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# COMPARATIVE ANATOMY AND HISTOLOGY OF DEVELOPMENTAL AND PARASITIC STAGES IN THE LIFE CYCLE OF THE LINED SEA ANEMONE *EDWARDSIELLA LINEATA*

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ABSTRACT: The evolution of parasitism is often accompanied by profound changes to the developmental program. However, relatively few studies have directly examined the developmental evolution of parasitic species from free-living ancestors. The lined sea anemone *Edwardsiella lineata* is a relatively recently evolved parasite for which closely related free-living outgroups are known, including the starlet sea anemone *Nematostella vectensis*. The larva of *E. lineata* parasitizes the ctenophore *Mnemiopsis leidyi*, and, once embedded in its host, the anemone assumes a novel vermiform body plan. That we might begin to understand how the developmental program of this species has been transformed during the evolution of parasitism, we characterized the gross anatomy, histology, and cnidom of the parasitic stage, post-parasitic larval stage, and adult stage of the *E. lineata* life cycle. The distinct parasitic stage of the life cycle differs from the post-parasitic larva with respect to overall shape, external ciliation, cnida frequency, and tissue architecture. The parasitic stage and planula both contain holotrichs, a type of cnida not previously reported in Edwardsiidae. The internal morphology of the post-parasitic planula is extremely similar to the adult morphology, with a complete set of mesenterial tissue and musculature despite this stage having little external differentiation. Finally, we observed 2 previously undocumented aspects of asexual reproduction in *E. lineata*: (1) the parasitic stage undergoes transverse fission via physal pinching, the first report of asexual reproduction in a pre-adult stage in the Edwardsiidae; and (2) the juvenile polyp undergoes transverse fission via polarity reversal, the first time this form of fission has been reported in *E. lineata*.

Parasitism has evolved repeatedly in every major organismal lineage, and parasites are thought to substantially outnumber free-living species (Windsor, 1998). Among extant animals, a recent minimum estimate counts more than 105,000 parasitic species from 12 different metazoan phyla (Poulin and Morand, 2004; Poulin, 2007). Some ancient metazoan lineages are dominated by parasitic species; e.g., more than 80% of the roughly 24,000 known platyhelminthes are parasites. However, other lineages of comparable antiquity are dominated by free-living species; e.g., of the roughly 6,200 extant species of anthozoan cnidarians that have been described, only 3 parasitic species have been documented, and all 3 are burrowing anemones whose larvae parasitize gelatinous zooplankton hosts. *Peachia parasitica* and *Peachia quinquecapitata* are parasites on scyphozoan medusae (Spaulding, 1972; McDermott et al., 1982), whereas *Edwardsiella lineata* infects the ctenophores *Mnemiopsis leidyi* and *Beroë ovata* (Crowell, 1976; Crowell and Oates, 1980; Bumann and Puls, 1996; Reitzel, Sullivan et al., 2007).

Animals with long histories as obligate parasites typically exhibit profound morphological, physiological, developmental, and behavioral changes relative to their closest free-living ancestors. However, recently evolved parasites may differ only modestly from their free-living ancestors, depending upon the degree to which the free-living ancestor was already pre-adapted for exploiting its host. Indeed, it is thought that parasitism cannot evolve except where the presumptive parasite exhibits pre-adaptations that allow it to exploit a host species in a manner that improves its fitness (Rothschild and Clay, 1952; Poulin, 2007). Given the prevalence of parasitism and its ecological and evolutionary significance, it is important to understand how free-living lineages undergo the transition to parasitism. This will require more data on recently evolved parasitic lineages and species that are just initiating a transition to parasitism (Poulin, 2007).

The lined sea anemone, *Edwardsiella lineata*, is a recently evolved parasite that belongs to the actinarian family Edwardsiidae (Daly, 2002b). Edwardsiid anemones are burrowers, using an extensible bulbous aboral end to bury themselves up to their tentacles in soft substrates. They lack the basal disc and basilar muscles that are used by many other anemones to adhere to hard substrates (Daly et al., 2002). Edwardsiids are relatively simple anatomically; e.g., they possess only 8 complete mesenteries, fewer than any other family of anemones (Daly et al., 2002). The complete mesenteries are sheets of endodermal tissue that connect the throat or pharynx to the outer body wall. Interestingly, many other sea anemones pass through an ''Edwardsia-stage'' in which they possess only 8 complete mesenteries and lack basilar muscles (Stephenson, 1935). The best-known member of the Edwardsiidae is the starlet sea anemone, *Nematostella vectensis*, a free-living estuarine species. *Nematostella vectensis* is an important new model system for both lab- and field-based studies of ecology, genomics, development, and evolution (Darling et al., 2005). Because of its close phylogenetic relationship with *E. lineata* and its highly similar adult body plan (Fig. 1), *N. vectensis* provides a valuable comparison with *E. lineata*.

Both phylogenetic and developmental evidence imply that the lined sea anemone *E. lineata* has only recently evolved a parasitic life cycle. *Edwardsiella lineata* nested within the Edwardsiidae and is the only member of the family that has been shown to be parasitic (although there is a single report of larval *Edwardsia carnea* living within the ctenophore *Bolinopsis* sp.; Stephenson, 1935), In all other Edwardsiid species that have been characterized, the life cycle comprises a free-living benthic adult stage and a free-swimming or -crawling larva (Fig. 2A); this is presumably the ancestral condition for the family. *Edwardsiella lineata* retains the ancestral larval body plan (the planula) and the ancestral adult body plan (the polyp; Fig. 2B). Furthermore, *E. lineata* exhibits signs of being a facultative parasite. When the parasite is excised from a host, it quickly

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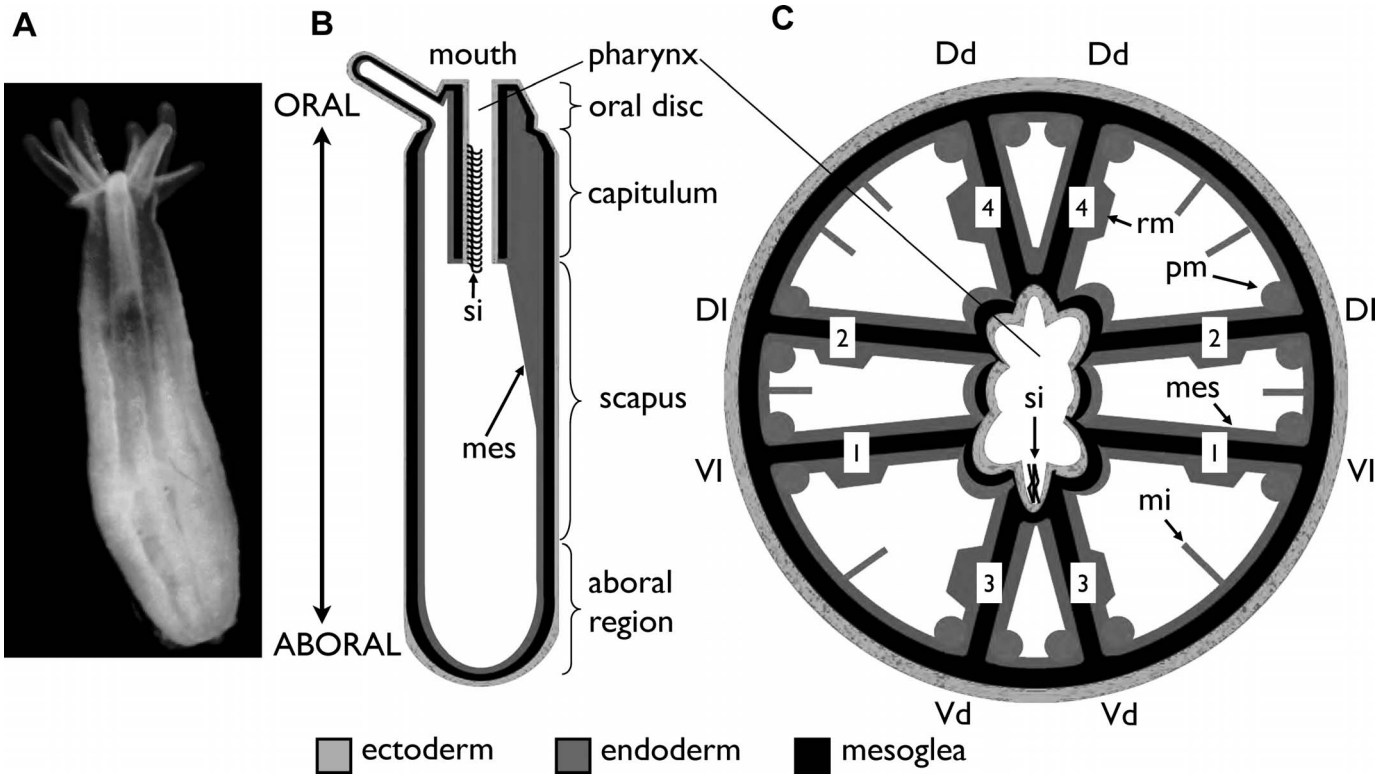


FIGURE 1. Anatomy of *E. lineata* polyp. (**A**) Photograph of adult polyp shown next to diagrammatic representation of longitudinal section (**B**) and transverse section taken at level of the pharynx (**C**). Macrocnemes are labeled to indicate order of development and their relative position: the ventro-lateral mesenteries (Vl) develop first, followed by the dorso-lateral mesenteries (Dl), the ventral directives (Vd), and the dorsal directives (Dd). Single microcnemes (mi) lie in the lateral and ventro-lateral mesenterial compartments; a set of paired microcnemes lie in each of the dorso-lateral compartments. The siphonoglyph (Si) is associated with the ventral directives. Abbreviations are: mi = incomplete mesentery, pm = parietal muscle, rm = retractor muscle, si = siphonoglyph.

