gland complex in the resting stages. Here again failure was encountered, since the animals, which had evidently undergone a long peroid of practical starvation in the icy waters, had resorbed all non-essential organs including, among others, the bulk of the nidamental gland. Probably final light must come from specimens reared to maturity in laboratory aquaria, a feat that never yet has been accomplished.

In spite of all the difficulties, however, certain facts were obtainable from the more favorable preparations, and certain regions of the gland could be distinguished and the path of the ova could be traced.

The ova pass from the hermaphrodite valve immediately into a slight expansion of the ciliated oviduct. In this small chamber the fertilization actually takes place. The wall of the fertilization chamber is contiguous with the wall of the upper portion of the vagina, that is, the portion most distant from the external orifice. Perforating the two walls is a minute ciliated duct (Pl. XXIX, fig. 4), which forms the connecting link between the female portion of the reproductive complex and the androgynous portion. Through this duct the motile spermatozoa pass to reach the ova. It is this short duct that makes of the system a triaulic type rather than diaulic. Further reference to its function is given in the consideration of the androgynous organs.

From the fertilization chamber the oviduct again narrows as far as the diameter of its lumen is concerned, but expands with reference to total diameter; thus the walls are much thickened. The walls from this point to the opening into the lower portion of the vagina are composed of high columnar gland cells disposed between thin-bodied ciliated cells all based on the same membrane of connective tissue. This disposition of the glandular cells has been described by Henneguy (1925) as general in the aeolidians that he has examined. He figures and describes three types of glandular cells in the course of the oviduct, each of which has a more or less complete "excretory organ" in the distal end. Alternating with these gland cells are thin elongated cells bearing cilia; cells the nuclei of which are highly compressed by the surrounding secretory cells.

In *Embletonia* I find no traces of the existence of this "special excretory organ" in the cells. As for the three gland types distinguishing three different regions of the duct, I find all three types,[1] with respect to relative height, staining capacity, and nuclear distribution, scattered

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[1] Described by L. F. Henneguy, see Bibliography.

indiscriminately throughout the convoluted course of the oviduct. I regard these as stages in the secretory cycle of the same type of secretory cell. This view seems strengthened by Henneguy's figures of the three types in which he shows the disposition of the nuclei. In Henneguy's type A the nuclei are rounded, relatively large, with distinctly visible linin network. This type of cell is figured as lightly colored in basic stains. In type C the nuclei have begun to show a compression due to the growing vacuolation of the cytoplasm. The staining reaction to basic dyes has increased. In type B the cell is completely filled with secretion product, the nucleus is forced to an extreme proximal position and is shrunken and irregular in outline. The presence of the "excretory organ" in type A seems to be truly a distinguishing feature, although I have failed to corroborate Henneguy's observation in this respect in the examination of a number of aeolids. Fixation in any case is so poor as to render uncertain any final conclusion.

Thus it was found impossible in the case of *Embletonia* to distinguish with certainty the portion of the oviduct that secretes albumen from the succeeding shell gland. The mucous gland, did not present the same difficulties. It is in two parts, the course of the ova passing through one, while the other finds an outlet for its product adjacent to the opening of the oviduct into the vagina. The entire channel of the oviduct is much convoluted, the first part of the mucous gland, which takes a dense blue stain, being folded upon itself three times. In the two distinguishable portions of the duct, albumen-shell and mucus, there are side pockets or reservoirs for the storage of secretion products. These seem to be of no regular occurrence and their size seems to depend · upon the amount of contained mucus or albumen.

This discussion of the oviducal gland complex leaves much to be desired, but little can be established with certainty until technical difficulties and the problem of getting material in suitable stages can be surmounted.

For the purposes of this present investigation it is sufficient to trace the course of the ova and to determine the combined functions of the oviduct and its connected glands.

The products of the second portion of the mucous gland are joined with the masses of ova as they pass into the vagina, and the whole is formed into a kidney-shaped ribbon which in the process of deposition is attached by its margin to a supporting object. This second portion of the mucous gland is composed solely of the high columnar secretory epithelium characteristic of the first part. The enclosing sheath is very

thin.   Various stages in the secretory cycle are invariably present in any one individual.   Plate XXXI, figure 1, shows the relation between mucous gland and oviduct on the one hand and vagina on the other. Plate XXX, figure 4, shows cells of the mucous gland in various phases of the secretory cycle.

