

**Larval Behavior and Post-Larval Development in *Parasmittina nitida*
Morphotype B (Bryozoa: Cheilostomata)**

E. M. HUMPHRIES

Zoology Department, University of Florida, Gainesville, Florida 32611

SYNOPSIS. Larval behavior and metamorphosis in *Parasmittina nitida* morphotype B from the Gulf of Mexico has been studied. The larvae have two basic types of movement: (1) a clockwise-counterclockwise movement about the aboral-oral axis of the lobular larval form resulting in either slow horizontal or rapid vertical movement, and (2) a directed horizontal movement of the creeping larval form, whereby either the oral lobe is pressed against the substrate or the aboral-oral axis is tilted forward. In both forms, the vibratile plume of the pyriform organ complex extends the leading edge of the larva. Metamorphosis was observed with Nomarski differential interference microscopy in living specimens and with scanning electron microscopy in fixed specimens. Polypide development—in particular, the formation and diminution of the nutritive mass, the differentiation of the polypide rudiment, diaphragm, vestibular glands, operculum, major components of the musculature and alimentary canal, and the early stages of astogenetic growth—is described. The tana ancestrula of this species is characterized by a frontal wall calcified distally to the aperture, which is surrounded by nine erect spines. The polypide feeds actively within seven to eight days after the onset of larval attachment and metamorphosis under laboratory conditions of 22°C.

INTRODUCTION

Morphological descriptions of both larvae and early developmental stages of ascophoran Bryozoa have been reported in the literature as early as 1877 (Barrois, 1877, cited by Marcus, 1939). However,

The present address of the author is: 1390 Milford Terrace, Teaneck, New Jersey 07666.

This research was supported by NSF Grant #GB31262 and the Division of Biological Sciences and the Department of Zoology, University of Florida. Portions of this paper are from a dissertation submitted to the Graduate Faculty of the University of Florida in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology.

I would like to thank Russel Zimmer, Frank Maturro, Jr., and Robert Woollacott for their critical reading of this manuscript; Paul Laessle for his constructive criticism and aid with the line drawings; members of the Undergraduate Research Apprentice Program, University of Florida for their help in field collections and data analysis; Ruth Sturrock, Veterans Administration Hospital, Gainesville, Florida for her invaluable instruction in ultrastructural techniques and for her aid in securing the loan of VAH equipment; and the Department of Biological Sciences, University of Delaware for the use of darkroom facilities.

the most frequently studied groups within the Bryozoa are the ctenostomes and the anascan cheilostomes, in particular *Alicyonidium* spp. and *Bugula* spp. respectively. Larval morphology and metamorphosis were originally described in detail for *Alicyonidium polyoum* by Barrois (1877) and Harmer (1887) respectively (cited by d'Hondt, 1972), and recently verified by scanning electron microscopy (Loeb and Walker, 1977). Larvae of *Bugula neritina* were described initially by Calvet (1898) and elaborated upon by Woollacott and Zimmer (1972), who also described the metamorphosis of this species (Woollacott & Zimmer, 1971).

Within the past three years, however, the naked planktonic lecithotropic larvae and the post-larval development of a few species of ascophoran cheilostomes have been described (Cook, 1973; Humphries, 1974). Early astogenetic growth in several species of ascophoran cheilostomes has also been described (Gordon, 1971; Mawatari, 1946; Moyano, 1972; Soule & Soule, 1972).

The objective of the present study was to

describe the differentiation of the primary disc into the actively feeding tata ancestrula in the mucronellid ascophoran *Parasmittina nitida* (Verrill, 1875) morphotype B. Larval morphology and behavior are described in order to complete the information presently known on the early life stages in this species.

Parasmittina nitida type material has two "varieties" designated A and B by Maturo and Schopf (1968). Larval morphology, post-larval development, astogenetic growth patterns and offspring variation studies, the latter initiated by Maturo (1973), have shown that these morphotypes are two distinct species (Humphries, 1974). The specific name *Parasmittina nitida* morphotype B is currently under consideration pending comparison of the recent specimens collected in Bogue Sound, North Carolina and in the Gulf of Mexico with that of the lecto- and paralectotypes.

Adult colonies of *Parasmittina nitida* morphotype B are readily found as encrustations on rocks and shell litter in shallow bays from Vineyard Sound, Massachusetts, to the Gulf of Mexico (Humphries, 1974). These colonies are characterized by zooids possessing single hyperstomial ovicells (brood chambers), which are apparently formed from evaginations of the body wall at the frontodistal margin of the maternal zooid subsequent to egg development in the ovary. Reproductively active colonies are observed to have a developing embryo in the ovicell and eggs in various stages of development in the ovary. Only one embryo develops in the ovicell at any one time; however, the ovicell is utilized repeatedly throughout that reproductive period and in subsequent reproductive periods following its formation as long as it remains intact, free of epifaunal organisms, and not covered by secondary growth of the colony.

Individuals of *P. nitida* morphotype B are reproductive throughout the year in nearshore waters of the Gulf of Mexico; however, most colonies are reproductive from spring through fall. No information is available as to the mode of fertilization employed by this species of bryozoan.

MATERIALS AND METHODS

In-vitro studies

Shell litter was obtained with a scallop dredge from the Gulf of Mexico in the vicinity of Cedar Keys, Florida in August 1973. Mature colonies of *Parasmittina nitida* morphotype B, with a minimum of twenty ovicells containing mature larvae, were separated with tile cutters from the remaining epifauna encrusting the substrate fragment. The attached colony was subsequently cleaned to remove all epifaunal organisms.

To ensure maximum larval release, colonies were placed in plastic petri dishes containing aerated seawater within hours of collection. The dishes were placed on a dark surface, the seawater aerated, and the organisms exposed to a light-dark cycle to enhance larval release and attachment. Colonies which released larvae were transferred to a second petri dish for later use.

Behavior of mature larvae, characterized by a pale peach color and distinct orange-red pigment spots, was studied within the ovicell, during extrusion, and upon release into the seawater. Twenty-six larvae were observed and photographed throughout metamorphosis and primary disc differentiation. Differentiation in the post-metamorphic individuals, maintained *in vitro* in nonaerated seawater at 22°C, was observed on a variable time frequency ranging from 20 minutes, for the early stages of the differentiation of the primary disc to the preancestrula, to 29 hours for the stages involving the completion of the differentiation of the alimentary canal and the development of the extrazoidal structures. The petri dishes were cleaned prior to each observation with a fine bristle artists' brush and the seawater changed following each observation period. For those individuals observed at 12-hour intervals, the seawater was changed at least twice in a 12-hour period.

SEM studies

Upon completion of the metamorphosis experiment, the tata ancestrula was fixed

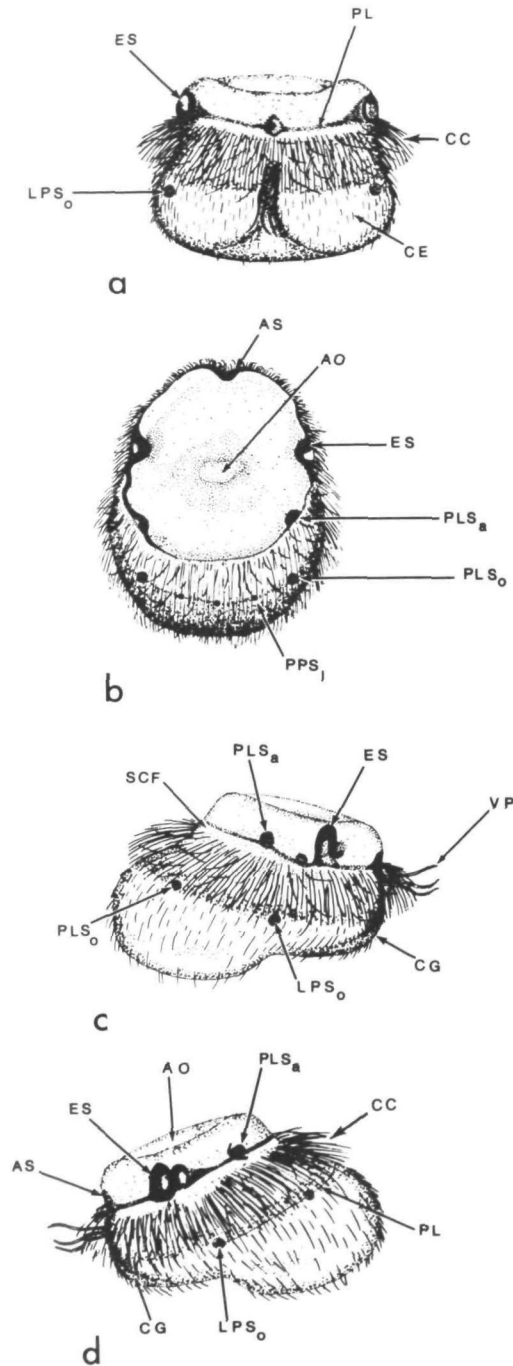


FIG. 1. Free hand drawing of the free-swimming lobulated form of the larva of *Parasmittina nitida* morphotype B. (Aboral cilia omitted.) Frontal view (a), aboroposterior view (b), profile view (c-d). AO, apical organ complex; AS, anterior pigment spot(s) of larva; CC, coronal cilia; CE, ciliated epithelium; CG, ciliated groove; ES, "eyespot" (lateral pigment spot of