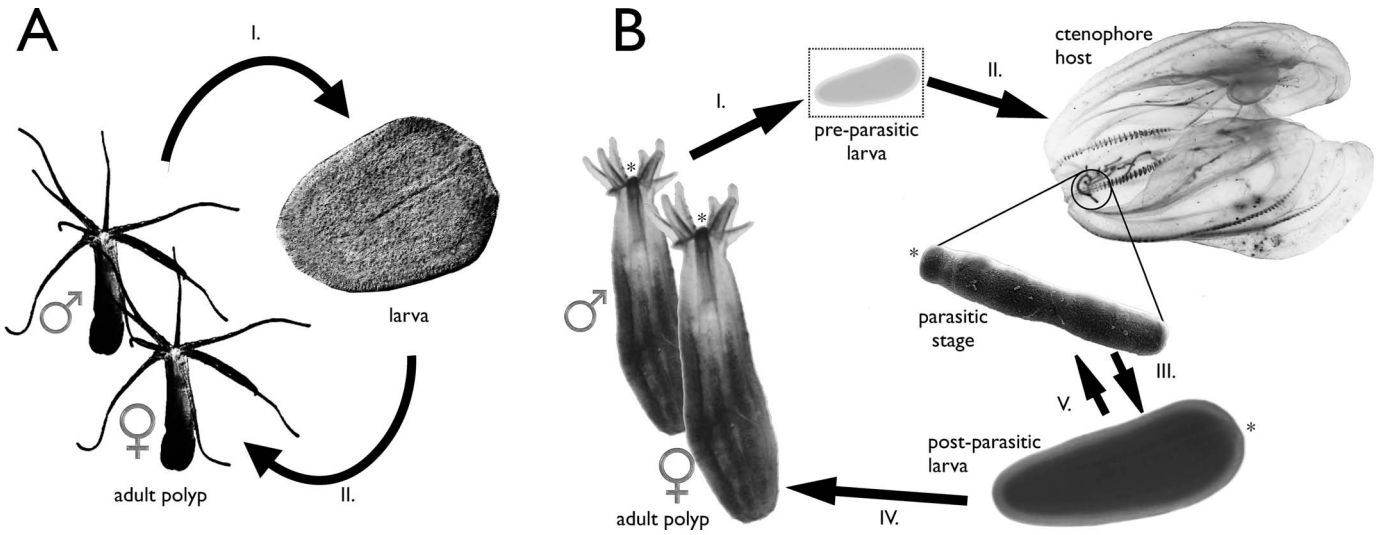


FIGURE 2. Comparative overview of life cycle of (**A**) *N. vectensis* and (**B**) *E. lineata*. In *N. vectensis*, sexual reproduction involves a free-living planula larva, which then develops into a polyp. In *E. lineata*, sexual reproduction generates a pre-parasitic larva. Upon infecting a host, the larva gives rise to the parasitic stage that exhibits a characteristic vermiform body plan. Upon exiting the host (or being experimentally excised), the vermiform parasite morphs into a ciliated, motile, post-parasitic larva. In the case of experimentally excised parasites, this larva can either develop into a polyp or it can re-infect a second host and re-develop the vermiform body plan of the parasitic life cycle stage.

develops into a free-swimming planula larva (Fig. 2B). This experimental manipulation presumably mimics the process that would occur in nature when the host dies or the parasite leaves the still living host of its own accord. At this juncture of the life cycle, at least in animals that were experimentally excised, the planula is capable of following 2 alternative developmental trajectories: it may re-infect another host and re-assume the parasitic body plan (Reitzel et al., 2006; Reitzel, Sullivan et al., 2007), or it may undergo settlement and develop into a free-living polyp (Crowell and Oates, 1980; Daly, 2002b; Reitzel et al., 2006; Reitzel, Sullivan et al., 2007).

Given that *E. lineata* is a relatively recently evolved parasite in which many aspects of the ancestral ontogeny are preserved, we can gain insights into its transition to parasitism by comparing its ontogeny to that of free-living outgroups, and by comparing ancestral and derived stages in its own life cycle. In an effort to understand how the ancestral developmental program was altered during the evolution of parasitism, we characterized the anatomy, histology, and cnidom (the complement of stinging cells, or cnidae) of *E. lineata* during 3 distinct life cycle stages (Fig. 2B): (1) the parasitic stage, (2) the post-parasitic planula stage, and (3) the adult polyp stage. In addition, based on laboratory observations, we describe novel aspects of asexual reproduction in the life cycle of this species. The parasitic stage differs from the adult polyp in several key morphological and histological traits, including the absence of tentacles, a reduction in the number of body cavity partitions (known as mesenteries), a reduction in the degree of body column differentiation, and an unusual complement of cnidae. Likewise, the parasitic stage differs from the post-parasitic planula in size, morphology of the column, number of mesenteries, musculature, and cnida frequency.

## MATERIALS AND METHODS

### Collection of *E. lineata* from the ctenophore *M. leidyi*

*Mnemiopsis leidyi* parasitized by *E. lineata* were collected from Great Harbor in Woods Hole, Massachusetts. Collections were made from a rock jetty that extends south-southeast for a distance of approximately 20 m from the shore. Ctenophores were gently lifted from the water in nylon plankton nets and placed in containers of seawater for transport to the laboratory of the Boston University Marine Program, Boston, Massachusetts.

### Culturing excised parasites in the absence of a second host

Hundreds of parasites were gently teased away from the mesoglea of field-collected *M. leidyi* with the aid of scalpel and forceps. The excised parasites were transferred to artificial seawater (salinity = 33 parts per thousand) and maintained at either 13 or 18 C. Parasites successfully developed into planulae larvae and later settled as polyps at both temperatures. The transition from the pelagic parasite to benthic polyp typically takes less than 1 wk and has been observed to occur in as few as 4 days (Reitzel, Sullivan et al., 2007). Polyps were cultured in bowls of non-circulating artificial seawater (33 ppt) or in re-circulating aquaria. They were fed freshly hatched *Artemia* sp. nauplii twice per week. We recorded and photographed organismal-level life cycle characters from animals in culture, including asexual reproduction via transverse fission in both the parasite and the polyp stages. Animals were photographed with a Nikon CoolPix camera coupled to an Olympus SV12 stereomicroscope.

### Histology

Individuals in the parasitic stage, post-parasitic larva stage, and adult stage were fixed in 10% buffered formalin, and later transferred to 70% ethanol. All material was processed for paraffin histology following standard protocols. Adults were incubated in each solution for 30 min, while parasites and planulae were incubated for 15 min. Transverse and longitudinal serial sections were cut at a thickness of 12 μm, mounted on slides, and stained using Masson's Trichrome (Presnell and Schreibman, 1997).

### Cnidae characterization

Cnidae were examined in situ in histological preparations and by squashing a small piece of adult tissue or a whole pre-adult individual on a microscope slide. Slides and squash preparations were examined under bright field microscopy at ×1,000 magnification using differential interference contrast optics. Cnidae were characterized from multiple individuals from 3 life cycle stages: parasitic stage (n = 4), larval stage (n = 3), and juvenile polyps (n = 3). The width and length of undischarged cnidae were measured utilizing a Boeckler measurement system coupled to a Leica MZ9.5 stereomicroscope with a Sony DXCS500 digital camera. Nematocyst terminology follows Mariscal (1974). These data were compared with cnidae measurements previously reported for adult *E. lineata* (Daly, 2002a).

