LARVAE AND POST-LARVAL DEVELOPMENT IN RELATION TO THE
SPECIES PROBLEM IN Parasmittina nitida (VERRILL)
[BRYOZOA: CHEILOSTOMATA]

By

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Abstract of Dissertation Presented to the Graduate Council
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A morphological-developmental approach was utilized to resolve the species problem posed by morphotypes A and B of Parasmittina nitida (Verrill, 1875). Specimens were collected from Bogue Sound, North Carolina and from the Gulf of Mexico, Cedar Keys, Florida, from June, 1972 through January, 1974, and studied by light and scanning electron microscopy. Post-larval developmental stages were analyzed over a 14-month period from colonies maintained in a running sea water system, and in a closed sea water system supplied with cultured algae. Parasmittina nitida, morphotype A, maintained in tanks supplied with running sea water reproduced from June through November, and P. nitida, morphotype B, from November through August in Beaufort, North Carolina. Parasmittina nitida, morphotype B, Florida reproduced throughout the year in the Gulf of Mexico.

Metamorphosis from larval attachment through the acquisition of a functional polypide, i.e., a polypide which obtains its nutrition solely from extra-zooidal sources, is given in detail for both morphotypes. The development of the orificial collar and spines in daughter zooids of morphotype A is presented for the first time in cheilostome bryozoans in terms of the formation of the surface topography of the exoskeleton. Larval morphology for both morphotypes is given in detail.

Offspring variation studies of both morphotypes for two generations revealed that all previously reported taxonomic characters for these morphotypes are genetic. The two morphotypes were further separated by the following new characters: (1) larval pigmentation, (2) aboral ciliation of larva, (3) distal communication pore complex, (4) distal bud, (5) orificial spines, and (6) colony form.

(1) Larvae of morphotype A have simple lateral pigment spots in the lower one-half of the lateral surface of the aboral lobe; whereas those of morphotype B have complex lateral pigment spots ("eyespot") in the lateral surface of the aboral lobe. Larvae of morphotype A have 4 or 6 pigment spots of an equal size in the supra-coronal furrow; whereas those of morphotype B have 5 or 6 and of 3 different sizes in the lateral surface of the aboral lobe. The pigment spots of morphotype B are at least twice the size of those of morphotype A. (2) The aboral cilia

INTRODUCTION

The taxonomy of bryozoans is based primarily on the morphology of the exoskeleton. Parasmittina nitida (Verrill) [Parasmittina trispinosa (Johnston) of earlier American authors] presents a panorama of diversity grossly discernable by light microscopy. Some authors (Osburn, 1910; Canu and Bassler, 1929; Osburn, 1940; and Shier, 1964) consider this species (= Smittina trispinosa (Johnston) in the monographs of Osburn, and Canu and Bassler) as a species complex having as many as fifteen "varieties." Harmer (1957) considers some of the "varieties" as species based solely on morphological features, and Maturo (1973) suggests that 2 of the morphotypes represent separate species. The literature does not resolve the question whether any or most of the variations in Parasmittina nitida (Verrill) represent separate species, or different phenotypes or genotypes within the same gene pool.

Parasmittina nitida (Verrill, 1875) type material has two "varieties," designated morphotypes A and B by Maturo and Schopf (1968). Parasmittina nitida is distributed from Cape Cod to Key West along the east coast of the United States (Maturo, 1957 and 1968). Off the coast of Florida (in particular at stations 1500, 1503, 1538, and 1539 of the Gosnold Expedition--WHOI, 1966) and in Bogue Sound,

North Carolina, the two morphotypes have been reported from the same dredge haul and frequently encrusting the same shell either on the same side or on opposite sides. The morphotypes are separable primarily by the structure and location of the avicularia, and the number and size of the pores on the frontal surface of the hyperstomial ovicells. Preliminary studies by Maturo (1973), during the summer of 1970, on the offspring variation in morphotypes A and B from known maternal stocks from North Carolina strongly suggested that the two morphotypes represented separate species. The maximum number of nonreproductive individuals within the first generation colonies studied were 11 zooids in diameter or approximately 94 total zooids.

To determine whether morphotypes A and B were definitely distinct species, analyses of larval morphology, metamorphosis, offspring variation from known maternal stocks followed for two generations, colony formation, and reproductive cycles in the two morphotypes were done. The morphological-developmental approach utilized in this work should be applicable to solving the problems posed by other "varieties" of this and other bryozoan species complexes.

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MATERIALS AND METHODS

Procurement of Larvae for Growth Studies, Development Analysis, and Microscopic Observation

Shell litter was obtained with a scallop dredge from Bogue Sound, North Carolina (southwest of Qk Fl R "MC" channel marker, in the shallows off Tar Bay Landing, nautical chart #833-SC), and the Gulf of Mexico, Florida (northwest of Snake Key in the vicinity of Cedar Keys). Shell fragments containing colonies of Parasmittina nitida, morphotypes A and B, were immediately separated out and placed in aerated sea water in styrofoam chests. Collections were made in the North Carolina area in late June 1972, early July 1973, and late August 1973. In the Gulf of Mexico, collections were made periodically from October 1971 through mid-January 1974.

Colonies of P. nitida containing a minimum of twenty full ovicells were separated with tile cutters from the other epifauna encrusting the shells, worm tubes, echinoderm tests and algae. The substrate fragment containing the colony was then scraped and brushed to eliminate extraneous epifaunal organisms. Shells containing boring sponges and polychaetes were discarded to avoid contamination. Each colony was identified to morphotype, assigned a number, and placed in individual sterile plastic disposable culture

dishes filled with sea water. The sea water used was collected in Nalgene carboys at the time the colonies were collected, and was later filtered through GAF Corp. 5 micron polypropylene calibrated filter strainer bags to remove planktonic organisms.

The size of the culture dishes used depended on the size of the colony. In all cases, colonies were completely immersed in sea water, which was slowly aerated through an angled micropipette located in one corner of the dish. These dishes were set up within hours from the time of collecting the parental colonies to insure maximum release of the mature larvae.

The dishes set up for development and growth studies were put either on a black or dark surface and exposed to a light-dark cycle to enhance larval release and attachment following Lynch (1947) and Ryland (1960). Lights (fluorescent, high intensity, or incandescent) placed immediately above the colonies were automatically turned on at sunrise. Within one hour, the majority of the mature larvae were released from the ovicells and had attached to the petri dish. Those which had not attached were prepared for scanning electron microscopy (hereafter referred to as SEM). For attached larva, the time of observation, a sketch or description of the state of the metamorphosing larva, and the location of the individual either by a number or a coordinate was recorded. In addition, a circle was inscribed around the metamorphosing larva or preancestrula

on the upper and lower side of the dish to insure relocation of the specific individual. Petri dishes with a grid of 36 squares proved to be ideal in helping to relocate larva which had set under experimental conditions.

Parental colonies which had released larvae that had subsequently set were transferred to a second petri dish for the following days release and attachment. The parental colony was kept under culture conditions with subsequent changes of sea water in new petri dishes for a maximum period of 10 days. However, after the fourth day the growth of a bacteria film over the colony appeared to limit larval release. In no case was the temperature controllable within the petri dish or the surrounding environment. Ultimately the parental colonies were dried and saved for morphological comparison with their progeny.

Petri dishes containing recently settled larva that were to be observed throughout metamorphosis were filled with sea water and placed on a table at room temperature. Individuals were studied, drawn, and photographed with a Unitron UMP-366 inverted phase contrast microscope as frequently as possible throughout their development. Observations were terminated upon the completion of metamorphosis, and the ancestrula formed preserved in 4% glutaraldehyde. Individuals representing various stages in the metamorphosis process were also preserved in 4% glutaraldehyde for future study with SEM.

The majority of petri dishes containing ancestrulae were set up in various environments for growth studies. Larvae released and metamorphosed from North Carolina parental stocks were either maintained in fiberglass aquaria in a variable temperature room at the Duke Marine Laboratory or in an outdoor wooden tank at Marineland, Florida. Both tanks were fed by partially filtered running sea water and thus simulated the natural environment with regard to type of food available. In both cases the dishes were suspended vertically along nylon fishing line strung the length of the tank to prevent silt build-up and to insure maximum water flow around the plates. To prevent the dishes from swaying in the water currents either the rows were separated by glass rods positioned approximately 1/2 way down the dish, or a nylon line was strung through the lower part of the dishes in a row and drawn taught.

In the field, dishes containing ancestrulae of morphotype B, Florida were vertically suspended by fishing line within a wooden crab trap anchored off Seahorse Key in the Gulf of Mexico. Additional petri dishes containing ancestrulae from parental stocks from the Gulf of Mexico were kept in culture tanks in my laboratory or in the sea water tables at the University of Florida Marine Laboratory.

Procurement of Second Generation Larvae

Colonies bearing ovicells containing larvae of morphotypes A and B, which had developed from ancestrulae in the aquaria at the Duke Marine Laboratory, were set up

for second generation larval release in late March 1973 and early July 1973 respectively. Sixteen petri dishes containing 82 colonies (70 colonies of known maternal stock) with 2,201 ovicells bearing mature larvae were set up for morphotype B. Thirty-two petri dishes containing 66 colonies (55 colonies of known maternal stock) with 4,040 ovicells bearing developing embryos and mature larvae were set up for morphotype A.

The procedure for collecting second generation larvae was similar to that described above for first generation larva with minor exceptions. Following the recording of all recently settled larvae, the water in the petri dishes was changed with water filtered from the growing tanks. As the first generation colonies were to be returned to the tanks for further growth, none were maintained in culture conditions for more than three days.

Preparation of Larvae for Observation

Narcotization

Larvae were narcotized prior to fixation in order to minimize contraction of the neuromuscular cord which runs vertically from the apical organ complex to the roof of the internal sac and horizontally to the pyriform organ complex (Woollacott and Zimmer, 1971). Chloretone (1,1,1 trichloro 2-methyl 2-propanol), $MgSO_4$, or tricaine methanesulphonate (hereafter referred to as tricaine) were utilized, with the latter anesthetic giving the most satisfactory results. The swimming larva was either

prepared for fixation in the petri dish in which it was released or transferred with a micropipette to another dish containing only sea water in order to prevent possible contamination of the parental colony by the narcotizing agent.

In narcotizing the larva, crystals of tricaine were picked up on a wet wooden toothpick and transferred to an area approximately 2 1/2 cm from the swimming larva. This facilitated diffusion of the chemical toward the larva. The larva was exposed to the drug for varying lengths of time, 1-20 min., depending on the concentration of the drug and the swimming stage of the larva I was interested in studying. In all cases, the larva showed the least distortion upon fixation when it was transferred to the fixative in a decreased state of either horizontal or vertical activity.

Fixation

Immediately following narcotization, larvae were transferred to 8 x 27 mm watch glasses containing a solution of 4% glutaraldehyde buffered with sodium cacodylate, which had been filtered through a .45 μ millipore filter just prior to use. The fixative was adjusted to correspond to the osmolality of the sea water in which the embryos had developed (See Table 1 for fixative preparation.) following the principles outlined by Maser, Powell, and Philpott (1967). This adjustment was essential as a preventive measure against tissue alteration by the fixative.

On those occasions when four or more larvae were simultaneously obtained from one petri dish, the larvae were transferred to a second solution of glutaraldehyde maintained at room temperature following the transfer of the last larva. This prevented dilution of the fixative with the additional sea water pipetted during transfer. The watch glasses were covered and stored in the refrigerator for approximately one hour.

Upon removal from the refrigerator, the larvae were rinsed twice in a cold .45 μ millipore filtered sodium cacodylate buffer solution of an osmotic concentration similar to the fixative used. Dextrose was added to elevate the concentration of the buffer. (See Table 2 for buffer preparation.) Rinsing was accomplished by pipetting the buffer solution into the watch glass at a faster rate than that of the simultaneous removal of the fixative. This was done several times to insure maximum removal of the fixative and, during the second rinse, removal of the first buffer. Rinses were at least 20 minutes apart between which the larvae were stored in the refrigerator.

Upon completion of the fixation process, the larvae were either photographed or transferred to 1/2 dram vials for storage in the refrigerator until subsequent preparation for SEM observation. Larvae were stored in the buffer solution up to two months with no discernable change in the tissue morphology.

Several larvae were observed with a Zeiss Nomarski differential interference microscope (Zeiss photomicrograph II) with a 35 mm automatic camera attachment. Photographs were taken as soon as possible following fixation in order to maximize observation of the pigment spots, which bleach with time. Observed larvae were placed in buffer solution on a depression slide, which facilitated rotation without flattening of the specimen under the coverslip. Black and white photographs were taken with Kodak Panatomic X (ASA 32), Ilford FP-4 (ASA 125), and Ilford Pan F (ASA 50) films; the last yielded the most information due to its extra fine grain and high speed. Kodak Ektachrome EH-B (ASA 125) was utilized for color photographs. Every effort was made to minimize handling the larvae during the photographing process.

Dehydration With the Polaron Critical Point Apparatus

The critical point method, introduced by Anderson in 1951, was utilized to bring larvae and ancestrulae from a wet to a dry state without their suffering the disruptive and distortive effects of surface tension characteristic of other methods of drying SEM specimens. Larvae were transferred to beem capsules lined with nylon plankton netting of a mesh opening of approximately $300\ \mu \times 450\ \mu$ in preparation for the critical point method. All larvae released from one colony were treated as one sample. The open end of the bag was pleated on itself and tied securely with #2 Mersilene polyester surgical suture.

The larvae were dehydrated through a prefiltered (.45 μ) 5% graded series of ethanol concentrations from 5% to 100% for one to two minutes in each concentration. The specimens were taken through a second change of 100% ethanol, and then through a 10% graded series of Freon 113-ethanol from 50% Freon 113 (1 pt. 100% ethanol: 1 pt. Freon 113) to 100% Freon 113. All changes were carried out as quickly as possible.

The larvae were then taken through a second change of 100% Freon 113, in which solution the mesh bags containing the specimens were tied securely to the specimen carrier of the Polaron Equipment Ltd. Critical Point Apparatus E3000. Recently Pelco Electron Microscopy Supplies has marketed a trough-shaped specimen holder with a mesh lid and a drain valve. This part would facilitate direct replacement of Freon 113, in which the larva is initially bathed, with Freon 13 and thus would prevent any possibility of air drying during the transfer of the larva from the 100% Freon 113 to the Polaron apparatus and Freon 13. The specimen carrier available at the time of my work was of a design such that the specimens within the mesh bag were exposed to the air briefly during the transfer of the bags from the 100% Freon 113 to the Polaron apparatus.

Freon 113 or TF [1,1,2 trichloro-trifluoro-ethane or Freon degreaser MS-182 (Miller-Stephenson Chem. Co., Inc.)] is an intermediate fluid in the critical point method as it is a fluid at ordinary temperature and pressure, and is

miscible with both ethanol and Freon 13 (chlorotrifluoromethane). Freon 13 is a transitional fluid with a critical pressure of 38 atm. or 561 lbs/in², and a critical temperature of 28.9°C.

Following the procedure of Cohen, Marlow, and Garner (1968), the specimen carrier was placed in the "bomb" (a term used to refer to the critical point apparatus), the chamber was closed, and the exhaust valve for the system slightly opened to vent the chamber of entrapped air as the Freon 13 entered the chamber. It was essential that the Freon 13 flowed in at a steady slow rate as to avoid boiling and thus tissue damage. After a stabilization period of approximately 10 minutes, the temperature of the "bomb" was slowly raised to a temperature slightly above the critical temperature and thus pressure of Freon 13 (approximately 34°C and 600 lbs./in²). Care was once again taken to prevent boiling. Following the phase change of Freon 13 and consequently the change in the specimen from a wet to a dry state, the chamber was stabilized for 5 minutes prior to removal of the specimen.

