I. The starlet sea anemone (*Nematostella vectensis*)—an "emerging model system"

A. The growing literature on *Nematostella*. A query of the *Scientific Citation Index* (conducted 06/26/07) identified 74 articles and reviews that contain "nematostella" in the title, keywords, or abstract. The number of such publications is increasing dramatically (Fig. 1a), as are the citations of these papers (Fig. 1b). Much of the *Nematostella* literature is not yet indexed; we identified another 66 published books, reviews, or articles published prior to the 1990's that mention *Nematostella*. An annotated list is housed at http://nematostella.org/Resources_References.

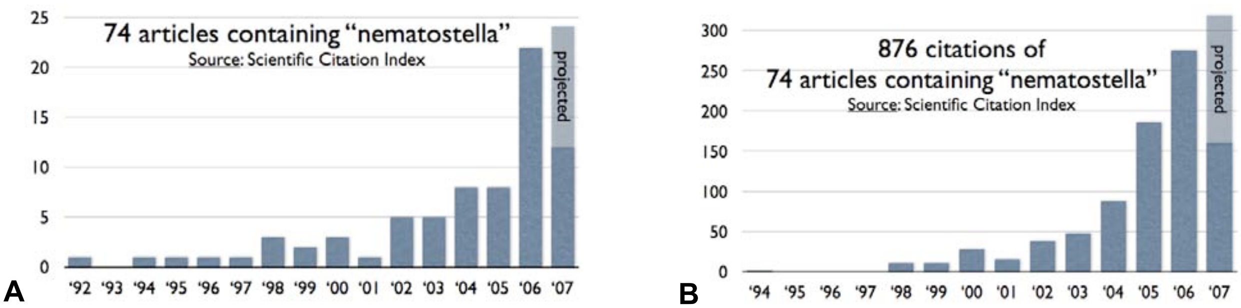


Figure 1. *Nematostella* publications (A) and citations (B) by year.

B. *Nematostella*'s Merits as a Model System

Nematostella is an estuarine sea anemone that is native to the Atlantic coast of North America. In the early 1990's, its potential value as a model system for developmental biology was first explicitly recognized by Hand and Uhlinger [1]. Over the last 10 years, its utility has extended far beyond developmental biology due to its informative phylogenetic position, and its amenability to field studies, organismal studies, developmental studies, cellular studies, molecular and biochemical studies, genetic studies, and genomic studies [2].

1. Phylogenetic relationships. *Nematostella* is a member of the Cnidaria, one of the oldest metazoan phyla. The Cnidaria is a closely related outgroup to the Bilateria, the evolutionary lineage that comprises >99% of all extant animals (Fig. 3). Comparisons between *Nematostella* and bilaterians have provided insights into the evolution of key animal innovations, including germ cell specification, bilateral symmetry, mesoderm, and the nervous system [3-7].

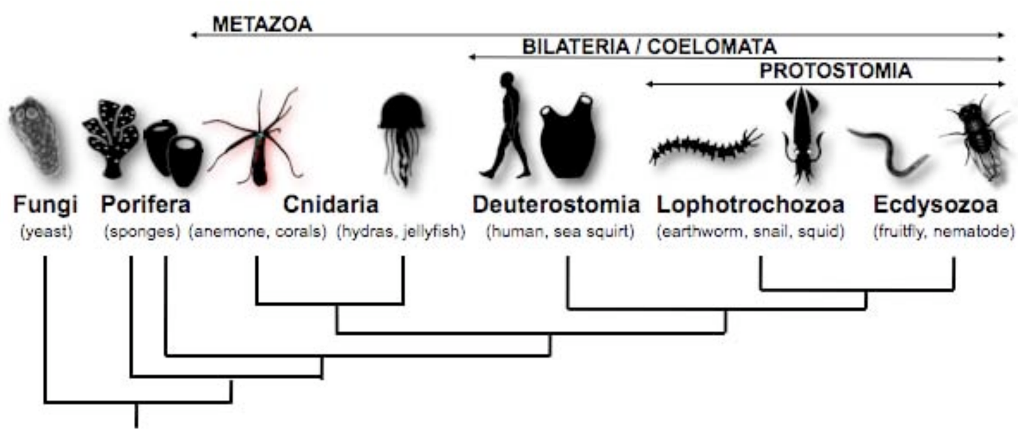


Figure 3. Metazoan phylogeny based on recent molecular phylogenetic studies [8-11].