ANDROGYNOUS ORGANS

Those structures in the reproductive organs having as their function the reception and transportation of incoming spermatozoa are three in number: the vagina, the receptacle, and the androgynous duct already mentioned.

The vagina (Pl. XXXI, figs. 1 and 5) is essentially a deep invagination of the body wall.   Its lumen is of approximately the same diameter and length as the penis which it is to receive.   The organ may be divided roughly into two halves with respect to the structure of the wall, the inner half being lined with large, cuboidal, non-ciliated, granular epithelial cells, the outer half with the same type of cells but bearing longer cilia, interspersed with goblet cells obviously secreting mucus.   In an individual that has not copulated for some time the product of these goblet cells forms a plug of coagulum closing the vaginal orifice.   The outer sheath of the vagina is a thin, slightly muscular membrane of connective tissue.

At the extreme inner end of the vagina a small aperture, regulated by a muscular sphincter, opens into a bulbous receptacle.   It seems likely that the tip of the penis penetrates into this structure during copulation, since the sperm masses are always found within it until some time after the mating process is completed.   The histological features of this storage chamber are simple, consisting of an outer muscular sheath and an inner lining of small, flat, granular epithelia.   The mass of spermatozoa entangled in the prostate fluid remains inactive for some time, after which the sperm become motile and begin to orient themselves toward the glandular structures on the wall of the receptacle.   At the proper interval, a relaxation of the sphincter, coupled with a contraction of the muscular sheath, forces the active spermatozoa into the inner portion of the vagina.   In plate XXXI, figure 5, the male germ cells may be seen definitely orienting themselves with respect to the walls of the vagina which probably secrete some nutritive substance.

Opening from the vagina at a point only a few micra from the aperture of the receptacle is the ciliated androgynous duct leading to the immediately adjacent fertilization chamber.   Through this duct the

spermatozoa eventually find their way and the fertilization of the ova is thus accomplished.

SUMMARY OF STRUCTURAL RELATIONSHIPS IN *EMBLETONIA*

Thus the anatomical evidence relates the reproductive system of *Embletonia* to Type IV of the classification given in an earlier part of this paper. The hermaphrodite duct arising from the hermaphrodite gland is finally divided into three passages: male, female, and androgynous. The male duct consists of a glandular prostatic portion, a muscular ductus ejaculatorius, and an extensible penis with a unique bulb mechanism for its erection. The female duct consists of a fertilization chamber followed by a much convoluted passage lined by alternating ciliated and glandular epithelial cells. The oviducal passage empties into the vagina near the copulatory orifice. Appended to the oviduct are an irregular number of storage reservoirs and one distinct mucous gland which provides the final gelatinous matrix of the spawn ribbon. The androgynous portion of the reproductive system is composed of a large pouch-like vagina of the proper shape to receive the extended intromittent organ during copulation, a muscular receptacle which also serves as a bursa copulatorix, and a very short ciliated duct through which the motile spermatozoa pass into the fertilization chamber of the oviduct.

The three-way valve effecting the selective separation of the spermatozoa from the ova is a feature heretofore undescribed and is presented as of possible general application to the hermaphroditic gastropods in which such a separation occurs, i.e., in most of the Tectibranchiata and all of the Nudibranchiata.

Embletonia seems to be peculiar among the aeolids in possessing a mechanism for the erection of the penis consisting essentially of a muscular bulb capable of ejecting its secreted contents into a spongy portion of the copulatory organ, the resultant pressure being limited in its operation to longitudinal extension of the member by appropriate arrangement of muscular layers.

The ova and spermatozoa are released from separate male or female acini of an hermaphrodite gland follicle into a common lumen. From there the products are forced through the non-ciliated but muscular hermaphrodite duct into the ampulla. Release of the eggs and the sperm never occurs at the same time, there being evidence of a definite series of protandrous cycles. From the ampulla the products are carried to the point of bifurcation of the hermaphrodite duct in the anterior

genital mass. The ciliated vas deferens makes a sharp angle ventrally while the oviduct passes through the hermaphrodite valve. The latter structure consists essentially of two sphincters which open successively to allow the passage of the large ova, either through the distending pressure of the ova themselves, or through some coördinating mechanism not discernible in anatomical preparations. From this point the ova pass through the fertilization chamber where they come in contact with the incoming spermatozoa, and into the nidamental gland complex where the albumen, shell, and mucous layers are successively laid down. As the fertilized eggs pass from the oviduct they receive the final matrix of gelatinous material and are formed into the spawn ribbon.