Mounting and Metal Coating

The portion of the specimen bag above the surgical suture was cut off and the contents dumped onto construction paper, which had been pretreated with alcohol and dried. A single larva was picked up with an eyelash mounted on a toothpick, and placed on double-sided Scotch tape fastened to a SEM aluminum specimen stub. No attempt was made to

orient the larva at either a particular angle or on a particular side on the stub. The stubs with their attached larva were then immediately placed in a vacuum desiccator to prevent rehydration of the specimens.

The specimens were coated, within one hour of their removal from the "bomb," with a film of gold-palladium (Au-Pd) alloy to prevent electrical charging and tissue damage while under the electron beam. Coating was carried out in a Denton high vacuum evaporator (Model DV-502) fitted with a liquid nitrogen cold trap and a tilting omni-rotary shadow caster. Gold-palladium is effective in the generation of secondary electrons, which form the image on the cathode ray tube. According to Jeol Ltd. (1972), maximum resolution is attained when the thickness of the evaporated film is less than the resolving power of the instrument; however, to prevent charging a film thickness slightly greater is recommended. In the present work, the larvae were coated with a minimum thickness of $300 \text{ \AA}$ of Au-Pd. Additional layers of the alloy, resulting in a film thickness of up to $600 \text{ \AA}$, were evaporated onto the specimens if they were to be observed a second time under the electron beam, or if they were not observed within two weeks of the initial coating. In all cases, the metal was evaporated slowly to give a uniform coating.

Preparation of Individuals in Various Stages of Metamorphosis

The fixation process for individuals in various stages of metamorphosis was similar to that previously reported for

the swimming larvae with only one major difference; the individuals were affixed to plastic petri dishes and thus the mechanics of the process had to be altered. One hundred and fifty microliters of an anti-bacterial agent (Grand Island Biological Co., Antibiotic-Antimycotic Mixture #524) were added to the sodium cacodylate buffer solution in the petri dishes for final storage in order to control the bacterial growth.

Specimens prepared for SEM were dehydrated following the procedure outlined for the larvae, with only minor modifications resulting from the nature of the specimens. Squares, containing the attached specimens were cut out from the plastic petri dish and were of a width slightly less than the specimen carrier of the "bomb." This was accomplished by inverting the experimental dish in a larger dish which contained sufficient buffer to cover the inner surface of the experimental dish. Repeated touching of the exposed dry surface of the plastic dish with hot scalpels would melt it, thus removing the desired area. Two sets of notches were made on opposite sides of the square in order to contain the suture used in tying the plastic square onto the specimen carrier of the "bomb."

The squares of plastic with the attached specimens were transferred through the ethanol series right side up, and through the Freon 113-ethanol series upside down. The plastic squares float in Freon 113. The specimens were oriented in the bomb upside down, with the back side of the square against the lower surface of the specimen

carrier. The transfer of the specimens from the 100% Freon 113 to the "bomb," and its subsequent filling was carried out as rapidly as possible.

Immediately following the critical point method, the individual specimens were cut out from the larger plastic square. It was essential that the size of the plastic containing the specimen be less than the surface of the SEM stub. E-Kote silver epoxy conductive paint was applied to the underside of the plastic squares to affix them to the stubs and to insure a conductive path from the specimen to the ground. The stubs were held in a vacuum desiccator for at least 12 hours before placing them in a vacuum evaporator for metal coating. Outgasing was prevalent if the metallic paint was not thoroughly dried. The specimens were coated with a minimum of $300 \text{ \AA}$ of gold-palladium alloy similar to the method previously reported for the larvae.

Scanning Electron Microscope (SEM) Observation

The surface morphology of individuals was viewed with a scanning electron microscope because of its wider range of useful magnification, its better resolution, and its far greater depth of field compared to that of a light microscope (Hay and Sandberg, 1967). The Cambridge Stereoscan II microscope having a resolution of $250 \text{ \AA}$ was utilized for this work. The scope was operated with a beam accelerating voltage of 10 to 20 KV, and an aperture of 100 to 200 μ . The lower KV and smaller aperture yielded the greatest surface detail and contrast as the electrons were not capable of penetrating the specimen.

Whenever possible, the specimens were viewed at a beam-specimen angle of 45° to insure maximum collection of the emitted secondary electrons from the surface of the specimen. It is the secondary electrons of low energy that convey the most information and are involved in image production; whereas, the reflected or back-scattered primary electrons of high energy originating from the electron gun are responsible for image contrast. Reorientation of a specimen to the incident electron beam was feasible without removing the specimen from the chamber due to the capability of the stage holding the stub of rotating, tilting around two axes, and moving along three axes. Thus, for example, I was able to observe each larva at various angles while still maintaining an ideal working distance and thus depth of focus.

The majority of the photomicrographs were taken in a magnification range of 200 to 2000 X using Polaroid Type 55 P/N film.

Colony Development and Growth Studies Carried Out in a Variety of Environments

Field

Petri dishes containing ancestrulae from maternal stocks of Parasmittina nitida, morphotype B, Florida were either suspended in crab traps in the Gulf of Mexico in the vicinity of Seahorse Key or placed in running sea water tables in the University of Florida Laboratory. Dishes housed within the crab traps were checked for growth after

15 days, and those in the sea water tables periodically over a 4 month period.

Laboratory at the University of Florida

The majority of the ancestrulae of morphotype B originating from maternal stocks collected from the Gulf waters were held in experimental tanks, within my laboratory or within a constant temperature room, and supplied with cultured algae. Since very little is known about the kinds and numbers of organisms captured by bryozoans, a variety of algae was cultured as a possible food source for the developing colonies. As impingement suspension feeders, bryozoans are capable of generating a feeding current and of transporting particles impinged on the tentacles to the pharynx (Bullivant, 1968); thus diatoms as well as flagellated forms were cultured as possible food sources.

The following algae were obtained through the courtesy of Dr. Paul Hargraves from the Narragansett Marine Laboratory, University of Rhode Island:

Isochrysis galbana
Monochrysis lutheri
Tetraselmis chui
Cricosphaera carterae
Phaeodactylum
Thalassiosira pseudonana (= Cyclotella nana)
Dunaliella
Cryptomonas
Katodinium rotundatum

Stephanoptera was obtained from the Culture Collection of Algae at Indiana University through Mrs. Alice Williams, curator; and Oxyrrhis marina was obtained from the Culture Collection of Algae and Protozoa, Cambridge, England through

Dr. E. A. George, director. All algae were initially cultured in the medium suggested by the culture centers from which they were obtained.

Because bryozoans are voracious eaters, a large amount of food suspension was required. Only Monochrysis lutheri, Phaeodactylum, Dunaliella, and Oxyrrhis marina were capable of being raised in sufficient quantities as food sources. It was essential that the type of food supplied was constant as the physiology of the polypide is governed by the food intake. Degeneration into a brown body and subsequent regeneration occurs with each change in the type of food (Jebram, 1968).

Initially the algae were separated from the nutrient medium and resuspended in sea water before being fed to the bryozoans. However, all methods of separation were found unsatisfactory either because of the large number of cells lost, the number of cells broken during the process, or the time required to carry out the procedure. Consequently the nutrient solution containing the algae was added directly to the experimental tanks. The quantity added depended on the size of the experimental tank and the concentration of algal cells. Five hundred milliliters of algal suspension containing 25×10^4 cells/ml were added to each 7 1/2 liters of sea water in the experimental tanks. The bryozoans were fed every other day, and the sea water in the tanks changed every sixth day. This was necessary as many marine species of bryozoans survive poorly in eutrophic media (Jebram, 1968).

The bryozoans were reared in aquaria ranging in size from 2 to 27 gallons. The sea water was of the same salinity in which parental colonies were obtained from the Gulf of Mexico, and was initially filtered through GAF Corp. 5 micron polypropylene calibrated filter bags. The water was circulated within the aquaria with aerators. The tanks were maintained either at room temperature (20-25°C), or in a constant temperature chamber (24°C) under indirect fluorescent lighting.

The developmental state of the ancestrula and its resulting colony was initially observed daily for a week. Notes were made on the morphological features of the ancestrula, such as spination and budding pattern, and the activity of the lophophore. Subsequently the colonies were observed weekly and notes recorded on the zooids formed and the condition of all polypides (actively feeding or transformed into a brown-body). Every 3-4 days the colonies and petri dishes were brushed clean of feces and bacteria.

Petri dishes were removed from the tanks when all individuals of a colony demonstrated brown bodies. Concurrent with this situation was the noticeable increase of contaminants of algae, protozoa, nematods, bacteria, and polychetes within the closed system.

Duke Marine Laboratory

Petri dishes containing ancestrulae of Parasmittina nitida, morphotypes A and B, originating from larval sets in June 1972 from Bogue Sound, North Carolina, were placed in

aquaria in a variable temperature aquarium room at the Duke Marine Laboratory. The dishes were surveyed in early August 1972 (approximately 4 weeks following larval set), in late November (approximately 16 weeks later), in late March 1973. (approximately 16 weeks later), in late June (approximately 12 weeks later), and in late August (approximately 8 weeks later). Initially the growth of each colony was recorded by number of zooids present, later by size as measured in millimeters along various axes, and finally by area in cm^2 .

The latter measurement was feasible only upon the return of the preserved specimens to my laboratory at the University of Florida. The circumference of the colony, denoted by the basal wall, was traced with a camera lucida set up on a Wild M-5 dissecting microscope. The colony outline was cut out from the tracing paper, and subsequently fed through the Hayashi Denko Co. Ltd Automatic Area Meter Type AAM-5.

During each observation period the dishes were scraped and brushed clean of the majority of epifaunal organisms which had set as a consequence of larvae being brought through the running sea water system. Those colonies of Parasmittina nitida, morphotypes A and B, originating as such were left on the dishes, encircled, and given a location designation. With additional colonies of P. nitida on the petri dish, it was speculated that the effects of competition would be observable. The

tanks holding the petri dishes were also cleaned of the silt build up, and the epifaunal and mobile organisms (primarily crabs, starfish, and scallops), whose larval stage had entered the tanks through the sea water system, discarded. Several plates were preserved in 95% ethanol from each observation period for detailed analysis of growth and morphological features of the colonies.

Morphological features particular to a colony such as (1) growth of secondary and tertiary layers, (2) location of zooids bearing spines, ovicells, and avicularia from the ancestrula or center of the colony and, (3) the presence of double ovicells and unusually shaped zooids and avicularia were noted whenever possible on living specimens. The number of empty ovicells and those containing embryos were also recorded. Whenever possible, the state of the developing embryo within the ovicell was noted. Typically young embryos and immature larvae appeared peach colored, with the shade varying among individuals of one colony. This was undoubtedly due to the developmental stage of the embryo, as well as the thickness of the calcareous wall of the ovicell. Mature larva are characteristically paler in color than the developing embryos and show conspicuous orange-red pigment spots. A record of an empty ovicell indicates one of three conditions: The mature larva has been shed and a second embryo has not yet been transferred to the ovicell from the ovary; the production of embryos within the zooid has ceased for a period of time; or the

first embryo has not yet entered the completely formed ovicell. No attempt was made in this study to differentiate among the three possibilities.

Growth in this environment was excellent, and thus it afforded me the opportunity to collect second generation larvae of both morphotypes, and to set up second generation colonies. (See section on "Procurement of second generation larvae.") As the filtering system for the sea water did not retain larvae of this species, only the maternal stock of the second generation was known.

Marineland of Florida

Several petri dishes, initially held in tanks at the Duke Marine Laboratory, were transported in aerated sea water in styrofoam chests to Marineland of Florida in order to make possible weekly observations. As the salinity of the sea water is approximately 35-37 o/oo at both locations during the summer months (personal communications, Dr. John Costlow, Director, Duke Marine Laboratory and Mr. Frank Miller, Curator, Marineland of Florida), it appeared that individuals brought from North Carolina could be reared successfully in outdoor tanks at Marineland. Sea water for these tanks was pumped in from off shore, and filtered through a gravel bed prior to entering the laboratory pipelines.

In early July 1972, 3-17 days after larval attachment, 20 petri dishes with a total of 158 morphotype A progeny, and 19 petri dishes with a total of 220 type B progeny of Parasmittina nitida were transported to Florida. Colonies

varied in size from 2-15 zooids for colonies of morphotype A, and 1-21 zooids for colonies of morphotype B. Another group of petri dishes were transported to Florida in early August 1972. The colonies attached to these petri dishes were 31-45 days old from the time of attachment and metamorphosis of the larvae. Colonies varied in size from 1 zooid to 40 zooids for colonies of morphotype A, and 1 to 79 zooids for colonies of morphotype B. It was hoped that the older colonies, and the speed in which they were relocated in running sea water would yield satisfactory results.

The July set of petri dishes was exposed to sunlight; whereas the August set of dishes was not. A wooden covering was placed over the outside tank in order to reduce the growth of filamentous algae. Initially, weekly observations were made. Subsequent observations were made every 2 weeks with the final observation in the 9th week.

RESULTS

Larval Morphology

Developing embryos of Parasmittina nitida are retained one at a time in a specialized brood chamber (an ovicell) produced from evaginations of the body wall at the fronto-distal margin of the maternal zooid. Typically, one ovicell is present per zooid; however, double ovicells have been recorded from first generation progeny of morphotype B, North Carolina. Five out of 82 colonies having zooids with ovicells bearing developing embryos showed two brood chambers originating simultaneously from one maternal zooid. Whether both chambers were occupied with developing embryos was not recorded. Mature eggs, which were determined by size, were observed in the ovary of a zooid at the same time the ovicell contained an embryo. Embryos are characterized by a peach color; whereas mature larvae are paler in color with distinct orange-red pigment spots. After completion of embryonic development, a larva escapes from the ovicell and spends 20 minutes to 2 hours, based on observations of 20 larvae under culture conditions, swimming prior to settling and metamorphosing.

To avoid ambiguities, the terminology and the internal morphology of the larva utilized in the description of the larval morphology of P. nitida follows that of Woollacott

and Zimmer (1971) for Bugula neritina. (Although larvae of Parasmittina nitida lack any vestige of the mouth, the terms oral and aboral are used in keeping with the terminology for bryozoan larvae which do possess an oral opening.) The larva of Parasmittina nitida is lobulated (Plates 1:A-1, B-1; 2:1; 3:3; 4:1; 6:3; 7:1) with the oral (OL) and aboral (ABL) lobes separated by a furrow (Plate 1:B-3). Since this furrow is not synonymous with the pallial furrow of Lynch (1947), which immediately surrounds the apical organ complex, the term supra-coronal furrow (SCF) is substituted. The larva of morphotype A measures approximately 110 to 130 μ in height, 100 to 125 μ in diameter at the widest region of the aboral lobe, and 125 to 140 μ in diameter in a similar region of the oral lobe. Larvae of morphotype B are slightly larger, measuring 135 to 180 μ in height, 100 to 135 μ in diameter at the aboral lobe, and 135 to 180 μ in diameter at the oral lobe. In both types, the aboral lobe is approximately 1/3 the height of the oral lobe when the larva is in the lobulated form characteristic of its spiral movement.

The corona (CC), a large dense ciliary field encircling the larva in the region between the oral and aboral lobes, is the chief locomotory organ (Plate 7:9). The coronal field is broken by the pyriform organ complex (POC) (Plate 1:A-3), which is a glandular sensory system consisting of an area of unicellular glands lacking cilia, a group of approximately 3 large flagella, and a ciliated groove. The

flagella, referred to as the vibratile plume (VP) (Plates 1:B-3; 4:1, 2; 6:6) measure approximately $288\ \mu$ in length and lie within the ciliated groove (CG) upon the completion of their effective stroke. The lateral borders of the ciliated groove are lined with dense cilia shorter than those cilia comprising the corona (Plates 1:B-3, B-4; 7:2). At its base the groove opens onto the surface of the oral lobe and appears to merge into the neck region of the internal sac (ISA) (Plate 4:6). This is particularly evident in the "creeping" form of the larva (Plates 3:4, 5; 4:8; 6:6). The creeping larva attains an elongated form 90° to its aboral-oral axis such that the ciliated groove and nonciliated basal portion of the oral lobe are parallel to the surface to which the larva will eventually attach. The vibratile plume usually projects forward when the larva is in this form, and this is in agreement with its function of selecting a suitable site for attachment (Ryland, 1970).

