Development and Metamorphosis of Cyphonautes Larvae in the Freshwater Ctenostome Bryozoan, *Hislopia malayensis* Annandale, 1916

Timothy S. Wood[1]

Abstract

The ctenostome bryozoan, *Hislopia malayensis*, is one of two hislopiid species in Thailand and one of the most common freshwater bryozoans in Southeast Asia. Colonies are hermaphroditic, forming sperm first and then oocytes, the two phases sometimes overlapping. Oocytes are released around nightfall in a way not yet determined. Coelomic oocytes initially measure 0.07 mm in diameter, expanding after release to 0.16 mm with the rise of a smooth fertilization membrane. Radial cleavage produces 4 tiers of 4 equal blastomeres after the fourth division. A distinct blastocoel appears at the 32-cell stage.

A swimming cyphonautes larva is formed within 14 hours at 28°C. It measures 0.10 mm along the base and 0.10 mm high. The apical organ is well developed. The stomach and intestine are combined in a single unit. Later, after the larva has doubled its size, a small pyriform organ appears together with a tuft of long vibratile cilia. No metasomal sac has been observed. Metamorphosis occurs after the larva reaches a length of about 0.2 mm at the base. Following attachment to a suitable substratum, strong muscular contractions pull the larval tissues away from the bivalve shell, and the shell valves are often discarded. The metamorphosing larva appears to expand, and the adhering base creeps outwards in all directions. As tissues reorganize the entire larval stomach remains intact, slowly digesting the food contents and apparently providing continuous energy for metamorphosis. The larval stomach is eventually integrated fully into the adult gut between the newly formed proventriculus ("gizzard") and intestine.

This is the first description of a cyphonautes larva in fresh water. The retention of a complete larval organ in the adult is interpreted as an ancient feature, suggesting significant evolutionary stasis in *Hislopia.* This developmental feature has been previously suggested but never before reported in any bryozoan.

Introduction

Like many other marine invertebrates, a bryozoan begins life as a motile larva developing from a fertilized egg. Typically, the egg is invested with major food reserves and retained by the parental zooid until hatching. Lacking a mouth or any digestive capability, the actively swimming larva is energized solely by its internal yolk supply, and does not remain long in the water column.

However, among several bryozoan species the eggs are dispersed directly into the water with only minor food supplies. They develop quickly into self-feeding larvae known as cyphonautes. How long cyphonautes larvae remain in the water column is unknown, but estimates range from 7 to 10 days to as long as 2 months, depending on the species (Dudley 1973, Marcus 1926). Feeding selectively on algal cells the larvae may grow to three times their original size before settling (Mawatari and Mawatari 1975).

All cyphonautes are essentially alike, as first described by Prouho (1890, 1892), then in greater detail by Kupelweiser (1905), and more recently with electron microscopy by Stricker et al. (1988a, 1988b). In side view the larva appears roughly triangular with rounded ends and the sides bowed slightly outwards. The body is laterally compressed and held between two thin unmineralized valves. The valves may gape slightly along the posterior and ventral margins. At the apex of the triangle is a rounded knob, the apical organ, bearing sensory cilia. Along the base is a ciliated corona serving primarily in locomotion. The oral region is deeply invaginated to form an interior vestibule with the mouth at its apex, just under the apical organ. The esophagus recurves to the stomach and intestine along the posterior third of the larva, and the anus opens near the posterior ventral corner. A pair of ciliary ridges projects slightly below the corona and then extends into the vestibule, curving towards the stomach. An internal metasomal sac, also called an adhesive sac, lies just anterior to

[1]Department of Biological Sciences, Wright State University, Dayton, OH 45435 U.S.A.

the stomach. A complex pyriform organ at the lower anterior corner includes a long tuft of vibratile cilia.

Cyphonautes is the only plankotrophic larva known among bryozoans, and it occurs within a small number of ctenostome and anascan cheilostome species. Among the Cheilostomata the most thoroughly studied species is *Electra pilosa*, with detailed accounts of early embryology (Prouho, 1892), metamorphosis (Kuplelwieser 1905), and the fully developed larva itself (Prouho 1892; Kupelwieser 1905; Atkins 1955). Species of *Membranipora* also have received some attention, with excellent treatment of early embryology (Mawatari and Mawatari 1975; Cook 1962), larval anatomy (O'Donoghue 1926; Stricker et al. 1988a, b), larval feeding (Atkins 1955), and metamorphosis (O'Donoghue 1926; Stricker 1988). By contrast, among ctenostome bryozoans planktotrophic cyphonautes are documented for only three species: *Alcyonidium albidum*, *Farella repens* and *Hypophorella expansa* (see Ström 1977; Zimmer and Woollacott 1977a). However, planktotrophic larvae have been inferred for several other *Alcyonidium* species based on the presence of lipid granules in the autozoid and/or the apparent absence of brooding (Porter and Hayward 2004).

In the exclusively fresh water phylactolaemate bryozoans the sexual union results in a transitory, motile structure containing 1 to several fully formed polypides (Reed 1991). Although larva-like in function, these free colonies bear no relation to the larvae of gymnolaemate bryozoans. In fact the only true larva reported for any freshwater bryozoan has been a simple stereogastrula belonging to the ctenostome *Paludicella articulata* (Braem 1896). Brooded embryos have been observed among other freshwater ctenostomes (Ström 1977; Smith et al. 2003), but the larvae are unknown. Planktonic larvae generally are unusual in fresh water habitats except for a few mollusks and microcrustaceans (Conn 1991).