All cnidarians are divided between two ancient lineages that diverged more than 500 million years ago. The Anthozoa includes sea anemones and corals (Fig. 3). The Medusozoa includes jellyfishes and hydras. The ancestral cnidarian life-history is thought to be conserved in contemporary anthozoans [10,12-14]. The polyp reproduces sexually to produce a planula larva, which then undergoes settlement and forms a new polyp. In the ancestral medusozoan, a novel life history stage originated—the medusa or "jellyfish" was interposed between the polyp and the planula. The medusa arises via asexual propagation from the polyp, and the medusa then assumes the role of sexual reproduction. Some Medusozoa such as *Hydra* have secondarily lost the medusa stage, and the polyp has once again assumed the task of sexual reproduction. Thus, *Nematostella* is an outstanding complement to *Hydra*, the next cnidarian model system whose genome will be sequenced in that (1) *Nematostella* represents the other major branch of cnidarian evolution, and (2) it represents the ancestral life history.

2. Development. Unlike the major developmental model systems (*e.g.*, fruitfly, nematode, zebrafish, mouse, *etc*.) *Nematostella* has a complex developmental repertoire. Adults can arise via (i) sexual reproduction, (ii) two forms of fission, or (iii) regeneration. All of these distinct developmental trajectories are amenable to lab analysis.

a. Embryogenesis and larval development. *Nematostella* is the only cnidarian in which the entire sexual life history and development are tractable in the laboratory year-round [1,15]. Following fertilization, embryogenesis and larval development proceed rapidly and reliably under laboratory conditions (Fig. 4). Throughout most of the life history (late blastula, gastrula, larva, and adult stages), the body wall is practically transparent, making it possible to visualize aspects of the internal anatomy throughout development.

b. Asexual fission. *Nematostella* can reproduce via two distinct modes of transverse fission [1,2,16-18]. In "physal pinching," a circumferential constriction becomes fixed near the aboral end of the animal. Fission at this site releases a small "foot" fragment, which soon regenerates missing oral structures (mesenteries, pharynx, mouth, and tentacles) [17]. In "polarity reversal" (Fig. 5), a novel head develops at the site of the existing foot. This two-headed animal then elongates as mesenteries regress from the center of the body column, forming a new foot region. Fission in this region generates two complete adult polyps [17].

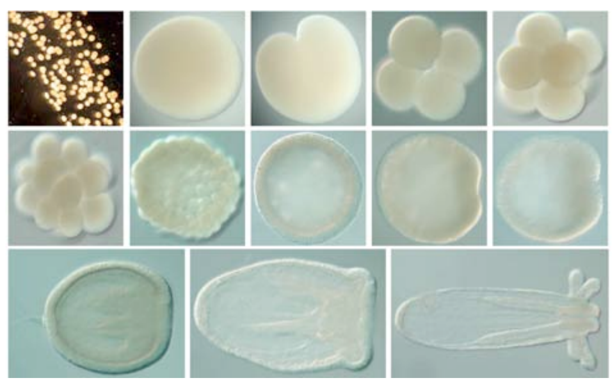


Figure 4. Development of the juvenile polyp following sexual reproduction. From upper left to lower right: egg mass, fertilized egg, first cleavage, 4-cell stage, 8-cell stage, 16-cell stage, early blastula, late blastula, early gastrula (blastopore to the right), late gastrula, early planula larva (internal endodermal structures including pharynx and mesenteries have begun to resolve), late planula larva with tentacles beginning to emerge, juvenile polyp with four tentacles (mouth to right). [Photos by Patricia Lee; http://*Nematostella*.org.]