The surface of the oral lobe is covered with cilia extending from the corona to the non-ciliated or sparsely ciliated, glandular appearing internal sac area (Plate 7:2, 3). This glandular region is particularly evident in oral views or basal views of the elongated form of the larva (Plates 3:4, 5; 6:6). A frontal view of the larva shows an outline of the internal sac within the oral lobe: this sac consists of an upper roof, lateral walls, and a neck region (Plates 2:1; 3:2; 5:1; 6:3).

The apical organ complex (AO) occupies the majority of the aboral lobe of the larva (Plates 1:A-1, B-2; 2:2; 4:9; 5:2, 3; 6:4, 5; 7:3, 8). Within the center of the complex lies an invaginated crescent shaped sensory cap composed of nonciliated cells continuing basally into the neuromuscular cord (Woollacott and Zimmer, 1971). In larvae of morphotype A the aboral surface immediately surrounding the sensory cap is covered by aboral cilia (ABC) arranged in a ring (Plate 4:9). Larvae of morphotype B, in contrast, show the aboral cilia as a transverse band which does not, however, extend into the sensory cap of the apical organ complex (Plate 7:3, 5, 8). The exact location of this band could not be determined; however, the band does continue onto the lateral edge of the aboral lobe at one end (Plate 7:1, 4). The aboral cilia of both morphotypes appears to be the shortest cilia covering the larval surface. The surface of the aboral lobe is more papillated and porous (Plates 4:7; 7:7) than that of the oral lobe (Plate 4:5).

The pigment spots of the larvae appear to be the most diagnostic character separating larvae of morphotypes A and B. In 1958, Ryland reviewed the taxonomic value of color in embryos as a valid and useful diagnostic character in determining species; however, little attention has been given to the location, number, and size of prominent pigment spots as taxonomic characters. This is undoubtedly a consequence of the spots fading and disappearing upon

fixation. The pigment spots within the larva represent areas of concentrated subepidermal pigment cells containing large amounts of pigment in the vacuolar system of the cytoplasm (Woollacott and Zimmer, 1971).

Two large pigmented areas are located in the equatorial region of the aboral lobe on each side of the pyriform organ complex in larvae of morphotype B. The size and shape of these pigmented fields vary between (Plates 5:1, 3; 6:2) and within (Plates 1:B-2--B-4; 5:2) individuals of this type. These pigmented areas referred to as "eyespot" (ES) range in size from $10\ \mu \times 13\ \mu$ in height to $21\ \mu \times 20\ \mu$ in width, and are of complex organization (Plates 1:B-1--B-4; 5:2). A pit approximately $5\ \mu$ in depth was observed in the center of the left eyespot of one larva which was extensively photographed. According to Woollacott and Zimmer (1972), a similar pigment spot complex was found in larvae of Bugula neritina with the pit of this region coming into close association with the equatorial nerve ring. The fact of its proximity to the underlying nerve tract and its organization suggested that this region had a sensory role according to these authors. The photoreceptive nature of these "eyespot" had been previously implied by Ryland (1960). The fact that large pigmented areas are lacking in the aboral lobe of larvae of type A is misleading if they are to explain the phototactic response of bryozoan larvae. Larvae of morphotype A have lateral pigment spots (LPS_a) measuring approximately $5\ \mu \times 7\ \mu$ in width,

and they lack the complexity of those observed in larvae of morphotype B (Plates 1:A-1, A-3).

Five or six pigment spots, joined by a pigment line (PL), are located in the lateral surface of the aboral lobe of larva of morphotype B. A frontal view of the larva shows either a single median diamond-shaped anterior pigment spot (AS) one half the height of the "eyespot" and bearing a pit (Plates 1:B-1, B-2; 5:1, 2), or two anterior pigment spots approximately one half the height of the "eyespot" (Plates 5:3; 6:1, 3, 4). Two postero-lateral pigment spots (PLS_a) complete the set of spots in the aboral lobe (Plates 1:B-2; 5:2, 3). Scanning electron micrographs indicate that the surface of these pigmented areas (Plates 7:3, 5, 6) is markedly distinct from the nonpigmented areas of the lobe. The pigment line in addition to the pigment spot appears nobular in surface detail compared to the smooth texture of the remaining areas of the aboral lobe.

Larvae of morphotype A possess 6 pigment spots of equal size joined by a fine pigment line in the lower half of the lateral surface of the aboral lobe (Plates 1: A-1--A-3; 2:1; 3:1). An infrequent occurrence is the location of the two anterior pigment spots in the supra-coronal furrow, one on either side of the aborally directed portion of the ciliated groove. On no occasion was I able to locate any of these pigmented areas in larvae of morphotype A with the SEM; thus I am assuming they are not surface structures in that morphotype as they appear to be in the larvae of morphotype B.

The oral lobe of larvae of morphotype B has 4 distinct pigment spots of equal size located immediately below the corona--2 lateral (LPS_O) and 2 postero-lateral (PLS_O) pigment spots (Plates 1:B-1, B-4; 5:2). Between the latter two are occasionally seen three smaller posterior pigment (PPS_l) spots interrupting the fine pigment line between the larger postero-lateral spots (Plate 1:B-2). Larvae of type A, in contrast, possess 6 pigment spots of equal size and all in the posterior region of the oral lobe--4 in the corona, and 2 medial and oral to the others (Plates 1:A-2, A-3; 2:2; 3:3). A fine pigment line runs between the postero-lateral pigment spot of the aboral lobe, the upper posterior pigment spot (PPS_u) and the postero-lateral spot of the oral lobe. The lower posterior pigment spots do not appear to be joined to any of the other pigment spots. The joining of the pigment spots between the two lobes is characteristic only of type A larva.

Metamorphosis of *Parasmittina nitida* Morphotype B, Florida

All descriptions of the ultrastructural aspects of the morphogenetic movements and tissue differentiation involved in metamorphosis is based on the work of Woollacott and Zimmer (1971). Their work is referred to throughout this section in order to present a fuller and more meaningful description of metamorphosis in *Parasmittina nitida*.

Immediately prior to larval attachment, there is an alternating clockwise and counterclockwise rotation of the lobulated form of the larva. This is followed by the

opening of the glandular neck of the internal sac (adhesive sac) at the point of attachment, possibly forming a suction. The internal sac subsequently everts, the roof of the sac forming the permanent junction with the substrate, i.e., the petri dish in vitro. Observations of living metamorphosing larvae indicate extensive morphogenetic movements as revealed by the relocation of the prominent pigment spots (previously noted in the free-swimming larva) with time.

Within 65 seconds from the time of attachment of the internal sac roof to the substrate, an inrolling of the aboral vesicular epithelium and corona are evident. Within 1 minute and 50 seconds from the onset of attachment the most prominent pigment spots, i.e., the "eyespot," have been passively carried into the interior of the metamorphosing larva with the vesicular oral epithelium, the ciliated oral epithelium, and the neck region of the internal sac. The pallial sinus epithelium of the larva subsequently evaginates and fuses with the wall region of the internal sac, thus

encasing the metamorphosing larva. Within 10 minutes from the onset of attachment and metamorphosis, the morphogenetic movements of the larval tissue (initially described for Bugula neritina by Woollacott and Zimmer, 1971) have been completed. The primary disc of the metamorphosed larva

(Plates 8:A-C; 9:A-C; 10:C) is formed with larval structures as the apical organ complex (AO) and pigments spots (PS) still evident.

The later stages in metamorphosis involve a thorough reorganization of the inner tissues of the primary disc to form the functional polypide. This phase of the process was observed from the dorsal surface of the individual with an inverted Unitron phase contrast microscope.

The primary disc, initially covered by the apical organ complex, the pallial sinus epithelium, and the wall region of the internal sac, is subsequently encased entirely by the wall of the internal sac resulting from the retraction of the apical organ complex and from the invagination of the pallial sinus epithelium. The wall of the internal sac forms the epidermis (E) of the preancestrula body wall (Plates 8:D; 9:D). The pigment spots evident in the initial attachment stage appear to break up into smaller masses; some are more evident than others, but all are connected by pigment lines (PL) (Plates 8:A-C; 9:A-C). With further disintegration of the larval tissue, the pigment masses disperse throughout the reorganizing tissue (Plates 8:D, E; 9:D, E). The apical organ complex undergoes ontogenetic changes upon its invagination into the primary disc. The upper blastema of the apical organ complex, which is ectodermal, differentiates into the polypide rudiment, which initially appears as an irregular mass (Plates 8:D; 9:D). The lower blastema of the apical organ complex,

which is ectomesodermal, differentiates into the lophophoral coelomic lining and the splanchnic peritoneum.

Within three hours from the onset of attachment, the polypide rudiment has acquired a distinct shape, the pigmented nutritive cells of the larva have begun to migrate toward the proximal region of the preancestrula, and the cuticle has been laid down by the epidermis (Plates 8:E; 9:E; 10:E). Further aggregation of the pigmented cells, the undifferentiated endodermal cells, and the degenerating cells of the larval transitory organs results in the formation of a discrete mass referred to as the nutritive mass (NM) (Plate 8:G). This mass is completely absorbed into the caecum portion of the polypide stomach prior to the feeding activity (Plates 8:Q; 9:Q).

Within 16 hours from the onset of attachment, the preancestrula consists of a cuticular cystid with a frontal membrane outlined by a weakly formed calcareous edge in the distal half of the ventral surface (Plate 8:G). Nine spines, all of which are at the same developmental stage, surround the frontal membrane (Plate 10:G). Initially they appear as cuticular protuberances, but within five hours a calcium carbonate core is established. This calcium deposition is at a considerably slower rate than the formation of the cuticular spine (SP_c) (Plate 10:Qc). Internally, the polypide rudiment (PR) which had formed from the upper blastema, appears as a "U" shaped structure (Plate 8:G). The base of the rudiment is within the nutritive mass with the upper

portion elevated toward the frontal membrane and within the forming tentacular sheath (TS). According to Woollacott and Zimmer (1971) the sheath possibly differentiates from the pallial sinus epithelium. Within five hours, the polypide rudiment appears as a lobed structure, the lobes being the primordia of the tentacles (TP) of the lophophore (Plates 8:H; 9:H).

Within 30 hours from the onset of attachment and metamorphosis, the primordia of the vestibular glands, diaphragm, and operculum are evident as a thickened mass of tissue (DP) at the disto-ventral extremity of the tentacular sheath (Plate 8:I). According to Lutaud (1964), the vestibular glands (VG) originate from the lateral folds of the wall of the embryonic tentacular sheath simultaneously with the differentiation of the diaphragm (D) and operculum (O) from the distal wall of the sheath (Plate 8:I-L). The glands are supported by the parietal vaginal frontal muscles (PVFM) which are continuous with the upper portion of the tentacular sheath (Plates 8:M; 9:M). The occlusor muscles (OM) of the operculum differentiate from mesenchymal cells in the distal region of the tentacular sheath and dorsal to the operculum. With time, the distal edge of the operculum is stiffened by sclerites (Soule and Soule, 1972).

Within 35 hours of the onset of attachment and metamorphosis of Parasmittina nitida morphotype B larva, the polypide rudiment has differentiated into an annular lophophore, with ciliated tentacles (T) surrounding a

mouth (M), and an alimentary canal (AC) (Plates 8:J; 9:J). Approximately 50 hours later (Plates 8:M; 9:M), the major components of the alimentary canal are discernable, i.e., mouth, ciliated pharynx (PX), cardia stomach (CAS), central stomach (CS), caecum (CM), pylorus (P), rectum (R), and anus (A). At this stage in the differentiation process, the tentacles of the lophophoral complex exhibit lateral movements within the tentacular sheath, and the pyloric cilia begin to beat. Subsequent development involves growth and differentiation of the various portions of the alimentary canal and absorption of the nutritive mass into the caecum.

The completion of metamorphosis is marked by the acquisition of a functional polypide, i.e., a polypide which obtains its nutrition solely from extra-zooidal sources. During the 3 to 4 days prior to the completion of metamorphosis movements within the polypide are accentuated. The intestinal region undergoes periodic volume changes with the retraction and eversion of the lophophore through the primary orifice (Plates 8:N-1, N-2; 9:N-1, N-2), and there is a continuous churning motion in the pyloric region of the stomach. On close observation, cells were observed pinching off from the nutritive mass into the caecum during the eversion of the lophophore. The nutritive cells were subsequently carried into the pyloric region, by cilia action of this region, where they were observed rotating clockwise 2 turns per second. With time the nutritive mass decreases in

volume, presumably supplying nutrients to the developing polypide. Whether plankton is engulfed and utilized by the polypide prior to the complete utilization of the nutritive mass was not observed. Within 8 days of the onset of attachment and metamorphosis of the larva, the primary disc had differentiated into a primary zooid or tata ancestrula 1.8 times the primary disc.

In addition to the occlusor muscles of the operculum, and the parietal vaginal and parietal vaginal frontal muscles associated with the vestibular glands, both sets of which have been discussed previously, the retractors (RM) of the lophophore are the most important muscles of the polypide. According to Woollacott and Zimmer (1971), the retractor muscles differentiate from elements of the large undifferentiated endodermal mass associated with the base of the polypide. The retractors inserting on the free lateral face of the lophophoral base, i.e., the face opposite to that enclosed by the alimentary canal, differentiate first and approximately at the same time the alimentary canal differentiates from the polypide rudiment (Plate 8:J). Thus the initial set of retractors are either located to the right or left of the alimentary canal depending on the position of the terminal portion of the canal within the cystid (Plates 8:K; 9:K). Within 50 hours, the second set of retractors are noticeable (Plate 8:M), and with further development are seen to extend along the dorsal and ventral surfaces of the upper portion of the

alimentary canal toward the proximal wall of the cystid (Plates 8:N-1; 9:N-2). By contracting, these muscles retract the extruded lophophore; however, their degree of contraction and elasticity appears to be limited as is indicated by their folded appearance when the lophophore is retracted into the cystid (Plates 8:N-1, P, Q-1; 9:N-1).

The funiculus (F), like the retractors, is differentiated from elements of the large undifferentiated endodermal tissue of the nutritive mass. The primordial structure is first discernable approximately 3 1/2 days from the onset of attachment and metamorphosis, and only when the lophophore is extruded (Plates 8:N-2; 9:N-2). With further absorption of the nutritive mass, the funiculus joins the stomach caecum to the proximal zooidal wall (Plate 8:O, P). According to Hyman (1959), the funicular cords seem to consist of connective tissue clothed with peritoneum, the latter probably originating from the lower blastema.

Concomitant with the deposition of calcium carbonate between the epidermis and the cuticle of the cystid, approximately 55 hours from the onset of attachment and metamorphosis, is the formation of calcified communication pore plates concave with respect to the "parent" zooid. Initially 3 pore plates and their respective chambers are present; one in the distal and one in each of the disto-lateral walls of the cystid (Plates 8:L; 9:K). Budding out of the communication pores follows as either a single median

distal bud or more typically as 3 buds; 1 distal and 2 disto-lateral (Plates 8:M; 9:M). The blastogenic face of the ancestrula is 180° out of phase with the pyriform organ complex of the larva. With growth the membranous buds join and initiate the formation of the lateral walls of the developing daughter zooids (Plate 8:O). The developing basal wall is observed beneath the buds against the substrate at approximately the same time as the appearance of the 2 proximo-lateral buds from their respective pores. By the completion of metamorphosis into a tata ancestrula, the exterior basal wall and the lateral walls (Soule and Soule, 1972) have become calcified for a considerable distance from the primary zooid (Plate 10:Qa, Qb), and there is evidence of a polypide rudiment in one of the buds (Plates 8:Q; 9:Q).