in either 95% ethanol or 4% glutaraldehyde buffered with sodium cacodylate and adjusted to the osmolality of the seawater in which the ancestrula developed. In addition, individuals in various developmental stages were fixed in glutaraldehyde and stored in a sodium cacodylate buffer solution.

Individuals were prepared for SEM using critical point drying (Anderson, 1951). Ancestrulae, attached to a plastic petri dish, were dehydrated through a 5% graded series of ethanol concentrations from 5% through 100%, placed in a second change of 100% ethanol, and then processed through a 10% graded series of Freon 113-ethanol from 50% through Freon 113. The individuals were then transferred to a clean solution of 100% Freon 113 (1,1,2 trichloro-trifluoro-ethane or Freon degreaser MS-182 [Miller-Stephenson Chem. Co., Inc.]). Freon 13 (chloro-trifluoro-methane) was the transitional fluid at a critical pressure of 38 atmospheres and a critical temperature of 28.9°C.

The tata ancestrulae fixed in 95% ethanol were air-dried following removal of the cuticle with 5% sodium hypochlorite.

All specimens were coated with a minimum thickness of 300 Å of gold palladium alloy and fastened to a SEM stub with E-Kote silver epoxy conductive paint. The Cambridge Stereoscan II microscope, with an optimal resolution of 250 Å, was utilized to study surface morphology.

RESULTS AND DISCUSSION

Larval morphology

Larvae of *Parasmittina nitida* morphotype B measure approximately 180 μm in the aboral-oral axis in the lobulated larval form (Figs. 1, 2). The corona, a large

aboral lobe of larva; LPS_o, lateral pigment spot of oral lobe; PL, pigment line; PLS_a, posterolateral pigment spot of aboral lobe; PLS_o, posterolateral pigment spot of oral lobe; PPS_l, posterior pigment spot of lower oral lobe; SCF, supracoronal furrow (=pallial sinus region); VP, vibratile plume. (After Humphries, 1975). Approximately × 143.

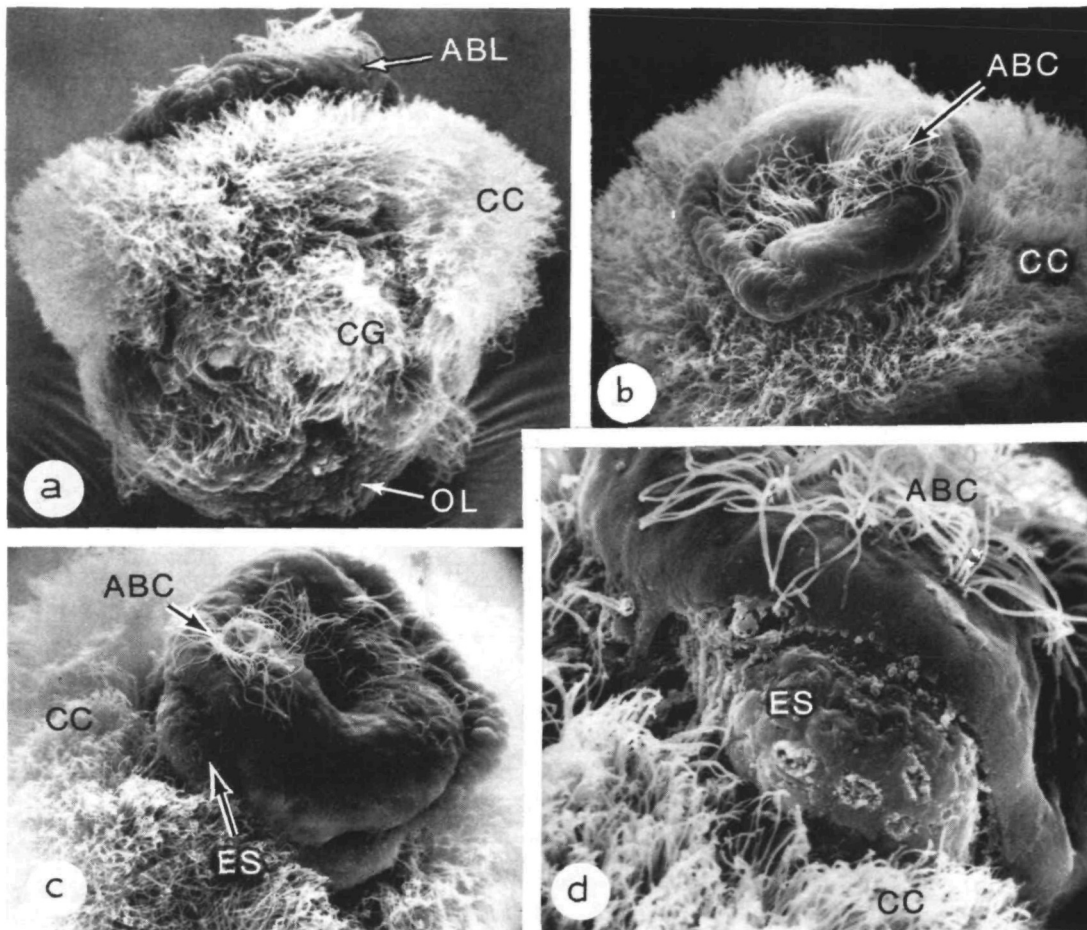


FIG. 2. Scanning electron micrographs of a larva of *Parasmittina nitida* morphotype B. (Larva attached to scanning stub on its oral lobe.)

- a) Frontolateral view of lobulated form of larva with aboral lobe (ABL), corona (CC), ciliated groove (CG) of vibratile organ complex, and glandular oral lobe (OL) indicated. $\times 530$.
 b) Aboral pole view of larva shown in Figure 2a with

transverse band of aboral cilia (ABC) and corona (CC) indicated. $\times 580$.

- c) Aboral pole view of larva rotated 180° clockwise on the aboral-oral axis from that shown in Figure 2b. Transverse band of aboral cilia (ABC), left "eyespot" (ES), and corona (CC) indicated. $\times 680$.
 d) Region of left "eyespot" shown in Figure 2c magnified. $\times 1580$.

dense ciliary field encircling the larva and superficially dividing it into two lobes, is the primary locomotory organ (Fig. 2a). Immediately oral to the corona in frontal view is the pyriform organ complex consisting of a conspicuous ciliated groove, lined with rows of cilia shorter than the coronal cilia, and vibratile plume (Fig. 2c). The plume extends forward during horizontal movement of the larva and comes to lie within the ciliated groove upon completion of its effective stroke.

The oral lobe of the larva is uniformly

covered with cilia on its lateral surface and sparsely ciliated in the glandular internal sac area of the oral pole. The cilia of the aboral lobe are limited to a band transversing the aboral pole (Figs. 2b, 2c); these are the shortest cilia on the larva. The nervous-sensory complex referred to as the apical organ complex occupies the majority of the aboral lobe of the larva (Fig. 2c).

The location, number, and complexity of pigment spots were proposed by Humphries (1975) as important diagnostic

characters differentiating larvae of morphotypes A and B of *Parasmittina nitida*. Larvae of *P. nitida* morphotype B have two large pigmented areas (Fig. 2d) located laterally on the aboral lobe. The shape of these pigmented fields, referred to as "eyespot," differs between the right and left view of an individual and also among individuals. Woollacott and Zimmer (1972) observed a similar pigment spot complex with transmission electron microscopy in *Bugula neritina*. These "eyespot" are thought to be responsible for the phototactic response of bryozoan larvae (Ryland, 1960).