This is why the recent discovery of a true cyphonautes larva in a fresh water ctenostome was quite unexpected. It began with the observation of mature oocytes inside the zooids of *Hislopia malayensis* Annandale, 1916. These were discharged freely into the water, where they quickly developed into fully planktotrophic cyphonautes. Thus began the series of observations summarized in the following preliminary report. *Hislopia malayensis* is a common species in Thailand (Fig. 1.1), occurring throughout the year in both still and flowing water on a variety of substrata (Wood et al. 2005).

Methods

The work described here was conducted on the Bangkhen campus of Kasetsart University in Bangkok, Thailand. A rectangular 0.6 ha pond used by the Faculty of Fisheries provided a convenient and reliable source of *Hislopia* colonies and larvae. Colonies providing eggs were grown on glass Petri dish lids suspended in an inverted position at a depth of about 1 m in a holder made from HDPE drainage pipe (Wood 2005). The glass substrata were populated naturally, and by 4 weeks *Hislopia* colonies lined the inside of the lid edge to edge. Both oocytes and sperm were easily visible when colonies were examined through the glass from the basal side using transmission light microscopy.

To obtain fertilized eggs, I collected the populated Petri lids about 1 hour before nightfall, brought them into the laboratory and placed them in darkness. The release of eggs into the water began almost immediately and continued for about 1 hour. (By contrast, larval release in marine bryozoans is normally triggered by light rather than darkness [Ryland 1960]). Water in the lid was swirled and decanted into a separate dish, where the eggs could be retrieved individually with a Spemann pipet. Observations and photos of egg development were made with a hanging drop preparation and a

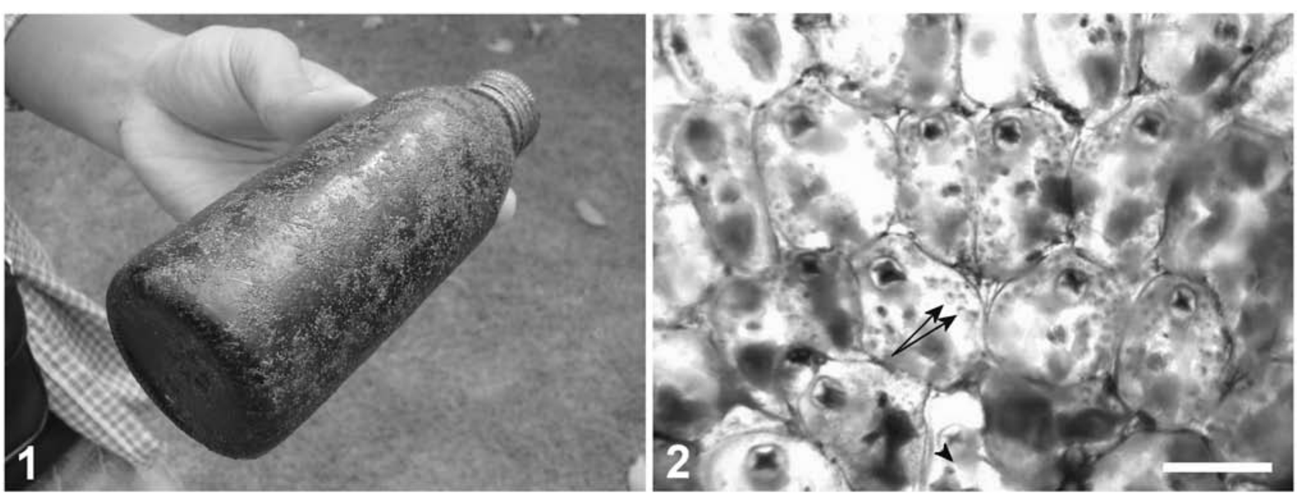

Figure 1. *Hislopia malayensis*. 1, Colonies on a glass bottle recovered from a pond in Thailand. 2, Zooids growing on a glass substratum with ova clearly visible (arrows), scale bar = 0.5 mm.

compound microscope. Embryos left to develop overnight at 25 to 28°C generally resulted in free-swimming cyphonautes by mid-morning the following day.

Older cyphonautes could be taken from the pond with a plankton net at any time of the day or night, although the largest individuals were most likely to be encountered after dark. My attempts to rear larvae in the laboratory have so far been unsuccessful.

To observe metamorphosis, larvae were allowed to settle on 4″ diameter disks of Aclar plastic embedding film (Electron Microscopy Services, Fort Washington, PA) attached by crossed threads to the underside of a corrugated polyethylene panel suspended horizontally in the pond. Disks were collected in the early morning and examined for the presence of newly settled larvae. The disks usually did not attract larvae until they had been aged in water for at least 5 days. Even then, the settling rate was never more than 1 larva per 90 cm^2 per day. The use of plastic film allowed the specimens to be manipulated and viewed from a variety of angles, and could also be used in preparations for electron microscopy. Both still photos and time-lapse video were recorded with a Nikon Coolpix 8700 camera with a microscope adapter from the Martin Microscope Company, Easley, SC.

