Nucleic Acids Research, 2006, Vol. 34, Database issue **D495–D499**
doi:10.1093/nar/gkj020

StellaBase: The *Nematostella vectensis* Genomics Database

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Received August 15, 2005; Accepted September 17, 2005

ABSTRACT

***StellaBase*, the *Nematostella vectensis* Genomics Database, is a web-based resource that will facilitate desktop and bench-top studies of the starlet sea anemone. *Nematostella* is an emerging model organism that has already proven useful for addressing fundamental questions in developmental evolution and evolutionary genomics. *StellaBase* allows users to query the assembled *Nematostella* genome, a confirmed gene library, and a predicted genome using both keyword and homology based search functions. Data provided by these searches will elucidate gene family evolution in early animals. Unique research tools, including a *Nematostella* genetic stock library, a primer library, a literature repository and a gene expression library will provide support to the burgeoning *Nematostella* research community. The development of *StellaBase* accompanies significant upgrades to *CnidBase*, the Cnidarian Evolutionary Genomics Database. With the completion of the first sequenced cnidarian genome, genome comparison tools have been added to *CnidBase*. In addition, *StellaBase* provides a framework for the integration of additional species-specific databases into *CnidBase*. *StellaBase* is available at http://www.stellabase.org.**

INTRODUCTION

In retrospect, the origin of the Bilateria may have been the most monumental event in the history of animal evolution. The Bilateria comprises more than 99% of all currently identified animal species. Bilaterian animals, including such major phyla as the chordates, arthropods, nematodes, annelids and mollusks have achieved far greater structural and behavioral complexity than non-bilaterian animals. However, in order to understand the genesis of bilaterian diversity and complexity, it is necessary to consult non-bilaterian outgroups taxa such as the phylum Cnidaria (sea anemones, corals, hydras, jellyfishes and their relatives).

Recent EST analyses on cnidarians have revealed surprising complexity in the genomes of these simple animals (1,2). For example, many genes that were previously thought to have originated within vertebrates due to their absence in the genomes of *Drosophila* and *Caenorhabditis elegans* have been found in cnidarians. These genes must have been present in the cnidarian-bilaterian ancestor, some 634 million years ago (3).

The starlet sea anemone, *Nematostella vectensis*, is a small burrowing sea anemone that is found in estuaries along the Atlantic and Pacific coasts of North America and the south of England (4–6). *Nematostella* is an important new model system for both lab-based and field-based studies of ecology, genomics, development and evolution [reviewed in (7)]. Importantly, *Nematostella* is the first member of the basal animal phylum Cnidaria, and the first basal animal generally, to have its genome sequenced (Joint Genome Institute; D. Rokshar, PI). Furthermore, among current cnidarian model systems, *Nematostella* is unsurpassed for the ease with which its entire life cycle may be cultured in the laboratory (8–10). An important advantage of *Nematostella* and other Cnidaria relative to the major animal models in developmental biology (fruitfly, soil nematode, zebrafish, etc.) is its extensive ability to regenerate. In fact, the adult *Nematostella* can originate via four distinct developmental pathways, including embryogenesis, regeneration and two forms of asexual

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors fission (7). A systematic comparison of regeneration and embryological development in animals that can regenerate should provide fundamental insights into the genomic basis of regenerative ability. The combination of its informative phylogenetic position, its exceptional experimental tractability, and its impressive developmental flexibility will ensure that *Nematostella* becomes a widely used genomic model system. As proof of its utility, *Nematostella* has already provided key insights into the evolution of metazoan body plan traits and developmental gene families (11–16).

NEMATOSTELLA GENE DATABASE

StellaBase houses a gene database comprising a gene library and an assembly of the full genome. The genome assembly was produced using the program Phusion (17); genes were predicted from the assembled genome using *GENSCAN* (18,19). The predicted genes were classified into putative gene families by comparing them against the complete *Pfam* library [release 17; (20)] using *HMMER*, version 2.3.2 [http://hmmer.wustl.edu; (21)]. Exon predictions and gene family predictions are accompanied by estimates of statistical significance (19,22).