Metamorphosis of Parasmittina nitida,
Morphotypes A and B, North Carolina

The procedure for the study of metamorphosis of individuals of P. nitida in culture dishes at the Duke Marine Laboratory was similar to that for individuals under observation in my laboratory at the University of Florida. Both studies were conducted at room temperature; however, temperatures within the Duke Laboratory fluctuated 4 to 8 degrees in 24-hour period. Comparison of the success of the completion of metamorphosis of larvae from the two localities reveals 43% of the individuals (6/14) of morphotype B from Florida developed into an ancestrula; whereas none of the individuals of either morphotype B (1) or

morphotype A (18 individuals) from North Carolina completed metamorphosis. The growth of bacteria in the experimental dishes was a severe problem in the in-vitro study carried out at the Duke Laboratory, and thus probably accounted for the limited success of metamorphosis among the individuals of both morphotypes from this locality.

Parasmittina nitida, Morphotype B

The basic outline of the cystid of morphotype B from North Carolina (Plate 11:P-1) was .8 times smaller than the smallest ancestrula of type B metamorphosed in-vitro from Florida waters. The irregularity of the size and location of the communication pores, and the poorly developed distal and lateral buds are additional features distinguishing this individual from similar aged preancestrulae from Florida waters. These differences are undoubtedly the result of the poor condition of the culture.

Parasmittina nitida, Morphotype A

The metamorphosis of larvae of morphotype A is similar to that reported for larvae of morphotype B, Florida except for the following features. The pigment spots characteristic of the larva are not readily discernable in either the primary disc (Plate 12:B) or in the nutritive mass of the preancestrula (Plate 12:J-M). This is probably related to the initial small size of the pigment spots which subsequently makes them invisible upon their disintegration and dispersal in the early phases of metamorphosis.

The formation of the distal communication pore and the distal bud is concurrent with the deposition of cuticle by the preancestrula epidermis (Plate 12:F). Thus the pore and its associated structures develop simultaneously with the formation and subsequent calcification of the distal wall. Within 60 hours of the onset of attachment and metamorphosis and prior to the differentiation of the polypide rudiment into the lophophore and alimentary canal (Plate 12:J), the formation of the communication pore and its associated plate and the separation of the bud from the coelomic space of the preancestrula are completed (Plate 12:I). The remaining buds around the fronto-lateral wall of the preancestrula are formed subsequent to the completion of their respective communication pores. Whereas the lateral walls of the developing daughter zooids were evident in individuals of morphotype B from Florida (Plate 8:O), none were formed in these individuals of a similar stage in metamorphosis.

Within approximately 142 hours, the primary disc of individuals of morphotype A had differentiated into the preancestrula with 5 buds and a reduced nutritive mass associated with the polypide (Plate 12:O). Within 133 hours of the onset of attachment and metamorphosis, the individual of morphotype B, North Carolina which metamorphosed under similar culture conditions to those of morphotype A, differentiated into a preancestrula with 3 buds and random nutritive cells within the polypide caecum. A comparison of the rate of metamorphosis in

individuals of metamorphic stage O of morphotype A, North Carolina with that of individuals of morphotype B from Florida, suggests that individuals of morphotype B complete metamorphosis at a faster rate than those of morphotype A: Morphotype A, 138 hrs-146 hrs; and, morphotype B, 103 hrs-118 hrs. In addition, 2/3 of the individuals of morphotype B, Florida had completely absorbed the nutritive mass by 146 hrs.

The formed preancestrula of morphotype A increased an average of 2.2 times the size of its primary disc. This is 1.2 times that reported for the average size increase for individuals of morphotype B, Florida. In individuals of both morphotypes A and B, budding occurred 180° out of phase from the pyriform organ complex of the larva, and the polypide alimentary canal showed either a dextral or sinistral turn.

Features of Colony Development and Growth in
Parasmittina nitida, Morphotypes A and B
in a Variety of Environments

Formation of Spines and Orificial Collar in Daughter Zooids
of P. nitida Morphotype A

Daughter zooids of Parasmittina nitida, morphotype A developing in the region of astogenetic change of a colony (terminology from Boardman and Cheetham, 1969) are characterized by a distinct orificial collar, and 3 to 5 spines projecting upward at least twice the height of the lateral lappets of the collar. The proximal margin of the collar is initially elevated above the frontal wall;

whereas the distal margin abruptly drops below it. Scanning electron micrographs of young colonies of P. nitida show gross morphological changes during the formation of these structures (Plate 13).

An early stage in the development of a daughter zooid is characterized by a walled structure, i.e., 1 distal and 2 lateral walls (LW), covered by a membrane (Plate 13:1). The lateral and distal walls of the developing exoskeleton are calcified early in development (Plate 13:2) as seen upon removal of the organic material.

Simultaneous to, but proceeding at a greater rate than the calcium carbonate deposition in the frontal surface (proximal region) of the developing zooid is the formation of the distal spines (SP) (Plate 13:3a). The spines form as evaginations in the upper one-third of the calcified distal wall (Plate 13:3b). The proximal side of the spine is later formed by a latero-medial growth of the evaginations which also forms the distal wall (DW_O) of the primary orifice (Plate 13:3b, 4). Calcium deposition continues in this region forming the distal and lateral walls of the primary orifice. The frontal surface at this stage (Plate 13:5) is characterized by a calcified proximal portion with areolae (AR) (marginal perforations of the frontal wall provided with intrazoooidal communication organs; Banta, 1973).

Skeletal protuberances such as condyles (CO) on which the operculum pivots are formed by the time of

completion of the frontal wall (Plate 13:6). Hence, the developing daughter zooid is characterized by a primary orifice bounded distally by spines and proximally by a convexed frontal wall (FW) (Plate 13:7a, 7b).

Subsequent development of the orificial region of the neanic zooid (first budded zooids in a colony; Boardman and Cheetham, 1969) involves lateral elevation of the orificial collar to form lappets (L) (Plate 13:7a, 7b). Upon completion of the development of the exoskeleton, the distal portion of the lappets have attained a pointed shape (Plate 13:8a, 8b), and the lyrule (LY), which projects horizontally into the primary orifice from the basal region of the proximal section of the collar, is formed. The five blunt projections on the medial side of the condyles which are characteristic of early development are later modified into distinct pointed projections (Plate 13:9a, 9b). Whether the number of these projections and their form are of taxonomic significance was undetermined.

The maximum height of the spines appears to be attained by the completion of growth of the orificial collar and its associated structures. Thus the completely developed exoskeleton of a daughter zooid for morphotype A is characterized by a frontal wall with areolae, an orificial collar raised at its proximal margin and elevated laterally into lappets, and three to five spines (typically 5) on the distal lower border of the primary orifice (Plate 13:10a, 10b).

Colonies of *P. nitida*, morphotypes A and B Maintained in
Tanks at the Duke Marine Laboratory

Parental colonies of *P. nitida* morphotype B on shell fragments collected from the Gulf of Mexico, Cedar Keys, Florida, and maintained in the experimental tanks at the Duke Marine Laboratory increased peripherally by 5 mm. between January and June, 1974. In all observed cases the diagnostic features of the zooids laid down in the Gulf of Mexico were maintained in the new marginal zooids laid down under different environmental conditions. The salinity in Beaufort harbor North Carolina between January and June, based on 1973 data, ranges between 27.8 o/oo and 34.6 o/oo (personal communications, Dr. William Kirby-Smith); whereas in the Gulf of Mexico it ranges between 19 o/oo and 27 o/oo. (Anonymous, 1973). The water temperature for the same period ranges between 9°C and 28.1°C in Beaufort harbor and between 13°C and 29.6°C in the Gulf of Mexico. These results support the contention that the diagnostic features utilized in the differentiation between *P. nitida* morphotypes A and B are genetically based rather than a consequence of the environmental factors of salinity or temperature.

A comparison of the growth rates of successful colonies of *Parasmittina nitida*, morphotypes A (75 colonies) and B (83 colonies) set up from parental colonies from North Carolina (Tables 3a, 3b) and maintained in North Carolina yielded the following results. In the early stages of growth and colony development, June through August 1972, colonies of morphotype B attained a size 3

times that of colonies of morphotype A. However, a comparison of the basal areas of the same colonies 12 months later (August 1973) indicated that colonies of morphotype A were 1.7 times that of colonies of morphotype B. In both cases, the average of the ratio of successful colonies to potentially successful colonies was similar: morphotype A 1:5.1 and morphotype B 1:4.3. However, the individual ratios for type B colonies indicated an overall greater success ratio.

Ancestrulae of P. nitida morphotype A are characterized by 8 to 10 spines, usually 9, and those of morphotype B by 9 or 10 spines. In both morphotypes there is a proximo-medial and 2 proximo-lateral spines surrounding the frontal membrane.

In morphotype A individuals, a median distal bud cut off from the ancestrula initiates colony growth followed by the formation of a latero-distal bud which dictates the initial direction of growth. These first generation daughter zooids produce additional buds in the one primary direction; however, eventually growth radiates 360° from the ancestrula, and a circular colony is formed (Plate 14:1). All zooids in the basal or primary growth layer are arranged in a regular radiating pattern (Plate 14:2, 3). Avicularia are lacking in the stage of astogenetic change or the center of the colony, but are conspicuous in the stage of astogenetic repetition in the marginal zone of older colonies (Plate 14:2). Colonies of morphotype B, in contrast, showed avicularia early in the astogeny of the colony.

A comparison of the growth form of colonies of morphotypes A and B (Tables 3a, 3b), of approximately the same age, indicates that adventitious or frontally budded zooids are prominent features of morphotype A colonies. One-hundred percent of the successful colonies of morphotype A formed a secondary layer of growth (Plate 14:1); whereas, only 85% demonstrated such in the successful colonies of morphotype B (Plate 15:1a, 2a). In both cases, space was not a limiting factor in the growth of the primary layer, and the zooids in the superimposed layers lacked orientation (Plates 14:4-6; 15:1b, 2a). Secondary layers in morphotype A colonies were typically centered over the ancestrula and were circular with 4 radiating extensions. In older colonies, scattered patches of zooids were evident in addition to the central secondary layer. In morphotype B colonies, secondary zooids were typically in scattered patches over the primary layer of growth (Plate 15:1a). On those occasions when a secondary layer occurred over the ancestrula, it was circular and entire at its periphery.

Fifty-nine percent (44/75) of the successful colonies of morphotype A formed tertiary layers of growth and 2.6% (2/75) of them quaternary layers (Table 3a). This is in extreme contrast to colonies of morphotype B which showed tertiary layers of growth in only 1.2% (1/83) of the successful colonies and no quaternary growth. Space was not limiting lateral growth of the lower layers. A comparison (Table 3a) of the frequency of tertiary layer

growth with the size of the primary layer as measured in cm^2 indicated that colonies possessing third layers had primary layers 1.6 times the size of those of colonies lacking third layers [29 colonies possessed secondary layers only, and had an average primary layer size of 8.6 cm^2 ($2.8\text{--}24.8 \text{ cm}^2$)--46 colonies possessed tertiary layers and had an average primary layer size of 13.3 cm^2 ($5.6\text{--}21.2 \text{ cm}^2$)]. It thus appears as if lateral and frontal growth occur simultaneously, and frontal growth is not affecting the growth of the primary layer. The vertical growth demonstrated in colonies of morphotype A is consistent with the nodular growth form of morphotype A in the type material for Parasmittina nitida (Maturo and Schopf, 1968).

A closer look at the second and third layers of growth in morphotype A colonies (Plate 14:4-6), show the formation of ovicells on these zooids as well as on the primary layer zooids, and the greater occurrence of the acute avicularium proximo-medial to the orifice. Avicularia on these superimposed layers appear to be larger than those of the first layer (Plate 14:3, 5, 6); however, quantitative data are lacking.

Colonies of morphotypes A and B which came into contact with colonies of a similar morphotype, fused at their growing edges and appeared to form one unit colony. The effect of competition for space between the two morphotypes was not observed.

Colonies of P. nitida, Morphotype B, Florida, Maintained in Culture at the University of Florida

No specimens of morphotype A were found in the vicinity of Snake Key in the Gulf of Mexico, and thus no morphological comparison could be made between it and morphotype B colonies maintained in a closed sea water system.

Reproductive colonies of P. nitida, morphotype B were collected throughout the year in the Gulf of Mexico, however, the frequency of mature larvae was lowest in January and February. Ancestrulae of morphotype B individuals which developed under culture conditions showed 7 to 12 spines, usually 9 or 10 spines, surrounding the frontal membrane.

Parasmittina nitida, morphotype B, Florida raised on Oxyrrhis marina, which was subcultured with Dunaliella and/or Monochrysis, grew into colonies containing a maximum of 200 zooids in 13 weeks. Whereas, ancestrulae provided with other cultured algae (listed in the materials and methods section) developed into colonies having a maximum of 4 zooids before death ensued. In all cases, mortality was extremely high and was probably a function of any or all of the following: type and quantity of food, the conditions under which the colonies were raised, the extremely thin calcium carbonate exoskeleton formed which was subject to breakage, and the weak adhesion of the basal wall of the colony to the petri dish.

No colony raised on Oxyrrhis marina attained a reproductive state, and only 2 (less than .5% of the 400

ancestrula started in culture) attained a size greater than 100 individuals (Plates 15:3a-3d; 17:1-6). A median distal bud cut off from the ancestrula initiates colony formation (Plate 16:1, 2a) followed by either a lateral or distal bud to it. The circular nature of the primary layer is attained early in colony formation. All zooids in the stage of astogenetic change showed 2 orificial spines and an orificial collar elevated into lappets (Plates 15:3a, 3b; 16:1, 2a; 17:1, 3). Infrequently (less than .5% occurrence) abnormal growth patterns were observed in the cultured colonies (Plate 16: 3a, 3b, 4). Zooids would form with two spines on the proximal and 4 spines on the distal margins of the primary orifice, or with 7 to 8 spines on the distal margin of the exoskeleton of a developing double zooid. In the latter case, two polypides occupy a single exoskeletal chamber until the completion of the frontal wall, (Plate 16:4) at which time they appear as two distinct zooids lateral to each other.

Zooids forming the primary layer showed a linear radiating pattern (Plates 15:3c, 3d; 17:1-3) from the ancestrula similar to that observed in colonies maintained in the running sea water system. However, colonies maintained in culture and of a maximum size of 200 zooids did not show avicularia nor secondary patches of growth. The lack of avicularia on the zooids is possibly extragenetic based on the work of Kaufmann (1968). Scanning electron micrographs of colony 2-11-D-3 #D-4, which demonstrated

maximum growth (Plate 17), showed that zooids from this colony possessed orificial collar and spination features similar to those in colonies of morphotype B, North Carolina. The distance between the lappets on the proximal side of the primary orifice appears narrower than those of the first generation zooids which develop in North Carolina. However, as this was the only colony grown under culture conditions that was viewed with the scanning electron microscope, it is impossible at this time to determine whether the collar form is a function of individual variation or of culturing conditions.

Colonies Maintained Elsewhere

Colonies from North Carolina and Cedar Keys, Florida, maintained in tanks at Marineland of Florida or colonies originating from Florida maternal stocks and maintained in the sea water tables at the University of Florida Marine Laboratory showed bare subsistence and no measurable growth. Those ancestrulae and young colonies placed in the Gulf of Mexico in crab traps were over-run by epifaunal organisms.

Offspring Variation from Known Maternal Stocks of *Parasmittina nitida*, North Carolina

In order to determine whether the specific diagnostic features noted by Maturo and Schopf (1968) to distinguish between *P. nitida*, morphotypes A and B were genotypic and thus result from separate gene pools, colonies were set up from known isolated maternal stocks (of both morphotypes) and specific features traced through two generations.