Results

In colonies reaching 20 mm or more in diameter it is usually possible to find regions where many of the zooids contain 20–30 oocytes (Fig. 1.2). Most of the oocytes are clustered in the ovary located around the inner periphery, while others may be suspended freely in the coelom. Ovulated oocytes have a distinctly pale blue color distributed in one hemisphere, and they measure about 0.07 mm in diameter.

Under natural conditions the free oocytes are released quickly around nightfall. While a number of ctenostomes possess an intetentacular organ, no such structure has yet been identified in *Hislopia*, nor have I been able to witness the moment when oocytes are released. I do know, however, that when mature colonies collected in late afternoon are placed in darkness, the water is soon filled with the near weightless eggs, their fertilization envelopes already raised. The time and place of fertilization have not be observed, although in other species fertilization has been shown to occur in the visceral coelom around the time of ovulation (Temkin 1994). The eggs and their surrounding smooth membrane envelopes are almost perfectly spherical (Fig 2.1) and about 0.16 mm in diameter. The egg contents appear granular, with a blue-gray pigmented mass localized in one hemisphere.

Embryology

At 25°C the first cleavage begins less than 30 minutes after egg release. It is a meridional division, bisecting the pigmented area. The blastomeres are concave along their inner faces, leaving a small central gap (Fig. 2.2). The second cleavage, coming about 20 minutes later, is again meridional and perpendicular to the first, and it isolates the pigmented region in two of the four new blastomeres (Fig. 2.3). The third cleavage is equatorial, producing two tiers of four blastomeres with the pigmented region distributed within one of the tiers (Fig. 2.4). The fourth division is now in the same cleavage plane as the first, leaving four tiers of 4 blastomeres in line, with the pigmented region centralized within the middle tiers (Fig.2.5). So far all blastomeres are equal in size and cleavage is biradial, similar to the pattern described for *Alcyonidium albidum* by Prouho (1892) and for *Membranipora serrilamella* by Mawatari and Mawatari (1975) (see also Reed 1991). Gastrulation begins after the fifth division, when blastomeres draw together in a nearly spherical cluster with pigmented cells enveloped by colorless ones (Figs. 2.6, 2.7). From this point onwards the divisions appear to be unequal and the margins of the inner cells become obscure. By the eighth division a blastocoel is apparent (Fig. 2.8), but it is soon obliterated Fig. 2.9). Six hours after its first cleavage the developing embryo loses its spherical shape, as one end begins to flatten and expand, like a small bell (Fig. 2.10). The contours of the future cyphonautes larva are now evident, and the pigmented area occupies space where the gut will be. A ridge of cilia develops around the margin of the bell, and slow beating of the cilia make the entire embryo wobble (Fig 2.11). About eight hours after the first cleavage the fertilization envelope ruptures and internal muscles of the larva can be seen to flex. The bell shape becomes laterally compressed. By 14 hours, the basic larva is well formed, swimming actively, and capable of ingesting small particles (Fig. 2.12).

Cyphonautes larva

The new cyphonautes is essentially a flattened cone, roughly triangular in side view and strongly compressed laterally. The base is almost straight, while the anterior and posterior margins bow outwards. A rounded apical organ protrudes from the aboral tip, with numerous long, immobile cilia projecting from it. Some time later a pair of smooth, colorless valves can be recognized enclosing the larva and providing external support for muscles and connective fibers.

A very prominent internal structure is the large, sac-like gut, about 3 times longer than wide, situated in the posterior third of the larva. The mouth is normally kept closed. The anus, at the opposite end, is most easily located at the moment when fecal material is discharged. A slight constriction in the middle of the sac-like gut might distinguish the stomach from the intestine, but there appears to be little functional separation of these regions. Ingested algal cells are clearly intact near the mouth and fully digested near the anus. The gut appears to handle only a small amount of food at a time except just before metamorphosis when it becomes filled with undigested algal particles. There is no apparent metasomal sac, but the neuromuscular cord and coronal cilia are highly visible.

