Justification for the Support of Echinobase: Enhancing the Impact of Echinoderm Model Systems for Biosciences

Gregory Cary, Carnegie Mellon University
Charles Ettensohn, Carnegie Mellon University
R. Andrew Cameron, California Institute of Technology & Carnegie Mellon University
Veronica Hinman, Carnegie Mellon University

1. PURPOSE

This document briefly outlines the current significance of echinoderm research models for understanding developmental mechanisms, with emphasis on their central importance in solving gene regulatory networks. It describes the set of genomic resources presently available to the echinoderm research community. Most importantly, it identifies the priorities of the research community concerning the future enhancement of these resources in support of core NIH missions.

2. IMPACT OF THE ECHINODERM COMMUNITY

In the late 1990s, the critical importance of the sea urchin as a research model spurred a sea urchin genome project. This NIH-funded project supported the sequencing and annotation of the genome of the purple sea urchin (*Strongylocentrotus purpuratus*) (Sea Urchin Genome Sequencing Consortium 2006a,b) Along with sequence information, many useful research materials such as macro-arrayed cDNA and genomic libraries were created. The growing popularity of other species of echinoderms as model systems, and the importance that comparative approaches have for illuminating genome and gene regulatory network (GRN) functions, have since prompted the inclusion of sequence data and library resources for several other echinoderm species. In addition, there have been continual improvements in the *S. purpuratus* genome assembly and gene annotations, and new research resources for this species have been produced (e.g., transcriptomes of many developmental stages and specific cell types as well as a more limited number of proteomes). Today, a comprehensive web information system, Echinobase (http://echinobase.org), offers a fully indexed view of the sequences as well as annotations and gene expression data. In addition, a library resource provides materials to researchers.

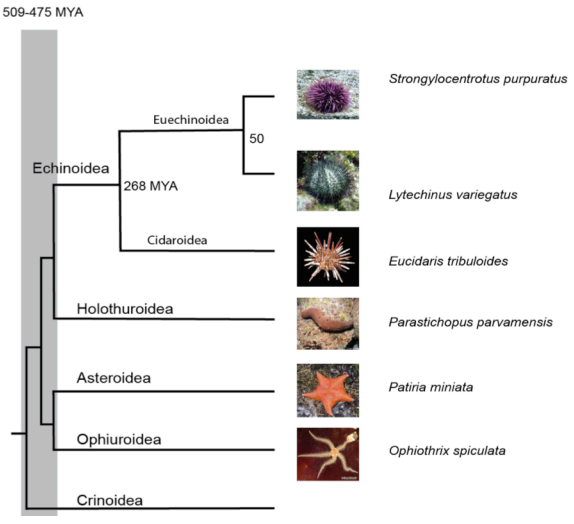

Fig. 1- Phylogenetic relationships, and times of divergence, of species of Echinoderms. The shaded region is the period of rapid divergence at the Cambrian boundary.

(A) Echinoderms

Early Cambrian stem group echinoderms, identified by their calcite endoskeletons and water vascular systems, were survived by stem group lineages that gave rise to the five extant classes (Figure 1). One of the most striking aspects of echinoderm evolutionary history is that since the Ordovician, 495-440 million years (MY) ago, the characters that define the echinoderm classes have been completely static. As established by modern phylogenomics (Telford et al. 2014): (i) the echinoderms plus hemichordates are the sister clade to the chordates within deuterostomes; (ii) the primitively stalked crinoids are basal to the other four extant echinoderm classes, all of which are motile as adults; (iii) the sea stars and brittle stars are sister groups; (iv) the sea cucumbers and sea urchins are sister groups; (v) the sea urchins themselves diverged from common ancestors that last lived at the latest about 268 MY ago, i.e., 18 MY before the great end-Permian extinction, and which were likely already separated into the two extant subclasses (cidaroids, “pencil urchins”, and euechinoids “modern sea urchins”).

(B) The Echinoderm Research Community

The investigators who use sea urchins and other echinoderms as research models for development and cell biology are an active and intellectually important community of researchers. Currently, this community comprises about 150 investigators, as measured by the number of people who attend the DBSU meeting (see below). There are about 50 laboratory directors on the current mailing list for this meeting. An NIH Reporter search for keywords: “urchin” or “Echinoderm” shows that there are awards totaled at $6.5 million for 2016-17. They reside in NICHD, NIGMS, NIEHS and OD institutes. Likewise, NSF lists 25 active awards for “Echinoderm” in Bio Directorate programs, funded at $9.5 million.

