The Developmental Biology of the Heteronemertean Worm *Cerebratulus californiensis*

Paul H. Dunn
Embryology, Spring 2007
Instructor: Craig Young
Oregon Institute of Marine Biology

Introduction

Containing approximately 900 species of unsegmented worms, the phylum Nemertea is represented in nearly every habitat on the planet. These carnivorous worms can be found crawling on intertidal rocks, swimming at great oceanic depths, stalking terrestrial prey in the tropics, and even parasitizing arthropod eggs. Despite this diversity, the nemerteans have been poorly studied as a group. One area that displays a particular paucity of information is the developmental biology of these animals. The recent work of Maslakova et al. (2003, 2004), in which the authors discovered a hidden trochopore larval stage that may lead to major changes in the taxonomic placement of the phylum, has revealed that much can be gained by looking at the development of these animals more closely.

Gibson (1972) summarized the typical reproductive strategies and development of the nemerteans. Most members of the phylum have separate sexes, develop saccular gonads along the sides of the body wall, and release their gametes into the environment for external fertilization. Cleavage is spiral and holoblastic, with the first cleavage being vertical and equal. The second cleavage is meridional and perpendicular to the first. The third cleavage is equatorial and results in animal micromeres that may be slightly larger than their vegetal macromere counterparts. Embryos develop into a coeloblastula, and gastrulation is most commonly accomplished by invagination.

After gastrulation, however, there is a major dichotomy in nemertean development. Species in the orders Paleonemertea, Bdellonemertea, and Hoplonemertea exhibit direct development, forming a juvenile worm that crawls within a few days. Most members of the order Heteronemertea, however, develop into the planktotrophic pilidium

larva, which may stay in the plankton for several months before metamorphosing dramatically into a juvenile worm (Gibson 1972).

The development of the heteronemertean genus *Cerebratulus* has been relatively well studied (Stricker 1987), although the majority of these studies have been carried out on one species, *Cerebratulus lacteus* of the American Atlantic coast. Beginning around the turn of the last century, scientists at Yale (Coe 1899) and Woods Hole (Wilson 1900) began to examine the details of *Cerebratulus* fertilization. Naohide Yatzu (1904, 1910), one of Wilson's students, carried out removal experiments to determine the movements and localization of developmental factors within the ovum prior to fertilization as well as the stages directly following the union of sperm and egg. This line of study was further pursued over a half century later by Hörstadius (1971) and Freeman (1978).

The developmental biology of the American Pacific coast congener of *C. lacteus*, *C. californiensis*, has not been extensively studied. Stricker (1987) observed that while viable oocytes of *C. lacteus* are easily obtained by cutting gravid females into short segments and filtering the exuded contents through cheesecloth or nylon mesh, *C. californiensis* oocytes obtained using this method are seldom fertilizable. In this study, embryos of *C. californiensis* were followed from fertilization through to pilidium larvae. Development was then compared to that of *C. lacteus*.

Materials and Methods

Specimens of *Cerebratulus californiensis* were collected during a low tide on April 24, 2007, from the mudflats near the Portside restaurant in Charleston, Oregon. The worms were taken to the laboratory at the Oregon Institute of Marine Biology. Sperm

was collected from male worms by placing each specimen in a bowl of seawater and cutting a posterior section of the worm with a razor blade. Muscular contractions of the body wall forced the sperm into the seawater. The sperm was then transferred using a pipette and diluted.

Eggs were collected by cutting several posterior sections from the female worms and gently prodding them with a pipette. This prodding combined with the contractions of the body wall sent the eggs into the surrounding water. They were then transferred into a bowl of new filtered seawater, and allowed to sit for 45 minutes prior to insemination.

Diluted sperm was added to the bowl containing the eggs. After fertilization had taken place, the excess sperm was poured off to prevent polyspermy and new filtered seawater was added. Cultures were monitored closely for the first 24 hours following fertilization and were then checked every other day for the following weeks. The filtered seawater was also replaced every other day for the duration of the study. Larvae were fed the green alga *Dunaiella tertiolecta* and the red alga *Rhodomonas lens* following each water replacement event. One culture was also treated with 100 mg/L of the antibiotic penicillin every other day (Strathmann 1987). All cultures were kept at 12-15°C in a seatable with flowing seawater.

Results

Upon release from the female worm, the oocytes had not completed meiosis, and a large germinal vesicle could be seen (Fig.1). Germinal vesicle breakdown occurred within 30-45 minutes after initial exposure to seawater.