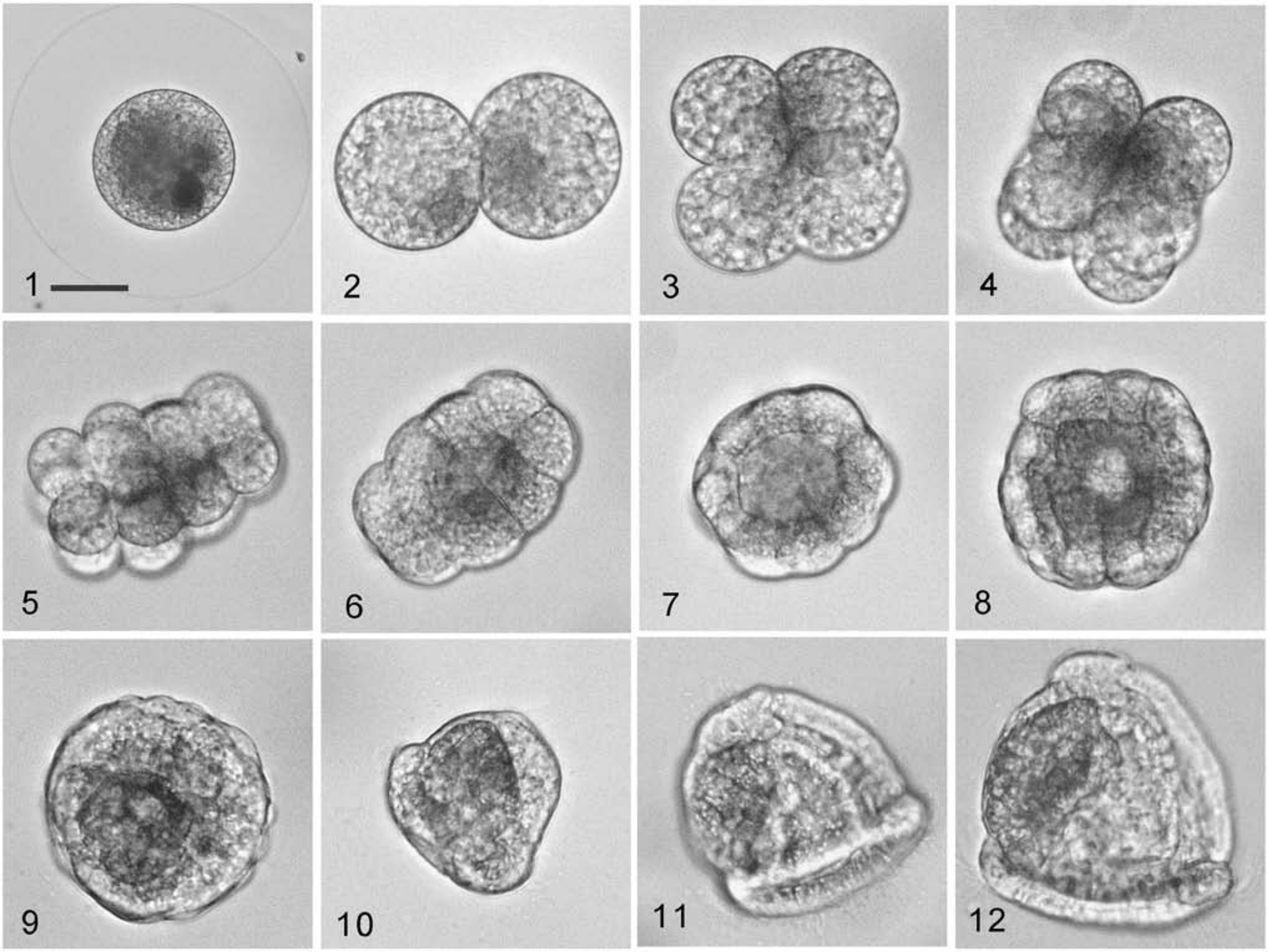

Figure 2. Developing eggs of *Hislopia malayensis*. 1, Fertilized egg at 0 minutes with fertilization envelope visible. 2, Two blastomeres at 21 min. 3, Four blastomeres at 40 min. 4, Eight blastomeres at 65 min. 5, Sixteen blastomeres at 1.50 hrs. 6, Sixteen blastomeres at 1.58 hrs. 7, Blastula at 2.25 hrs. 8, Gastrula at 3.3 hrs. 9, Embryo at 6.3 hrs. 10, Embryo at 7.5 hrs. 11, Embryo at 9.75 hrs. 12, Actively swimming cyphonautes at 12 hrs. Scale bar = 0.04 mm for Fig 1 and 0.02 mm for Figs 2-12.

The cyphonautes larva is relatively small, initially measuring 0.10 mm long at the base and 0.10 mm in height. Its maximum size is seldom more than 0.2 mm long and 0.16 mm in height. As the larva approaches the time for metamorphosis a small pyriform organ develops at the basal anterior corner. Associated with this organ is a tuft of long cilia that can be either recurved inside the vestibule or extended in the opposite direction straight outwards from the base. These so-called vibratile cilia appear to be deployed as a sensory device, especially during substrate exploration prior to settlement.

Increasing size also brings about a change in the valves. A darkly pigmented band develops along the basal posterior margin near the gut. At the same time the lower portion of the posterior edge grows slightly outwards and the valves separate to form a gently rounded lobe along the lower third of the larva. Small denticles line the edges of the flared valves.

When swimming rapidly the larva reaches a speed of 8–10 times its length per second, leading variably with the anterior edge or the apical organ, and rotating around the leading axis. For most of the time, however, it swims much more slowly, tumbling through the water without any particular orientation, constantly changing direction, sometimes hovering almost stationary. There appears to be no directional response to light or gravity. The valves are strongly hydrophobic, allowing the larva to be easily caught in the surface tension or trapped against any nonpolar surface, such as freshly submerged polyethylene.