In addition to the two prominent pigment fields, there are three to four smaller pigmented areas in the lateral surface of the aboral lobe; all pigmented areas are connected by a pigment line. At least four pigment spots are located in the lateral surface of the oral lobe (Fig. 1a-d), and are connected laterally and posteriorly by a pigment line. The pigmented areas are probably areas of concentrated subepidermal pigment cells similar to that reported in *Bugula neritina* (Woollacott & Zimmer, 1971).

Larvae of *P. nitida* morphotype B differ from other species of ascophoran larvae. The umbonuloid ascophoran *Metrarabdotos unguiculatum* larvae are larger (average diameter 500 μm), of a deeper body color (reddish purple as opposed to pale peach), ovoid in shape, and lack pigment spots. The cryptocystidean ascophoran *Stylopoma duboisii* larvae are yellowish-pink, measure 250 μm in diameter, possess four large bright red pigment spots oral to both the pallial furrow (=supracoronal furrow) and a ring of smaller pigment spots, and are usually ovoid in shape (Cook, 1973). The larvae of *Savignyella lafontii* (120 μm) show the greatest color diversity; the center of the apical organ complex is carmine, the pallial zone (=supracoronal furrow) has numerous black spots, and the oral lobe has three distinct color bands, two large ocular pigment spots and two slightly smaller pigment spots (Marcus, 1939). Pigmentation patterns in lecithotropic ascophoran larvae have also been described in detail for *Catenicella contei*,

Schizoporella carvalhoi, and *Hippoporella gorgonensis* (Marcus, 1939). However, additional research on all aspects of the morphology of lecithotropic larvae is needed before larval characters may be used as taxonomic and phylogenetic parameters, as proposed by Nielsen (1977).

Larval behavior

The developing embryo rotates within the membranes of the ovicell prior to its release in the larval stage. Extrusion of the larva through the aperture of the ovicell into the surrounding seawater occurs within one to two minutes, during which time the larva twists and constricts. Following extrusion, the larva is positively phototropic.

Three forms of movement were observed *in vitro* for released, free-swimming larvae of *Parasmittina nitida* morphotype B: (1) an alternating slow clockwise-counterclockwise spiral movement about the aboral-oral axis of the lobulated larva, with either the oral or aboral lobe oriented toward the substrate, resulting in horizontal movement; (2) a rapid spiral movement about the aboral-oral axis with the oral lobe oriented toward the substrate, resulting in a directed, but sporadic, vertical movement through the water column; and (3) a slow, but extensive, creeping movement along the bottom of the petri dish or beneath the surface film of the seawater. In this horizontal type of movement, the larva has either the oral lobe or the frontal surface (*i.e.*, pyriform organ complex) oriented toward the substrate. In the former case, the larva is depressed along the aboral-oral axis and the frontal surface of the oral lobe is directed forward and extends beyond the aboral lobe. In the latter case, the lobulated larva is compressed laterally and tilted forward at an angle approximately 40° to the substrate, resulting in the extension of the frontal surface of the aboral lobe beyond that of the oral lobe. It is hypothesized that creeping movement persists during site selection (Humphries, 1974) and that the vibratile plume "selects" the site for attachment (Ryland, 1970). Intermittent between the

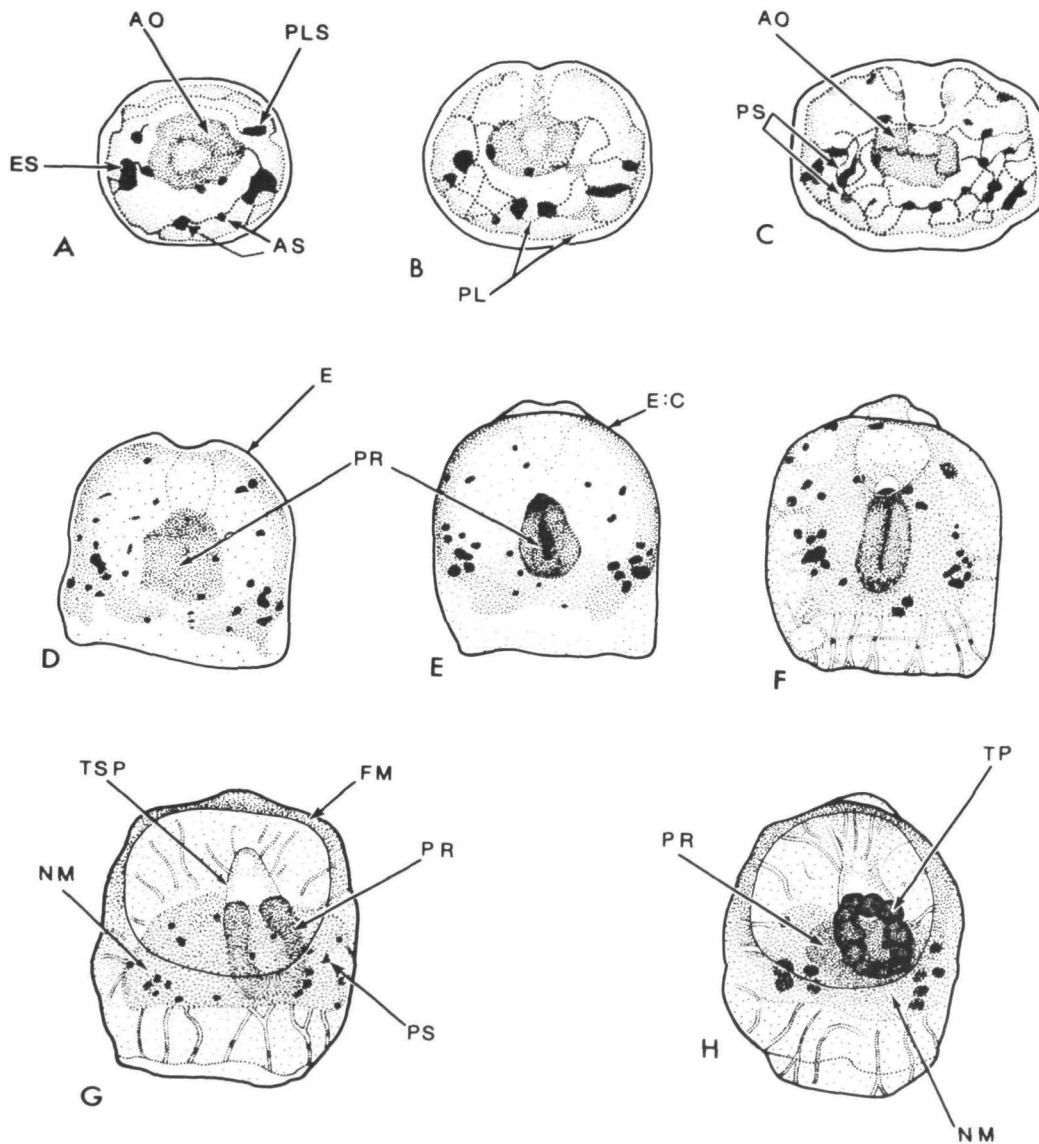
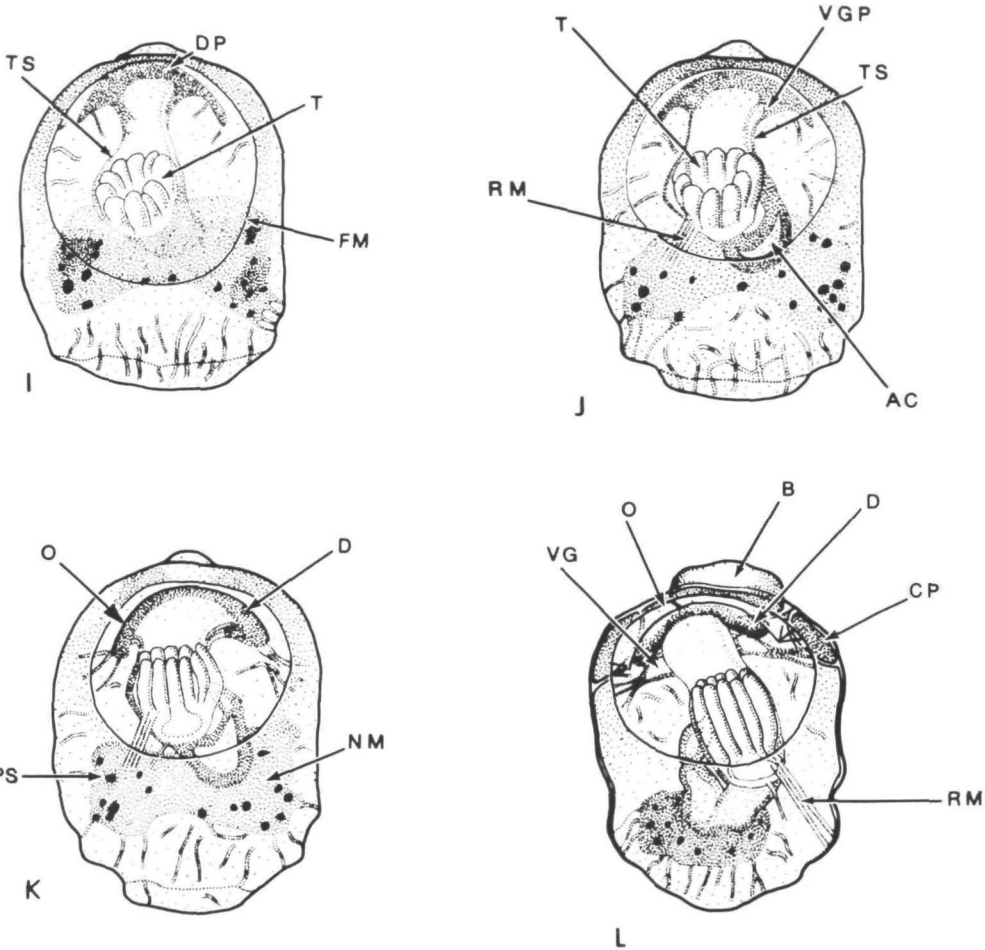