Development of *C. californiensis* proceeded as recorded in Table 1. The first cleavage occurred two hours following fertilization. The second cleavage occurred nearly two hours after the first, and the third cleavage happened between four and six hours after fertilization. At the 8-cell stage, the typical spiral cleavage of the group was evident (Fig. 2 & 3). By 20 hours post-fertilization, a ciliated coeloblastula had formed and could be observed swimming (Fig. 4). Gastrulation was by invagination and occurred two days after fertilization.

The first pilidium larvae were observed on April 29, 2007, five days after fertilization. These pilidia were small and had stubby, cilia-lined lappets on their ventral side (Fig. 5 &6). The gut of the pilidium was incomplete, consisting of an esophagus and a blind stomach. A well-formed apical organ with an apical tuft could be seen on top of the larval "helmet." As the pilidia grew, both the lappets and "helmet" elongated. On May 18, 2007, 24 days after fertilization, pilidium larvae were observed with mesenchymal cells moving about in the "helmet" region (Fig. 7).

The last culture of *Cerebratulus californiensis* larvae crashed May 19, 2007. No immediate reason was apparent for this failure; however, it may have been attributable to bacterial infection, as the antibiotic treatment had ceased on May 17.

Discussion

The early development of *Cerebratulus californiensis* followed the typical nemertean blueprint, marked by spiral, holoblastic cleavage, coeloblastula formation, and gastrulation by invagination. Development of the pilidium larva was also typical for this

heteronemertean species. As described by Costello & Henley (1971), the development of *C. lacteus* and that of *C. californiensis* appear to be very similar. The major divergence between the two groups occurs within the timing of developmental events. According to Wilson (1900), development in *C. lacteus* proceeds as shown in Table 2.

The observed development of *C. californiensis* in this study occurred at a much slower rate than *C. lacteus*, with the first cleavage happening 120 minutes after fertilization and the second and third cleavage events happening two and four hours later, respectively. A swimming blastula was not seen until 20 hours post-fertilization for *C. californiensis*, five hours later than its Atlantic coast congener. But the most striking difference between the two is seen with the emergence of young pilidia by 38 hours in *C. lacteus* while young pilidia were not seen in *C. californiensis* until five days after fertilization.

These differences in developmental timetables could be directly attributable to variation in temperature and nutrition. Regrettably, Wilson (1900) did not include the temperature at which he kept his developing embryos, nor did he document what he fed them. It may also be that the difference is real and has something to do with the life histories and larval strategies of the two species.

Although obtaining any viable eggs from *C. californiensis* via the methods described above can be viewed as fortuitous (Stricker 1987), a larger sample size and replication of cultures are needed to discover if these developmental results are truly typical of the species.

Literature Cited

Coe, W.R. 1899. The maturation and fertilization of the egg of *Cerebratulus*. Zool. Jahrb., abt. Anat. u. Ontog. 12: 425-476.

Costello, D.P. & C. Henley. 1971. Methods for obtaining and handling marine eggs and embryos. 2nd edition. Marine Biological Laboratory, Woods Hole. 247 pp.

Freeman, G. 1978. The role of asters in the localization of factors that specify the apical tuft and gut of the nemertine *Cerebratulus lacteus*. J. Exp. Zool. 206: 81-108.

Gibson, R. 1972. Nemerteans. Hutchinson & Co. LTD, London. 224 pp.

Hörstadius, S. 1971. Nemertinae. In: Experimental Embryology of Marine and Freshwater Invertebrates (G. Reverberi, ed.), pp. 164-174. Elsevier-North Holland Publ. Co., Amsterdam.

Maslakova, S., M.Q. Martindale, & J.L. Norenburg. 2003. Fundamental properties of the spiralian develpmental program are displayed by the basal nemertean *Carinoma tremaphoros* (Paleonemertea, Nemertea). Dev. Biol. 267: 342-360.

Maslakova, S., M.Q. Martindale, & J.L. Norenburg. 2004. Vestigial prototroch in the basal nemertean *Carinoma tremaphoros* (Nemertea; Paleonemertea). Evolution & Development 6 (4): 219-226.

Strathmann, M. 1987. Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae. University of Washington Press, Seattle. 670 pp.

Stricker, S.A. 1987. Phylum Nemertea. In: Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae (M. Strathmann), pp.129-137. University of Washington Press, Seattle.

Wilson, E.B. 1900. The habits and early development of *Cerebratulus lacteus* (Verrill). A contribution to physiological morphology. Quart. J. Micr. Sci. 43: 97-198.