## RESULTS

### Parasitic and post-parasitic stages of the life cycle

During the parasitic stage of its life history, *E. lineata* embeds itself in the mesoglea of its ctenophore host, typically in the vicinity of the host's stomach or pharynx (Reitzel et al., 2006). From this location the anemone can extend its mouth into the host's gut and feed on partially digested food (Fig. 3A, B). Upon excision, the vermiform parasitic stage (Fig. 3C, D) undergoes a rapid and dramatic remodeling of the body plan that generates a fusiform planula larva (Fig. 3E). This post-parasitic planula can follow 2 distinct developmental trajectories (Fig. 2B; Reitzel, Sullivan et al., 2007). It can re-infect another ctenophore host, in which case it morphs back into the parasitic body plan. Alternately, it can develop into a polyp and undergo settlement (Fig. 3F–J). When we cultured post-parasitic planulae with potential host ctenophores, we observed that planulae infect ctenophores via 2 different routes: epidermal burrowing (Crowell, 1976) and burrowing across the gastrodermal wall after being ingested by the ctenophore (Reitzel, Sullivan et al., 2007). Once the planula has become embedded in the mesoglea of a second ctenophore host, it quickly redevelops the vermiform body plan characteristic of the parasitic stage, in as little as 1 hr. Planulae reared in cultures lacking possible hosts developed into polyps in as few as 3 days following excision from the ctenophore.

### Morphological and histological differences among life cycle stages

*Overall dimensions, body plan, and tentacles:* The 3 life cycle stages of *E. lineata* differ markedly with respect to body plan. An adult specimen of *E. lineata* is typically slender and vermiform, 15–20 mm long and 5 mm in diameter, with 18–24 smooth, gradually tapering tentacles. All recently settled juvenile polyps had 8 primary tentacles (Fig. 3F–H). Although planulae typically settle and develop into adults with a single oral crown (Fig. 3J), a proportion of settled juveniles developed oral crowns asynchronously at both ends of the primary body axis (Fig. 3I). These individuals developed into adults with 2 oral crowns (Fig. 3K). In the parasitic stage, the animals are approximately 1 mm in diameter, and range in length from 1 to >10 mm. They lack tentacles but may exhibit 4 tentacle

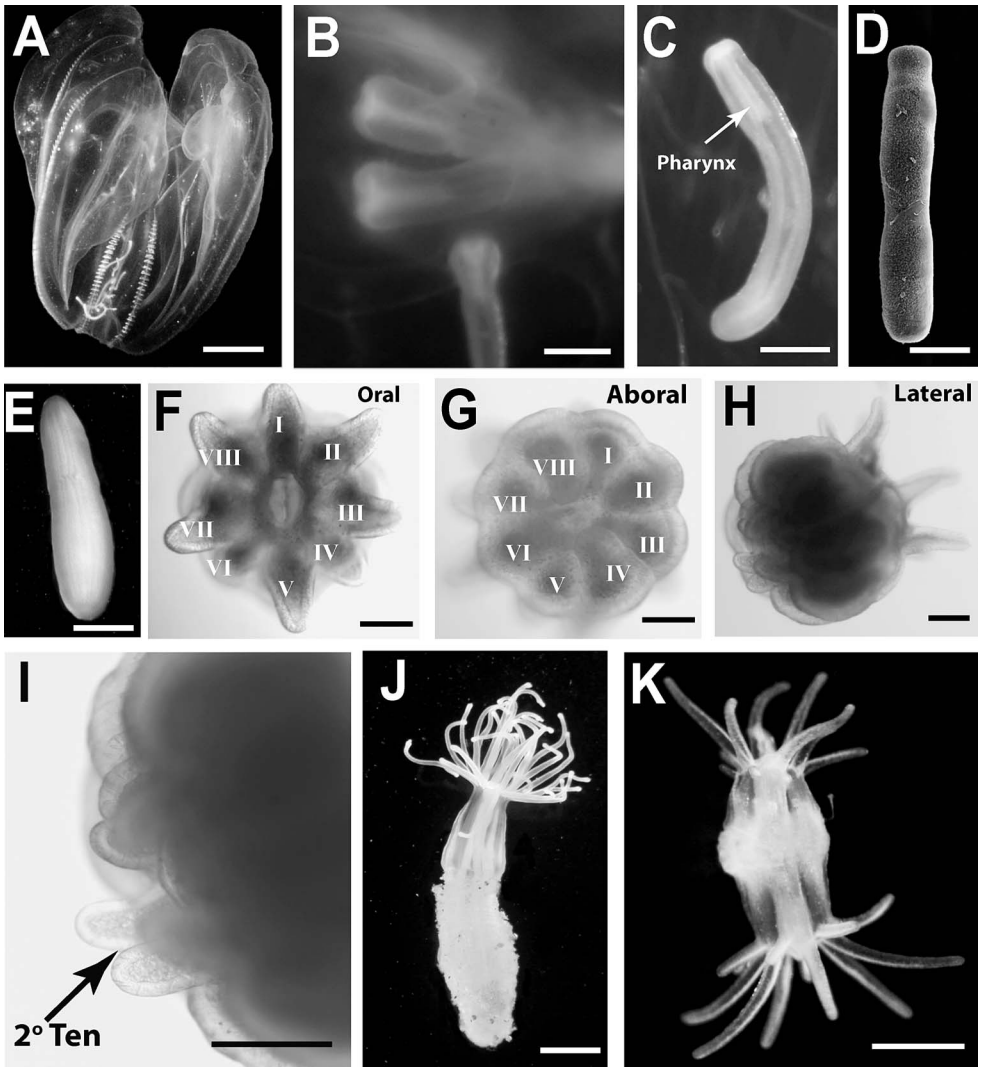


FIGURE 3. Life cycle stages of *Edwardsiella lineata*. We have not witnessed spawning, so observations begin with the parasitic stage in the ctenophore *M. leidyi* (**A–B**). (**C**) Parasites are long and cylindrical with a prominent pharynx. (**D**) SEM of parasitic stage showing extensive ciliation. (**E**) Post-parasitic planula stage. (**F**) Oral view of recently settled juvenile polyp. Tentacles numbered with roman numerals. (**G**) Aboral view of juvenile polyp in panel F. Mesenteries numbered with roman numerals. (**H**) Lateral view of juvenile polyp with asynchronous development of second oral crown (magnified in **I**, 2° Ten = Secondary Tentacles). (**J**) Adult polyp. (**K**) Two-crowned adult polyp. *Scale bar:* 0.5 cm in panel A, 0.5 mm in panels B–I, 3 mm in panels J–K.

mounds, reminiscent of the protrusions that form when tentacles first begin to emerge during the larval development of *N. vectensis*, a closely related non-parasitic sea anemone (Daly, 2002a). The post-parasitic larvae are considerably shorter than animals in the parasitic stage, being roughly spherical and having a diameter of 1–2 mm. There are no externally distinguishable tentacles or tentacle mounds at this stage in development.

*Actinopharynx:* In the polyp the post-parasitic planula, and the parasitic stage, the mouth is the only opening to the gastrovascular cavity (the coelenteron), and it leads into a prominent throat, or actinopharynx (Figs. 1B–C, 3C). The internal lumen of the actinopharynx is lined by ectodermal epithelium, while the outer, coelenteronic surface consists of endoderm. A layer of largely acellular mesoglea lies between these 2 epithelia.

In the adult polyp the actinopharynx appears star-shaped in cross-section, with 8 deep folds corresponding to the spaces between the 8 complete mesenteries or macrocnemes (Figs. 1C, 4A). The star-shape is created by the inpocketing of the lumen of the actinopharynx and variation in the height of the cells in the columnar epithelium lining the lumen. The deepest fold, which lies between the ventral pair of macrocnemes (the ventral directives), is lined with considerably longer cilia. This ciliated groove, the siphonoglyph, is 1 of the structural elements that imposes bilateral symmetry on the cross-sectional anatomy of *E. lineata* (Stephenson, 1928). In the parasitic stage, the epithelium of the actinopharynx has dense cilia and numerous gland cells (Fig. 5B). In cross-section the pharynx of the parasitic stage does not exhibit the star-shape that is typical of adult actinarian (including the polyp stage of *E. lineata*) because cells lining the actinopharynx of the parasite are more uniform in height. In the parasitic stage the siphonoglyph is not yet differentiated. In the post-parasitic planula the actinopharynx is well developed, resembling that of an adult, but much smaller in size (Fig. 5A).