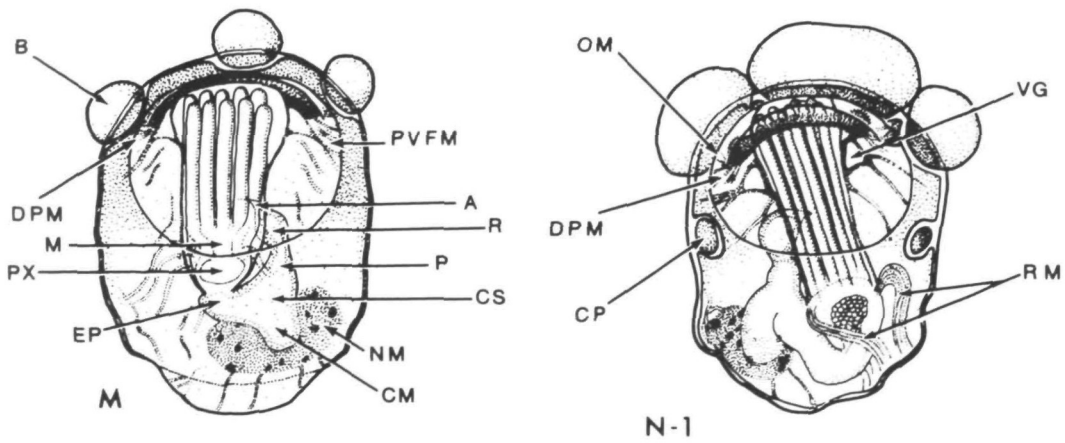
FIG. 3. Polypide development in *Parasmittina nitida* morphotype B from the primary disc to the feeding tana ancestrula. Figures were drawn from a composite of living individuals as viewed from the basal and frontal surfaces. Approximate times of the completion of specific developmental stages from the onset of attachment and metamorphosis of larvae are for experimental conditions of 22°C. Spines were omitted from the frontal surface to enhance clarity of the internal anatomy of the developing zooid. Magnification approximately $\times 144$. -1, lophophore retracted; -2, lophophore extruded, and tentacles and operculum omitted.

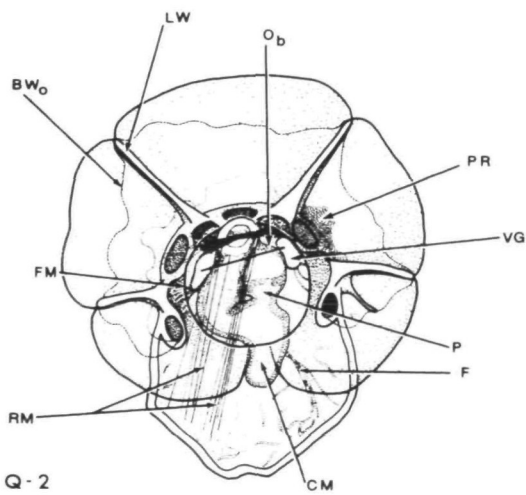
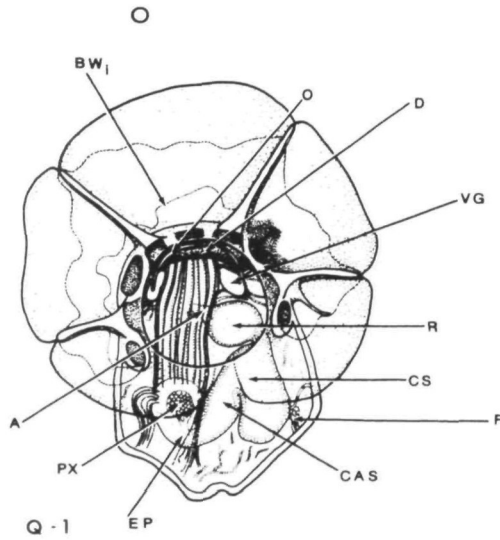
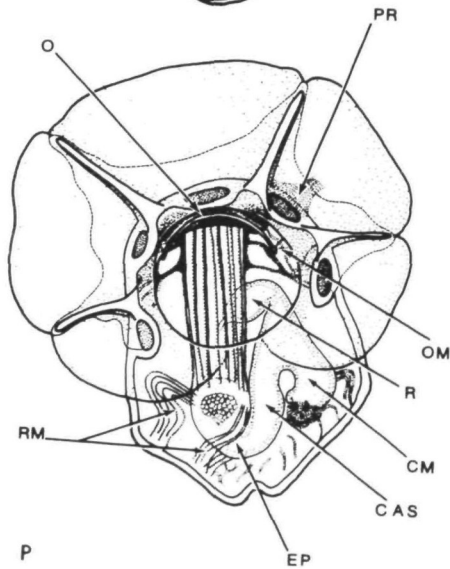
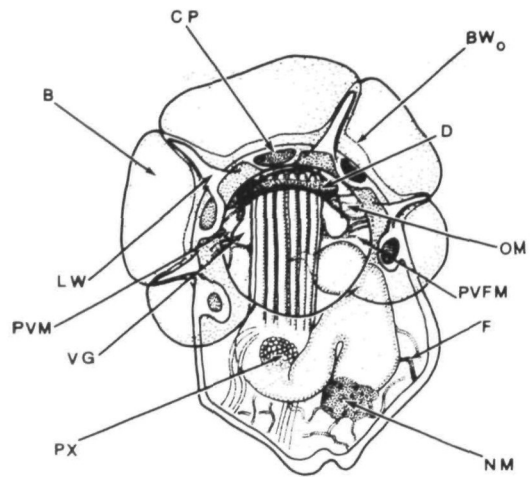
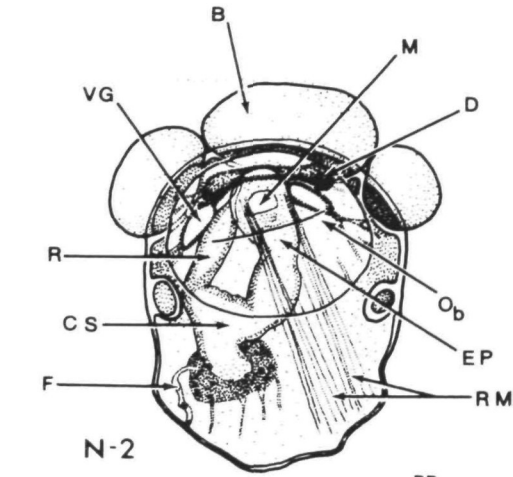
stages	time
Primary disc—larval pigment spots and lines evident	
A	10–17 minutes
B	30–37 minutes
C	43–50 minutes
Preancestrula—differentiation of polypide rudiment	
D	1 hour
E	3 hours
F	6–7 hours
G	21–22 hours
H	26–27 hours



Preancestrula—differentiation of diaphragm, vestibular glands, and operculum
I 30 hours

J 34-35 hours
K 37-39 hours
L 50-56 hours





Preancestrula—differentiation of alimentary canal of polypide

M 79–85 hours
N-1/N-2 88–94 hours (approx. 3½–4 days)

Preancestrula—differentiation of extrazooidal structures

O 103–118 hours
P 127–167 hours

Ancestrula—differentiation of alimentary canal complete and nutritive mass completely absorbed.