c. Complete bidirectional regeneration. Following bisection through the body column, *Nematostella* rapidly undergoes complete bidirectional regeneration—within a matter of days, the aboral fragment develops a new "head" and the oral fragment develops a new foot or physa [17]. Partial cuts through the body column near the mouth can trigger the formation of supernumerary heads, while partial cuts through the body column near the tip of the foot can trigger the formation of supernumerary physae [17]. This remarkable regenerative ability sometimes results in dramatic "developmental anomalies" such as a specimen with three oral crowns and two physae that developed in laboratory culture (Fig. 6).

Figure 5. Adult *Nematostella* polyp (right), egg mass (center), and "two-headed" adult undergoing fission by polarity reversal (left).

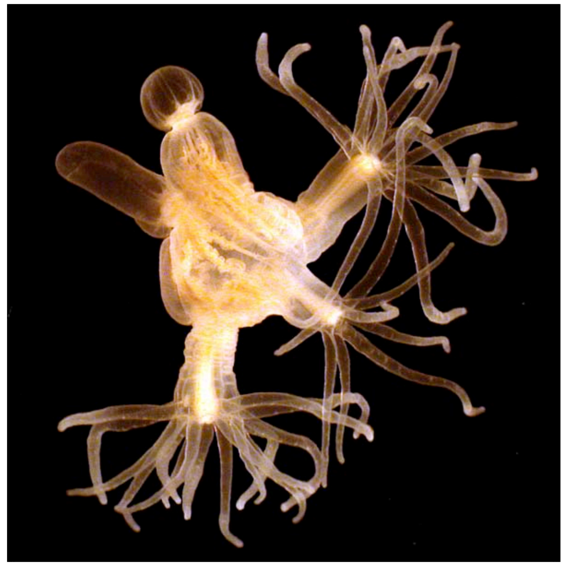


Figure 6. Nicks to the body column typically heal rapidly, but they sometimes lead to the development of supernumerary heads or physae [17].

3. Organismal Laboratory Studies. *Nematostella* is small (~1 cm in length), hardy, and gregarious, making it ideal for lab-based studies of ecology, behavior, and physiology. It can be cultured at high densities in finger bowls of standing artificial seawater [1]. Its ability to regenerate facilitates the production of living genetic stocks with the same genotype. We have developed curricular materials using this species [19], and we have supplied *Nematostella* to teachers who have used the animal in their biology curricula.

4. Molecular and Biochemical Studies. Unlike many marine invertebrates, it is easy to isolate clean DNA, RNA, or protein. *In situ* hybridization produces results of spectacular clarity, so the spatiotemporal expression of genes can be monitored with great precision (Fig. 7). DNA binding studies can easily be performed using *Nematostella* protein extracts in electrophoretic mobility shift assays (EMSA; Fig. 8). Specific transcripts can be "knocked-down" using morpholinos [20], and labeled proteins can be expressed *in vivo* to examine gene function [21]

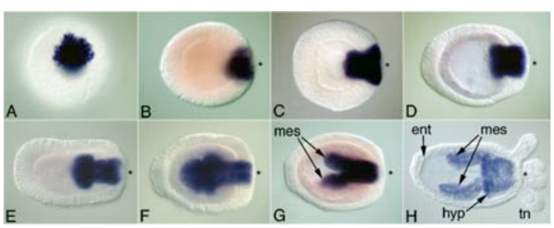
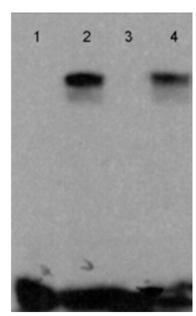


Figure 7. Expression of *forkhead* during *Nematostella* larval development assayed by *in situ* hybridization. [5]

Figure 8. EMSA reveals specific binding of *Nematostella* protein(s) to an NF-kappaB binding site. **Lane 1**: labeled kappaB site; **Lane 2**: + *Nematostella* protein extract; **Lane 3**: + 10-fold excess of unlabeled kappaB site; **Lane 4**: + 10-fold excess of unlabeled estrogen-responsive binding element.