Yatzu, N. 1904. Experiments on the development of egg fragments in *Cerebratulus*. Biol. Bull. 6: 123-136.

Yatzu, N. 1910. Experiments on the germinal localization in the egg of *Cerebratulus*. J. Coll. Sci. Imp. Univ. Tokyo. 27 (Art. 17): 1-37.

Tables

Stage	Time
First cleavage	120 minutes
Second cleavage	4 hours
Third cleavage	4-6 hours
Blastula	20 hours
Gastrula	48 hours
Young pilidium	5 day
Pilidium with mesenchymal cells in the "helmet"	24 days

Table 1. Developmental timetable of *Cerebratulus californiensis*

Stage	Time
First cleavage	75 minutes
Second cleavage	155 minutes
Third cleavage	170 minutes
Blastula	15 hours
Gastrula	20 hours
Hatching, young pilidium	38 hours
Well-formed pilidium	108 hours

Table 2. Developmental timetable of *Cerebratulus lacteus* as described by Wilson (1900).

Figures

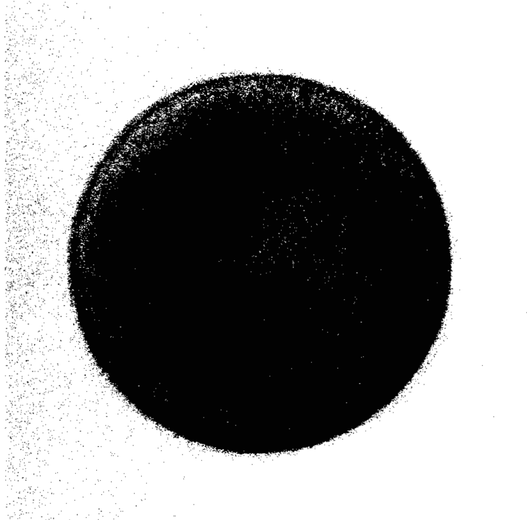

Figure 1. Unfertilized egg of *Cerebratulus californiensis* with large germinal vesicle. (4/24/07)

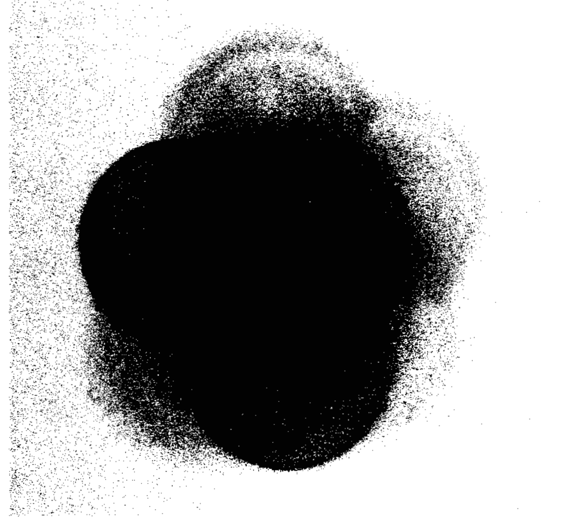

Figure 2. 8-cell embryo of *Cerebratulus californiensis* showing spiral cleavage. (4/24/07)

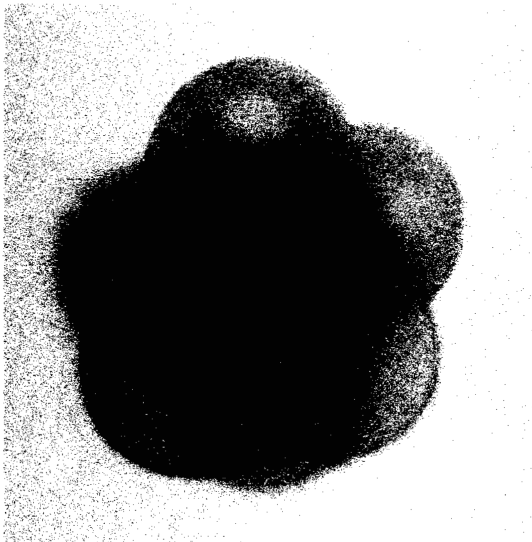

Figure 3. 8-cell embryo of *Cerebratulus californiensis* showing spiral cleavage. (4/24/07)