Q-1/Q-2 176–217 hours (7–9 days)

A, anus; AC, alimentary canal; AO, apical organ complex; AS, anterior pigment spot(s); B, bud of developing daughter zoid; BW_i, inner layer of basal wall; BW_o, outer layer of basal wall; CAS, cardiac stomach; CM, caecum; CP, communication pore complex; CS, central stomach; D, diaphragm; DP,

various types of movement, the larva maintains a stationary position with the oral lobe usually oriented toward the substrate, although occasionally in the reverse orientation, and with the coronal cilia beating. An additional activity observed is the expansion and contraction of the neck region of the internal sac during both vertical and horizontal movement. Similar types of movements, other than the clockwise-spiral movement, have been reported in the literature for larvae of *Eurystomella foraminigera* (Gordon, 1970) and *Bugula neritina* (Lynch, 1947; Ryland and Stebbing, 1971).

Larval attachment

One to three minutes after the release of the larva from the ovicell, and up to one hour later under experimental conditions, negative phototropism replaces positive phototropism and the larva settles, attaches, and metamorphoses in an area which it had previously "searched." Approximately 68% of the larvae observed settled and attached successfully as determined by the differentiation of the primary disc into a normal tata ancestrula. Although attachment and metamorphosis generally occur on a hard substrate, larvae of *P. nitida* morphotype B were observed to metamorphose on the undersurface of the surface film of the seawater in a petri dish.

Metamorphosis of Parasmittina nitida morphotype B

Immediately prior to attachment, the *P. nitida* morphotype B larva rotates clockwise and counterclockwise on the oral pole. The attachment process, observed in a live larva in profile view, initially involves

the opening of the glandular neck region of the internal sac (adhesive sac) and the attachment of the lateral edges to the substrate (*i.e.*, petri dish). The roof of the internal sac of the larva subsequently "lowers" to the level of the substrate and forms a permanent junction with it. The extensive morphogenetic movements of the metamorphosing individuals were studied by observing the relocation of the prominent larval pigment spots.

Within 65 seconds from the time of attachment of the internal sac roof to the substrate, an inrolling of the aboral vesicular epithelium and coronia occurs and is evident from the lateral movement of the "eyespot." After two minutes, the "eyespot" are located within the interior of the metamorphosing larva. After ten minutes, the major morphogenetic movements of the larval tissue are completed.

The apical organ complex, the pallial sinus epithelium, and the wall region of the internal sac initially encase the larval structures as observed with SEM (Fig. 5C). Subsequently, the larval form of the apical organ complex is retracted and observed within the center of the primary disc as a dark circular mass around which are distributed numerous pigment spots (Figs. 3A-C, 4A-C). The integrity of the body surface is apparently maintained by a fusion of the epithelia of the wall region of the internal sac and of the pallial sinus, similar to that described in *Bugula neritina* Woollacott and Zimmer, 1971).

The later stages in metamorphosis involve a thorough reorganization of the inner tissues of the primary disc to form the preancestrula and ultimately the tata ancestrula. These developmental phases were observed from the basal and frontal surfaces of the differentiating individual.

The epithelium of the larval internal sac appears to form the epidermis of the

diaphragm, operculum, and vestibular gland primordium; DPM, diaphragm parietal muscle; E, epidermis; E:C, epidermis + cuticle; EP, esophagus; ES, "eyespot" (lateral pigment spot of aboral lobe of larva); F, funiculus; FM, border of frontal membrane; LW, lateral wall of daughter zooid; M, mouth; NM, nutritive mass; O, free edge of operculum; O_b, base of operculum; OM, occlusor muscles of oper-

culum; P, pyloric stomach; PL, pigment line; PLS, posterolateral pigment spot; PR, polypide rudiment; PS, pigment spot; PVFM, parietal vaginal frontal muscle; PVM, parietal vaginal muscle; PX, pharynx; R, rectum; RM, retractor muscle; T, tentacle; TP, tentacle primordium; TS, tentacle sheath; TSP, tentacle sheath primordium; VG, vestibular gland

preancestrula body wall, which is evident approximately one hour after the onset of attachment and metamorphosis. The pigment areas of the initial attachment stage, *i.e.*, the primary disc, appear to disassociate into smaller masses which are connected by pigment lines (Figs. 3A-C, 4A-C). With further degeneration of the larval tissue, the pigment areas disperse throughout the reorganizing tissue (Figs. 3D-E, 4D-E). The apical organ complex, which has maintained its integrity to this stage, gives rise to the polypide rudiment, which appears as an irregular mass (Figs. 3D, 4D). The upper blastema of the complex differentiates into the ecto- and entodermal derivative of the polypide, and the lower blastema forms the lophophoral coelomic lining and the splanchnic peritoneum (Woollacott and Zimmer, 1971).

About three hours after attachment begins, the polypide rudiment acquires a distinct shape, the pigmented nutritive cells which were evident in the larva as pigment spots begin to migrate toward the proximal region of the preancestrula, and the transparent cuticle laid down by the epidermis is visible (Figs. 3E, 4E, 5E). Aggregation of pigmented cells, undifferentiated entodermal cells, and degenerating cells of the larval transitory organs results in the formation of the nutritive mass (Fig. 3G).

About 16 hours after the onset of attachment, the preancestrula consists of a cuticular cystid with a distally located frontal membrane which is outlined by a weakly formed calcareous edge (Fig. 3G) and nine equally developed cuticular spines—a proximomedian spine, a set of proximolateral, lateral, and distolateral spines, and two distomedian spines (Fig. 5G). Although rare, 10 and 11 spines have been observed in the tata ancestrula of *Parasmittina nitida* morphotype B. The additional spines are located around the distal margin of the frontal membrane as either a single spine between the distome-

dian spines, or as a set of spines between the distomedian and distolateral spines. Calcium deposition within the cuticular spine proceeds at a slower rate than the formation of the cuticular spine (approximately five hours later) (Figs. 5G, 5L, 5Qc), but is concomitant with the calcification of the proximal portion of the frontal surface of the cystid.

Internally, the polypide rudiment, which has formed from the blastema, appears as a U-shaped structure (Fig. 3G). The base of the rudiment is within the nutritive mass, with the upper portion elevated toward the frontal membrane and within the forming tentacle sheath. According to Woollacott and Zimmer (1971), the tentacle sheath in *Bugula neritina* differentiates from the pallial sinus epithelium; this is not the case in all cheilostomes (Zimmer, personal communication). About 26 hours after the onset of attachment and metamorphosis, the polypide rudiment appears as a lobed structure, the lobes being the primordia of the tentacles.

After 30 hours, the primordium of the vestibular glands, diaphragm, and operculum are evident as a thickened mass of tissue at the distofrontal extremity of the tentacle sheath (Fig. 3I). Lutaud (1964) reported that vestibular glands in cheilostomes originate from the lateral folds of the wall of the embryonic tentacle sheath simultaneously with the differentiation of the diaphragm and operculum from the distal wall of the sheath (Figs. 3I-L). The form and dimension of these glands are species-specific in ascophoran cheilostomes. The glands are supported by parietal vaginal and parietal vaginal frontal muscles, the latter inserting on the distal region of the tentacle sheath (Figs. 3M, 4N-2). The operculum occlusor muscles are also evident (Fig. 3N-1), having differentiated from mesenchymal cells in the region distal to the tentacle sheath and dorsal to the operculum (Lutaud, 1964).