Parasmittina nitida, Morphotype A, North Carolina

Mature colonies of Parasmittina nitida, morphotype A are characterized by the following conspicuous features as initially outlined by Maturo and Schopf (1968):

- (1) quadrate zooids approximately .52 mm in length and .40 mm in width, the frontal wall of which is perforated by areolae (Plate 18: 1a, 1b);
- (2) primary orifice squarish, with prominent condyles toothed on their medial side (Plate 18:2a, 2b), and the lyrule $1/3$ to $1/2$ the width of orifice (Plate 18:4);
- (3) hyperstomial ovicell with frontal area perforated by at least 20 small round or irregular pores, and orificial border lacking (Plate 18:3-6);
- (4) during secondary calcification, ovicell surrounded distally and laterally by roughened tuberculate rim, and ridges form between pores of the frontal surface (Plate 18:6);
- (5) avicularium acute, single, and directed proximally on the frontal wall usually to one side of the midline and proximal to the orifice (Plate 18:1a, 4); and,
- (6) two to three spines on distal border of primary orifice in marginal zooids of colonies greater than 50 zooids.

Maternal colonies of Parasmittina nitida, morphotype A set up for larval release in June 1972 yielded 718 ancestrulae. The maximum number of larvae set from one parent colony was 92, of which only 11% (10/92) developed into colonies still present at the termination of the experiment 14 months later. Fifty-four percent (390/718) of the total number of first generation ancestrulae were

present in August 1972, and had developed into colonies of up to 111 zooids. Of the colonies present in August 1973, only 19% (75/390) of them had been successful in reproducing during the experiment (June 1972-August 1973).

One-hundred percent of the first generation ancestrulae developed into colonies (Plate 19) having characters resembling those of their maternal parent. Typically, a zooid in a colony of approximately 100 zooids showed an avicularium located proximal to the orifice in the midline (Plate 14:6) or to one side of the midline (Plate 19:1, 3a) of the frontal wall. The orificial collar is elevated into lappets which slopes toward the distal border of the primary orifice lined by 3 to 4 spines (Plate 19:2, 3b). Secondary calcification migrating proximally from a distal zooid as well as from the distal wall of marginal zooids is characteristic of Parasmittina nitida (Banta, 1973) and results in breakage of the orificial spines and their subsequent elimination as a surface detail from other than newly formed zooids (Plates 18:1b; 19:1). The elimination of the lappets of the orificial collar proceeds similarly, but involves secondary calcification of the frontal wall.

Periodic observations over a 14-month period of the reproductive state (Table 4) of the first generation colonies maintained in the experimental tanks at the Duke Marine Laboratory indicated that July is the height of the reproductive period (June through November) for P. nitida, morphotype A. At this time, ovicells were

recorded in 105 colonies, 76 of which had not been previously reported as bearing ovicells. Of the 105 colonies, 66 colonies were set up for larval release in order to follow diagnostic features of the second generation. One hundred and seven larvae were released (Table 5a) and metamorphosed into normal ancestrulae. This was less than 7% (107/1465) of the number of embryos present in the colonies used for the experiment. Twenty-eight ancestrulae or their colonies, 26% (28/107) of the initial set, were present the next observation period approximately 2 months later. Twenty-seven of them were from known first generation maternal stock. Nine zooids (Plate 20:3) were the maximum size of the second generation colonies. The limited growth was due to the termination of the experiment rather than any biological-environmental factor.

One hundred percent of the second generation ancestrulae developed into colonies (Plate 20) with traits resembling those of their first generation maternal parents. Zooids in the stage of astogenetic change of a developing colony were characterized by 3 to 5 spines on the distal border of the primary orifice, with typically 5 on the median distal bud or the first formed bud of the colony. In colonies of P. nitida, morphotype A, the number of spines per zooid tended to decrease away from the ancestrula. In all cases, the spines originated from a socket type structure (Plate 20:3g). The frontal walls lacking avicularia in the zone of astogenetic change were convex and rise to the

proximal edge of the orificial collar (Plate 20:3e). Secondary calcification was evident early in colony formation and resulted in camouflaging the basal portion of the orificial spines (Plate 20:3c, 3d).

Parasmittina nitida, Morphotype B, North Carolina

Mature colonies of Parasmittina nitida, morphotype B are characterized by the following conspicuous features as initially outlined by Maturo and Schopf (1968): (1) quadrate zooids approximately .60 mm in length and .37 mm in width, the frontal wall of which is perforated by areolae (Plate 21:1, 2); (2) primary orifice round with condyles short and toothed (Plate 21:3a, 3b), and the lyrule $1/4-1/3$ the width of the orifice (Plate 21:3a); (3) hyperstomial ovicell with frontal area perforated by less than 10 large round or irregular pores, with acute avicularium raised on the distal or lateral side of the ovicell (Plate 21:4a-5) and with proximal border formed by orificial collar (Plate 21:4a); (4) secondary calcification of the ovicell similar to that described for P. nitida morphotype A (Plate 21:5); and, (5) one to three avicularia per zooid, and if 2 or more, then of 2 different types. Avicularium is either acute and placed lateral to the orifice, usually with the rostrum elevated on the side of the collar and in a disto-medial orientation (Plate 22:4), or oval and placed lateral or proximal to the orifice in variable orientations but opposite the acute avicularium if the latter is present. Giant avicularia of various shapes are occasionally present.

(6) Two spines are located on the distal border of the primary orifice of marginal zooids in the stage of astogenetic repetition of a colony.

Maternal colonies of Parasmittina nitida, morphotype B set up for larval release yielded 926 ancestrulae in June 1972. The maximum number of larvae set from one parent colony was 286, of which only 10% (28/286) developed into colonies still present at the termination of the experiment 14 months later. Forty-nine percent (451/926) of the total number of first generation ancestrulae were present in August 1972 and had developed into colonies of up to 153 zooids. Of the colonies present in August 1973, only 18% (83/451) of them had successfully reproduced during the experiment (June 1972-August 1973). The percent survival and success of first generation colonies set up from known maternal stocks of morphotype B were similar to that for morphotype A.

One hundred percent of the first generation ancestrulae developed into colonies (Plate 22) having characters resembling those of their maternal parent. Typically a zooid in a colony of approximately 100 zooids had 1 to 2 avicularia with usually an oval type if only one was present (Plate 22:1). In all cases, the acute avicularium was oriented with its rostrum elevated on the side of the pointed lappets of the orificial collar (Plate 22:4). The distal border of the primary orifice had two spines (Plate 22:2, 5). Secondary calcification was minimal and

did not appear to obliterate the orificial spines in most cases.

Periodic observations of the reproductive state (Table 4) of first generation colonies of P. nitida, morphotype B maintained in the experimental tanks at the Duke Laboratory indicated March was the height of the November through June reproductive period. At this time, ovicells were recorded in 92 colonies; 56 of which had not been previously reported as bearing ovicells. Of the 92 colonies, 81 were set up for larval release in order to follow diagnostic features of the second generation. Forty-three larvae were released (Table 5b) and metamorphosed into normal ancestrulae. This was less than 2% (43/2279) of the number of mature larvae observed in the ovicells of the colonies used for the experiment. Seven ancestrulae or their colonies, 16% of the initial set, were present the next observation period approximately three months later; however, only 1 (Table 5b: dish no. 25B-3-2) was from a definitely known first generation maternal stock, and it did not develop past the ancestrula stage. The remaining ancestrulae and colonies could have originated from mature colonies either originating from larvae brought into the tanks or set up under culture conditions and of known maternal stock. No scanning electron micrographs are available for the second generation colonies of P. nitida, morphotype B, as all colonies remaining in the experimental tanks until the end of August were dead.

DISCUSSION

Colony Variation

The high level of organization of individual zooids and of bryozoan colonies makes available many phenotypic characters for the taxonomic study of all groups of Bryozoa. As individuals in a colony are produced in a sequential series by asexual budding from the ancestrula, all zooids of a colony share a common genotype and are theoretically genetically identical individuals. Intracolony variation does occur and can be assigned to extragenetic factors. The zone of astogenetic change of a colony reflects ontogenetic changes in zooids during the course of their development, astogenetic changes in the morphology and budding pattern of a colony in a directed series from the ancestrula, and polymorphic differences between zooids occupying a similar position in the colonial budding pattern. The remaining morphologic variation within a colony occurring either in isolated regions or in scattered zooids results from such microenvironmental factors as nutrition, crowding by growth of the colony itself or competitive growth of other organisms, irregularities in the substrate, and differential sediment accumulation. Some environmental changes such as differences in temperature, salinity, light intensity and duration may

be expressed in the phenotype of the colony as a whole and thus in zooid morphology, budding pattern, and colony form (Boardman, Cheetham, and Cook, 1969).

As certain colonies of morphotypes A and B of Parasmittina nitida were maintained in the same environment, and as individual colonies are genetically uniform; it can be assumed that the phenotypic differences observed between the first generation colonies of the two morphotypes were primarily genetic differences. The fact that the diagnostic characters of the maternal parent of each type were reported in 100% of the colonies of the first generation progeny of that morphotype demonstrates further that the characters are genetic and result from separate gene pools. This is in agreement with the findings of Maturo (1973). In no case were any of the characters of one morphotype or intergrades of the characters of both morphotypes observed in colonies originating from maternal stocks of the other morphotype. Colonies of the second generation progeny for both morphotypes demonstrated diagnostic features of the first generation maternal stock and the maternal parent in 100% of the cases. There is a possibility, although rare, that the genes are separating in a Mendelian fashion, but the phenotype is controlled by a cytoplasmic factor according to Maturo (1973). Assuming that the colonies collected from the field are the parents (maternal zooids identifiable) of successive generations.

studied in the laboratory, a change in the diagnostic features of the exoskeleton of zooids in the third generation but not in the second or first (the latter two generations under observation in the present study) would possibly resolve the question of a cytoplasmic factor as the true mechanism of inheritance for these individuals.

The fact that morphotype A colonies tend to lay down frontally budded zooids more readily than those of morphotype B under identical environmental conditions, and not withstanding space as a limiting factor, suggests that colony form is also genetically controlled.

The mode of fertilization in bryozoans remains unknown for the most part. Silén (1972) reported the release of sperm through the tips of the tentacles in 5 species of Cheilostomata; thus indicating that cross fertilization is possible in marine bryozoans. If this is the case for P. nitida also, then eggs of zooids of the first generation were fertilized by: (1) sperm either from zooids within the same colony; (2) from zooids in adjacent colonies on the same petri dish and thus of the same morphotype and maternal stock; (3) from zooids of colonies on other petri dishes and thus of the same or different morphotype and a different maternal stock; or (4) from sperm of P. nitida brought into the experimental tanks through the running sea water system. I am assuming that if sperm of one morphotype fertilized eggs of the

second morphotype, the embryos were inviable. Sperm originating from zooids in one colony and fertilizing eggs in zooids of the same colony could result if monocious-zooid colonies exist for this species. In the case of monocious zooids, self-fertilization could also occur; however, Silén (1966) reported a tendency towards protandry in zooids simultaneously containing mature sperm and developing egg cells. Parthenogenesis is also a possible means of reproduction in this species; although it has not been reported in other bryozoans.

Reproductive Period

It is a demonstrated fact (Orton, 1920) that the breeding and distribution of many marine invertebrates is directly related to sea temperature. According to Hyman (1959) bryozoans have an annual breeding season extending over 2 to 6 months. Fouling studies conducted in Bogue Sound and Beaufort Harbor, North Carolina from May 1954 through May 1955 (Maturo, 1959) indicated that Bugula californica reproduced 9 months out of the year; whereas, Parasmittina trispinosa (= P. nitida; morphotypes not designated at the time) reproduced from mid-May through mid-November. P. nitida, morphotype A raised in experimental tanks at the Duke Marine Laboratory, Beaufort, North Carolina, from June 1972 through August 1973 showed a reproductive period from June through early November, and P. nitida morphotype B from late November through June.

According to Maturo (1973) larvae of P. nitida morphotype B were readily obtained from mid-July through mid-August in 1970. From the reproductive data available on populations of P. nitida morphotypes A and B, it appears as if their breeding seasons overlap slightly, i.e., June through August. The 2 populations are in a very large part, however, temporally reproductively isolated because individuals of morphotype A reach the height of their production of embryos in July; whereas, individuals of morphotype B reach their height in March. According to this hypothesis, new colonies of P. nitida should have been reported from the fouling plates throughout the year in the 1954-1955 study. The reproductive period reported for P. trispinosa (= P. nitida) by Maturo (1959) would suggest that the individuals settling on the fouling plates were primarily P. nitida morphotype A because of the coincidence of breeding periods in both studies.

The sea temperatures in Beaufort Harbor, North Carolina averaged 15.8°C (10-19°C) in March 1973, and 29°C (27.4-31.5°C) in late June-early July, 1973. It appears as if individuals of P. nitida morphotype B in North Carolina are reproducing at the lower limit of the temperatures experienced by individuals of morphotype B in the Gulf of Mexico, Cedar Keys, Florida. Individuals of morphotype B are reproductive all year round in the Gulf of Mexico.

From the scanty distributional data available, it appears as if P. nitida morphotype B ranges from Vineyard Sound (specimens originally collected 1874, and reclassified as to species and assigned morphotypes by Maturo and Schopf, 1968) to the Gulf of Mexico. Colonies of P. nitida morphotype A range from Long Island Sound (specimens originally collected 1874, and reclassified by Maturo and Schopf, 1968) to Vero Beach, Florida (P. nitida morphotype A recognized from specimens collected during the Gosnold Expedition; Maturo, personal communications). Published reproductive information for these two morphotypes within their respective ranges is lacking, except for Beaufort, North Carolina.

Larvae

Ryland (1958) published a list of the embryo color of 41 British species following the color terminology of Silén (1943), who recognized the diagnostic value of color in the taxonomy of bryozoans. The present work is the first to indicate the taxonomic value of pigmentation and distribution of aboral cilia in pelagic lecithotropic bryozoan larvae. Larvae of P. nitida morphotype B are seen to be distinct from larvae of P. nitida morphotype A based on the complexity, size, number, and location of the pigment spots, and the arrangement of the aboral cilia on the aboral lobe, (see below). A review of bryozoan larva of a variety of species should prove these features to be of taxonomic value for the group. The variation in

the shape of the larvae of P. nitida, depending on the phase of movement in the interval prior to metamorphosing, has been similarly described for larvae of Bugula neritina (Lynch, 1947) and Scrupocellaria reptans (Ryland and Stebbing, 1971).

Post-Larval Development

Our knowledge of post-larval stages of development in cheilostome bryozoans is based on the description of metamorphosis in Bugula neritina. The initial work was done by Lynch (1947) and later verified and enumerated by transmission electron microscopy (Woollacott and Zimmer, 1971). Developmental aspects of the differentiation of the primary disc, the first formed structure, into a tata ancestrula are described here for the first time in any cheilostome bryozoan.

Soule (1954) reviewed post-larval development in the ctenostomes, and suggested that the development of the musculature was of taxonomic significance in the classification of this group. It thus appears as if the formation of the distal communication pore complex and the distal bud off the ancestrula, which is different in the two morphotypes of P. nitida, could be of taxonomic significance in the smittinids. The rate of development of the two morphotypes could not be compared directly as they were studied under slightly different environmental conditions.