Each gene in *StellaBase* is associated with a unique ID number. Through its ID number, detailed information about the gene may be retrieved including (i) its predicted exon structure, (ii) the statistical significance of the exon predictions and (iii) a listing of all *Pfam* protein families that match the gene in question with an *E*-value $\leqslant 10$. Nucleotide and amino acid sequences may be returned to the user in a FASTA format. The definition line of FASTA sequences downloaded through *StellaBase* includes the ID number, genomic location, best HMM match to a protein family at *Pfam* (the match with the lowest *E*-value) and indication of experimental confirmation, if applicable. Genes are considered to be experimentally confirmed if a BLAST search indicates a match against an expressed *Nematostella* sequence housed at NCBI with an *E*-value $\leqslant 10^{-10}$.

SEARCHING THE GENE DATABASE USING BLAST

StellaBase uses NCBI's BLAST program (23) to allow users to perform sequence similarity searches against the *Nematostella* gene database and genome sequence. Results from queries of the gene database return the following information: (i) *StellaBase* ID number; (ii) the genomic contig on which that gene resides; (iii) the location of the gene on the contig; (iv) whether or not the gene's expression has been confirmed; (v) best Pfam protein family match and associated *E*-value (as determined by HMMER) and a (vi) blast score with associated *E*-value. Other information about the gene, including exon structure and other *Pfam* motif matches may be retrieved through the *StellaBase* ID number search function.

PROTEIN FAMILY SEARCH

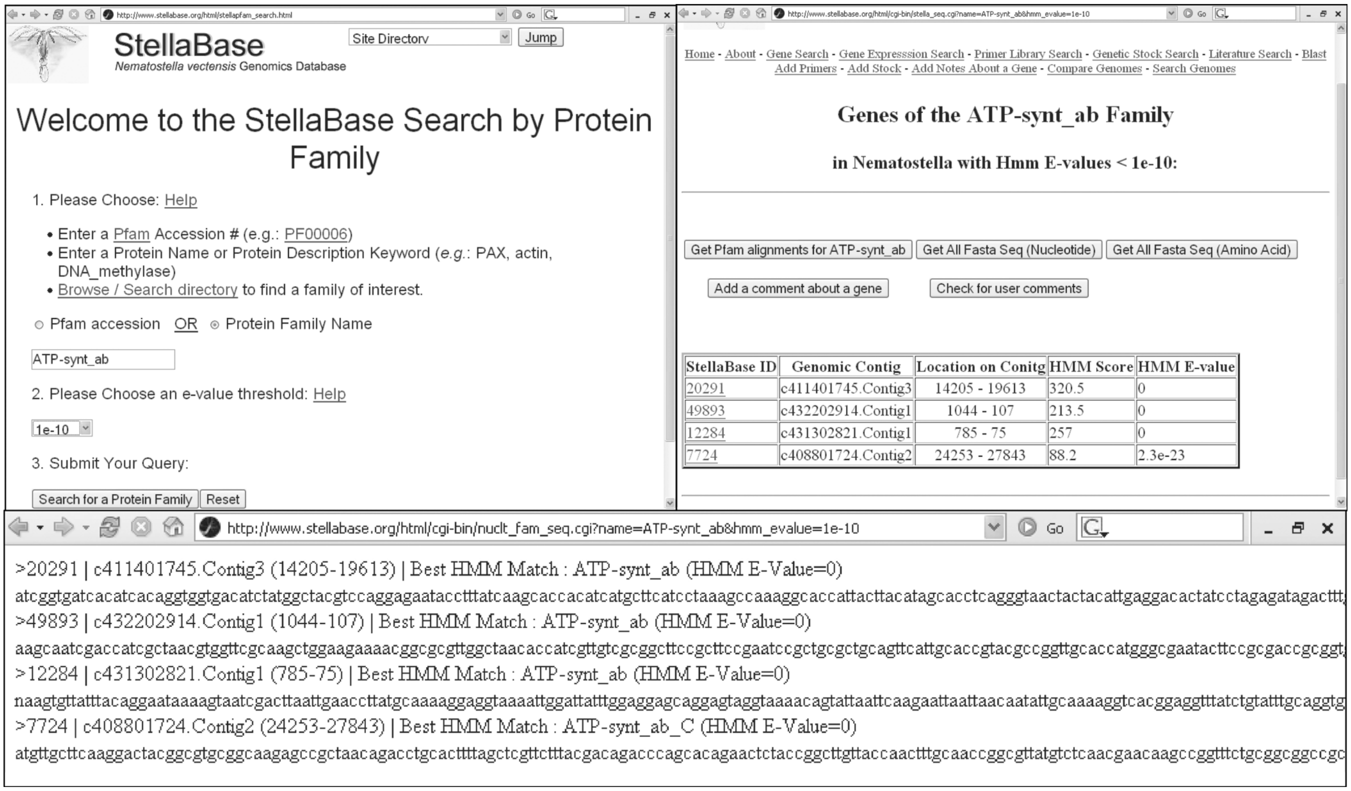
There is great interest in the membership of various protein families in the Cnidaria due to their status as a closely related outgroup to the Bilateria. To identify *Nematostella* sequences from a particular gene family, users may enter the name or accession number for any of the 7868 protein families included in the *Pfam* database [http://pfam.wustl.edu/; (20)]. The stringency of the search is determined by selecting a threshold 'expectation value' (ranging from 10^1 to 10^{-100}). The search returns a summary of the predicted genes that match the protein family of interest. For each matching gene, the following information is provided: (i) *StellaBase* ID number; (ii) the genomic contig on which the gene is found; (iii) HMMER score and associated *E*-value. Gene sequences in FASTA format may be retrieved for individual genes or for all matches to a particular query. Cross-references to *Pfam* provide information on the gene family and protein function (Figure 1).