During copulation the sperm mass, a tangled bundle of spermatozoa embedded in the prostatic secretion, is deposited in the receptacle. After a period of time the prostatic coagulum dissolves and the spermatozoa become motile probably for the first time. At least they show the first evidence of any sort of orientation. By contraction of the muscular wall of the receptacle the spermatozoa are ejected into the upper portion of the vagina from which place they are carried, or find their way through their own motility, into the androgynous duct and finally into the fertilization chamber.

The spermatozoa are forced through the convoluted prostatic portion of the vas deferens and at the proper moment are ejected through the penis by the contractions of the ductus ejaculatorius.

The complexity of such a system of muscular sphincters, contracting duct and receptacular walls, secretory structures, and ciliary mechanisms, requires the operation of some coördinating mechanism the nature of which presents problems of amazing difficulty because of the small size of the organism. All that can be said of the nervous control is that Dreyer (1910) has found the innervation of the gentalia of pedal ganglionic origin.

NATURAL HISTORY OF REPRODUCTION IN *EMBLETONIA* AND OTHER NUDIBRANCHS

Little is known of the life histories of the Nudibranchiata since specimens are rarely taken at other than the spawning period, i.e., during a brief period in the summer months. *Embletonia fuscata* was found in enormous numbers on the piles of the bridge across Barnegat Bay near Beach Haven, N. J., on November 11, 1928. All the individuals were engaged in reproductive activity of one sort or another, either copulating or depositing ova, or sectioning of them showed evidence of

recent matings. The short kidney-shaped spawn ribbons were found in large numbers, each ribbon attached by its margin to a stalk of one of the numerous hydroids upon which the animals were living.

Copulation in *Embletonia* is like that observed in many different species of aeolids by dozens of authors and by myself during the summer of 1929 at the Mt. Desert Island Biological Laboratory in Salisbury, Cove, Maine. In the case of two individuals kept in a glass finger-bowl each seemed aware of the presence of the other even while separated by a space of eight or ten centimeters. The intromittent organ at once begins its process of extension and the two animals proceed in a straight line toward each other. Eventually the oral tentacles come into contact and there follows a moment of hesitation after which the course is slightly altered to permit further progress with the right sides of the two nudibranchs in contact. When the external genital orifices are adjacent the erection of the penis is quickly accomplished and reciprocal union takes place, the spermatozoa from each individual being deposited in the receptacle of the other.

The actual union sometimes lasts many minutes. A substance secreted from the goblet cells of the lower part of the vagina seals the animals together in such a way that it is difficult to separate them during copulation without causing severe injury to the genital structures.

Deposition of the spawn ribbon usually occurred twelve to twenty four hours after copulation in those aeolids which were studied with the time relationships in mind. Thus ample time was allowed for dissolution of the serminal mass or "spermatophore" and for the motile spermatozoa to reach the fertilization chamber.

Two weeks after the capture of several hundred specimens of *Embletonia*, efforts to find even a single individual in the same locality proved fruitless. The sudden disappearance gave the impression of a migration of the entire colony. In the systematic literature there is a widespread acceptance of supposed migratory phenomena as preliminary to and succeeding the actual spawning and related processes among the Nudibranchiata. Such an assumption is particularly to be found in the writings of Alder and Hancock (1845–55), of Risbec (1928), and especially of Hecht (1895). In all cases the theory is based upon just such mysterious disappearances and sudden appearances of colonies at a locality as obtained in the case of *Embletonia*. Acceptance of the view has been so general as to make it almost universal.

In general terms the migratory theory assumes that at stated periods large "swarms" of nudibranchs of a single species find their way

from deep water to shallow where copulation and deposition of egg masses take place. There follows after a few weeks a sudden disappearance of the species from the place of spawning. Hecht concludes from his observation of the spawning habits of *Aeolidia papillosa* that death follows very shortly after the spawning has been accomplished, and thus he accounts for the sudden and mysterious disappearance. His laboratory observations seem to bear out this theory, since he reports that all specimens kept in captivity died immediately after spawning, while no difficulty was experienced in keeping alive those individuals not sexually active. Garstang (1890), dealing with the British species *Goniodoris nodosa*, also concluded that spawning was followed by death.