Species Designation

Based on the data presented, morphotypes A and B of Parasmittina nitida (Verrill, 1875) represent 2 species. As the lectotype of Discopora nitida Verrill is the type specimen of P. nitida (Verrill, 1875), and it shows characters of morphotype A; P. nitida morphotype A shall retain the name Parasmittina nitida. Parasmittina nitida, morphotype B must be designated as a distinct species (Maturo and Schopf, 1968). The following characters differentiate the 2 species:

Diagnostic Characters	<u>Parasmittina nitida</u> (= <u>P. nitida</u> morphotype A)	<u>Parasmittina n.sp.</u> (= <u>P. nitida</u> morphotype B)
Larva		
Size (height)	110-130 μ	135-180 μ
Lateral pigment spot (aboral)	simple equal in size to other pigment spots	complex larger than other pigment spots
Aboral pigment spots	4 or 6, small (~25x34 μ), in supra-coronal furrow	5 - 6, large (~ 50x60 μ), lateral in aboral lobe
Lateral pigment spot (oral)	absent	present
Aboral cilia orientation	scattered over entire aboral surface of aboral lobe	transverse band on aboral surface of aboral lobe
Ancestrula		
Spines	8-10	9-10
Metamorphosis		
Distal communication pore complex formation	simultaneous to formation of pore plate and distal wall of tata ancestrula	secondary to pore plate formation and distal wall

Diagnostic Characters	<u>Parasmittina nitida</u> (= <u>P. nitida</u> morphotype A)	<u>Parasmittina n.sp.</u> (= <u>P. nitida</u> morphotype B)
Metamorphosis		
Distal bud	precocious to pore plate formation	secondary to pore plate formation
Size increase (primary disc to ancestrula)	2.2	1.8
Zooid		
Primary orifice condyles lyrula	squarish prominent-toothed 1/3-1/2 proximal edge of orifice	rounded reduced-toothed 1/4-1/3 proximal edge of orifice
orificial spines	2-5	2
Orificial collar	low in old colonies ¹ proximal portion above frontal wall in young zooids proximal margin free of projections present in young zooids--elevated	elevated in old colonies ¹ proximal portion equal in height to frontal wall projections around proximal margin elevated and pointed
lappets		
Secondary calcification	extensive eliminates spines and lappets from surface morphology early in colony astogeny	limited
Avicularium	acute, single proximal to orifice lateral to midline or in midline of frontal wall proximally directed	ovoid, acute, 1-3 acute: lateral to orifice in proximal orientation or with rostrum elevated on lappet ovoid: lateral or proximal to orifice opposite to that of acute avicularium in variable orienta- tions
Ovicell		
frontal surface	≥20 small, round or irregular pores	≤10 large, round or irregular pores
orificial border	lacking	present
avicularium	none	acute

Diagnostic Characters	<u>Parasmittina nitida</u> (= <u>P. nitida</u> morphotype A)	<u>Parasmittina n.sp.</u> (= <u>P. nitida</u> morphotype B)
Colony form	adventitious layers (up to 4 layers reported) nodular growths	only secondary layers of growth, and in 85% of the colonies
Secondary layers of growth	circular over center of colony and with radiating extensions scattered patches in older colonies ¹	scattered patches entire over center of colony in older colonies ¹

¹zooids showing ovicells and having secondary calcification

SUMMARY

1. It has been demonstrated that morphotypes A and B of Parasmittina nitida (Verrill, 1875) represent two species. Parasmittina nitida morphotype B must be designated as a distinct species.
2. The morphological features of the larvae of P. nitida, morphotypes A and B were studied in detail. The larvae of the two types are distinguishable by size; location, number, size, and complexity of the pigment spots; and the orientation and distribution of the aboral cilia on the aboral lobe.
3. Metamorphosis of larvae of P. nitida morphotype A, North Carolina and P. nitida morphotype B, Florida is given in detail. The formation of the distal communication pore and bud is different in the two morphotypes and may be of taxonomic significance in the Smittinidae.
4. A description of the formation of the orificial spines and collar in daughter zooids of P. nitida morphotype A is given in detail.
5. Growth of colonies of P. nitida morphotype B, Florida was limited when provided with cultured algae in a closed sea water system. Colonies fed Oxyrrhis marina showed the greatest growth.

6. Colonies of P. nitida morphotype B maintained in a running sea water system at the Duke Marine Laboratory grew to a maximum primary layer size of 10.2 cm²; colonies of P. nitida morphotype A to a maximum primary layer of 23.8 cm².
7. Colony form in P. nitida, i.e., superimposed layers of growth, was demonstrated to be a genetic factor and of possible taxonomic significance. Colonies of P. nitida morphotype A formed secondary layers in 100% of its colonies and third layers in 59% of its colonies; whereas, colonies of morphotype B formed only secondary layers and in only 85% of its colonies.
8. Zooids of P. nitida, morphotype B were observed to have two orificial spines in all newly formed zooids and in zooids in all stages of a colony; whereas, those of P. nitida morphotype A had 2 to 5 spines, the number decreasing in zooids further from the ancestrula and increasing in neanic zooids of older generation progeny. Secondary calcification was initiated early in colony formation in morphotype A and masked the presence of the orificial spines.
9. Offspring variation studies of the two morphotypes of P. nitida raised under identical environmental conditions for two generations demonstrated that the diagnostic features separating the two morphotypes are genetic. In all cases the characters bred true to the maternal stock.

10. Individuals of P. nitida morphotype B reproduce all year round in the Cedar Keys, Florida vicinity, Gulf of Mexico. Individuals of the same morphotype maintained in running sea water aquaria in an aquarium room without controlled temperature, in North Carolina, were reproductive from November through June with the peak in March. Natural populations of morphotype B were reproductive through mid-August. Individuals of P. nitida morphotype A occurring sympatrically with those of morphotype B in their natural environment were reproductive in the running sea water aquaria from June through November with their peak in July.

APPENDIX I

CULTURING ALGAE

All large quantities of algae were grown in 2-liter low form culture flasks. The final quantity of culture media within each flask did not exceed 1 1/2 inches in depth in order to maximize surface to volume ratios, and thus algal growth (Myers, 1962). Dunaliella, Monochrysis lutheri, Phaeodactylum, and Oxyrrhis marina were cultured as food for Parasmittina nitida, morphotype B, Florida. Whenever possible, the algae were grown in a salinity comparable to that in the tanks holding the developing colonies, i.e., approximately 26 o/oo.

Dunaliella sp. is a biflagellated alga approximately 6 μ X 8 μ . It is a euryhaline organism capable of growing in a range of salinities from .75 - 120 o/oo (McLachlan, 1960). Initially Dunaliella was grown in a Guillard "C" sea water medium of approximately 26 o/oo; however, growth was found to be more rapid in salinities of 32 o/oo. The cultures were adjusted to the appropriate salinity prior to feeding the colonies of P. nitida. Cultures of Dunaliella used as food for the heterotroph Oxyrrhis marina were grown in an Erdschreiber medium.

Oxyrrhis marina is a euryhaline phagotrophic dinoflagellate with a body length between 22 μ and 32 μ , and a cross diameter between 12 μ and 20 μ . It is a

cosmopolitan species (Schiller, 1933), and has been reported from the eastern Gulf of Mexico (Steidinger and Williams, 1970). According to Dr. George (personal communications), Oxyrrhis marina grows well in a Erdschreiber media supplied with bacteria, small diatoms or a very dilute suspension of Saccharomyces as particulate food. Dr. Hargraves (personal communications) found a mixture of bacteria and a 2 μ flagellate adequate as a food source. In the present work, O. marina was cultured in approximately a 32 o/oo Erdschreiber media prepared with a Texas cotton soil extract. The medium prepared with soil supernatant from soils collected in the vicinity of the University of Florida campus was found to be unsatisfactory for maximum growth of the dinoflagellate.

Monochrysis and Phaeodactylum were cultured in 26 o/oo Guillard "F" sea water media. The former algae was utilized as a food source for Oxyrrhis when quantities of Dunaliella were depleted. No attempt, however, was made to adjust the salinity of the culture to that of the higher salinity of the O. marina culture.

Cell counts for Oxyrrhis were made periodically with a hemocytometer.

APPENDIX II

TABLE 1

Preparation of 20 ml. quantity of 4% Glutaraldehyde (.4 M) buffered with sodium cacodylate and adjusted to the osmolality of sea water. (Sodium cacodylate and sodium chloride are dissolved in 10 ml. of distilled water to which 10 ml. of 8% Glutaraldehyde is subsequently added.)

Salinity of sea water o/oo	Milliosmoles of sea water	Sodium Cacodylate g/20 ml	Sodium Chloride g/20 ml	Molality of final solution
22	655.9	.205	.075	.464
24	715.1	.252	.092	.479
25	741.9	.274	.100	.485
26	774.2	.300	.110	.494
28	833.3	.347	.127	.508
29	862.9	.371	.135	.516
30	892.5	.394	.144	.523
31	922.5	.418	.153	.531
32	951.6	.442	.161	.538
33	982.0	.466	.170	.546
34	1011.0	.490	.179	.553
35	1041.6	.514	.188	.560

TABLE 2

Preparation of 100 ml. quantity of sodium cacodylate buffer solution which is adjusted to the osmolality of sea water.

Salinity of sea water o/oo	Milliosmoles of sea water	Sodium Cacodylate g/100 ml	Dextrose g/100 ml	Molality of final solution
22	655.9	3.500	3.94	.438
24	715.1	3.817	4.30	.478
25	741.9	3.955	4.45	.494
26	774.2	4.131	4.65	.516
28	833.3	4.448	5.00	.556
30	892.5	4.763	5.36	.596
32	951.6	5.078	5.71	.634
34	1011.0	5.395	6.07	.674

TABLE 3a

Growth of successful¹ colonies of *Parasmittina nitida*, morphotype A, North Carolina, grown in experimental tanks at the Duke Marine Laboratory. Larvae were released from known maternal stocks and subsequently metamorphosed on petri dishes between June 19 and June 27, 1972.

Plate No.	No. of ancestrulae in initial set	No. successful colonies	No. colonies with 2nd layer of growth	No. colonies with 2nd and 3rd layer of growth	No. zooids per colony August 1972		Area (cm ²) of primary growth layer August 1973	
					Average	Range	Average	Range
16A-16-2	12	3	3	3	29.3	19-47	12.9	5.6-23.8
16A-16-4	1	1	1	1	14	14	5.8	5.8
16A-16-6	6	3	3	32	21.3	10-29	12.8	9.7-16.8
16A-16-7	6	5	5	2	17.2	7-23	11.7	6.9-18.7
17A-1	2	1	1	1	79	79	--	--
17A-1-2	2	1	1	1	13	13	12.9	12.9
17A-2-1	1	1	1	1	72	72	21.2	21.2
18A-1-2	5	1	1	1	34	34	14.2	14.2
18A-3	2	1	1	1	17	17	12.9	12.9
19A-8	3	1	1	0	32	32	10.2	10.2
21A-2	6	3	3	1	23	21-25	18.7	18.7 ¹
21A-3	2	1	1	1	12	12	5.1	5.1
21A-3-2	2	1	1	1	17	17	19.6	19.6
21A-4	4	3	3	0	29.3	22-34	7.8	7.4-8.1 ²
22+A-7-2	2	1	1	0	47	47	24.7	24.7
22+A-13	1	1	1	1	17	17	43.1	43.1
22+A-13-2	2	1	1	1	14	14	9.5	9.5
22+A-13-3	7	4	4	1	34.8	13-54	6.6	3.5-8.8
22+A-13-4	3	3	3	1	95	82-111	10.1	7.8-11.3
23+A-5	4	3	3	2	19.3	13-23	11.3	6.1-16.4 ²
23+A-5-2	12	3	3	1	15.5	4-17	8.2	6.5-9.8

TABLE 3a (Continued)

Plate No.	No. of ancestrulae in initial set	No. successful colonies	No. colonies colonies with 2nd with 3rd layer of layer of growth growth	No. zooids per colony August 1972	Area (cm ²) of primary growth layer August 1973			
				Average	Range	Average	Range	
23 ⁺ A-5-3	17	2	2	1	13	10-16	9.3	8.8-9.8
23 ⁺ A-5-4	12	2	2	1	9	8-10	6.2	5.9-6.4
25A-8	4	1	1	1	31	31	17.3	17.3
25 ⁺ A-12	3	1	1	0	60	60	10.6	10.6
26 ⁺ A-14	4	1	1	1	31	31	10.0	10.0
29 ⁺ A-5	23	2	2	2	6.5	5-8	19.7	18.2-21.2
29 ⁺ A-5-2	33	1	1	1	11	11	7.7	7.7
29 ⁺ A-5-3	33	3	3	1	8.3	6-13	11.1	10.6-11.5
29 ⁺ A-12-2	16	1	1	1 ²	24	24	7.4	7.4
30 ⁺ A-2	48	3	3	3	4.3	1-6	11.3	8.3-14.3
30 ⁺ A-5	29	4	4	2	7.8	5-12	6.6	3.7-9.1
30 ⁺ A-5-3	39	8	8	2	29.4	12-52	8.4	3.7-14.2 ⁷
30 ⁺ A-6	5	2	2	2	5	5-5	17.0	15.7-18.3
30 ⁺ A-7	2	1	1	1	23	23	11.2	11.2
3 ⁺ A-34	1	1	1	1	26	26	20.5	20.5 ¹

¹The success of a colony was measured by the presence of ovicells and the subsequent development of embryos within the ovicells.

²One colony showed a fourth layer of growth.

³Exponent indicates number of colonies involved in determining range of area measurements.

⁴Photographed in Plate 14:1, 3-6.

TABLE 3b

Growth of successful¹ colonies of Parasmittina nitida, morphotype B, North Carolina, grown in experimental tanks at the Duke Marine Laboratory. Larvae were released from known maternal stocks and subsequently metamorphosed on petri dishes between June 18 and June 27, 1972.

Plate No.	No. of ancestrulae in initial set	No. successful colonies	No. colonies with 2nd layer of growth	No. zooids per colony August 1972			Area (cm ²) of primary growth layer August 1973		
				Average	Range	Average	Average	Range	Range ³
16B-7-1	5	2	1	114.5	39-190	--	--	--	--
16B-7-3	12	1	1	55	55	6.58	6.58	6.58	6.58
16B-2-2	2	1	1	204	204	10.3	10.3	10.3	10.3
16B-2-4	8	2	2	111.5	92-131	7.7	7.7	5.1-10.6	5.1-10.6
16B-4-2	7	4	4	106	85-153	6.4	6.4	2.3-10.4 ³	2.3-10.4 ³
16B-4-4	38	11	9	78	48-123	5.0	5.0	1.8-7.4 ²	1.8-7.4 ²
16B-4-6	53	14	9	34.6	8-82	3.9	3.9	3.0-5.5 ⁶	3.0-5.5 ⁶
16B-4-7	32	22	19	57.0	30-119	6.0	6.0	3.9-8.6 ¹¹	3.9-8.6 ¹¹
16B-4-9	17	7	6	34.6	15-58	7.7	7.7	5.1-8.9 ⁵	5.1-8.9 ⁵
16B-4-10	11	3	3	7.3	6-10	11.5	11.5	6.6-14.1	6.6-14.1
16B-5-2	2	1	1	73	73	4.8	4.8	4.8	4.8
16B-5-4	10	3	3	76.3	53-102	4.9	4.9	4.1-5.8 ²	4.1-5.8 ²
16B-5-5	20	3	3	64.7	23-115	6.9	6.9	5.3-9.1	5.3-9.1
16B-5-6	1	1	1 ²	51	51	15.6	15.6	15.6	15.6
16B-8	9	1	1	34	34	7.6	7.6	7.6	7.6
16B-9	1	1	1	156	156	8.7	8.7	8.7	8.7
24 ⁺ B-1	11	3	3	44.7	34-58	7.1	7.1	5.8-8.5	5.8-8.5
25B-3-2	5	1	1	50	50	10.2	10.2	10.2	10.2
30 ⁺ B-1-2	27	2	2	12.5	10-15	5.9	5.9	5.9 ¹	5.9 ¹

¹The success of a colony was measured by the presence of ovicells and the subsequent development of embryos within the ovicells.