Metamorphosis

The settling process has not yet been observed, but in Thailand the newly settled larvae are most often found around daybreak. By that time the basal edge is firmly held

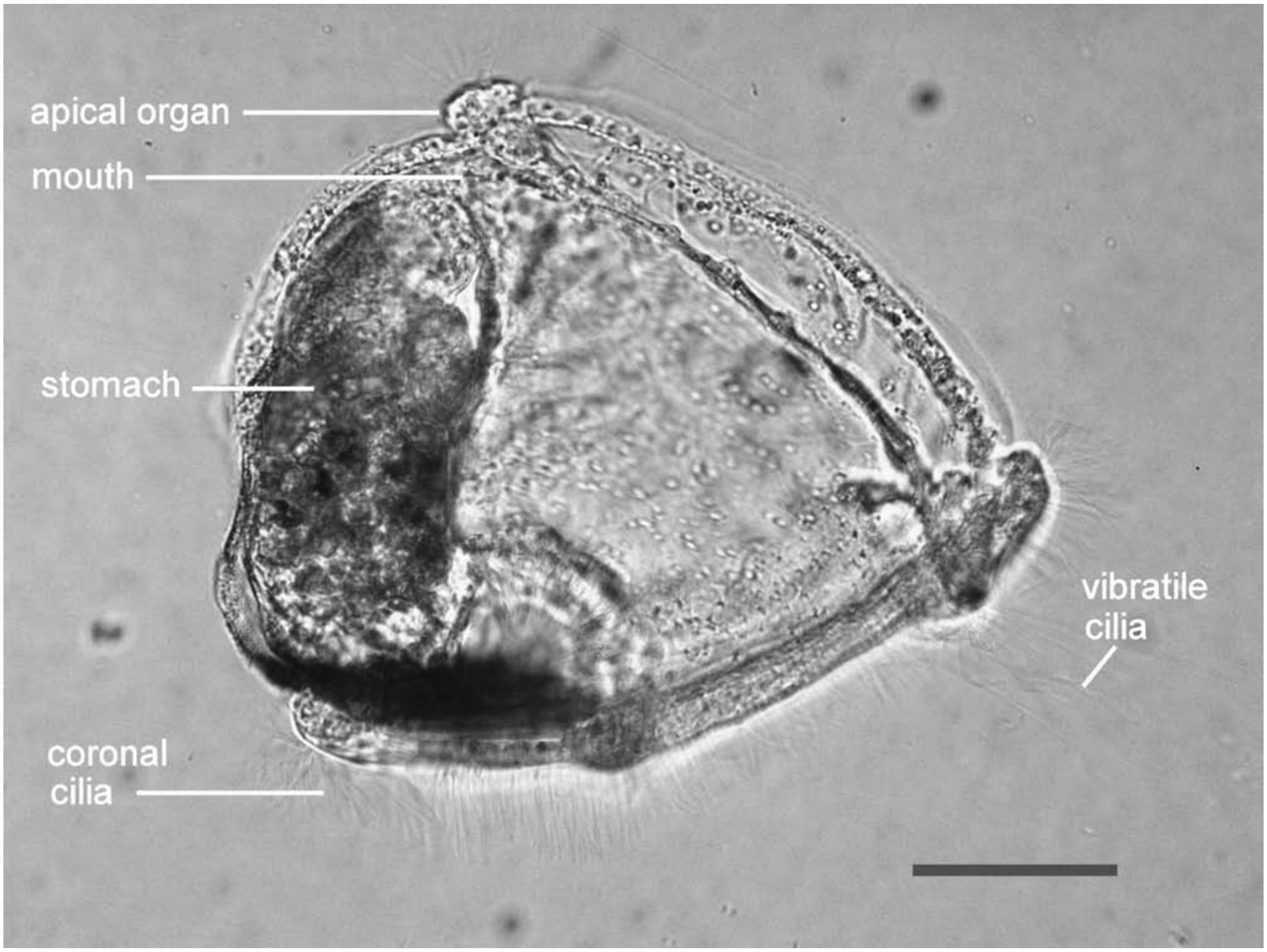

Figure 3. Cyphonautes larva of *Hislopia malayensis* with a full gut, ready for metamorphosis. Scale bar = 0.05 mm.

to the substratum. In apical view the metamorphosing larva is roughly oval in shape, measuring 0.18 mm long and 0.09 mm wide (Fig 3.1). The internal tissues have pulled away from the apex, but the valves are usually still in place. The anterior edges taper to a point and the posterior edges overlap slightly. The coronal tissues are withdrawn into the interior with cilia still beating weakly; the gut remains completely intact and full of undigested algal cells.

Time-lapse video shows considerable internal activity as the tissues reorganize. The stomach pulls away from the posterior edge and hugs the basal region while other tissues move along the sides in the opposite direction (Fig 4.1). The paired valves are forced apart as an expanding base is thrust between them at both ends and extended outwards. Following the initial axial expansion, the base begins to expand laterally, starting at the two ends and progressing towards the middle. Valves often become detached at this point, although they may persist for several more hours. When fully expanded the metamorphosing larva measures about 0.24 mm long and 0.18 mm in width. What had been the posterior end of the larva will become the anterior (distal) end of the new colony ancestrula (Fig. 4.2).

Meanwhile, nearly all the cellular material migrates towards the center and comes to rest directly over the intact gut. A wedge-like thickening of the body wall appears distally and will become the vestibulum (Fig. 4.2). Other tissues become organized into recognizable stubby tentacles of the lophophore (Figs 4.4, 4.5). Several series of still photos and time-lapse videos appear to show the future proventriculus and intestine acquiring a lumen continuous with the larval stomach. The stomach remains active throughout this process, and its contents diminish considerably. Muscular contractions often push some of the stomach contents into the newly formed intestine (Fig 4.6). As muscle fibers form throughout the rest of the ancestrula they too begin to flex at regular intervals. Approximately 18 hours after settling, following dozens of tentative thrusts and retreats, the polypide finally extends and begins collecting food to replenish a nearly empty gut (Figs. 4.7, 4.8).