There are important objections to complete acceptance of the theory of migrations, and particularly to the accounting for the sudden disappearance by death incident to spawning.

In the first place there is the relative immotility of the Nudibranchiata to be considered. Even granting the secondary type of "leaping motion" described by Agersborg (1922) in one species, there is little justification for according to them the ability to travel considerable distances under their own propulsion, even under conditions of comparative or absolute calm. Their feeble ciliary motility cannot possibly be conceived as carrying them through the shallows adjacent to the seashore where their laborious progress would be hindered by the powerful currents and the battering action of the surf. It seems far more reasonable to assume that such nudibranchs as find their way to a spawning ground near the shore or within the tidepool zone are carried there from their normal deep water habitat by the action of the waves and tidal currents. Having once secured a foothold inshore under favorable environmental circumstances such as prevail during the spawning season, it is easily conceivable that within a few weeks a few specimens could account for the large colonies that are reported so frequently.

This theory of the fortuitous establishment of colonies as opposed to any mass migration finds abundant support in fact. The researches of the past two years show clearly that inshore migration is not requisite to spawning. During the summer of 1929 several colonies of *Doto coronata* were discovered at various localities on the shores of Mt. Desert Island, Maine. The individuals in these colonies were observed to copulate and spawn during July and the greater part of August. Observations were made at frequent intervals. During this same period dredgings from depths of twenty-five to fifty fathoms showed specimens of the same species engaged in precisely the same type of reproductive activity.

Egg masses taken with them showed that development of the embryos was proceeding normally and many fully formed veligers were seen in process of releasing themselves from the spawn ribbon.

Exactly the same observation was made with respect to *Lamellidoris aspera*, a species which was active inshore during all of July, and which again appeared in the early days of September. The dredge on one occasion brought up a pair of this species adhering to a bit of bryozoan. The two were copulating during their long trip from a depth of thirty fathoms and beside them was a freshly deposited ribbon of spawn.

The case of *Cadlina obvelata* was somewhat different but equally significant. No members of this species were found on the shores of the island during the entire summer although they had been reported as abundant the previous season. However, the dredge revealed several specimens all reproductively active.

Certain individuals of a species, under favorable circumstances, survive at the site of the previous season's spawning activity. This was indicated by the capture of several specimens of *Polycerella emertoni* on the bridge piles across Barnegat Bay on February 20, 1930. In November of the previous year large numbers of the same species were taken at the same place, all sexually active. Histological section of the winter survivors showed the fully formed hermaphrodite glands, liver, and other internal structures in process of resorption. There is every reason for believing that the organisms had remained in the area of the old spawning ground since the previous season. It should be noted that the water depth was sufficient to prevent the formation of ice in the immediate vicinity of the hibernating nudibranchs. The survival through the winter of one such fertilized individual would probably be sufficient to account for enormous numbers the following spring.

In order that this rapid multiplication may come about we should expect to find a rapid development from ovum to mature nudibranch. Such a rapid development in fact is invoked by Hecht and by Garstang as evidence that the life span is but a single year. Observation of colonies of *Dendronotus frondosus* at Mt. Desert Island in the summer of 1929 showed that three separate generations become reproductively mature in the period from July 1 to September 15. It is probable that five or more broods were produced during the entire summer in a single locality, all of which were reproductively mature at the close of the season.

Unfortunately, data as to the time of development of any typical nudibranch is lacking. It has been found almost impossible to keep

them alive in captivity through the metamorphosis. However, reports of Pelseneer on the atypical *Cenia* (1899) indicate a development from the one-cell stage to the mature adult in approximately eighteen days.

Anatomical evidence is cited elsewhere in this paper to the effect that each individual nudibranch may pass through repeated reproductive cycles in the same season, cycles of the protandric type. With so many broods, and with every individual of each brood depositing numbers of spawn masses, each single mass containing several hundred ova, there is considerable possibility of deriving the presumed migrations from a single individual cast on the shore by chance. Certain it is that inshore spawning grounds are not essential.