²One colony showed a third layer of growth.

³Exponent indicates number of colonies involved in determining range of area measurements.

TABLE 4

Summary of the reproductive state of colonies of Parasmittina nitida, morphotypes A and B, grown in experimental tanks at the Duke Marine Laboratory.

	late Nov. '72		late Mar. '73		early July '73	
	A	B	A	B	A	B
No. plates recorded for the first time with ovicell bearing colonies	8	12	4	6	30	1
Total ² no. plates with ovicell bearing colonies	8	12	9	18	43	18
No. colonies bearing ovicells for first time	10	35	4	56	76	3
Total no. colonies bearing ovicells in good condition	10	35	11	92	105	58
No. ovicells bearing embryos in various stages of development in colonies bearing ovicells for the first time	6	225	0	1037	1882	0
Total no. ovicells bearing embryos in various stages of development	6	225	0	8976	4248 ³	0
Range -- no. ovicells bearing embryos per colony	1-2	1-68	0	1-341	-	No record -

TABLE 4 (Continued)

	<u>late Nov. '72</u>		<u>late Mar. '73</u>		<u>early July '73</u>	
	<u>A</u>	<u>B</u>	<u>A</u>	<u>B</u>	<u>A</u>	<u>B</u>
No. mature larvae per ovicell in colonies bearing ovicells for the first time	4	39	0	614	-	No record -
Total no. mature larvae in ovicells	4	39	0	2284	-	No record -
Ratio of mature larva to developing embryo in ovicells	1:1	1:7	0	1:3.0	-	No record -

¹Colonies originated from metamorphosed larvae from known maternal stocks, June 1972, or from metamorphosed larvae brought into the tanks in the sea water system.

²The total number of counts do not necessarily follow from the previous total counts as colonies were lost and/or colonies originating from larvae brought into the experimental tanks attained maturity in the interim.

³This number is based on the 66 colonies used in the second generation larval release experiment and not from the total number of ovicells bearing embryos in July 1973.

TABLE 5a

Summary of the reproductive state of specific colonies¹ of *Parasmittina nitida*, morphotype A, North Carolina in July 1973. Colonies were raised in experimental tanks at the Duke Marine Laboratory and released second generation larvae that metamorphosed into normal ancestrulae.

Plate No.	Date of larval release (1973)	No. colonies with ovicells bearing embryos in various stages of development	No. ovicells containing embryos in various stages of development	No. second generation ancestrulae August 1973	No. ancestrulae present in generation colony	Maximum No. zooids per second generation colony
16A-16-16	7-6	3	178	1	0	-
16A-16-7	7-3	3	124	1	1	1
16A-7	7-8	(1)	(36)	1	0	-
17A-1-2	7-2	2+(1)	125+(46)	1	1	1
17A-2-1	7-6	1	84	3	0	-
18A-1-2	7-8	1	65	3	0	-
18A-5-6	7-4	1	47	3	1	1
21A-3-2	7-3	1	172	5	1	1.5
22 ⁺ A-13-2	7-9	1	209	11	104	3
23 ⁺ A-5-3	7-8	2	45	2	1	1
30 A-5	7-9	3	34	2	1	2
3 ^A A-32	7-9	1	≥300	74	123	9

¹Data includes information for colonies of known maternal stock, and for colonies of unknown maternal stock originating from larvae brought into the tank through the running sea water system. Information on the latter is included within parenthesis where discernable.

²Photographed in Plate 14:1, 3-6.

³Photographed in Plate 20:2-3.

⁴Photographed in Plate 20:1.

TABLE 5b

Summary of the reproductive state of specific colonies¹ of *Parasmittina nitida*, morphotype B, North Carolina in March 1973. Colonies were raised in experimental tanks at the Duke Marine Laboratory and released second generation larvae that metamorphosed into normal ancestrulae.

Plate No.	No. colonies with bearing ovicells	No. embryos in various stages of development	No. ovicells containing embryos in various stages of development	No. ovicells containing mature larva	No. second generation ancestrulae July 1973	No. zooids per second generation	Maximum
16B-7-1	3	42	18	3	0	-	-
18B-2-2	1	52	16	1	0	-	-
18B-4-4	9+(2)	57+(23)	49+(12)	6	0	-	-
18B-4-6	12+(6)	87+(45)	67+(30)	4	0	-	-
18B-4-7	19+(3)	7933+(535)	1633+(343)	19	5	47	-
18B-4-10	3+(1)	30+(1)	13+(0)	1	1	5	-
18B-5-4	3	16	10	4	0	-	-
18B-9	1	17	5	1	0	-	-
25B-3-2	1	8	5	4	1	-	1

¹Data includes information for colonies of known maternal stock, and for colonies of unknown maternal stock originating from larvae brought into the tank through the running sea water system. Information on the latter is included within parenthesis where discernable.

APPENDIX III

ABBREVIATIONS

These abbreviations are utilized throughout this dissertation in all plates and figures.

A	anus
ABC	aboral cilia
ABL	aboral lobe
AC	alimentary canal
AN	ancestrula
AO	apical organ complex
AR	areolae
AS	anterior pigment spot(s) of larva
AV	avicularium
B	bud of developing daughter zooid
BW _i	inner layer of basal wall
BW _o	outer layer of basal wall
C	cuticle
CAS	cardia stomach
CC	corona cilia
CE	ciliated epithelium
CG	ciliated groove
CM	caecum
CO	condyle
CP	communication pore complex
CS	central stomach

ABBREVIATIONS (Continued)

D	diaphragm
DP	diaphragm, operculum, and vestibular gland primordium
DPM	diaphragm parietal muscle
DW _O	distal wall of orifice
E	epidermis of ancestrula body wall
E:C	epidermis + cuticle
ES	"eyespot" (lateral pigment spots of aboral lobe of larva morphotype B)
F	funiculus
FM	border of frontal membrane
FW	frontal wall
ISA	internal sac area
L	lappets of orificial collar
LPS _a	lateral pigment spot - aboral lobe
LPS _O	lateral pigment spot - oral lobe
LW	lateral wall of daughter zooid
LY	lyrule
M	mouth
NM	nutritive mass
O	free edge of operculum
O _b	base of operculum
OL	oral lobe
OM	occlusor muscles of operculum
OV	ovicell
OV _p	proximal border of ovicell
P	pylorus

ABBREVIATIONS (Continued)

PL	pigment line
PLS _a	posterio-lateral pigment spots of aboral lobe of larva
PLS _o	posterio-lateral pigment spots of oral lobe of larva
PO	primary orifice
POC	pyriform organ complex: superior glandular field, vibratile plume, and ciliated groove
PPS _l	posterior pigment spot - lower oral lobe
PPS _u	posterior pigment spot - upper oral lobe
PR	polypide rudiment
PS	pigment spot
PVFM	parietal vaginal frontal muscle
PVM	parietal vaginal muscle
PX	pharynx
R	rectum
RM	retractor muscle
SCF	supra-coronal furrow
SP	spine
SP _c	cuticular spine
T	tentacle
TP	tentacle primordium
TS	tentacular sheath
TSP	tentacle sheath primordium
VG	vesibular gland
VP	vibratile plume

Plate 1

Figures A-1 through A-3

Larva of Parasmittina nitida, morphotype A, shown in three views. Prominent pigment spots indicated.

Figures B-1 through B-4

Larva of Parasmittina nitida, morphotype B, shown in four views. Prominent pigment spots indicated. Aboral cilia omitted.

- View:
1. Frontal view showing pyriform organ complex (superior glandular field, vibratile plume, and ciliated groove) and pigment spots.
 2. Posterior (morphotype A) and aboro-posterior (morphotype B) views showing pigment spots and the adjoining pigment lines.
 3. Right profile view showing the lateral pigment spot (morphotype A) and the "eyespot" (morphotype B) in direct lateral view. Vibratile plume and ciliated groove of the pyriform organ complex evident.
 4. Left profile view.

Plate 2

Parasmittina nitidia, morphotype A, larva (#1-2) photographed with the Zeiss Nomarski differential interference microscope.

1. Frontal view. Ciliated groove of pyriform organ complex, anterior pigment spots, and lateral pigment spots evident. X 310
2. Aboro-posterior view. Pigment spots of the aboral and oral lobes evident. X 297

Plate 3

Parasmittina nitida, morphotype A, larvae photographed at various angles with the Zeiss Nomarski differential interference microscope.

1. Larva #m-2--right latero-frontal view. Anterior and postero-lateral pigment spots evident. X 275
2. Larva #m-4--fronto-oral view. Ciliated groove area and anterior and lateral pigment spots evident. X 270
3. Larva #l-2--posterior view. Postero-lateral pigment spots of the aboral and oral lobes and posterior pigment spots evident. Note the pigment line joining the above mentioned pigment spots. X 300
- 4-5. Larva #G-1--basal view of larva in swimming phase of movement
 4. Focus is on the external portion of the ciliated groove. X 330
 5. Focus is on the corona and inner edges of the ciliated groove. X 327

Scanning electron micrographs of larvae of Parasmittina nitida, morphotype A.

1. Larvae #63 attached to scanning stub on its aboral lobe. Right lateral view of larva with ciliated groove evident. Artifact in upper left corner. X 155
2. Aboral region of ciliated groove of Figure 1 magnified to show vibratile plume. X 486
3. Posterior view of larva #63 with corona evident between the aboral and oral lobes. X 270
4. Latero-oral region of ciliated groove of Figure 1 magnified. X 538
5. Papillated surface of oral lobe of larva #63. X 480
6. Larva #46 attached to scanning stub on its left lateral surface. Neck region of internal sac evident in center of oral lobe. X 322
7. Larva #47. Papillated surface of descending wall of apical organ complex. Arrow points to sensory cap area. X 2419
8. Larva #42 attached to scanning stub on right edge of oral lobe and projecting at a 45° angle from the stub. Left lateral view of swimming larva with ciliated groove evident on functional basal surface. X 310
9. Aboral view of swimming larva of Figure 8 showing aboral cilia following the contour of the aboral lobe, and the invaginated sensory cap area of the apical organ complex. Artifact upper right corner. X 311

Plate 5

Parasmittina nitida, morphotype B, larva photographed with the Zeiss Nomarski differential interference microscope.

1. Larva #d. Frontal view showing anterior pigment spot and "eyespot." X 335
2. Larva #d. Aboro-posterior view showing the five prominent pigment spots of the aboral lobe and the postero-lateral pigment spots of the oral lobe. X 377
3. Larva #W-2. Aboro-posterior view showing the six prominent pigment spots of the aboral lobe. X 375

Plate 6

Parasmittina nitida, morphotype B, larvae photographed at various angles with the Zeiss Nomarski differential interference microscope.

Larva #Q-1.

1. Frontal view of larva showing "eyespot" and anterior pigment spots in the aboral lobe. X 270
2. Frontal view focused on "eyespot." X 270
3. Fronto-oral view showing ciliated groove area, anterior pigment spots and left "eyespot." X 250
4. Aboral view showing the 6 pigment spots of the aboral lobe, and the invagination of the sensory cap of the apical organ complex. X 260

Larva #O-1.

5. Aboral view showing invagination of sensory cap in center of apical organ complex, and corona. X 310
6. Oral view of the spiral form of the larva with the ciliated groove merging into the internal sac area. X 340

Plate 7

Scanning electron micrographs of larvae of Parasmittina nitida morphotype B. (Note: All larvae attached to scanning stub on their oral lobe.)

1. Larva #33a with corona evident between the sparsely ciliated aboral lobe and the more densely ciliated oral lobe. X 481
2. Fronto-lateral view of larva #31 with ciliated groove and glandular nature of oral lobe evident. X 374
3. Aboro-posterior view of larva #31b. Corona and transverse band of aboral cilia evident. X 414
4. Pigment spot and adjacent ciliated region shown in Figure 1 magnified. X 1140
5. Aboral view of larva shown in Figure 2. Transverse organization of aboral cilia evident. X 528
6. Region of the left "eyespot" shown in Figure 3 magnified. X 1303
7. Papillated surface of pigment spot of Figure 4 magnified. X 2571
8. Larva rotated approximately 180° on the aboral-oral axis from that shown in Figure 5. X 573
9. Upper portion of corona showing cilia (larva #27b). X 1079

Plate 8

Stages of development of *Parasmittina nitida*, morphotype B, Florida from the primary disc to the ancestrula with a functional polypide. Figures were drawn from a composite of living individuals as viewed from the ventral surface. Times of development from the onset of attachment and metamorphosis are given for each stage. Spines were omitted from the figures to enhance clarity of the internal anatomy of the developing zooid. Magnification approximately X 150.

<u>Stages</u>	<u>Time</u>
Primary disc -- larval pigment spots and lines evident	
A	.10 - 17 min.
B	30 - 37 min.
C	43 - 50 min.
Preancestrula -- differentiation of polypide rudiment	
D	50 - 57 min.
E	2 hrs. 55 min. - 3 hrs. 2 min.
F	6 hrs. 10 min. - 6 hrs. 52 min.
G	21 hrs. 35 min. - 22 hrs. 17 min.
H	26 hrs. 20 min. - 27 hrs. 2 min.
Preancestrula -- differentiation of diaphragm, vestibular glands and operculum	
I	29 hrs. 50 min. - 30 hrs. 32 min.
J	34 hrs. 15 min. - 35 hrs. 12 min.
K	37 hrs. 30 min. - 39 hrs. 12 min.
L	50 hrs. - 56 hrs. 2 min.
Preancestrula -- differentiation of alimentary canal of polypide	
M	79 hrs. 20 min. - 85 hrs. 22 min.
N-1 ^a	88 hrs. 20 min. - 94 hrs. 22 min.
N-2 ^b	(3.7 - 3.9 days)
Preancestrula -- differentiation of extra-zooidal structures	
O	103 hrs. 40 min. - 117 hrs. 57 min.
P	127 hrs. 25 min. - 166 hrs. 59 min.
Ancestrula -- differentiation of alimentary canal complete and nutritive mass completely absorbed	
Q-1 ^a	176 hrs. - 217 hrs. (7.3 - 9 days)
Q-2 ^b	

^alophophore retracted

^blophophore extruded, and tentacles and operculum omitted

Plate 8 (Continued)

Preancestrula --	differentiation of diaphragm, vestibular glands and operculum
J	34 hrs. 15 min. - 35 hrs. 12 min.
K	37 hrs. 30 min. - 39 hrs. 12 min.
L	50 hrs. - 56 hrs. 2 min.
Preancestrula --	differentiation of alimentary canal of polypide
M	79 hrs. 20 min. - 85 hrs. 22 min.
N-1 ^a	88 hrs. 20 min. - 94 hrs. 22 min.
N-2 ^b	(3.7 - 3.9 days)

Plate 8 (Continued)

Preancestrula -- differentiation of extra-zooidal structures

O	103 hrs. 40 min. - 117 hrs. 57 min.
P	127 hrs. 25 min. - 166 hrs. 59 min.

Plate 8 (Continued)

Ancestrula -- differentiation of alimentary canal complete
and nutritive mass completely absorbed
Q-1^a 176 hrs. - 217 hrs. (7.3 - 9 days)
Q-2^b

Plate 9

Various stages in the development of five individuals of Parasmittina nitida, morphotype B, Florida. Photographs were taken from the dorsal surface of living organisms with an inverted Unitron Phase Contrast Microscope. Letters of the stages correspond to those in Plate 8.