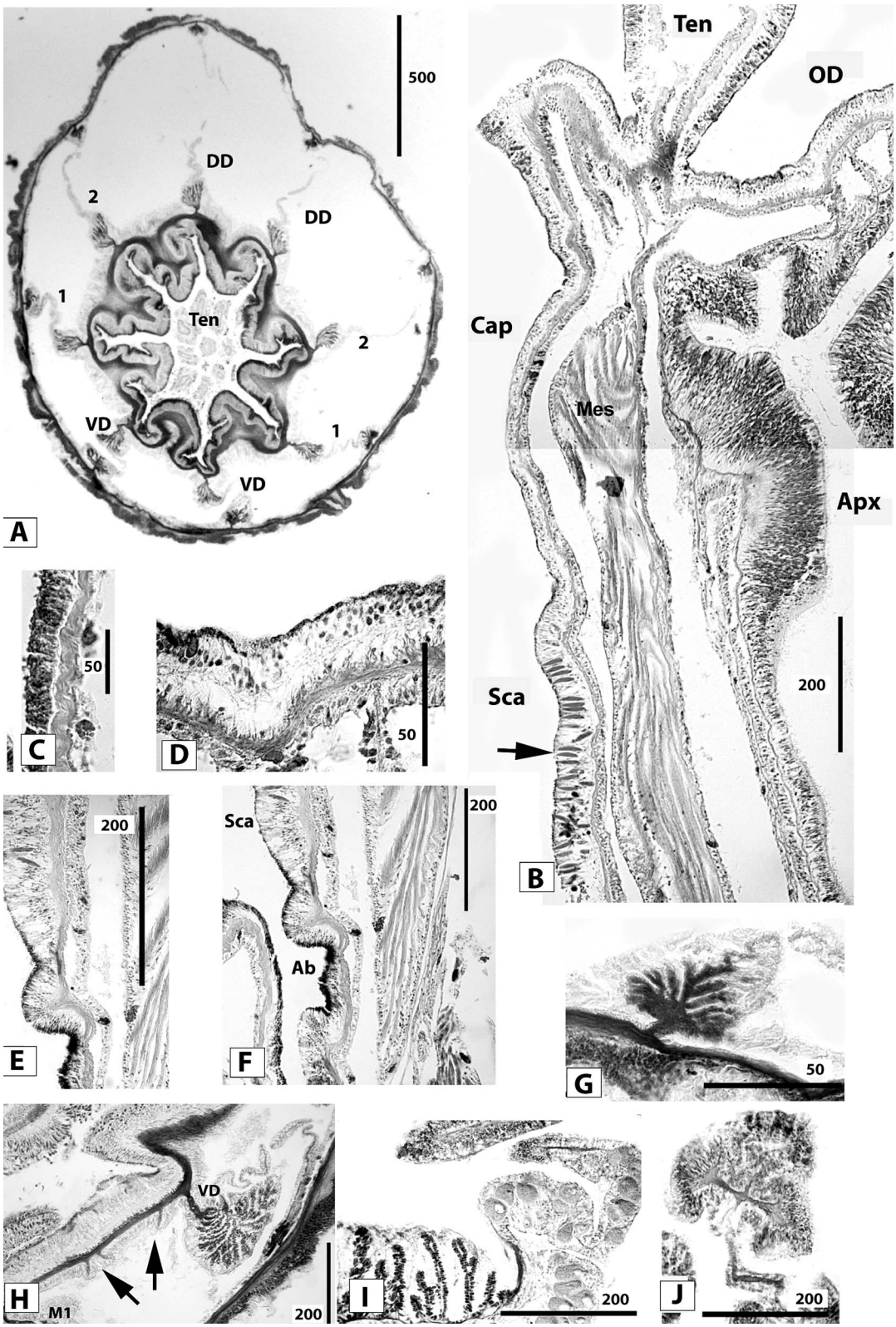


FIGURE 4. Anatomy and histology of adult *E. lineata*. (**A**) Transverse section through actinopharynx of a contracted individual. Tentacles (Ten) are retracted inside actinopharynx. Mesenteries are labeled to indicate sequence of development; 1 = first couple, 2 = second couple, DD = dorsal directive, VD = ventral directive. (**B**) Longitudinal section through oral region of the column, showing oral disc (OD), tentacles (Ten), Capitulum (Cap), actinopharynx (Aphx), and Scapus (Sca). Dark-staining oval structures in the scapus are basitrichous nematocysts (indicated by arrow). (**C**) Longitudinal section through aboral region of the column, showing detail of ectoderm of aboral end. (**D**) Longitudinal section through oral disc and base of tentacle, showing ectodermal longitudinal musculature. (**E**) Longitudinal section through the column at transition between the thick scapus and thinner, more glandular aboral end. (**F**) Longitudinal section through the column showing the scapus (Sca) and the aboral

*Body column:* The column of an adult is composed of 4 histologically distinct regions: the oral disc (which bears the tentacles), the capitulum (which houses the actinopharynx), the scapus (which accounts for the majority of the body column), and the aboral end (Figs. 1B, 4B, F). The tentacles and oral disc (Fig. 4B) are well endowed with endodermal musculature (Fig. 4D) and 2 kinds of cnidae: spirocysts and basitrichs (Fig. 6). The capitulum closely resembles the tentacles and oral disc in terms of the overall tissue thickness, presence of endodermal musculature, and general appearance of the ectoderm (Fig. 4B). However, the capitulum lacks spirocysts and contains fewer basitrichs. The scapus has a thicker ectoderm and more abundant cnidae than the capitulum (Fig. 4B). In the aboral end (Fig. 4E, F) the ectoderm is slightly thinner than in the scapus and approximately the same thickness as in the capitulum. The aboral ectoderm contains more gland cells than the ectoderm of the scapus, capitulum, oral disc, or tentacles. It lacks spirocysts and has relatively few basitrichs.

In contrast to the adult, the body column of the parasitic stage is not differentiated into distinct regions. The body wall is uniform with respect to its histological composition and thickness along its entire length (Fig. 5C, D), except for a slight thickening in the oral-most region, which corresponds to the tentacle mounds (Fig. 5D). The body column has no discernable musculature (Fig. 5C, D). The ectoderm has numerous glands and long cilia, and it bears cnidae of 3 types: spirocysts, basitrichs, and holotrichs (Figs. 5E, 6).

In the post-parasitic planula the column is also not clearly differentiated into regions, at least initially (Fig. 5F, G), although the beginnings of differentiation are visible in some specimens. For example, in older larvae that are undergoing the developmental transition into a polyp (Fig. 5G), the mid-column has many fewer spirocysts than the region around the mouth (the presumptive oral disc and tentacles). Holotrichs are not numerous but can be seen throughout the ectoderm. There are no obvious differences in the thickness or cellular composition of body wall at different points along the body column; the thickenings at the oral-end of the column seen in the parasite are not visible in even late-stage post-parasitic planula. No columnar muscles are visible in the oral or aboral column.

*Mesenteries:* The mesenteries are membranous lamellae that consist of 2 layers of endodermal epithelium surrounding a central core of extracellular connective tissue known as mesoglea (Stephenson, 1928). There are 2 main types of mesenteries in anthozoan cnidarians, e.g., corals and sea anemones: macrocnemes and microcnemes. In the adult polyp of edwardsiid anemones, the larger macrocnemes are all perfect (or complete), meaning they span the gap between the body wall and the actinopharynx. Each macrocneme projects inward from the body wall to the endodermal epithelium of the actinopharynx and extends the entire length of the animal, from the inside of the base to the inner surface of the oral disc (Stephenson, 1928). Where they join the body wall, each macrocneme bears a pair of parietal muscles, longitudinally oriented endodermal muscles symmetric with respect to the mesenterial lamella. Approximately halfway between the body wall and the actinopharynx, each macrocneme also bears a single retractor muscle on 1 surface. Contraction of these endodermally derived muscles causes the body column to shorten along the oral-aboral axis. Proximal to the pharynx, between the retractor muscles and the free edge of the mesentery, a macrocneme may bear gametic tissue. Along the free edge of each mesentery (aboral to the actinopharynx), there is a thickened rim called the mesenterial filament that contains ciliary or cnidoglandular tracts comprised of numerous stinging capsules (nematocysts) and secretory cells.

Microcnemes are mesenteries that are shorter and less complex morphologically than macrocnemes. They project inward from the body wall but fail to meet the actinopharynx, and they extend aborally from the oral disc only a few mm. Microcnemes lack mesenterial filaments, muscles, and gametic tissue (Stephenson, 1928).

With respect to its complement of mesenteries, the adult polyp of *E. lineata* is typical for an edwardsiid sea anemone (Stephenson, 1928). Adult *E. lineata* have 8 perfect macrocnemes with clearly demarcated retractor and parietal muscles (Figs. 1C, 4A, G, H, I). Proximal to the actinopharynx, the macrocnemes bear gametes. The free edge of each macrocneme is bounded by a mesenterial filament (Fig. 4J). The macrocnemes extend the length of the body column, to just distal to the base, with the muscles becoming slightly smaller but no less clearly demarcated proximally.