The sudden disappearance of the great swarms of nudibranchs is a more difficult problem to resolve. In some cases where the vanishing occurs late in the fall the reduction in food supply (living hydroids and bryozoa) may be a factor. In other cases failure of food supply at one point, together with the inability of the organisms to move long distances in search of more abundant sources, may bring about the destruction of the colony and its total extinction. One further factor may be found in the rapid multiplication of crustacean enemies, particularly *Caprella*, in the area occupied by the swarm. Such a condition was found to exist among the *Polycerella* taken in Barnegat Bay in the fall of 1929. *Caprella* was rapidly becoming the dominant life form among the masses of hydroids, and the nudibranchs were being slaughtered in enormous numbers.

The conclusion of Hecht and of Garstang that the life of a nudibranch extends over a period of one year only seems to me unjustified. Hecht's argument is based largely on the supposed death of *Aeolidia papillosa* following deposition of the spawn, while Garstang draws his view from purely speculative evidence.

Several specimens of *Aeolidia papillosa* kept in laboratory aquaria at the Mt. Desert Island Laboratory were observed to deposit spawn ribbons. In no case did death ensue immediately after the deposition.

At the Princeton University laboratory a specimen of *Coryphella* species was kept in a glass tank from October 28, 1929, to January 1, 1930. During this period three separate spawn masses were deposited at intervals of at least two weeks. Death occurred only when the supply of suitable food was exhausted. Not only does this case indicate that death is not the inevitable price of reproduction in the species, it also indicates that the reproductive processes may continue so long as favorable temperature and other environmental conditions are continued.

Hecht also sees the sexual maturation of parasitic copepods concurrent with the death of the nudibranchs as a phenomenon giving important evidence as to the duration of the life cycle. If such a case could be demonstrated, its bearing on the problem would be in doubt. The phenomenon could as easily be explained on the assumption that the coming to maturity of the copepods so devitalizes the nudibranchs as to make death inevitable.

To summarize: it seems probable that no mass migrations of nudibranchs occur at the spawning season; that the appearance of large numbers of a single species at a particular locality is traceable to the chance displacement or survival of a few individuals and their rapid multiplication, together with their normally rapid development; and that the mysterious disappearance of entire colonies may be due to the fatal intervention of some environmental factor such as cold, lack of food, or inability to compete successfully with other life forms which spring into prominence among them. At present the evidence does not justify any estimate of the duration of the life cycle.

Such a view would account for all the known facts relative to the "migrations." The peculiar irregularity in occurrence of a certain species over a period of years is particularly well taken care of, since fortuitous establishment of inshore colonies cannot be expected to occur with any regularity.

Adequate investigation of the natural history of the Nudibranchiata is much to be desired. Deep-water dredges in common use are so constructed that small soft-bodied mollusks are easily washed through the meshes of the net before it has reached the surface. This probably accounts for much of the great gap in our knowledge. With the use of specially constructed apparatus one might expect more satisfactory results.

SUMMARY

1.—A fundamental similarity existing among the complex reproductive systems of the Nudibranchiata is pointed out and a classification is presented based on the extent of primary and secondary bifurcation of the hermaphrodite duct. Four types of system are indicated, any one of which might conceivably be the phylogenetic derivative of any other.

2.—The histology and anatomy of the reproductive system in *Embletonia fuscata*, a typical aeolid, are given, and the functional implications of the existing anatomical relationships discussed.

3.—Some phases of the natural history of reproduction in the Nudibranchiata are discussed indicating that the mass migrations supposed to precede and follow spawning probably do not occur.

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Fig. 1. Longitudinal section of hermaphrodite gland follicle showing mature ovum released from the female acinus and lying in the follicular lumen. ×65.

o—ovum
ff—female acinus

Fig. 2. Photomicrograph of ovum just after release from the female acinus of the hermaphrodite gland. ×90.

The spindle of the first maturation division is in a central position. No change occurs until the entrance of a spermatozoan.

Fig. 3. Cross-section of mature hermaphrodite gland follicle showing arrangement of developing spermatozoa, spermatids, and mother cells. Photomicrograph ×65.

The large vacuolated nurse cells appear on the ventral wall of the follicle. The close connection between follicular and body walls is well shown.

cb—cytoplasmic bodies sloughed off during spermiogenesis
nc—nurse cell
bw—external body wall

Fig. 4. Photomicrograph of section through three female acini of an hermaphrodite gland. ×65.