GENETIC STOCKS OF *NEMATOSTELLA*

Genetic stock data can assist users to identify and obtain isolated DNA or live animals of known geographic origin or genotype. Populations are identified by collection locale, by laboratory and by genetic distance as indicated on a recent intraspecific phylogeny constructed using AFLP data (24). Contact information and population genetics data indicating clonality of the population of interest is returned to the user if available, as are any unique phenotypes represented by that population. The easy availability of genetic stocks is necessary to foster laboratory-based research. To maximize the utilization of this resource, those who collect or culture *Nematostella* are encouraged to make their own genetic stocks available through *StellaBase*. This resource will prove particularly useful as unusual phenotypes of particular interest are identified in natural populations or are produced in the laboratory. Currently, *StellaBase* houses data from 24 available populations of *Nematostella*.

LITERATURE SEARCH

The rise of *Nematostella* as a model system is a very recent phenomenon. In the early 1990s, Cadet Hand and Kevin Uhlinger highlighted the merits of this species as a possible model system for developmental biology (8–10). In 1997, the first gene sequences from *Nematostella* were published (25). The first molecular analyses of *Nematostella* development were published in 2003 (26). However, while much of the interest in *Nematostella* is quite recent, there exists a substantial literature on this anemone that would be of great use to the community. From the first published species account in 1935 (4) through 2005, no fewer than 79 publications have directly referenced *Nematostella*. A wealth of information on the morphology, development and natural history of this species is contained in these articles and book chapters. However, only a small minority of these existing citations (currently 17 of 79) is indexed in electronic literature databases such as PubMed. *StellaBase* indexes all of them and allows users to perform keyword searches on the complete texts. As future publications on *Nematostella* are indexed by PubMed, these will automatically be added to the *StellaBase* literature database. Existing publications that have not been identified, as well as future publications that are not indexed by PubMed, will be added manually.



http://www.stellabase.org/html/stellapfam_search.html

StellaBase
Nematostella vectensis Genomics Database

Welcome to the StellaBase Search by Protein Family

1. Please Choose: Help
   - Enter a Pfam Accession # (e.g.: PF00006)
   - Enter a Protein Name or Protein Description Keyword (*e.g.*: PAX, actin, DNA_methylase)
   - Browse / Search directory to find a family of interest.

○ Pfam accession OR ● Protein Family Name

ATP-synt_ab

2. Please Choose an e-value threshold: Help

1e-10

3. Submit Your Query:

http://www.stellabase.org/html/cgi-bin/stella_seq.cgi?name=ATP-synt_ab&hmm_evalue=1e-10

Home - About - Gene Search - Gene Expresssion Search - Primer Library Search - Genetic Stock Search - Literature Search - Blast
Add Primers - Add Stock - Add Notes About a Gene - Compare Genomes - Search Genomes

Genes of the ATP-synt_ab Family

in Nematostella with Hmm E-values < 1e-10:

Get Pfam alignments for ATP-synt_ab
Get All Fasta Seq (Nucleotide)
Get All Fasta Seq (Amino Acid)
Add a comment about a gene
Check for user comments