Discussion

Following the discovery of the *Hislopia* larva in Thailand, another cyphonautes was announced from South America. For some years these larvae had been recovered from plankton samples in the Rio Negro in the Amazon basin by Gerd-Oltmann Brandorff and Edinaldo Nelson dos Santos-Silva (2005, unpublished). The larvae had been known to investigators as *coraçãozinhos*, or "little hearts" because a large posterior lobe gave them a heart-shaped appearance. The measured length, including the lobe, ranged from 200 to 380 µm. Although the source has not been confirmed, the larvae may belong to *Hislopia corderoi* Mané-Gazon 1960, which has been reported over a wide area of the Amazon basin (Wiebach 1967, 1970).

Five elements combine to suggest that the developmental process in *Hislopia* reflects that of primitive marine bryozoans. There are, first of all, comparative studies of invertebrate larvae indicating that true self-feeding larvae are of great antiquity, and that once lost, the ability to capture and digest external food is unlikely to be recovered (Strathmann 1985). Among living bryozoans the cyphonautes is the only self-feeding larva. The majority of known bryozoan larvae are lecithotrophic with no digestive tract (Reed 1991), or else possess a digestive tract that is vestigial or nonfunctional (Zimmer and Woollacott 1977a).

Secondly, the appearance of cyphonautes in both ctenostome and cheilostome bryozoans suggests that the larva existed before the division of these two groups (Prouho 1892; Zimmer and Woollacott 1977a). Since these larvae are all nearly identical in structure, it is reasonable to suppose that they have remained essentially unchanged at least since that time.

Third, the apparent retention of the larval stomach in the adult may be considered a primitive feature. In all other bryozoan species where metamorphosis has been studied, including a ctenostome with cyphonautes, the larval organs are essentially histolyzed after settlement. They are replaced by entirely new structures arising from clusters of undifferentiated blastema cells. Zimmer and Woollacott (1977b) suggest that, considering metamorphic patterns in other phyla, "… one would expect planktotrophic larvae of ancestral and of modern gymnolaemates to retain the food gathering and processing machinery of the larva and utilize it. That this does not occur in modern species is well known, but what happened in primitive gymnolaemates can only be hypothesized." In *Hislopia malayensis* this pattern in bryozoans may no longer be hypothetical. However, there is also the possibility that in early metamorphosis the primoria of an adult gut envelope the larval one. In this interpretation, the larval gut might be treated essentially as a brown body, as already described in certain adult zooids (Marcus 1926; Gordon 1977). In the case of brown bodies, all of the material is normally ejected when the polypide evaginates for the first time. In *Hislopia*, by contrast, there remains little material to eject. However, whether the larval stomach is truly retained or whether it is treated as a brown body is an issue that can only be resolved by careful sectioning.

Fourth, with the invasion of fresh water a *Hislopia* ancestor, population may have faced conditions that favor evolutionary stasis in this group. According to Eldridge et al. (2005), those conditions could include

- highly variable environment restricting the range of variant genotypes;
- spatially induced barriers to dispersal, slow gene flow;
- high population turnover rates.

Finally, the excellent cladistic analysis by Todd (2000) supports a basal position for hislopiids in bryozoan phylogeny, well below that of other ctenostome groups represented in fresh water: Victorelloidea, Paludicelloidea, and Arachnidioidea. Based on colony and zooid morphology of recent and fossil taxa, Todd places Hislopioidea between Alcyonidioidea and the cheilostomes, two groups in which cyphonautes larvae are also known to occur.

Thus it is an intriguing possibility that this larva and this genus represent one of the most primitive known among living bryozoans. The easy availability of cyphonautes larvae in *Hislopia* now opens many opportunities for detailed studies on its embryology, morphology, metamorphosis, physiology, ecology, behavior, and more.

Acknowledgments

This study was made possible by generous logistical support from the Department of Environmental Science and the Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. In particular I thank J. Mahujchariyawong, P. Anurakpongsatorn, and S. Yimsri for their invaluable assistance. Funding was provided in part by the National Research Council of Thailand, the Faculty of Science at Kasetsart University, and the Fulbright Fellowship Program of the United States.