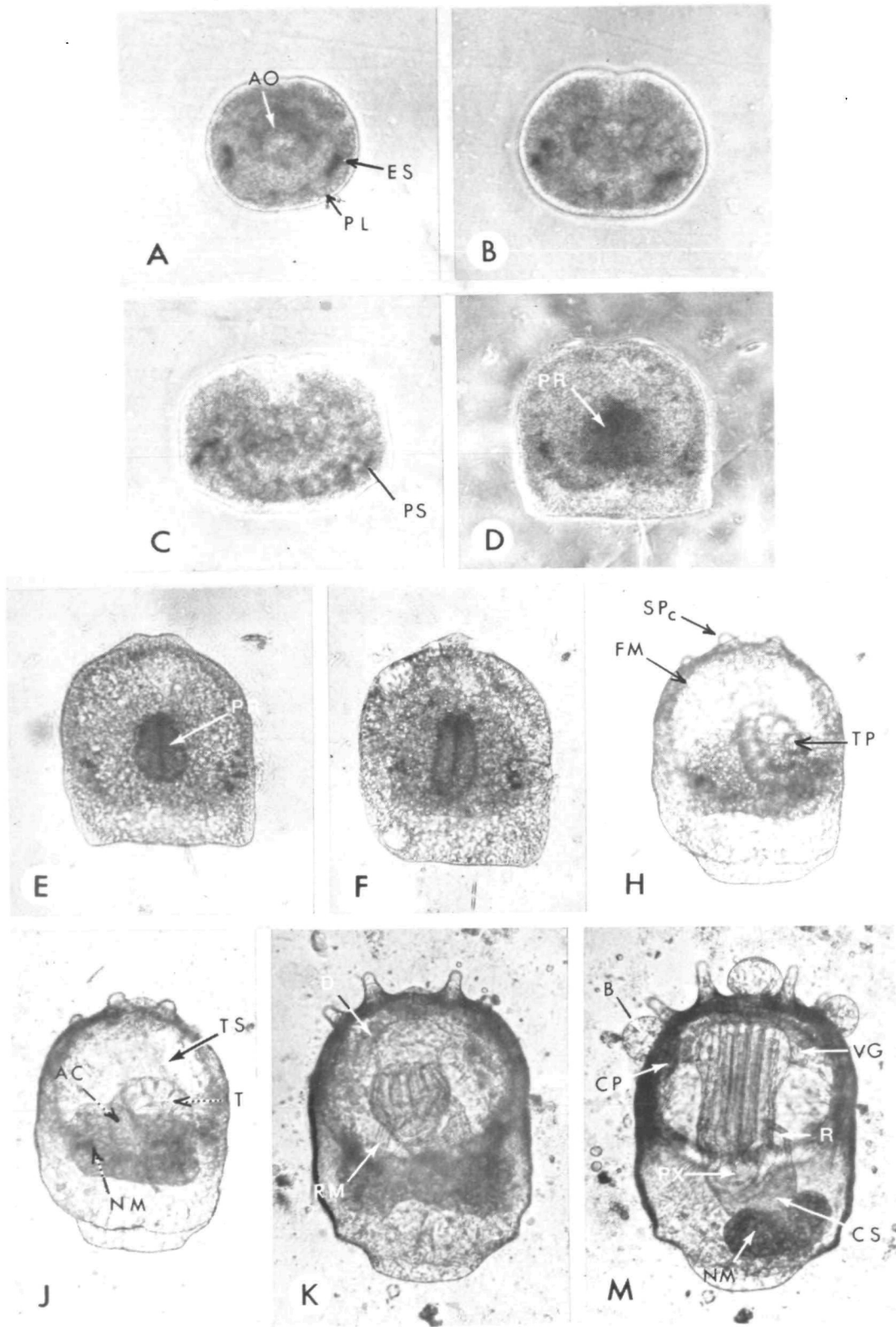
FIG. 4. Polypide development in five individuals of *Parasmittina nitida* morphotype B. Photographs were taken through an inverted Unitron phase contrast microscope from the basal surface of the living organisms. Letters of the stages correspond to those in

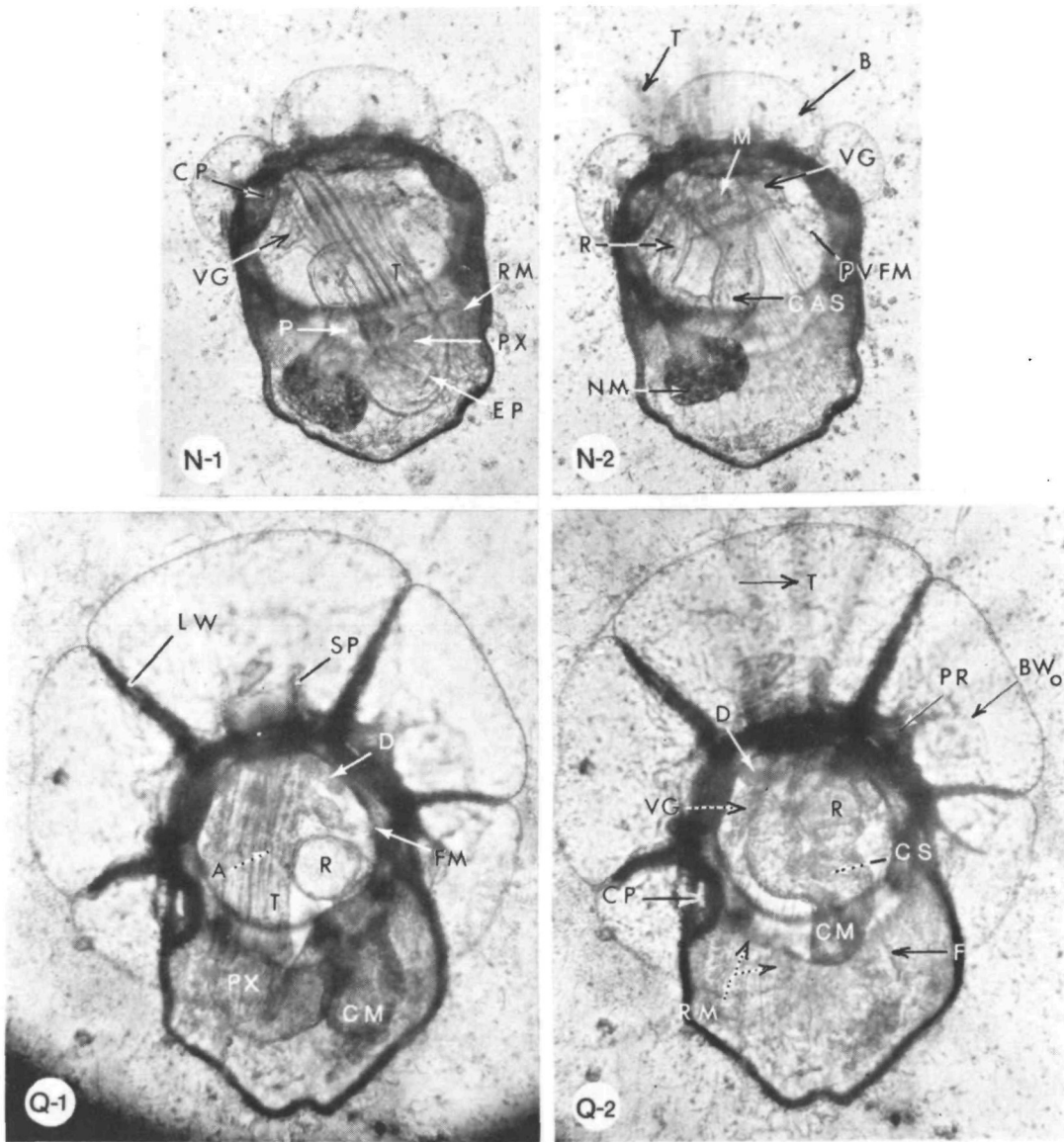
Figure 3. $\times 144$.