The 8 macrocnemes exhibit bilateral symmetry about the directive axis, which bisects the pharyngeal lumen. Each macrocneme on the left half of the directive axis has a matching counterpart on the right. The directive couples, i.e., the dorsal and ventral directive pairs, lie closest to the directive axis, with the first (ventrolateral) and second (dorsolateral) mesenterial couples located laterally.

Below the tentacles in the polyp, in the oral-most portion of the capitulum, are 8 small microcnemes that lack muscles, gonadal tissue, or filaments (Fig. 4H). Individual microcnemes are located adjacent to each of the 2 dorsolateral macrocnemes and each of the 2 ventrolateral macrocnemes. Additionally, there is a pair of microcnemes in each ventrolateral mesenterial compartment.

The parasitic stage has 8 mesenteries orally. These will develop into the macrocnemes of the adult, but in the parasitic stage they lack retractor muscles, parietal muscles, gametogenic tissue, and mesenterial filaments (Fig. 5B, H). Aboral to the actinopharynx, only the first mesenterial couple is clearly visible, with the second and directive couples being visible as depressions in the material that fills the coelenteron (Fig. 5I). No microcnemes were observed in any of the parasites sectioned.

In the youngest post-parasitic planulae, the macrocnemes are

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(Ab) regions of column. Aboral end is flattened so that it bulges to the side of the column. (**G**) Transverse section through column aboral to the actinopharynx, showing parietal muscle. Compare with Fig. 4I. (**H**) Transverse section through oral region of the column of a contracted individual. Oral-most column is contracted and inverted, such that microcnemes (arrows) between the ventral directive (VD) and first couple mesentery (M1) are on the inside of the column. (**I**) Transverse section through a mesentery in the scapus showing retractor muscle and gametes. (**J**) Transverse section through a mesentery in the scapus showing mesenterial filament. All scale bars are in μm.

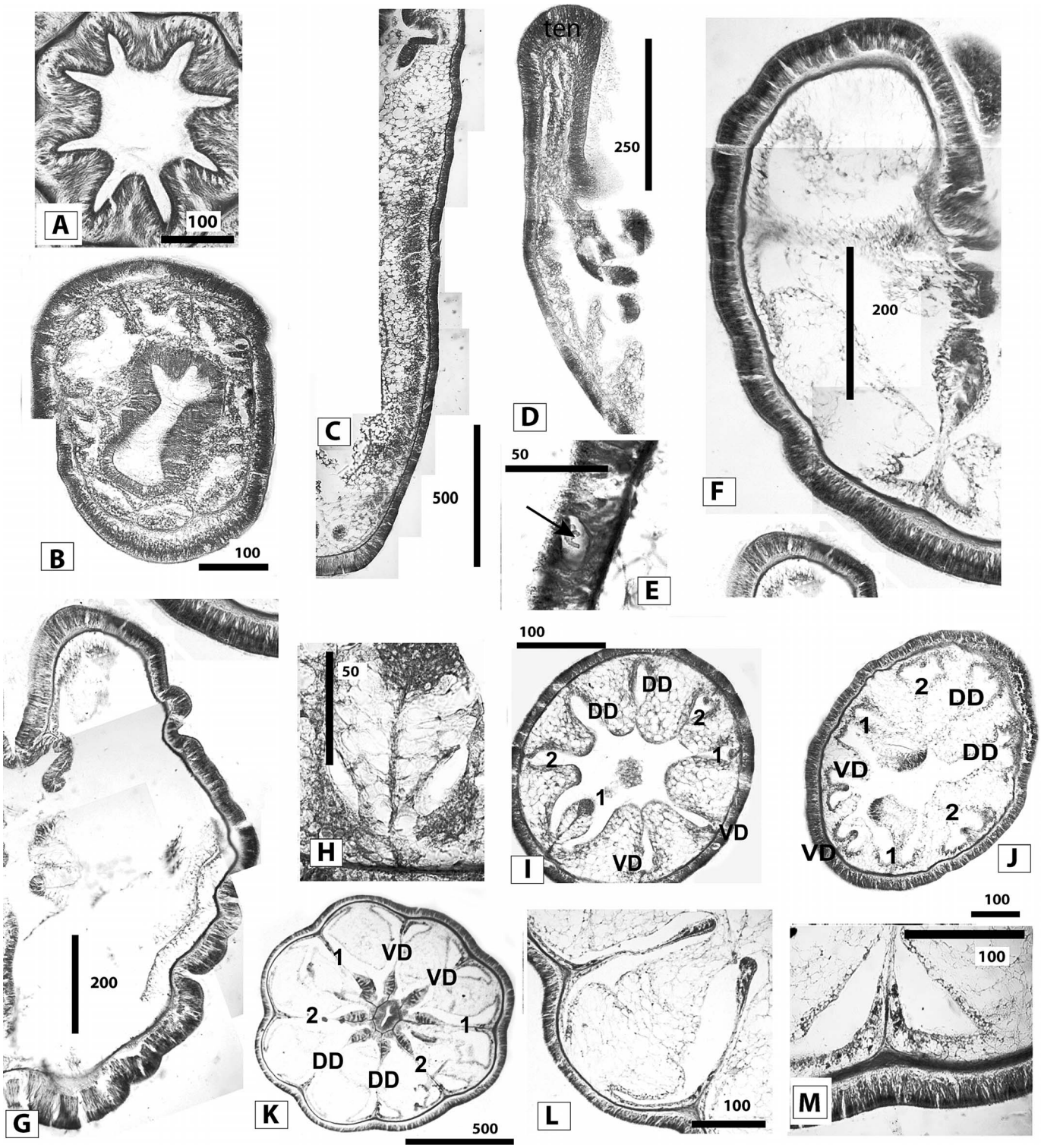


FIGURE 5. Anatomy and histology of parasitic stage and larva of *E. lineata*. (**A**) Transverse section through actinopharynx, post-parasitic planula. (**B**) Transverse section through parasitic stage. (**C**) Longitudinal section through aboral column, parasitic stage. (**D**) Longitudinal section through oral column, parasitic stage. Note slight thickening of the body wall in the oral-most column where a tentacle (ten) will form. (**E**) Close-up of longitudinal section through column, parasitic stage. Note holotrichous nematocyst (indicated by arrow) and absence of differentiated columnar musculature. (**F**) Longitudinal section through early post-parasitic planula. (**G**) Longitudinal section through older post-parasitic planula. Tentacle and oral disc region are starting to differentiate from the rest of the column, but muscles and nematocysts are not quantitatively different throughout the column. (**H**) Transverse section through mesentery of parasite, aboral to the actinopharynx. Note absence of retractor or parietal musculature, and abundance of vacuolated tissue around developing mesentery. (**I**) Transverse section through column aboral to the actinopharynx, parasitic stage. Mesenteries are labeled to indicate sequence of development; 1 = first couple, 2 = second couple, DD = dorsal directive, VD =

only slightly more developed than in the parasitic larva (compare Fig. 5I, J). All 8 macrocnemes are present, although each may consist of only a short lamella (Fig. 5J). The macrocnemes are most fully developed orally (Fig. 5K), with well-demarcated retractor muscles. Aboral to the actinopharynx, the retractor muscles are smaller (Fig. 5L). The muscles of the older, e.g., first and second, mesenterial couples are larger and more fully developed than the muscles of the directive couples. In contrast with the adult polyp, parietal musculature cannot be distinguished on any of the macrocnemes (Fig. 5M), and microcnemes were not observed.

*Coelenteron:* The coelenteron of adult polyps is a hollow space that acts as a digestive cavity and hydrostatic skeleton. In the parasitic stage the coelenteron is partially filled with a loose, vacuolated material, probably the remnants of maternally provided yolk or lipid stores accumulated while feeding inside the ctenophore host. In comparison to the parasitic stage or adult, the coelenteron of the post-parasitic planula is crowded. The body cavity is subdivided into compartments by 8 longitudinal partitions of endodermal tissue and still contains some vacuolated material (Fig. 5F, L). The coelenteronic space expands as the post-parasitic planula develops into an adult because of the growth of the body and the disappearance of the vacuolated material.

*Cnidom:* Each life cycle stage exhibited a unique complement of stinging cells (Table I, Fig. 6). In all 3 stages we identified spirocysts, microbasic *b*-mastigophores, microbasic *p*-mastigophores, and basitrichs in both the squash preparations and the histological preparations. However, when examining the squash preparations, holotrichs were identified only in the parasitic stage; these cnidae were relatively rare but were evident in squash preparations from all 4 parasites examined. Histological examination confirmed that holotrichs were also present in the post-parasitic planula, although they were less abundant than in the parasitic stage. Long, thin basitrichs were identified in the juvenile and adult stages, but not the parasitic. This type of cnida was rare in the juvenile polyps we examined and was recorded in only 1 of 3 individuals. Daly (2002b) reported these same long, thin basitrichs in mesenterial filaments of adult *E. lineata*.