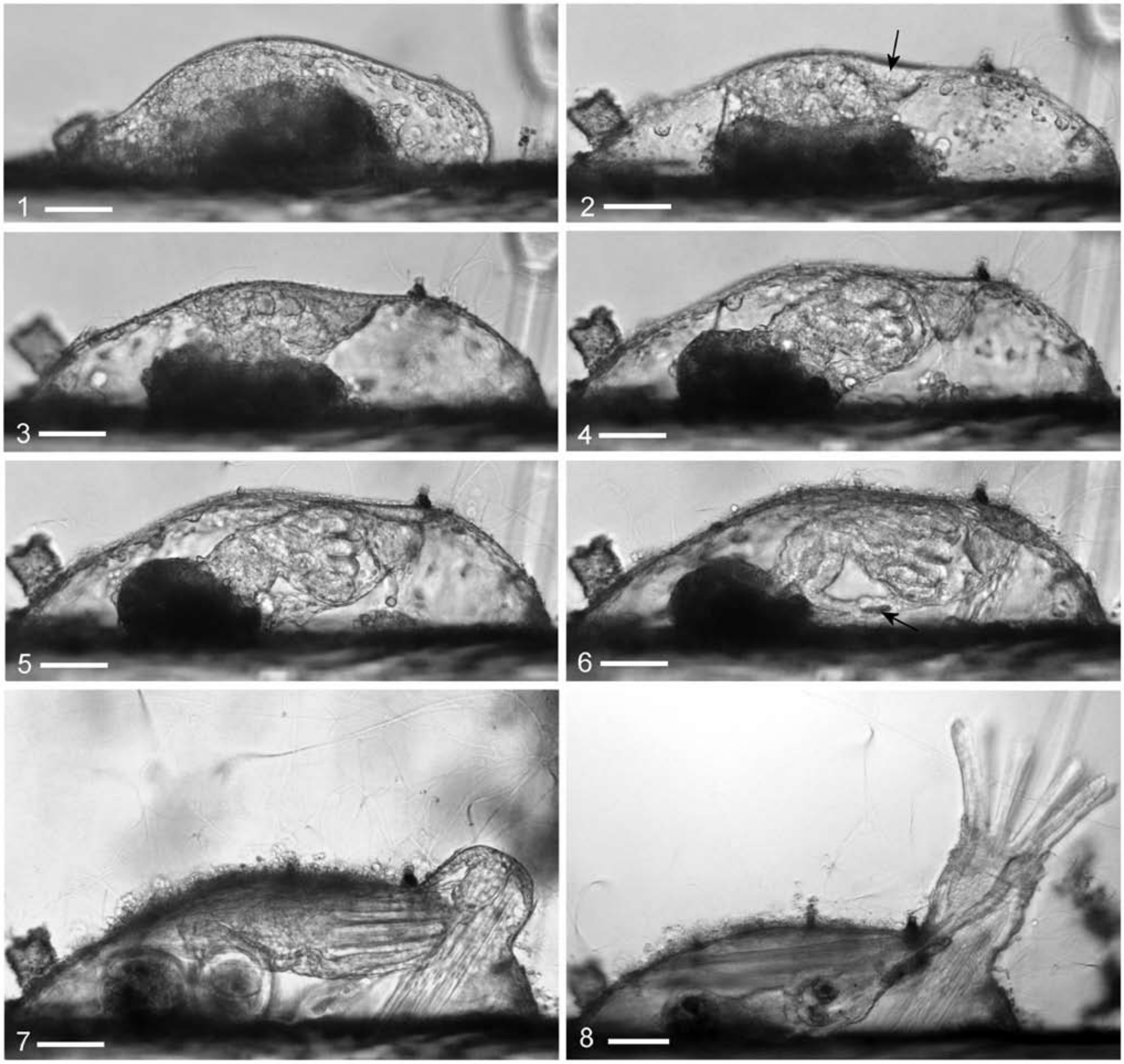

Figure 4. Stages of metamorphosis in lateral view, with larval valves already discarded. (1) Approximately 1 hour after settling, but considered 0 hrs with reference to subsequent observations. Dark mass in the center is larval stomach. Loosely organized material is moving actively within the embryo. (2) 4 hrs. The embryo flattens and spreads outwards in all directions. Loose cells cluster above the stomach. The compact mass (arrow) represents the future vestibulum. The chimney-like projection at the posterior end is an unfinished rotifer tube. (3) 5.5 hrs. The beginning of polypide bud formation. (4) 8.5 hrs. The polypide bud projecting anteriodorsally from the stomach is the future polypide. Strands of tissue extending between vestibulum primoridium and zooid base will become retractor muscles. (5) 9.5 hrs. Stubby primordia of tentacles become visible. (6) 12 hrs. The intestine is now clearly visible and has already received particles (arrow) pushed from the stomach. (7) 18 hrs. A fully formed lophophore pushes the vestibular structure, finally rupturing the membranes that had separated them. Polypide and peristome are now very active. The stomach contents are almost expended. (8) 24 hrs. The extended polypide has been feeding for several hours; this image is shown with at slightly smaller scale to accommodate the lophophore. Scale bars = 0.3 mm.

References Cited

Atkins, D., 1955, The cyphonautes larvae of the Plymouth area and the metamorphosis of *Membranipora membranacea* (L.). Journal of the Marine Biological Association of the United Kingdom, 34:441-449.

Brandorff, G.-O. and dos Santos-Silva, E.N., 2005, Note about a planktonic larva of freshwater Bryozoa from the Amazon basin. Lab. de Plâncton-CPBA/INPA, Manaus-AM, Brazil. (Unpublished).

Braem, F., 1896, Die geschlectliche Entwicklung von *Paludicella Ehrenbergii*. Zoologischer Anzeiger, 19:54-57.

Conn, D.B., 1991, Atlas of Invertebrate Development. Wiley Liss, New York, 300 pp.

Cook, P.L., 1962, The early larval development of *Membranipora seurati* (Canu) and *Electra crustulenta* (Pallas), Polyzoa. Cahiers de Biologie Marine, 3:57-60.

Dudley, J., 1973, Observations on the reproduction, early larval development, and colony astogeny of *Conopeum tenuissimum* (Canu). Chesapeake Science, 14:270-278.