The arrangement of the mature ova (large and more densely stained), nurse cells, and oöcytes, is typical.

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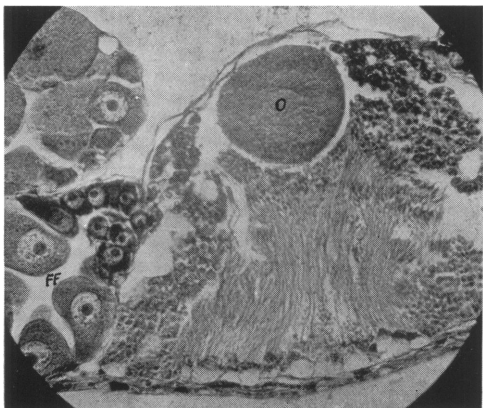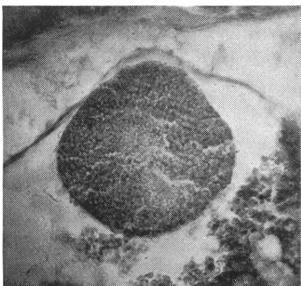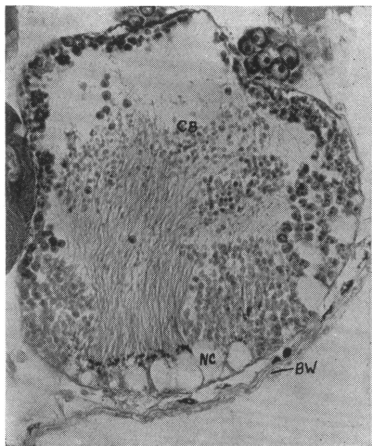
Fig. 1.   Photomicrograph of a section through the ampulla showing the entrance of the hermaphrodite duct and its continuation into the anterior genital mass.   ×80.

Arrows indicate the direction of the effective ciliary strokes and consequently, of the spermatozoa.

Fig. 2.   Photomicrograph of a section through a follicle of the hermaphrodite gland at the moment of expulsion of the mature spermatozoa and undifferentiated cells.   ×65.

A longitudinal section of a portion of the hermaphrodite duct is also shown.

Fig. 3.   Photomicrograph of section through the hermaphrodite valve.   ×130.

The inlet sphincter (s), the outlet sphincter (s'), and the intravalvular lumen (c) with its wall of conical gland cells are shown.   (hd) indicates the termination of the hermaphrodite duct.   The outer sphincter (s') leads directly into the fertilization chamber not included in this section.

Fig. 4.   Photomicrograph of section through the androgynous duct (ad) connecting the vagina (va) with the fertilization chamber (fc).   ×130.

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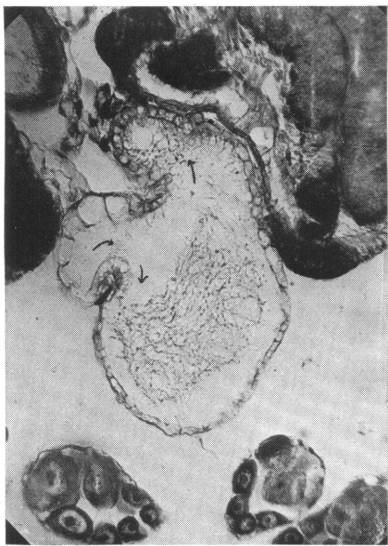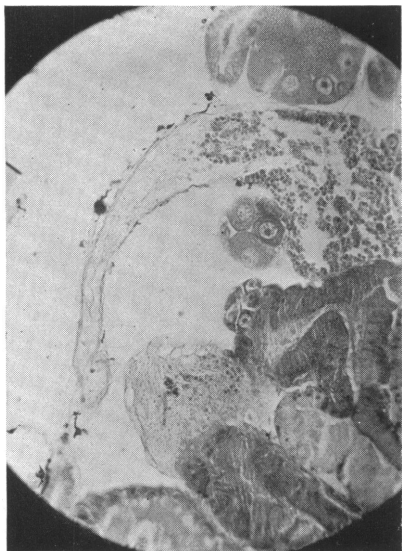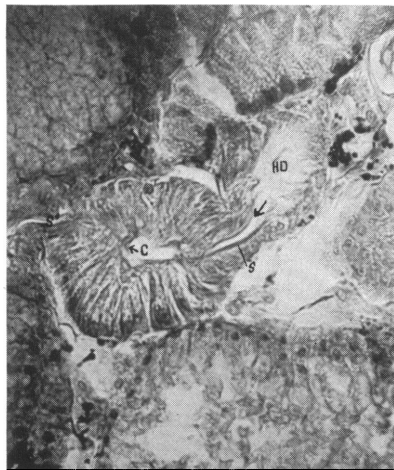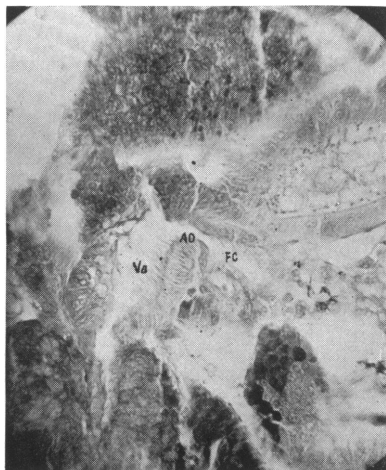
Fig. 1.   Photomicrograph of cross-section of the penis in its most distended state.
×65.