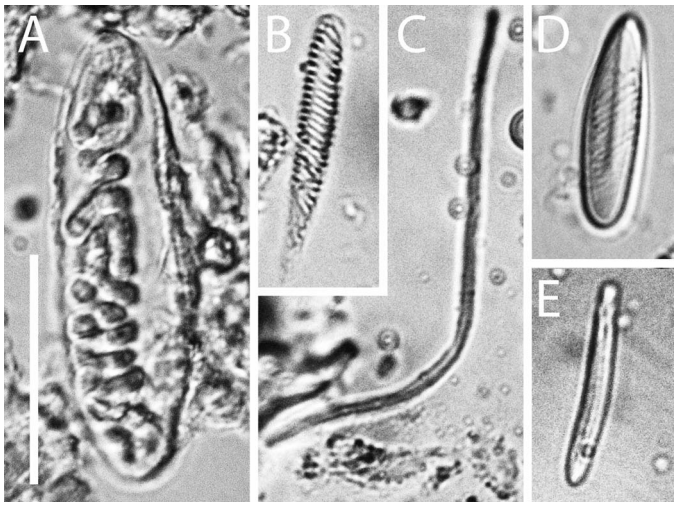


FIGURE 6. Cnidae of *E. lineata*. (**A**) holotrich, (**B**) spirocyst, (**C**) a long, thin basitrich, (**D**) microbasic b-mastigophore, (**E**) small basitrichs. Scale bar: 20 μm for all panels.

### Asexual reproduction in parasitic and adult *E. lineata*

We observed 2 distinct forms of asexual reproduction in *E. lineata*, ''physal pinching'' and ''polarity reversal,'' both culminating in transverse fission. Both modes of reproduction have been previously reported in adults of the closely related free-living sea anemone, *N. vectensis* (Darling et al., 2005). In physal pinching a circumferential constriction arises in the aboral (= physal) region of the animal, and a small fragment pinches off from the rest of the animal. This fragment then regenerates missing oral structures to generate a complete polyp. In polarity reversal, oral structures develop at the aboral end of the animal, resulting in an individual with 2 sets of oral structures and no physa. Subsequently, a physal region emerges roughly midway between the 2 mouths, and a constriction in this region separates 2 individuals, each possessing a complete oral-aboral axis. Both of these modes of transverse fission have been described in adult *N. vectensis*.

In *E. lineata* we documented transverse fission via physal pinching during the parasitic stage and the polyp stage. Upon excising individual parasites from their ctenophore hosts, we observed a number of individuals exhibiting pronounced transverse constrictions (Fig. 7A–D). These animals underwent fission at the site of the constriction, separating the individual into 2 pieces. We did not systematically monitor the frequency of parasites undergoing fission, but we did record 1 individual parasite that underwent fission 3 times in the course of 24 hr.

We observed physal pinching in adult *E. lineata*, as originally reported by Crowell and Oates (1980). The aboral end of the polyp undergoes a transverse constriction (Fig. 7E). After a number of days, a small physal fragment pinches off and regenerates missing oral structures. Contrary to the original description (Crowell and Oates, 1980), at the completion of fission, an oral cavity was present (Fig. 7F). The small fragment that is cleaved retains the complete complement of 8 macrocnemes that were present in the pre-fission adult. During regeneration of the oral disc, 8 to 16 tentacles erupted simultaneously (Fig. 7G, H). In laboratory cultures, when fed regularly, adults frequently undergo this form of transverse fission.

We observed polarity reversal in a small number of settled juveniles. In these individuals 2 oral crowns developed asynchronously on opposite ends of an individual (Fig. 3I). These individuals developed into adults with 2 oral crowns (Fig. 3K). Each double-crowned individual eventually underwent trans-

←

ventral directive. (**J**) Transverse section through the column of young post-parasitic planula, aboral to the actinopharynx. Labeling of the mesenteries follows that of Fig. 4I. (**K**) Transverse section through capitulum, late post-parasitic planula. Labeling of mesenteries follows that of Fig. 5I. (**L**) Transverse section through aboral column, post-parasitic planula. (**M**) Transverse section through mesentery at level of the actinopharynx, showing junction between body wall and mesentery in post-parasitic planula. Parietal muscles are not yet differentiated. All scale bars are in μm.

TABLE I. Size (μm) and number of cnidae in 3 developmental stages of *E. lineata*. Five different cnida types were counted in squash preparations of 3 or 4 individuals from each life history stage. The fraction of animals that were found possess each cnida type is shown (Ind no.). N refers to the number of each type of cnidae counted. For each cnida type and life cycle stage, the maximum and minimum dimensions (length and width) of the capsules (Cap.) were measure in μm.

| Stage | Ind no. | Cnida type | N | Cap. of smallest length | Cap. of smallest width | Cap. of greatest length | Cap. of greatest width |
|---|---|---|---|---|---|---|---|
| Parasite | 4/4 | Spirocyst | 52 | 12.05 × 2.21 | 15.50 × 1.97 | 29.83 × 2.63 | 25.67 × 6.68 |
| | 4/4 | Micro b-mast | 90 | 18.08 × 4.07 | 18.59 × 2.25 | 28.95 × 5.66 | 23.35 × 6.54 |
| | 4/4 | Micro p-mast | 51 | 17.60 × 4.65 | 17.91 × 2.17 | 27.97 × 5.51 | 26.68 × 6.50 |
| | 4/4 | Basi | 70 | 12.10 × 2.75 | 12.18 × 1.50 | 26.34 × 5.13 | 24.37 × 6.14 |
| | 4/4 | Holo | 10 | 32.11 × 12.02 | 40.50 × 8.29 | 47.68 × 12.93 | 46.28 × 13.53 |
| | 0/4 | Long basi | 0 | NA | NA | NA | NA |
| Post-parasitic planula | 3/3 | Spirocyst | 27 | 17.50 × 3.07 | 20.71 × 2.94 | 27.41 × 4.13 | 18.40 × 4.78 |
| | 3/3 | Micro b-mast | 97 | 20.42 × 5.44 | 23.85 × 4.21 | 31.26 × 5.27 | 26.37 × 7.97 |
| | 3/3 | Micro p-mast | 14 | 25.44 × 5.25 | 26.28 × 4.88 | 32.85 × 6.40 | 28.63 × 7.40 |
| | 3/3 | Basi | 47 | 6.68 × 2.30 | 18.41 × 1.30 | 25.67 × 5.46 | 25.17 × 9.86 |
| | 0/3 | Holo | 0* | NA | NA | NA | NA |
| | 0/3 | Long basi | 0 | NA | NA | NA | NA |
| Juvenile | 3/3 | Spirocyst | 40 | 11.10 × 3.04 | 12.40 × 2.05 | 20.48 × 3.99 | 13.93 × 5.13 |
| | 3/3 | Micro b-mast | 67 | 13.06 × 4.12 | 13.78 × 2.93 | 28.29 × 6.12 | 23.21 × 6.52 |
| | 3/3 | Micro p-mast | 61 | 15.39 × 3.03 | 15.39 × 3.03 | 27.67 × 4.86 | 27.05 × 7.20 |
| | 3/3 | Basi | 14 | 12.10 × 3.93 | 13.65 × 2.14 | 22.23 × 5.13 | 19.46 × 5.32 |
| | 0/3 | Holo | 0 | NA | NA | NA | NA |
| | 1/3 | Long asi | 2 | 26.98 × 1.37 | 33.11 × 0.70 | 33.11 × 0.70 | 26.98 × 1.37 |

* Observed in histological sections.

verse fission, resulting in 2 individuals. We did not observe asexual fission via polarity reversal in the parasitic stage.

## DISCUSSION

### Recently evolved parasitic stage of *Edwardsiella lineata*

Incipient parasites must rely on existing pre-adaptations to exploit their hosts. Specific adaptations to a parasitic life history evolve subsequently. Based on our anatomical and histological comparisons of the parasitic stage, the post-parasitic planula, and the polyp, we can conclude that the vermiform parasite exhibits a novel body plan, distinct from other life cycle stages with respect to several character traits, including overall shape and dimensions, body wall histology, mesenteries, musculature, and cnidom (Table II, Fig. 8). The parasite does not appear to possess novel organs or structures specifically adapted to parasitism as can be identified in other more ancient parasitic lineages, e.g., the scolex of tapeworms or the suckers of leeches and monogenean flatworms. However, it exhibits distinctive body proportions, ciliation patterns, and cnidom relative to the post-parasitic planula and the polyp. Thus, the novel body plan of the parasite could have been achieved via differential growth in combination with heterotopic and heterochronic shifts in the development of cell types and structures that already existed in the larva and/or the polyp of the free-living ancestor. The morphological and histological distinctiveness of the parasite is discussed below with respect to the demands of its parasitic existence.