Primary disc: stages A-C

Preancestrula: stages D-F, H, J, K, M, N-1, N-2

Ancestrula: stages Q-1, Q-2





About 35 hours after the onset of attachment and metamorphosis of *Parasmittina nitida* morphotype B larva, the polypide rudiment differentiates into an annular lophophore, with ciliated tentacles

surrounding the mouth, and an alimentary canal (Figs. 3J, 4J). Approximately 50 hours later, the major components of the alimentary canal are discernible, *i.e.*, mouth, pharynx, esophagus, cardiac

A, anus; AC, alimentary canal; AO, apical organ complex; B, bud of developing daughter zooid; BW_o, outer layer of basal wall; CAS, cardiac stomach; CM, caecum; CP, communication pore complex; CS, central stomach; D, diaphragm; EP, esophagus; ES, "eyespot" (lateral pigment spot of aboral lobe of larva); F, funiculus; FM, border of frontal mem-

brane; LW, lateral wall of daughter zooid; M, mouth; NM, nutritive mass; P, pyloric stomach; PL, pigment line; PR, polypide rudiment; PS, pigment spot; PVFM, parietal vaginal frontal muscle; PX, pharynx; R, rectum; RM, retractor muscle; SP, spine; T, tentacle; TP, tentacle primordium; TS, tentacle sheath; VG, vestibular gland.

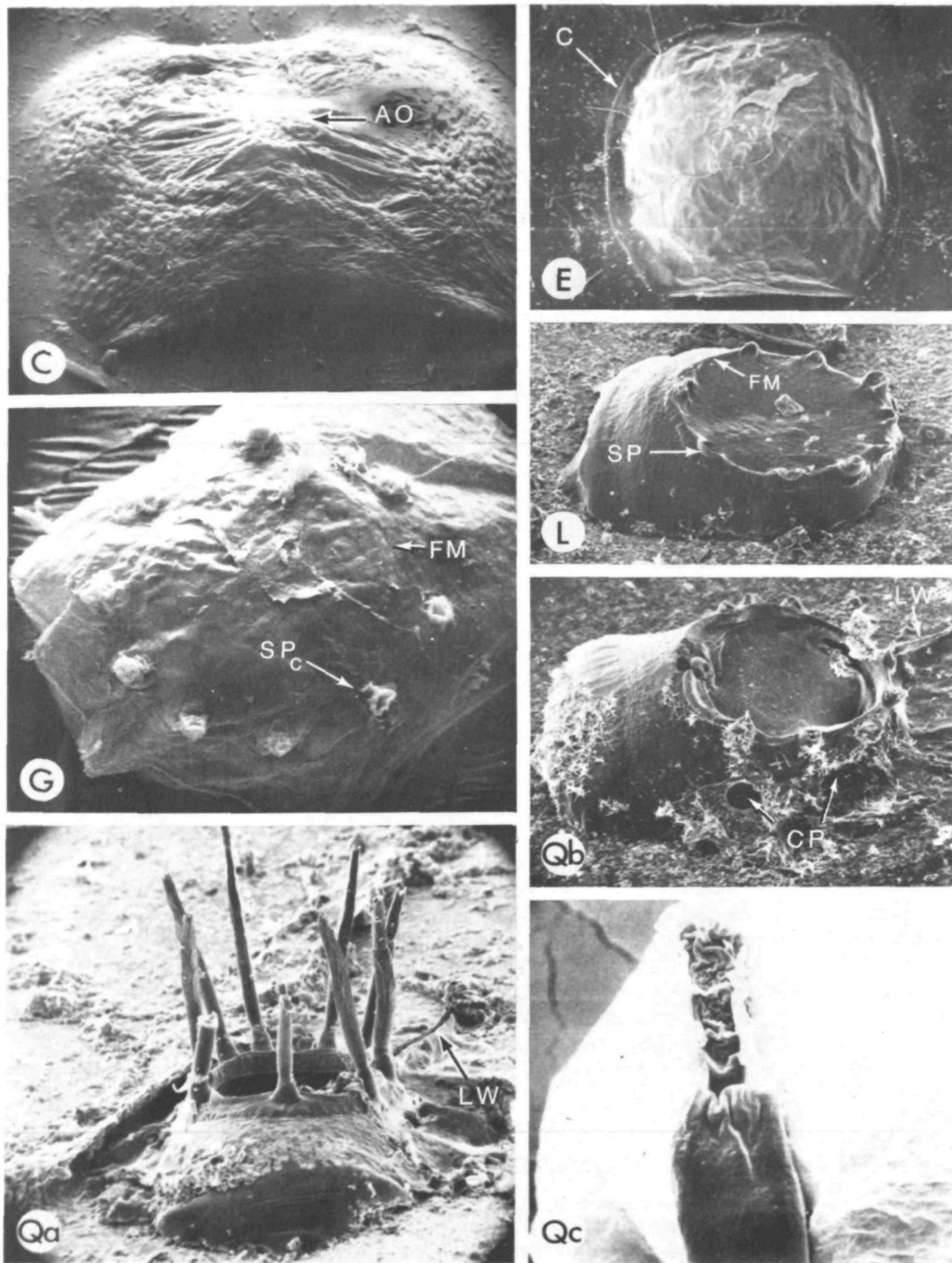


Fig. 5. Scanning electron micrographs of various stages of development in *Parasmittina nitida* morphotype B. Letters of the stages correspond to those in Figure 3.

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

Stage C. Primary disc showing region of apical organ complex (AO), pallial sinus

stomach, central stomach, caecum, pyloric stomach, rectum, and anus (Figs. 3M, 4M). At this stage in the differentiation process, the tentacles show lateral movement within the tentacle sheath and the pyloric stomach cilia beat. Subsequent development involves growth and differentiation of the various portions of the alimentary canal and diminution of the nutritive mass.

During the three to four days prior to the completion of metamorphosis, movements of and within the polypide are accentuated. The alimentary canal undergoes periodic volume changes with the retraction and protrusion of the lophophore through the primary orifice (Figs. 3N-1, 3N-2, 4N-1, 4N-2); and there is a continuous churning motion in the pyloric region of the stomach, which is attributed to the action of the cilia covering the pyloric walls (Silén, 1944). Particles which appear to "pinch off" from the nutritive mass during protrusion of the lophophore are carried by ciliary action into the pyloric stomach where they rotate clockwise at a rate of two turns per second. Gradually, the nutritive mass decreases in volume (Figs. 3Q-1, 4Q-1, 4Q-2). It is not known whether phytoplankton are ingested during protrusion of the lophophore and utilized by the polypide prior to the complete utilization of the nutritive mass. Within eight days of the onset of larval attachment and metamorphosis, the primary disc differentiates into the primary zooid or tata ancestrula which is 1.8 times the size of 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, the retractor muscles of the lophophore are the most conspicuous muscles of the

polypide. Woollacott and Zimmer (1971) suggest that the retractor muscles in *Bugula neritina* differentiate from elements of the nutritive mass. The retractor muscles of *P. nitida* morphotype B inserting on the free lateral face of the lophophoral base, *i.e.*, the face opposite the internal portion of the U-shaped alimentary canal, differentiate first and at approximately the same time as the alimentary canal differentiates from the polypide rudiment (Fig. 3J). The point of origin of these muscles on the cystid wall depends on the orientation of the alimentary canal relative to the frontal surface of the cystid, *i.e.*, dextral or sinistral (Figs. 3K-L). After 50 hours, the second set of retractor muscles are evident (Fig. 3M). With further differentiation, the muscles extend along either the frontal or basal surface of the upper portion of the alimentary canal toward the proximal wall of the cystid (Figs. 3N-1, 3N-2, 4N-1, 4N-2). The retractor muscles retract the extended lophophore; however, they do not appear to remain in a contracted state as evident from their folded appearance when the lophophore is within the cystid (Figs. 3N-1, 3P, 3Q-1, 4N-1).

The funiculus primordium is discernible approximately 3½ days after the onset of attachment and metamorphosis, but only when the lophophore is protruded (Figs. 3N-2, 4N-2). With diminution of the nutritive mass, the funiculus is seen to extend as a distinct cord (of mesenchymatous tissue [Ryland, 1970]) from the stomach caecum to a proximal wall of the zooid (Figs. 3-O, 3P). Woollacott and Zimmer (1971) suggest that the funiculus differentiates from elements of the nutritive mass in *Bugula neritina*.