The section is in a plane midway between base and tip of the organ.
From center to periphery the following layers of tissue are shown: cuboidal epithelial cells, circular muscle, spongy tissue, longitudinal muscle, circular muscle, external epithelium.   The penis sheath is also included in the section.

Fig. 2.   Photomicrograph of cross-section of the penis near the base.   ×65.

In addition to the layers of tissue shown in Fig. 1, the duct from the penial bulb into the spongy layer is illustrated.   The section passes through the ductus ejeculatorius (de).

Fig. 3.   Photomicrograph of longitudinal section through the retracted penis and expanded penial bulb.

The relation existing between bulb and penis is well illustrated.   The bulb shows a muscular sheath lined by cuboidal secretory cells.   An acid staining coagulum occupies its lumen.   A section through the prostate gland lies just above the bulb.

vd—continuation of vas deferens
st—spongy tissue layer
ps—penis sheath
pb—penial bulb
pr—prostate gland

Fig. 4.   Photomicrograph of secretory cells of the mucous gland in various phases of the secretory cycle.   ×130.

In the upper left-hand corner are cells at the beginning of the cycle with central nuclei; cells in the lower half of the picture are filled with secretion product and the nuclei are deformed and basal in position.

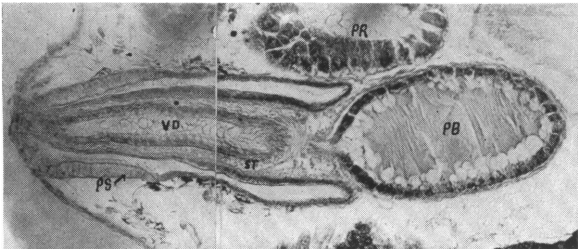Fig. 5.   Photomicrograph of longitudinal section of retracted penis.   ×130.

The six tissue layers and the structure of the sheath are well illustrated.

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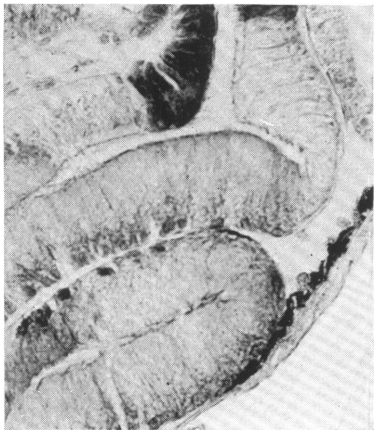2

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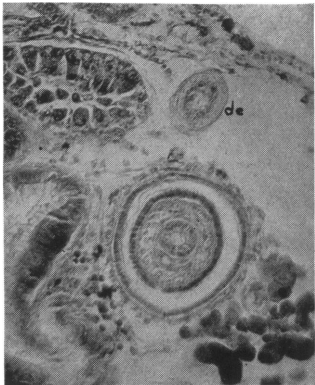
Fig. 1.   Photomicrograph of the section showing juncture of oviduct and vagina near the external female orifice.   ✕80.