### Dimensions and overall body plan

The parasitic stage and the subsequent post-parasitic planula are markedly different in size. Although the 2 are similar in diameter, the parasite is 2 to 10 times longer than the post-parasitic planula. The difference in size between the 2 raises questions about the fate of the parasitic stage's tissues, and whether the post-parasitic planula is using these tissue ''reserves'' to make internal structures. The post-parasitic planula is more developed internally, having retractor muscles, and a relatively longer and more differentiated actinopharynx. The vacuolate matter in the coelenteron diminishes in relative proportion as the larva develops but is present in the larval stage, which suggests that the larvae of *E. lineata* do not feed, but depend upon energy reserves acquired during the parasitic stage. Previous experimental observations measuring success of development of the polyp from parasites of different sizes showed a significant, positive relationship of parasite size and settlement success (Reitzel, Sullivan et al., 2007). The histological comparison of parasitic stages and post-parasitic planulae suggests that this relationship is likely related to the quantity of energetic reserves in the parasitic stage necessary to fuel development through settlement.

### Musculature and movement

Of the 3 life cycle stages examined, the parasitic stage has the least extensive musculature. The polyp is extensively endowed with muscles in the endoderm of the body wall in addition to the parietal muscles and retractor muscles that are present on the mesenteries. These animals are able to telescope up and down by alternating contraction of circumferential muscles in the body wall with longitudinal muscles on the mesenteries. The post-parasitic planula lacks body wall musculature, but it does possess retractor muscles on the mesenteries. The parasitic stage lacks both body wall muscles and retractor muscles. Concordant with its paucity of musculature, excised parasites appear incapable of locomotion. The embedded parasites

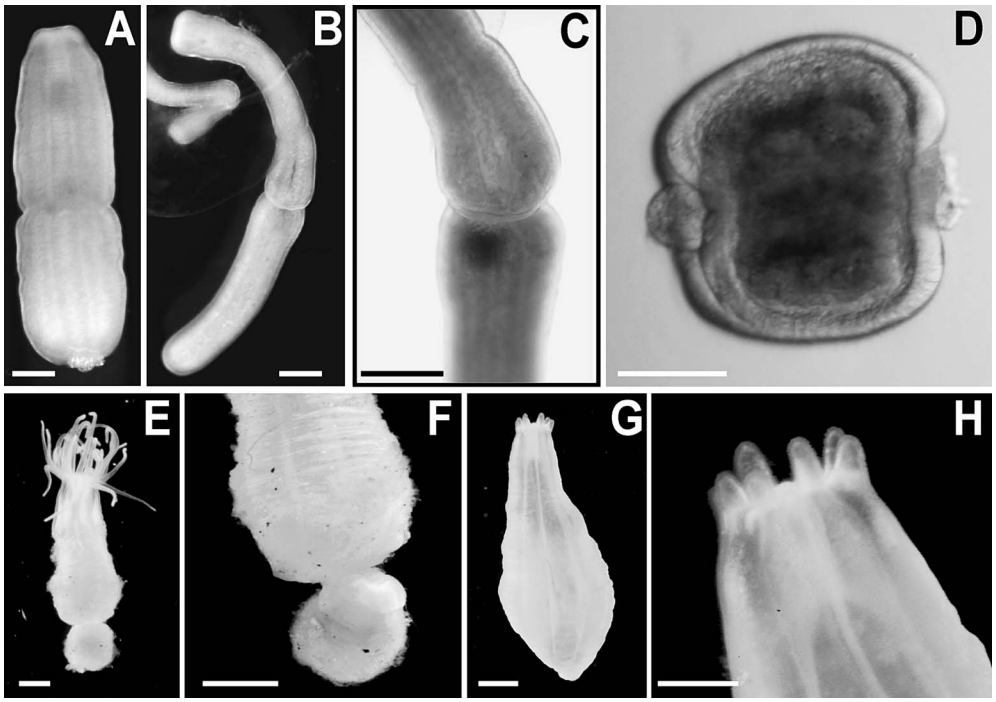


FIGURE 7. Asexual reproduction via transverse fission. Transverse fission was observed in both parasite (**A–D**) and adult (**E–H**) stages of *E. lineata*. Scale bar: 1 mm in A–D, 5 mm in E–G, 2 mm in H.

are capable of limited movement within their hosts, but they typically remain still.

### Cnidom

The parasitic stage and the post-parasitic larva of *E. lineata* have holotrichs, a type of nematocyst not seen in adults (Fig. 6A). This is a surprising finding given that the Edwardsiidae, to which *E. lineata* belongs, is among the actinarian families not reported to have holotrichs (Carlgren, 1949). The discovery of this type of cnidae in larvae of *E. lineata* suggests either that (1) the cnidom can evolve convergent similarity, i.e., holotrichs were missing in the edwardsiid ancestor, but the holotrichs of *E. lineata* were secondarily acquired, or (2) we need to systematically investigate the cnidae complement in larvae as well as adults, because the cnidae complement of larvae may be an important source of phylogenetic or taxonomic information, as it is in some Medusozoa (Calder, 1971; Mariscal, 1974; Calder, 1982; Östman, 1982).

Holotrichs are among the most widespread type of cnida in the Cnidaria (Mariscal, 1974), being found in some members of each class. Because of this broad phylogenetic distribution, and because holotrichs have many of the features seen in other types of nematocysts, but are relatively unspecialized in the spination pattern or tubule morphology, Cuttress (1955) suggested that these might represent the plesiomorphic cnida. In other actinarians holotrichs facilitate attachment or are used for intraspecific aggression (Francis, 1988). Spirocysts are used in attachment and in prey capture by adhesion (Mariscal, 1974). Together, holotrichs and spirocysts may facilitate attachment of the *E. lineata* larva to its ctenophore host, or help it maintain its position inside the host.

A further comparison of the cnidom for each stage indicates

TABLE II. Morphological and histological traits exhibited by distinct life history stages of *E. lineata.*

| | Parasite | Postparasite larva | Juvenile and adult |
|---|---|---|---|
| Cnidom | Spirocysts; basitrichs, holotrichs; microbasic *p*-mastigophores | Spirocysts; basitrichs; long, thin basitrichs; holotrichs; microbasic *p*-mastigophores | Spirocysts; basitrichs; long, thin basitrichs; microbasic *p*-mastigophores |
| External morphology | Long, slender, vermiform; body not divisible into regions; 4 tentacle mounds | Stout, oblong; body not divisible into regions; no tentacle mounds | Long, slender; body divisible into capitulum, scapus, aboral end; 8–24 tentacles |
| Internal morphology | Primary mesentery pair present, second and third may be rudimentary; no pairs of mesenteries perfect; siphonoglyph not differentiated | All 4 pairs macrocnemes present, at least 1 primary pair perfect; siphonoglyph differentiated | All 4 pairs macrocnemes present, perfect; 8 microcnemes present; siphonoglyph differentiated |