The formation of the communication pore complex occurs approximately 55

	epithelium, and wall of the internal sac. × 373.	Stage Qc. Spine showing calcium carbonate core and outer cuticular sheath. Daughter bud shown in background. × 1487.
Stage E.	Preancestrula stage with cuticle (C) evident at lateral and distal edge. × 168.	
Stage F.	Distal portion of preancestrula stage showing cuticular spines (SP _e) surrounding border of frontal membrane (FM). × 353.	Specimens fixed in 95% ethanol and cuticle removed with 5% sodium hypochlorite.
Stage L.	Preancestrula stage with base of spines (SP) calcified. × 146.	Stage Qa. Tata ancestrula with nine spines. × 158.
		Stage Qb. Lateral view of tata ancestrula with communication pores (CP) and lateral walls (LW) evident. × 152.

hours after the onset of attachment and is concomitant with the deposition of calcium carbonate between the epidermis and cuticle of the cystid. Initially, three pore plates and their respective chambers form, one in the distal and one in each of the distolateral walls of the cystid (Figs. 3L, 4K). Daughter zooid buds emerge through these communication pores; a single distomedian bud may develop first (Fig. 3L), or three buds—one distal and two distolateral—may develop concurrently (Figs. 3M, 4M). Although rare in *Parasmittina nitida* morphotype B, one distolateral bud may develop prior to the second and concomitant with the distomedian bud. The most common ancestrula budding pattern in Hawaiian smittinids, however, is a median distal bud and one distolateral bud at right angles to it (Soule and Soule, 1972). The region of growth of the buds in *P. nitida* morphotype B is 180° opposite the location of the pyriform organ complex in the larva.

At the area of contact between each pair of membranous buds, the lateral wall of the developing daughter zooids form (Figs. 3-O, 5Qb). The calcification of the basal wall of the daughter zooids occurs after the initiation of lateral wall formation, but concomitant with the appearance of the proximolateral buds. The buds develop synchronously and follow distolateral bud formation. The formation of five buds from the tata ancestrula is reported in at least one other species of smittinid—*Smittina papillifera* (Stach, 1938). The initial formation of three buds also occurs in smittinids—*Smittina collidera* (Mawatari, 1946).

The culmination of larval metamorphosis and polypide development in *Parasmittina nitida* morphotype B, occurring approximately seven to nine days after the onset of attachment, is the tata ancestrula or primary zooid (Figs. 3Q-2, 4Q-2, 5Qa). Astogenetic growth, resulting from asexual reproduction, begins prior to the completion of primary zooid differentiation and is indicated by lateral and basal wall formation and the appearance of a polypide rudiment in daughter zooids (Fig. 4Q-2). Colony formation, *i.e.*, bud-

ding pattern and morphological variation between the zooids of a colony, is species-specific in gymnolaemate bryozoans (Boardman, Cheetham and Cook, 1969).

The description of larval behavior and post-larval development of the ascophoran *Parasmittina nitida* morphotype B adds to the limited amount of current knowledge of early development in gymnolaemate bryozoans, and indicates potential areas for future investigation, in particular, polypide development at the ultrastructural level.

REFERENCES

- Anderson, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *New York Acad. Sci. Trans.* 13:130-133.
- Barrois, J. 1877. Mémoire sur l'embryologie des Bryozoaires. *Trav. Inst. Zool. Wimereux* 1. Cited in Marcus, 1939.
- Boardman, R. S., A. H. Cheetham, and P. L. Cook. 1969. Intracolony variation and the genus concept in Bryozoa. *Proc. North Amer. Paleontol. Conv. Part C*, pp. 294-320.
- Calvet, L. 1898. Sur le développement et la structure de la larve de quelques bryozoaires chélostomes. *C. R. Acad. Sci. Paris* 127:79-81.
- Cook, P. L. 1973. Settlement and early colony development in some Cheilostomata. In G. P. Larwood (ed.), *Living and fossil Bryozoa: Recent advances in research*, pp. 65-71. Academic Press, New York.
- Gordon, D. P. 1970. Reproductive ecology of some northern New Zealand Bryozoa. *Cahiers de Biol. Mar.* 11:307-323.
- Gordon, D. P. 1971. Colony formation in the cheilostomatous bryozoan *Fenestulina malusu* var. *thyreophora*. *New Zealand J. Mar. Freshw. Res.* 5(2):342-351.
- Harmer, S. F. 1887. Sur l'embryogénie des Bryozoaires Ectoproctes. *Arch. Zool. Exp. Gén.* 2, Sér. 5:443-458. Cited in d'Hondt, 1972.
- Hondt, J. -L. d' 1972. Métamorphose de la larve d'*Alcyonidium polyoum* (Hassall). *Bryozoaire Cténostome. C. R. Acad. Sci. Paris, Ser. D* 275:767-770.
- Humphries, E. M. 1974. Larvae and post-larval development in relation to the species problem in *Parasmittina nitida* (Verrill) (Bryozoa: Cheilostomata). Ph.D. Diss. Dept. Zoology, Univ. Florida.
- Humphries, E. M. 1975. A new approach to resolving the question of speciation in smittinid bryozoans (Bryozoa: Cheilostomata). In S. Poyet (ed.), *Bryozoa 1974*, proceedings of the third conference, International Bryozoology Assoc., pp. 19-36. *Docum. Lab. Géol. Fac. Sci. Lyon, H.S.* 3 (fasc. 1), France.
- Lutaud, G. 1964. Sur la structure et le rôle des glandes vestibulaires et sur la nature de certains organes de la cavité cystidienne chez les

- Bryozoaires Chilostomes. *Cahiers de Biol. Mar.* 5:201-231.
- Lynch, W. F. 1947. The behavior and metamorphosis of the larva of *Bugula neritina* (Linnaeus): Experimental modification of the free-swimming period and the responses of the larvae to light and gravity. *Biol. Bull.* 92(2):115-150.
- Loeb, M. and G. Walker. 1977. Origin, composition, and function of secretions from pyriform organs and internal sacs of four settling cyclo-ctenostome bryozoan larvae. *Mar. Biol.* (In press)
- Marcus, E. 1939. Bryozoários marinhos brasileiros III. *Bol. Fac. Filos. Ciências e Letras, Univ. São Paulo, Zoologia* 3:111-354.
- Maturo, F. J. S., Jr. 1973. Offspring variation from known maternal stocks of *Parasmittina nitida* (Verrill). In G. P. Larwood (ed.), *Living and fossil Bryozoa: Recent advances in research*, pp. 577-584. Academic Press, New York.
- Maturo, F. J. S., Jr. and T. J. M. Schopf. 1968. Ectoproct and entoproct type material: Re-examination of species from New England and Bermuda named by A. E. Verrill, J. W. Dawson, and E. Desor. *Postilla* (Peabody Mus. Natur. Hist., Yale Univ.) No. 120.
- Mawatari, S. 1946. Metamorphosis and formation of the young colony in *Smittina collifera* Robertson. *Misc. Rep. Res. Inst. Natur. Resour.* 9:21-28.
- Moyano, H. I. 1972. Aspectos sistematicos astogeneticos y reproductivos de *Inversulia nutrix*. *Bol. Soc. Biol. Concepcion* 45:105-116.
- Nielsen, C. 1977. The relationships of Entoprocta, Ectoprocta and Phoronida. *Amer. Zool.* 17:149-150.
- Ryland, J. S. 1960. Experiments on the influence of light on the behaviour of polyzoan larvae. *J. Exp. Biol.* 37(4):783-800.
- Ryland, J. S. 1970. *Bryozoans*. Hutchinson & Co., Ltd., England.
- Ryland, J. S. and A. R. D. Stebbing. 1971. Settlement and orientated growth in epiphytic and epizoic bryozoans. In D. J. Crisp (ed.), *Fourth Eur. Mar. Biol. Symp.*, pp. 105-123. Cambridge Univ. Press, England.
- Silén, L. 1944. On the division and movements of the alimentary canal of the Bryozoa. *Ark. Zool.* 35A(12):1-40.
- Soule, D. F. and J. D. Soule. 1972. Ancestrulae and body wall morphogenesis of some Hawaiian and eastern Pacific Smittinidae (Bryozoa). *Amer. Microsc. Soc. Trans.* 91(3):251-260.
- Stach, L. W. 1938. Colony formation in *Smittina papillifera* (MacGillivray, 1869) (Bryozoa). *Proc. Zool. Soc. London* 108:401-415.
- Woollacott, R. M. and R. L. Zimmer. 1971. Attachment and metamorphosis of the Cheilo-ctenostome bryozoan *Bugula neritina* (Linné). *J. Morphol.* 134(3):351-382.
- Woollacott, R. M. and R. L. Zimmer. 1972. Fine structure of a potential photoreceptor organ in the larva of *Bugula neritina* (Bryozoa). *Z. Zellforsch.* 123:458-469.