The external portion of the vagina (*va*), the oviduct (*ov*), and the common female aperture (*fa*) are indicated. The goblet cells of the vaginal wall are well shown.

Fig. 2.   Photomicrograph of the upper end of the vagina (*va*) and the sperminal receptacle (*sp*) in section.   ✕85.

The muscular wall of the receptacle (*rw*), and the receptaculo-vaginal sphincter (*rv*) are shown. The sperm mass is beginning to break up.

Fig. 3.   Photomicrograph of cross-section through the prostate gland showing cells in process of emptying contents into lumen of the gland.   ✕265.

Fig. 4.   Photomicrograph of longitudinal section through the hermaphrodite duct and ampulla showing the ciliary valve at the point of juncture.   ✕65.

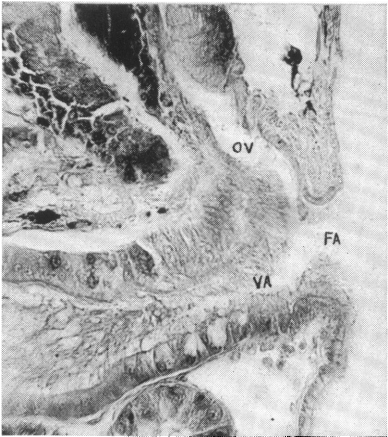The peculiar vacuolation of the cells forming the duct wall is illustrated.
hd—hermaphrodite duct
am—ampulla
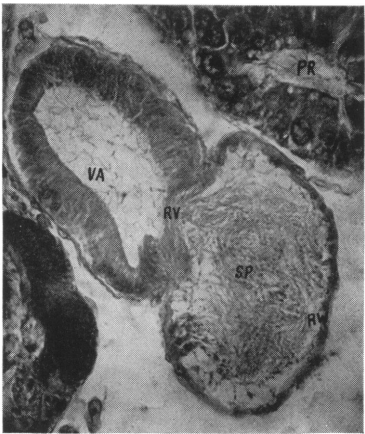*cv*—ciliary valve

Fig. 5.   Photomicrograph showing the entire vagina in longitudinal section. ✕100.
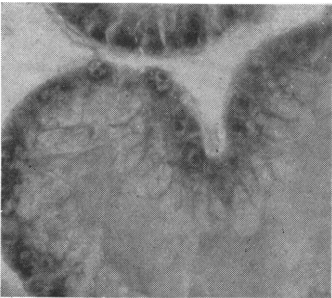
The outer muscular sheath is slightly separated from the epithelial lining. The cells nearest the orifice are heavily ciliated and distributed among them are goblet cells which secrete mucus. The inner half of the vagina is composed of ciliated cells with central nuclei.
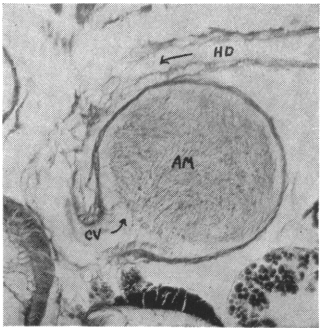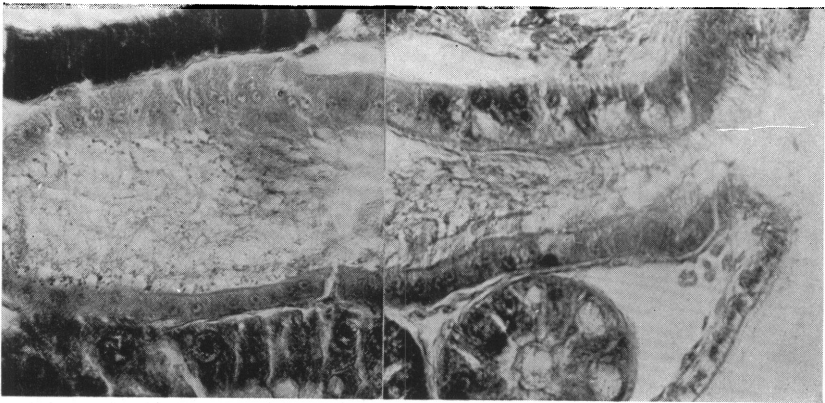
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