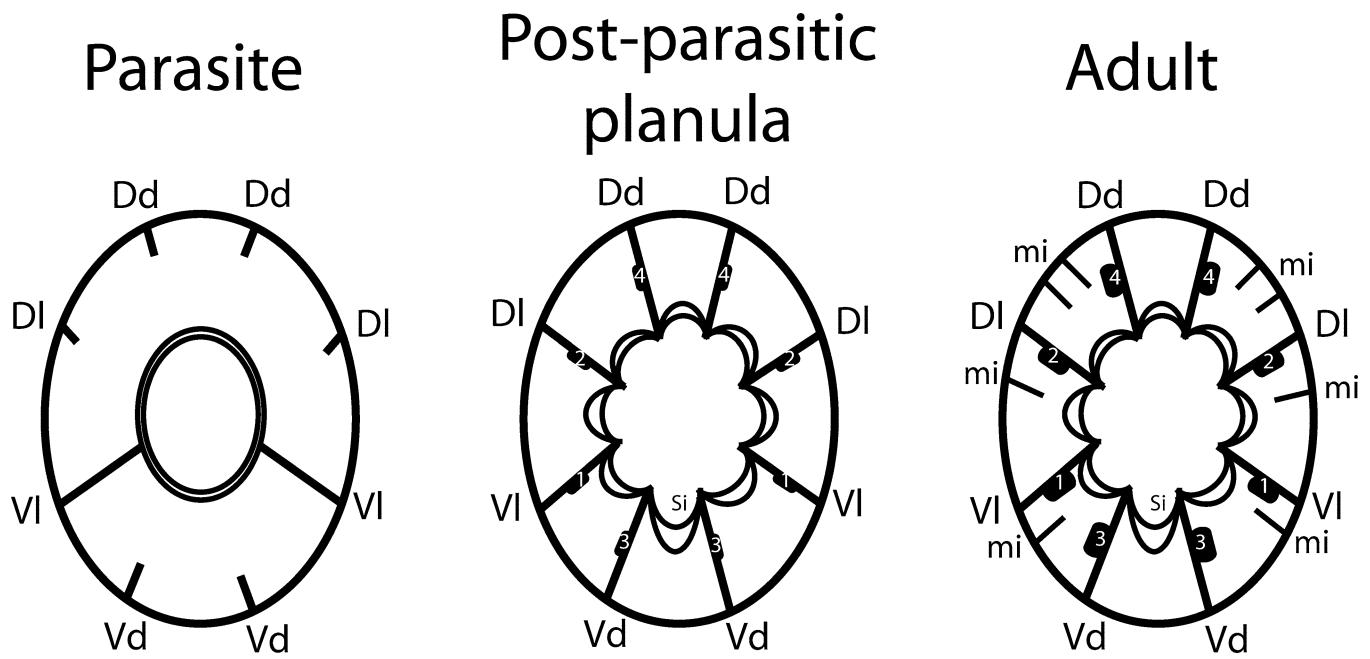


FIGURE 8. Anatomical differences between developmental stages of *E. lineata*. Development from parasitic stage to the post-parasitic planula involves formation of all 8 mesenteries, each with a small retractor muscle on its face. Younger post-parasitic planulae were observed with only some of their mesenteries attached to the pharynx, an observation consistent with most of the development occurring during the pelagic phase. During this transition the pharynx become further differentiated into a star-shaped opening with a siphonoglyph. The transition from the post-parasitic planulae to the adult polyp involves development of microcnemes, thickening of the retractor muscles, development of parietal muscles (not shown), and greater differentiation of tissue in the body column (not shown). Labels follow those in Fig. 1C.

they are discrete developmental stages. In juvenile polyps we found all the types of cnidae reported from adults (Daly, 2002b), including long, thin basitrichs. However, we did not observe these long, thin basitrichs in the post-parasitic planula or the parasitic stage, which suggests that the shift from larva to juvenile polyp is a discrete transitional stage in the life cycle.

### Asexual reproduction

*Edwardsiella lineata* undergoes transverse fission via physal pinching in both the parasitic stage and adult stage of the life cycle. Crowell and Oates (1980) previously reported that physal pinching (''transverse fission'') occurs during the adult stage. Our observations are consistent with their description, with the exception that we observed a distinct oral opening at the time of fission.

Our observations of transverse fission during the parasite stage are novel for this species. Asexual reproduction in pre-adult stages has not been reported in any other edwardsiid anemones, but it has been observed in other cnidarians (Ayre and Resing, 1986) and other animal phyla, particularly echinoderms (Jaeckle, 1994; Vickery and McClintock, 2000; Vickery et al., 2001). In *E. lineata* we suspect that the success rate for colonizing a suitable host is low. If the infection process represents a bottleneck in the reproduction of *E. lineata*, and because single hosts could support multiple individual parasites (Reitzel, Sullivan et al., 2007), selection may favor individuals that could undergo asexual reproduction while embedded within the host. A similar selective advantage would pertain to many other endoparasites, and, indeed, asexual reproduction is a common life history strategy for endoparasitic species (Craig et al., 1997). The relative impact of asexual fission by *E. lineata* on host parasite load has not yet been determined, but infection data suggest that asexual reproduction is commonplace. In a 3-yr study on *E. lineata* infection prevalence in ctenophores collected at Woods Hole, Massachusetts (Reitzel, Sullivan et al., 2007), most infected ctenophores were found to harbor multiple parasites. A single ctenophore was found to contain 20 parasites, even as the proportion of uninfected ctenophores almost always exceeded the proportion of infected ctenophores.

We also observed asexual reproduction via polarity reversal. A proportion of post-parasitic planulae that had settled developed oral crowns on opposite ends. These individuals later underwent transverse fission, producing 2 adults with a single oral crown. We have not observed adults spontaneously producing this 2-headed phenotype, which has been reported in *N. vectensis* (Reitzel, Burton et al., 2007).

### Comparative development between 2 edwardsiid anemones

*Nematostella vectensis* and *E. lineata* are closely related. They are members of Milneedwardsiinae, a monophyletic subfamily of Edwardsiidae (Daly, 2002a). Despite the reasonably close phylogenetic relationship, these species have very different life cycles (Fig. 2A, B), at least partly because of the evolution of the parasitic stage in *E. lineata*. A comparison of the development in these 2 species shows that the internal development is similar in sequence, e.g., the first mesenteries emerge prior to the development of the actinopharynx, which is followed by the emergence of tentacles. However, the overall pace of development is far more rapid in *N. vectensis*. The devel-

opmental trajectory of *E. lineata* contains an additional life cycle stage, and the development of some structures is shifted between stages, even though the sequence is similar. *Nematostella vectensis* is also relatively precocious when it comes to settlement. At the time of settlement, *E. lineata* juveniles have developed all 8 macrocnemes, which form during the pelagic post-parasitic planula stage. By contrast, *N. vectensis* typically have at most 4 macrocnemes when they undergo settlement. Similarly, *E. lineata* juveniles have 8 primary tentacles at the time of settlement, while *N. vectensis* have 2 or 4. In *N. vectensis* planulae, prior to settlement and concomitant with the development of the mesenteries, tentacle bud development is initiated, and these buds then elongate into tentacles. However, during the parasitic stage of *E. lineata*, tentacle buds are initiated but then regress upon exiting their host. We have not observed tentacles in any of the thousands of parasites we have excised from ctenophores, and it is thus unlikely that the buds initiated during the parasite stage ever elongate. Another apparent heterochronic shift between *N. vectensis* and *E. lineata* concerns holotrichs. This cnida type is present in pre-adult stages of *E. lineata*, but absent from pre-adult stages of *N. vectensis* (M. Daly, pers. obs.). This nematocyst may have a life cycle stage-specific function in *E. lineata*. A final heterochronic shift concerns the ability to reproduce asexually. In *N. vectensis* the ability to undergo transverse fission appears to be restricted to the adult polyp; in *E. lineata* the pre-adult parasite undergoes transverse fission via polarity reversal. Comparisons with additional edwardsiid anemones will be required to determine whether the traits observed in *E. lineata* or *N. vectensis* are derived, or primitive, within the family, especially the presence of holotrichs in pre-adult stages.

By characterizing and comparing the life history stages of *E. lineata*, our data reveal qualitative differences between the derived parasitic stage and the primitive adult and larval stages. The parasitic stage originated via an overall simplification of the anatomy, including the suppression of muscle and tentacle development and a retarding of mesentery development. There also appears to have been a heterochronic acceleration of the ability to initiate asexual reproduction. Finally, the parasitic stage possesses at least 1 potentially unique character (holotrichs).

As we have not yet characterized the pre-parasitic larval stage (Fig. 2B), we do not know if this larva is simplified relative to the planula of related free-living anemones, and we cannot determine the point in the life cycle at which the ontogeny of *E. lineata* begins to depart from that of its free-living ancestors. We have shown that the post-parasitic larva shares with the pre-parasitic larva the ability to infect a new host and develop the characteristic body plan of the parasitic stage. Thus, at least in terms of its developmental competence, the post-parasitic larva may be very similar to the pre-parasitic larva. However, relative to other anthozoan planulae, the post-parasitic larva of *E. lineata* is unusual in lacking an apical tuft of elongated cilia at its aboral pole. As the apical tuft is associated with an apical sensory organ, a structure that has been analogized to ''the brain'' in a range of invertebrate larvae, the absence of the apical tuft may implications for the development of the nervous system.

Considering its unusual experimental tractability, its close relationship to a free-living species with a fully sequenced genome (*N. vectensis*), and the potential importance of *E. lineata* as a biological control of the highly invasive *M. leidyi*, *E. lineata* represents a valuable new model system for investigating developmental, morphological, and life cycle innovations associated with the origin of parasitism. Important questions raised by this work include how the development of muscle, tentacles, and mesenteries has been suppressed during development of the parasitic stage. Planned molecular studies will compare the spatiotemporal expression of candidate developmental regulatory genes in *E. lineata* and *N. vectensis* in an attempt to identify which gene regulatory networks have been altered in the developmental evolution of this novel life cycle stage. Finally, additional whole-organism and histological studies will be required to determine whether the larvae of non-parasitic Edwardsiidae might be somehow pre-adapted to parasitize lobate ctenophore hosts, and if holotrichs are specifically adapted to *E. lineata*'s parasitic existence.

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