StellaBase ID	Genomic Contig	Location on Conitg	HMM Score	HMM E-value
20291	c411401745.Contig3	14205 - 19613	320.5	0
49893	c432202914.Contig1	1044 - 107	213.5	0
12284	c431302821.Contig1	785 - 75	257	0
7724	c408801724.Contig2	24253 - 27843	88.2	2.3e-23

http://www.stellabase.org/html/cgi-bin/nuclt_fam_seq.cgi?name=ATP-synt_ab&hmm_evalue=1e-10

```
>20291 | c411401745.Contig3 (14205-19613) | Best HMM Match : ATP-synt_ab (HMM E-Value=0)
atcggtgatcacatcacaggtggtgacatctatggctacgtccaggagaatacctttatcaagcaccacatcatgcttcatcctaaagccaaaggcaccattacttacatagcacctcagggtaactactacattgaggacactatcctagagatagacttt
>49893 | c432202914.Contig1 (1044-107) | Best HMM Match : ATP-synt_ab (HMM E-Value=0)
aagcaatcgaccatcgctaacgtggttcgcaagctggaagaaaacggcgcgttggctaacaccatcgttgtcgcggcttccgcttccgaatccgctgcgctgcagttcattgcaccgtacgccggttgcaccatgggcgaatacttccgcgaccgcggt
>12284 | c431302821.Contig1 (785-75) | Best HMM Match : ATP-synt_ab (HMM E-Value=0)
naagtgttatttacaggaataaaagtaatcgacttaattgaaccttatgcaaaaggaggtaaaattggattatttggaggagcaggagtaggtaaaacagtattaattcaagaattaattaacaatattgcaaaaggtcacggaggtttatctgtatttgcaggtg
>7724 | c408801724.Contig2 (24253-27843) | Best HMM Match : ATP-synt_ab_C (HMM E-Value=0)
atgttgcttcaaggactacggcgtgcggcaagagccgctaacagacctgcacttttagctcgttctttacgacagacccagcacagaactctaccggcttgttaccaactttgcaaccggcgttatgtctcaacgaacaagccggtttctgcggcggccgc
```

Figure 1. Screens showing *StellaBase* interface, clockwise from top left: (i) User interface to search for gene families in the *Nematostella* genome by keyword and expectation values; (ii) Output for a particular query; (iii) All sequences matching query in FASTA format.

PRIMER LIBRARY

StellaBase houses a library of 698 oligonucleotide primer sequences. The primer sequences and associated information were gleaned from the literature or obtained directly from researchers. Users may search for primers by gene name; the primer sequence, its melting temperature and usage notes will be returned to the user. Users are encouraged to submit additional primer sequences to this database.