Eldridge, N., Thompson, J.N., Brakefield, P.M., Gavrilets, S., Jablonski, D., Jackson, J.B.C., Lenski, R.E., Lieberman, B.S., McPeek, M.A. and Miller, W. III, 2005, The dynamics of evolutionary stasis. Paleobiology 31(2, Supplement): 133-145.

Gordon, D., 1977, The aging process in bryozoans. pp. 335-376, In Woollacott, R.M. and Zimmer, R.L. (eds.), Biology of Bryozoans. Academic Press, New York.

Kupelweiser, H., 1905, Untersuchungen über den feineren Bau und die Metamorphose des cyphonautes. Zoologica, 19:1-50.

Marcus, E., 1926, Beobachtungen und Versuche an lebenden Meeresbryozoen. Zoologische Jahrbücher. Abteilung für Systematic Oekologie und Geographie der Tiere, 52:1-102.

Mawatari, S., and Mawatari, S.F., 1975, Development and metamorphosis of the cyphonautes of *Membranipora serrilamella* Osburn. Documents des Laboratoires de Géologie de la Faculté des Sciences de Lyon, HS 3 (1):13-18.

O'Donoghue, C.H., 1926, Observations on the early development of *Membranipora villosa* Hincks. Contributions to Canadian Biology and Fisheries 3(8):249-263.

Porter, J., and Hayward, P., 2004, Species of *Alcyonidium* (Bryozoa: Ctenostomata) from Antarctica and Magellan Strait, defined by morphological, reproductive and molecular characters. Journal of the Marine Biology Association of the United Kingdom 84:253-265.

Prouho, H., 1890, Recherches sur la larve de *Flustrella hispida* (Gray) structure et metamorphose. Archives de Zoologie Expérimentale et Générale, 2nd Series, 8:409-459.

Prouho, H., 1892, Contribution à l'histoire des Bryozoaires. Archives de Zoologie Expérimentale et Générale, 2nd Series, 10:557-656.

Reed, C.G., 1991, Bryozoa. pp. 85-245, In Giese, A.C., Pearse, J.S., and Pearse, V.B. (eds.), Reproduction of Marine Invertebrates. VI. Echinoderms and Lophophorates. The Boxwood Press, Pacific Grove, California.

Ryland, J., 1960, Taxes and tropisms of bryozoans. pp. 411-436, In Woollacott, R.M. and Zimmer, R.L. (eds.), Biology of Bryozoans. Academic Press, New York.

Smith, D.G., Werle, S.F., and Klekowski, E., 2003, The anatomy and brooding biology of *Pottsiella erecta* (Potts, 1884) (Ectoprocta: Gymnolaemata: Ctenostomata), with an expanded diagnosis of the Pottsiellidae. Hydrobiologia, 490:135-145.

Strathmann, R.R., 1985, Feeding and nonfeeding larval development and life history evolution in marine invertebrates. Annual Review of Ecology and Systematics, 16:339-361.

Stricker, S.A., 1988, Metamorphosis of the marine bryozoan *Membranipora membranacea*: an ultrastructural study of rapid morphogenetic movements. Journal of Morphology, 195:53-72.

Stricker, S.A., Reed, C.G., Zimmer, R.L., 1988a. The cyphonautes larva of the marine bryozoan *Membranipora membranacea*. I. General morphology, body wall, and gut. Canadian Journal of Zoology, 66:368-383.

Stricker, S.A., Reed, C.G., Zimmer, R.L., 1988b, The cyphonautes larva of the marine bryozoan *Membranipora membranacea*. II. Internal sac, musculature, and pyriform organ. Canadian Journal of Zoology, 66:384-398.

Ström, R., 1977, Brooding patterns of bryozoans. pp.23-55, In Woollacott, R.M. and Zimmer, R.L. (eds.), Biology of Bryozoans. Academic Press, New York.

Temkin, M., 1994, Gamete spawning and fertilization in the gymnolaemate bryozoan *Membranipora membranacea*. Biological Bulletin, 187:143-155.

Todd, J.A., 2000, The central role of ctenostomes in bryozoan phylogeny. pp. 104-134, In Herrera Cubilla, A. and Jackson, J.B.C. (eds.), Proceedings of the 11th International Bryozoology Association Conference. Smithsonian Tropical Resarch Institute, Panama.

Wiebach, F., 1967, Amazonische Moostiere (Bryozoa). Amazoniana, 1:173-188.

Wiebach, F., 1970, Amazonische Moostiere (Bryozoa) II. Amazoniana, 2:353-362.

Wood, T.S., 2005, Study methods for freshwater bryozoans. Denisia, NS 28 (2005):103-110.

Wood, T.S., Anurakpongsatorn, P. and Mahujchariyawong, J., 2005, Freshwater bryozoans of Thailand (Ectoprocta and Entoprocta). The Natural History Journal of Chulalongkorn University, 6(2):83-119.

Zimmer, R.L. and Woollacott, R.M., 1977a, Structure and classification of gymnolaemate larvae. pp. 57-89, In Woollacott, R.M. and Zimmer, R.L. (eds.), Biology of Bryozoans. Academic Press, New York.

Zimmer, R.L. and Woollacott, R.M., 1977b, Metamorphosis, ancestrulae, and coloniality in bryozoan life cycles. pp. 91-142, In Woollacott, R.M. and Zimmer, R.L. (eds.), Biology of Bryozoans. Academic Press, New York.