	<u>Stages</u>	<u>Individuals</u>
Primary disc:	A - C	F-3 # 1a
Preancestrula:	D - F, H, J	F-3 # 4
	K, M	F-3 # 2
	N-1, N-2	F (8-20-73) # 2
Ancestrula:	Q-1, Q-2	F (8-6-73) # 1b

Plate 9 (Continued)

	<u>Stages</u>	<u>Individuals</u>
Preancestrula:	E - F, H, J	F-3 # 4
	K, M	F-3 # 2

Plate 9 (Continued)

	<u>Stages</u>	<u>Individuals</u>
Preancestrula:	N-1, N-2	F(8-20-73) # 2
Ancestrula:	Q-1, Q-2	F(8-6-73) # 1b

N-1

N-2

Q-1

Q-2

Plate 10

Scanning electron micrographs of various stages of development of Parasmittina nitida, morphotype B, Florida. Letters of the stages correspond to those in Plate 8.

Specimens fixed in 4% glutaraldehyde and dehydrated with the critical point method.

Stage C -- Late primary disc with apical organ complex still evident as a partial covering of the disc. X 403

E -- Preancestrula stage with cuticle evident at lateral and distal edge. X 182

G -- Distal portion of preancestrula showing cuticular spines surrounding frontal membrane. X 331

L -- Preancestrula with base of spines calcified. X 158

Q_C-- Spine with calcium carbonate core and cuticular sheath. Daughter bud shown in background. X 1606

Specimens fixed in 95% ethanol and cuticle removed with 5% sodium hypochlorite.

Q_a-- Ancestrula with spines. X 171

Q_b-- Lateral view of ancestrula with communication pores evident. X 164

Plate 11

Various stages in the development of one individual of *Parasmittina nitida*, morphotype B, North Carolina. Photographs were taken from the dorsal surface of the living organism with an inverted Unitron Phase Contrast Microscope. Letters of the stages correspond to those in Plate 8. Approximate times of development from the onset of attachment and metamorphosis are given for each stage. X 150

<u>Stages</u>	<u>Time</u>
Preancestrula -- differentiation of polypide rudiment	
F	7 hrs.
G	34 hrs.
I	53 hrs.
Preancestrula -- differentiation of alimentary canal of polypide	
M	78 hrs.
P-1 ^a	133 hrs
P-2 ^b	

^alophophore retracted

^blophophore extruded

Plate 12

Various stages in the development of six individuals of *Parasmittina nitida*, morphotype A, North Carolina, from the primary disc of a metamorphosed larva to that of the completed preancestrula. Photographs were taken from the dorsal surface of the living organisms with an inverted Unitron Phase Contrast Microscope. Letters of the stages correspond to those in Plate 8. Times of development from the onset of attachment and metamorphosis are given for each stage. X 150

<u>Stages</u>	<u>Time</u>	<u>Individuals</u>
Primary disc		
B	30-37 min. ^C	I-30
Preancestrula -- differentiation of polypide rudiment		
D	--	J-28
E	15 hrs. 35 min.	J-30 #1
F	30 hrs. 15 min. - 30 hrs. 45 min.	J-30 #1
H	53 hrs. 45 min. - 54 hrs. 15 min.	J-28
Preancestrula -- differentiation of diaphragm, vestibular glands and operculum		
I	--	B-31 #2
J	61 hrs. 30 min. - 62 hrs.	O-3
K	--	B-31 #2
L	73 hrs. 20 min. - 73 hrs. 50 min.	B-31 #1
Preancestrula -- differentiation of alimentary canal of polypide		
M	90 hrs. 50 min. - 91 hrs. 20 min.	O-3
N-1 ^a	116 hrs. - 121 hrs. 15 min.	B-31 #1
N-2 ^b		
O	138 hrs. 5 min. - 146 hrs. 25 min.	B-31 #2

^alophophore retracted

^blophophore extruded

^Cinitial time from that of the corresponding stage of morphotype B larva, Florida

Plate 12 (Continued)

<u>Stages</u>	<u>Time</u>	<u>Individuals</u>
Preancestrula -- differentiation of diaphragm, vestibular glands, and operculum		
K	--	B-31 #2
L	73 hrs. 20 min. - 73 hrs. 50 min.	B-31 #1
Preancestrula -- differentiation of alimentary canal of polypide		
M	90 hrs. 50 min. - 91 hrs. 20 min.	O-3
N-1 ^a	116 hrs. - 121 hrs. 15 min	B-31 #1
N-2 ^b		
O	138 hrs. 5 min. - 146 hrs. 25 min.	B-31 #2

Plate 13

Sequence of scanning electron micrographs showing spine and orificial collar formation in daughter zooids of Parasmittina nitida morphotype A, North Carolina. (Ancestrula indicated where evident.)

1. Colony of three zooids with lateral wall of developing daughter zooid indicated. X 66
2. Lateral view of colony showing lateral and distal walls of a developing daughter zooid. (Organic material partly removed.) X 142
- 3a. Daughter zooid showing initial stage in spine formation. X 177
- 3b. Area of spine formation shown in Figure 3a magnified. X 525
4. Bases of orificial spines completely formed, and primordial distal wall of primary orifice complete in second zooid of colony. (Organic material partly removed.) X 109
5. Daughter zooid with partly calcified frontal surface possessing areolae. Distal portion of the primary orifice completely formed. X 205
6. Primary orifice characterized by a well formed distal wall and condyles. Bases of spines surrounding distal wall indicated. (Organic material partly removed.) X 411

Plate 13 (Continued)

- 7a. Frontal view of colony showing daughter zooids in two stages of development. Daughter zooid (upper left corner in photograph) characterized by orificial spines on the distal border of the primary orifice and a convex frontal wall. Second daughter zooid (upper right) with early collar formation. X 98
- 7b. Lateral view of colony shown in Figure 7a. (Daughter zooid in lower right corner of photograph corresponds to zooid in upper right corner of photograph in Figure 7a). X 140
- 8a. Lateral view of completely formed orificial collar lappets rising above the distally located spines. X 346
- 8b. Fronto-ventral view of primary orifice shown in Figure 8a. X 365
- 9a. Right condyle showing toothed structure on medial side. X 1526
- 9b. Left condyle showing toothed structure. X 1425
- 10a. Formed daughter zooid showing orificial spines, areolae, and orificial collar lappets in frontal view. X 177
- 10b. Lateral view of zooid shown in Figure 10a. X 181

Plate 14

Morphological features of colonies of Parasmittina nitida, morphotype A, North Carolina. Organic material removed. Colonies photographed with a Wild dissecting microscope with a Pentax attachment. Colony 3^AA-3 was initiated July 3, 1972, and preserved July 1, 1973. (All photographs are of this colony unless otherwise stated.)

1. Entire colony showing secondary and tertiary layers of growth. X 1.8
2. Region of astogenetic repetition in colony #A-5 dish 23A-5-4 (Growth period: June 29, 1972 through August 29, 1973). Center of colony is toward the lower right hand corner of photograph. X 18.9
3. First layer of growth showing linear arrangement of zooids, acute avicularia and ovicells. X 39
4. Second and third layers of growth showing lack of zooid orientation. X 19.6
5. Second layer zooids showing arrangement of zooids bearing ovicells. X 39.2
6. Second layer of growth showing nonreproductive zooids with medially located avicularium. X 39.2

Plate 15

Morphological features of colonies of Parasmittina nitida, morphotype B, North Carolina and Florida.

North Carolina. Organic material removed from zooids.

- 1a. Colony 18B-4-4 #E-6 -- Growth period: June 21, 1972 through July 7, 1973. X 7.6
- 1b. Increased magnification of two adventitious zooids shown in Figure 1a. X 43.5
- 2a. Colony 18B-4-9 #C-6 -- Growth period June 26, 1972 through July 4, 1973. Initial stage of second layer of growth indicated. X 7.9
- 2b. Increased magnification of zooids from the first layer of growth (Figure 2a) showing multiple avicularia per zooid. X 56.6

Florida. Colony 2-11-D-3 #D-4 photographed live (See Plate 17 for SEM.)

- 3a. Growth period: February 17, 1973 through April 6, 1973. Initial five zooids marked by a number 1; however, they did not form simultaneously. X 17
- 3b. Increased magnification of zooids (Figure 3a) showing orificial spines (2 per zooid). X 8.5
- 3c. Growth period: February 17, 1973 through April 26, 1973. X 8.5
- 3d. Increased magnification of central portion of colony shown in Figure 3c. Note the increase in structural detail in the frontal walls of the zooids. X 35.4

Plate 16

Young colonies of Parasmittina nitida, morphotype B, Florida maintained in an aquarium in a constant temperature room and fed cultures of Oxyrrhis marina (Dunaliella/ Monochrysis). Growth period indicated. Budding pattern of ancestrula indicated by numbers where possible.

1. Colony 4-11-G #E-2 (April 11, 1973 through May 2, 1973). X 60.0
- 2a. Colony 4-9-C #B-1 (April 9, 1973 through May 1, 1973). X 60.5
- 2b. Colony shown in Figure 2a 10 days later. X 30.0
- 3a. Colony 4-9-C #E-2 (April 9, 1973 through May 13, 1973). X 29.7
- 3b. Increased magnification of Figure 3a showing spination and polypides of double zooid. X 59.4
4. Colony 4-9-C #B-6 (April 9, 1973 through May 13, 1973) showing completed double zooids. X 60.0

Plate 17

Scanning electron micrographs of first generation colony 2-11-D-3 #D-4 Parasmittina nitida, morphotype B, Florida which was raised in an aquarium supplied with Oxyrrhis marina and maintained in a constant temperature room. Growth period: February 17, 1973 through May 20, 1973. (Organic material partly removed.)

1. Lateral view of marginal zooids with lappets evident. X 85
2. Lateral view of zooids approximately midway in linear growth from ancestrula. X 43
3. Frontal view of zooids with narrow area between orificial collar lappets evident. X 97
4. Increased magnification of a typical zooid. Note the orificial spines are not covered by secondary calcification. X 190
5. Right condyle with toothed structure evident. X 4800
6. Left condyle with toothed structure evident. X 4786

Plate 18

Parasmittina nitida, morphotype A, North Carolina,
parental colonies.

1. View of zooids with avicularia and lyrule indicated.
 - a. Frontal view. X 113
 - b. Latero-frontal view. X 116
2. Condyles magnified to show toothed structure on medial side.
 - a. Right. X 1061
 - b. Left. (Membrane joining condyle and lyrule broken at condyle end.) X 1077
3. Frontal view of ovicell bearing zooids. X 75
4. Zooid with ovicell, which is characterized by many small pores in the frontal surface. X 117
5. Lateral view of hyperstomial ovicells showing secondary calcification on distal and lateral edges. (Arrow points to the region.) X 119
6. Frontal surface of ovicell with pores and secondary calcification. X 289

Plate 19

Parasmittina nitida, morphotype A, North Carolina,
first generation colonies (less than 100 zooids per
colony).

1. Frontal view of marginal zooids showing three distal spines. Secondary calcification obscures spination of lower medial zooid. X 133
2. Frontal view of zooids bearing four spines on the distal border of the primary orifice. X 179
- 3a. Fronto-ventral view of primary orifice showing lyrule and condyles. X 332
- 3b. Frontal view of zooid shown in Figure 3a showing orificial collar lappets. X 363
- 3c. Increased magnification of Figure 3a showing prominent condyles with their toothed structure. X 926

Plate 20

Parasmittina nitida, morphotype A, North Carolina, second generation colonies. (Ancestrula (AN) and order of budding indicated where possible.)

1. Colony #22⁺A-13-2 three zooids present. X 127
2. Colony 3^aA-3a #A-4 five zooids present. X 59
3. Colony 3^aA-3a #A-4 edge nine zooids present.
 - a. Frontal view of whole colony. X 66
 - b. Left lateral view of whole colony. X 69
 - c. Frontal view showing ancestrula and early buds. X 133
 - d. Frontal view of marginal zooids. X 135
 - e. Lateral view of marginal zooids showing orificial collar lappets and spines. X 134
 - f. Fronto-ventral view of primary orifice. X 319
 - g. Increased magnification of base of spines shown in Figure 3f. X 684
 - h. Left condyle magnified to show toothed structure. X 1655

Plate 21

Parasmittina nitida, morphotype B, North Carolina,
parental colonies.

1. Frontal view of zooid with ovoid avicularium and two orificial spines. X 151
2. Lateral view of zooids showing orificial collar lappets. X 158
- 3a. Primary orifice magnified to show lyrule, condyles, and orificial spines. X 326
- 3b. Narrow lyrule evident between toothed condyles. X 881
- 4a. Fronto-ventral view of ovicells each with an avicularium on the distal edge, and an ovoid avicularium lateral to the orificial border of the primary orifice. X 135
- 4b. Lateral view of ovicells shown in Figure 4a. X 133
5. Frontal surface of ovicell magnified to show pore structure and impinging secondary calcification. X 328

Plate 22

Parasmittina nitida, morphotype B, North Carolina,
first generation colonies.

1. Frontal view of zooids showing two distal spines. X 129
2. Fronto-ventral view of primary orifice showing lyrule and condyles. X 342
- 3a. Proximal margin of primary orifice magnified to show lyrule and condyles. X 897
- 3b. Right condyle showing toothed projections. X 2111
4. Lateral view of zooids with the rostrum of an avicularium elevated on the orificial lappet of the zooid in the foreground. X 150
5. Frontal view of orifice showing elevated collar and bases of two orificial spines. X 408

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BIOGRAPHICAL SKETCH

Edythe Marie Humphries was born in Brooklyn, New York on October 6, 1944, and was raised in Teaneck, New Jersey. Upon graduation from Teaneck High School in 1962, she entered Wittenberg University, Springfield, Ohio and received a Bachelor of Arts degree in Biology in June 1966. In September 1966 she entered the University of Delaware, Department of Marine Biology to work for a Master of Science degree. From September 1966 through August 1967, she was in charge of a shellfish survey in Indian River Bay and Rehoboth Bay, Delaware sponsored by the Northeast Marine Health Science Laboratory. A publication entitled "Shellfish survey of Indian River Bay and Rehoboth Bay, Delaware, June 1967. University of Delaware Marine Lab. and Northeast Marine Health Science Lab. Tech. Rep. 85 p." resulted from this study. She graduated with a Master of Science in Biology in June 1970. From June to August 1970 she worked on the taxonomy of mollusks at the Shellfish Laboratory, University of Delaware Marine Laboratory.

In September 1970 she began studies toward a Doctor of Philosophy degree in Zoology at the University of Florida. She held a research assistantship for one year under the direction of Dr. Frank Maturo, Jr. to work

on a taxonomic study of the Bryozoa collected from the Gulf of Mexico by the Dept. Natural Resources, Fla. In October 1971 she was awarded a NSF doctoral dissertation research grant in the amount of \$4,100 for the period October 1971 through March 1974. During the summer of 1971 she studied invertebrate embryology at the Friday Harbor Marine Laboratory, University of Washington as a NSF trainee. From September 1971 through June 1974 she worked as a graduate assistant in the Department of Zoology.

She is a member of the biology honorary Beta Beta Beta, and Sigma Xi. She is also a member of the following professional societies: Atlantic Estuarine Research Society, American Fisheries Society, American Littoral Society, Florida Academy of Science, American Society of Zoologists, and the International Bryozoology Association. She has presented two papers--one at the Florida Academy of Science 35th Annual Meeting in Melbourne, Florida, March 1971; and one at the International Bryozoology Association 2nd International Conference in Durham, England, September 1971--both of which have resulted in publications. "Mollusks at Seahorse Key" Quart. J. Florida Acad. Sci. 34 (Suppl. 1):5 (Abstract), and "Seasonal settlement of bryozoans in Rehoboth Bay, Delaware," p. 115 to 128. In G. P. Larwood (ed.) Living and Fossil Bryozoa. Academic Press, New York.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank J. S. Maturo, Jr., Chairman
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John W. Brookbank
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Henry C. Aldrich
Associate Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank G. Nordlie
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A handwritten signature in dark ink, appearing to read 'J. Reiskind', is written over a horizontal line.

Jonathan Reiskind
Associate Professor of Zoology

This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1974

Dean, Graduate School