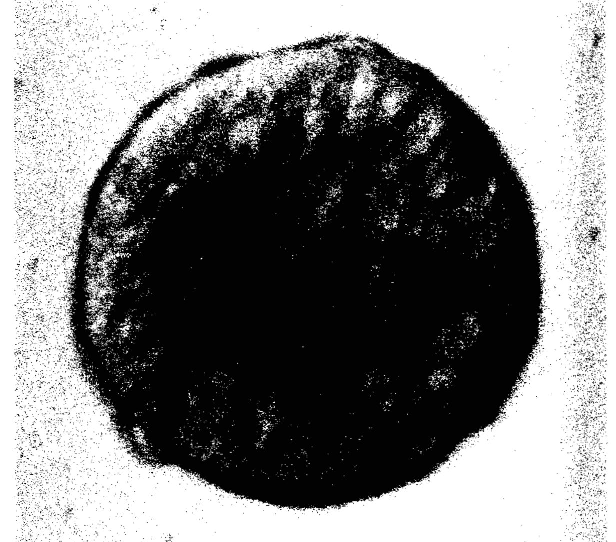

Figure 4. Coeloblastula of *Cerebratulus californiensis*. (4/25/07)

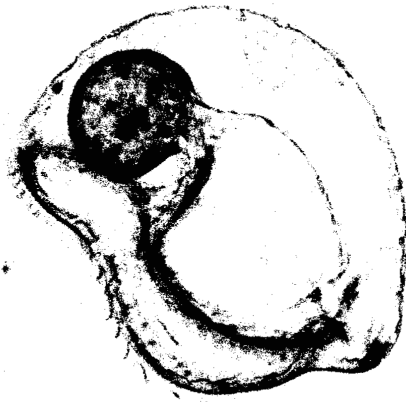

Figure 5. Early pilidium larva of *Cerebratulus californiensis*. Blind gut, apical organ, and ciliated lappets are visible. (4/29/07)

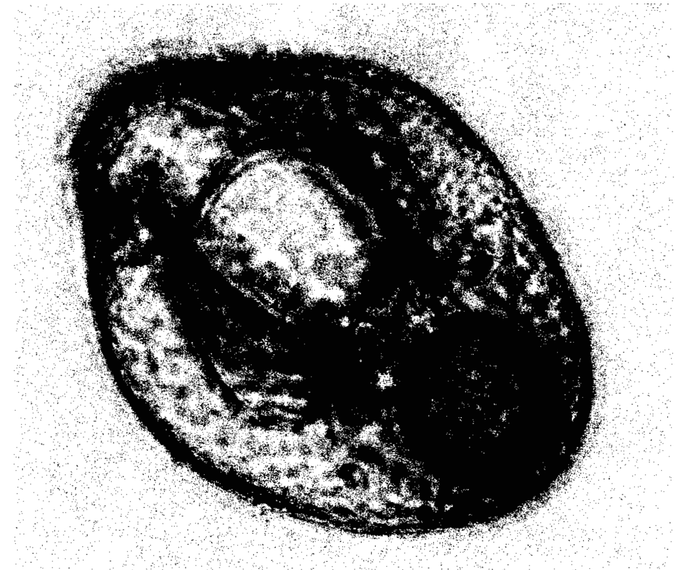

Figure 6. Early pilidium larva of *Cerebratulus californiensis*, ventral view. Mouth is to the left of center. Blind stomach appears reddish. (4/29/07)

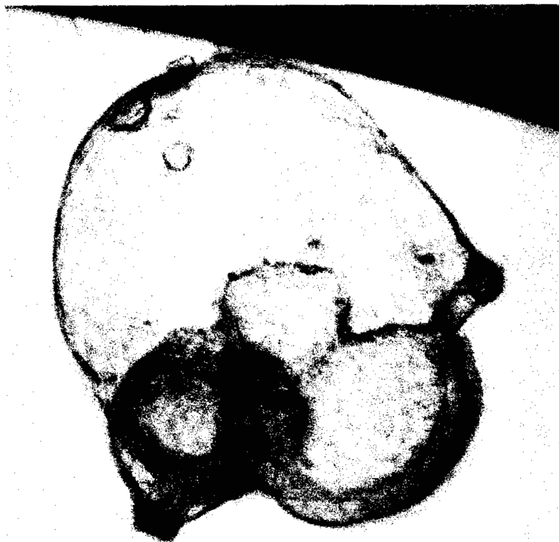

Figure 7. Pilidium larva of *Cerebratulus californiensis*. Mesenchymal cells are seen as circles in the "helmet" region. (5/18/07)