GENE EXPRESSION QUERIES

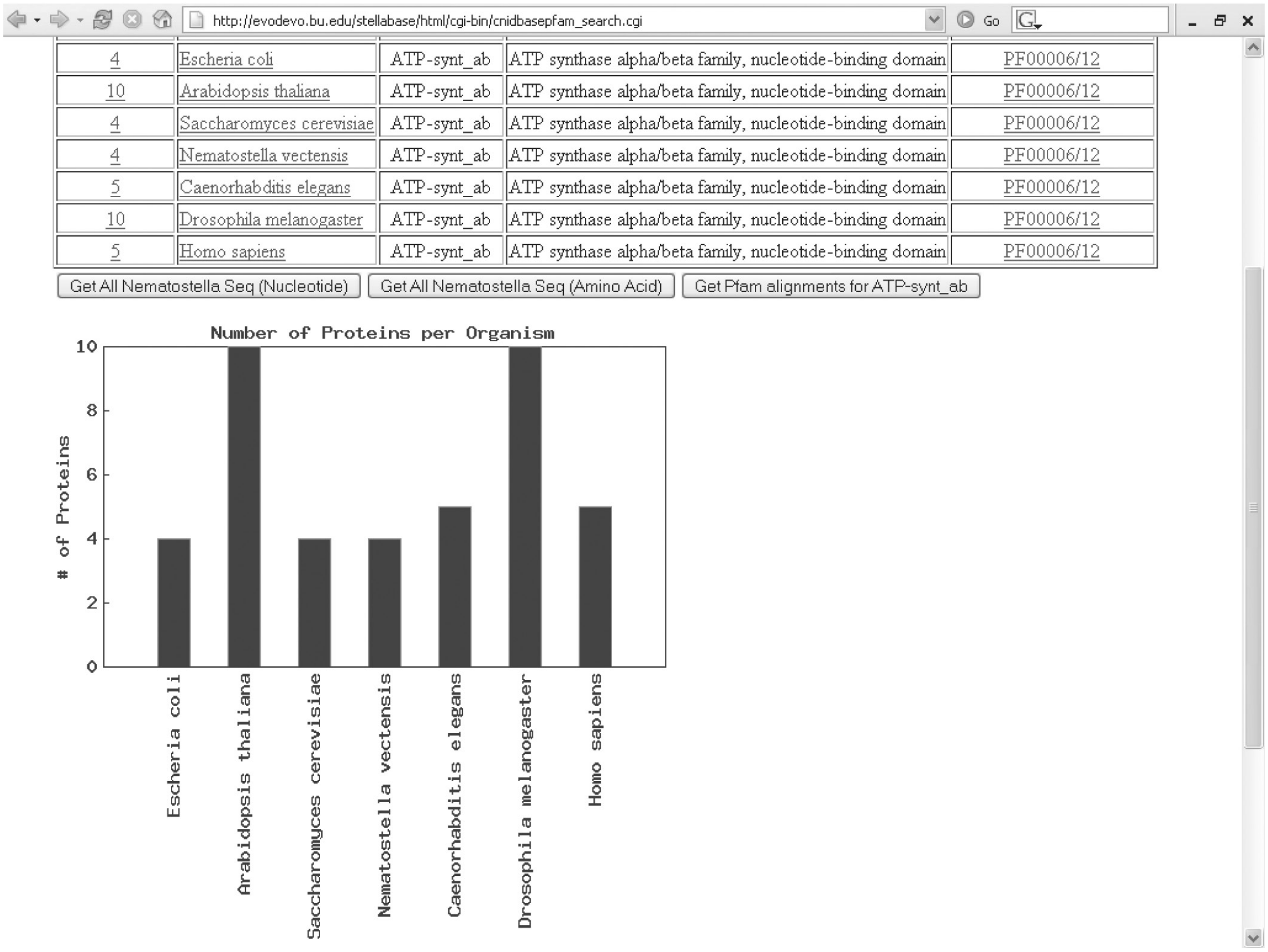
StellaBase has been integrated into the gene expression search function on *CnidBase, The Cnidarian Evolutionary Genomics Database* [http://cnidbase.bu.edu; (27)]. Users can search for gene expression data in *Nematostella* by specifying seven different gene expression parameters: gene name, expression level, life history stage, body region, body layer, assay type and cell type. The gene expression search function of *CnidBase* facilitates direct comparisons of gene expression between *Nematostella* and other members of the phylum Cnidaria.

INTEGRATION OF *STELLABASE* AND *CNIDBASE*

Genomic data from the Cnidaria are accumulating at a rapid rate. The EST database at NCBI currently lists well over 190 000 cnidarian ESTs from a phylogenetically diverse range of species including corals, jellyfishes, sea anemones and hydras. *CnidBase* was developed to organize various forms of cnidarian genomic data into a single repository that would facilitate comparative studies among species (27). To further support comparative cnidarian research, we chose not to develop *StellaBase* as an isolated entity, but rather, we have integrated *StellaBase* with *CnidBase*. The synergistic relationship between *CnidBase* and *StellaBase* provides a model for the incorporation of future species-specific cnidarian databases into a *CnidBase* centered network. As more species-specific experimental data is obtained from cnidarians, the need for more species-specific cnidarian databases is likely to arise. Large amounts of data are being amassed for a number of other informative cnidarian species, including *Acropora* (28), *Hydra* (29), *Hydractinia* (30) and *Podocoryne* (31). To facilitate the inclusion of other species-specific cnidarian databases, we have posted the table structure and an entity-relationship diagram (32) for all genomic data stored within *StellaBase*.