5. Genomic Studies. Recently, the starlet sea anemone became the first "basal" animal whose entire genome was sequenced [22]. The most stunning finding of the genome-sequencing project is the remarkable resemblance between *Nematostella* and humans. For example, intron locations are more highly conserved between human and *Nematostella* than between human and any other non-vertebrate whose genome has been sequenced (Fig. 9, [23]). Furthermore, *Nematostella shares* more orthologous genes in common with human than any other non-vertebrate animal examined, including the sea squirt *Ciona*, which, like human, is a deuterostome bilaterian (Fig. 10, [7]). This unexpected similarity between human and sea anemone also extends to those genes implicated in human disease [24].

	Pf	At	Sp	Nv	Ce	Dm	Ag	Hs
Pf	159	3%	3%	3%	2%	2%	3%	2%
At		1076	5%	17%	7%	5%	5%	16%
Sp			173	6%	4%	7%	6%	7%
Nv				1459	14%	9%	10%	47%
Ce					560	11%	11%	15%
Dm						260	43%	13%
Ag							269	12%
Hs								1246

Figure 9. Percentage of introns in 343 orthologous genes shared among 8 eukaryotes. The total number of introns in each species is shown along the diagonal [23].

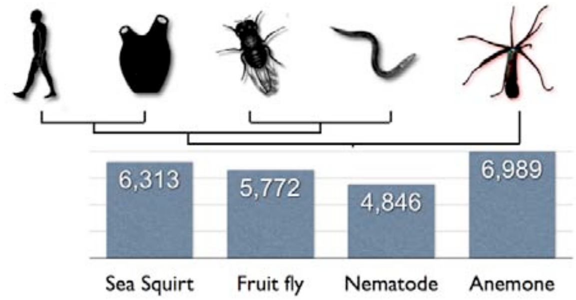


Figure 10. Orthologous genes shared between human and each of 4 animals whose genomes have been sequenced. Orthologs were identified by pairwise reciprocal Blast searches [7].

6. Cell Culture Studies. *Nematostella* is amenable to cell culture techniques that have been developed for corals (Fig. 11). Access to such cultures will facilitate studies on cellular physiology, toxicology, and differentiation.

7. Field Studies. *Nematostella's* environment (tidal creeks and salt marsh pools) is easily accessible from shore, and we have conducted extensive field surveys and collections throughout the species' expansive range (Fig. 12; [25-27]). *Nematostella* is found at both relatively pristine sites and heavily impacted sites. In its native range, along the Atlantic coast of North America from Nova Scotia to Florida, the species spans a pronounced latitudinal temperature gradient. Several recently introduced populations have been identified, both inside and outside the native range, and the genetic fingerprints of these populations are markedly distinct from long-standing populations within the native range [27]. The semi-permanent nature of high marsh pools and tidal creeks enables multi-year longitudinal sampling of subpopulations.

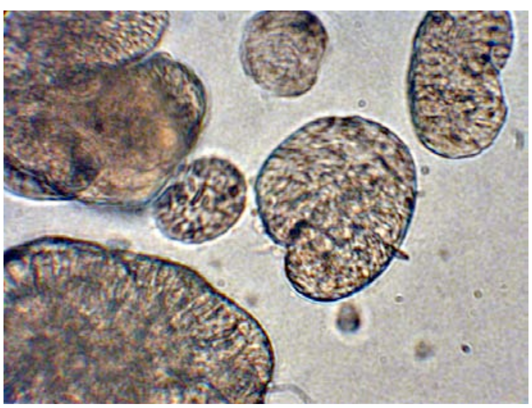


Figure 11. *Nematostella* cells in culture (Traylor-Knowles, unpubl).

Figure 12. Collecting *Nematostella* from pools at Sippewissett Marsh on Cape Cod. (http://nematostella.org/Resources_Collecting).

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