GENE FAMILY COMPARISONS

As the phylum Cnidaria enters the genomic age, it will become possible to uncover the full complement of particular gene families present in selective cnidarian species and to compare the complexity of particular gene families in cnidarian and bilaterian models. We have added new functions to *CnidBase* that facilitate rapid and thorough comparisons of (i) genome content and (ii) gene family content among distantly related organismal lineages. The completed proteomes of *Homo sapiens*, *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Escheriachia coli* and *Nematostella*



4	Escheria coli	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
10	Arabidopsis thaliana	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
4	Saccharomyces cerevisiae	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
4	Nematostella vectensis	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
5	Caenorhabditis elegans	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
10	Drosophila melanogaster	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12
5	Homo sapiens	ATP-synt_ab	ATP synthase alpha/beta family, nucleotide-binding domain	PF00006/12

http://evodevo.bu.edu/stellabase/html/cgi-bin/cnidbasepfam_search.cgi
Go
Get All Nematostella Seq (Nucleotide)
Get All Nematostella Seq (Amino Acid)
Get Pfam alignments for ATP-synt_ab
Number of Proteins per Organism
# of Proteins
0
2
4
6
8
10
Escheria coli
Arabidopsis thaliana
Saccharomyces cerevisiae
Nematostella vectensis
Caenorhabditis elegans
Drosophila melanogaster
Homo sapiens

Figure 2. Output from gene family query using the genome comparison tool.

vectensis were compared to the *Pfam* database to identify the number of genes from a particular family present in each lineage. Users can query these data by *Pfam* protein family name and by genome comparison. The stringency of each search is controlled by specifying a threshold expectation value. The search returns all sequences that match the user defined criteria in each selected species. The full complement of each gene family may quickly be compiled from each taxon, providing a broad overview of the evolution of genomes and particular gene families and a convenient launch point for detailed phylogenetic studies (Figure 2).

COMMUNITY PARTICIPATION IN *STELLABASE* AND *CNIDBASE*

It is a challenge to support researcher initiated databases, regardless of their potential value to the research community—'consortium-based' databases are generally more successful in disseminating information than are databases maintained by individual labs or research groups (33). Both *StellaBase* and *CnidBase* incorporate explicit opportunities for the cnidarian research community to supply critical content. Users are invited to submit primer sequences and genetic stock. Users are also encouraged to submit comments regarding specific gene sequences or families, such as suggestions for gene annotations. User comments will become available with sequence information as they are added.

In addition to supplying content to these existing databases, we supply relatively simple guidelines for cnidarian researchers to use our model and generate species-specific databases that can be seamlessly integrated into *CnidBase*. Table structure and an entity-relationship diagram are available on the *StellaBase* website; code for query interfaces, annotation and database construction are available upon request. As additional cnidarian genomes are sequenced, it is our hope that this model will allow for data to be available to the community quickly and in such a way that interphylum comparisons are facilitated.

FUTURE DIRECTIONS

A number of improvements to both *StellaBase* and *CnidBase* will occur in the near future. (i) As *StellaBase* is used to mine the *Nematostella* genome, information stored within *StellaBase* will be updated and new information will be added. We are currently in the process of annotating genes from a number of families; these annotations will be added to

StellaBase as completed. (ii) The *CnidBase* proteomic search function will increase in utility in the future as more species are added. *Porifera* and *Ciona intestinalis* will prove to be valuable additions due to their interesting phylogenetic positions and *Drosophila melanogaster* and *Mus musculus* will increase the confidence with which estimates are made regarding the proteomes of protostomes and deuterostomes, respectively. (iii) We intend to add a 'classic literature' search function to *CnidBase*. Scanned versions of texts no longer protected by copyright law containing valuable information on cnidarian morphology, development and natural history will be made available.

CONCLUSIONS

StellaBase is the genomics database of *Nematostella vectensis*. Through it, users may search a wide range of data types, including genomic data, genetic stocks, primers, literature and gene expression patterns. *StellaBase* provides a launching point for performance of both desktop phylogenetic and genomic analyses and bench-top laboratory research. By developing *StellaBase* within the framework of *CnidBase*, we have utilized the inherently comparative nature of *CnidBase* to develop search functions that facilitate detailed phylogenetic analyses of extremely divergent lineages. We have posted a roadmap for cnidarian researchers to follow in the development of additional species-specific databases that will integrate seamlessly with *CnidBase*.

ACKNOWLEDGEMENTS

The authors are grateful to the many researchers who published on *Nematostella* long before it entered the genomic age (for a complete list, see http://nematostella.org). We are especially grateful to Cadet Hand and Kevin Uhlinger who introduced the PI to this species. This research was funded by the National Science Foundation (grant IBN-0212773 to JRF) and by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health. Funding to pay the Open Access publication charges for this article was provided by the National Science Foundation.

Conflict of interest statement. None declared.

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