The echinoderm research community is a remarkably cooperative group. This collection of investigators has held an international meeting (Developmental Biology of the Sea Urchin; DBSU) every 18 months for 37 years (since 1981) with only a rotating, ad hoc committee of organizers and no other official structure. Many investigators, graduate students, and post-doctoral scholars have spent time in echinoderm research laboratories other than their own for sabbaticals or short training experiences. Interdisciplinary work ranging from paleontology to molecular developmental biology is evident within the community. The exchange of unpublished sequence data and reagents occurs seamlessly among laboratories. This group of scientists exhibits “coopetitive” behavior, a mixture of competition and cooperation discussed in game theory and social organization (Ghobadi and Ambra 2012). In this context, the support of community resources returns much more than it costs while still supporting innovative efforts.

(C) Broader Impacts of the Echinoderm Community

Sea urchins have been an important research model for more than 150 years (McClay 2011). Currently, studies with sea urchins and other echinoderms are making far-reaching contributions to many fields of biology. For example, a Google Scholar search reveals that the term,

"Echinoderm" has been used in the text of ~14,500 publications since 2011. Most significantly, echinoderms have contributed uniquely to understanding the controlling role of the genome in the process of development. Still today, the best understood regulatory logic control processing is known for genes acting during sea urchin development. The strengths of this experimental model will continue to make it a preeminent system for the analysis of the genomic control of embryogenesis.

Research with echinoderms is currently impacting several major areas, including:

Gene Regulatory Networks: Presentation of the first detailed animal gene regulatory network model for development (Davidson et al. 2002) heralded the beginning of a new and ever more important research role for sea urchins. This work was tremendously augmented by the genome sequence announced in 2006 (Sea Urchin Genome Sequencing Consortium et al. 2006) and by the use of arrayed library resources. The sea urchin embryo is now the leading model system for analyzing the regulatory networks that underlie all animal development. Current work in this important field is expanding our understanding of the architecture of GRNs (Li et al. 2014; Andrikou et al. 2015; Peter and Davidson 2017; Cui et al. 2017), GRN evolution (Cheatle Jarvela et al. 2014; Erkenbrack and Davidson 2015; Cary et al. 2017), linkages between GRNs and tissue morphogenesis (Rafiq et al. 2014; Saunders and McClay 2014), the regulation of GRNs by intercellular signaling pathways (Cui et al. 2014; Sun and Ettensohn 2014), and the potential utility of GRNs in re-engineering the process of embryogenesis (Damle and Davidson 2012). This work has pioneered the use of systems and GRN approaches into many other models of biology (Dutkowski and Ideker 2011; Sánchez Alvarado 2012; Wilson et al. 2008; Zmasek and Godzik 2013). **As evidence of impact in this cardinal area, there are ~11,600 Google Scholar citations using the terms "sea urchin gene regulatory network" since 2011.**

Immunology: Sea urchins have potent non-adaptive immune systems that utilize hundreds of receptors of classes such as Toll-like Receptors (TLR) (Buckley and Rast 2015). Elucidation of echinoderm immune function has the potential to inform our understanding and evolution of vertebrate immune function (Buckley et al. 2017; Yue et al. 2014).

Cell Signaling: The genome sequence has also made possible important new insights regarding cell signaling processes in early development and their role in embryonic patterning (Haillot et al. 2015; Materna and Davidson 2012; McIntyre et al. 2013; Sethi et al. 2012; Chang et al. 2017; Range and Wei 2016; Whittaker et al. 2006; Warner et al. 2014). Current research on detoxification biochemistry, as well as response abiotic stressors relies heavily on the sea urchin (Bošnjak et al. 2013; Hamdoun and Epel 2007; Campanale and Hamdoun 2012; Chan et al. 2015). The sea urchin has a long history of contributions to fertilization biology (reviewed in (Briggs and Wessel 2006)) and echinoderms continue to be an important model system for the study of this biological phenomenon (Ramos and Wessel 2013; Guo et al. 2015). Further, because of the high resolution imaging capabilities, cell cleavage processes continue to be informed by studies with echinoderms (Abruzzese et al. 2017; Henson et al. 2017).

Neurobiology: In the realm of neurobiology, the genome sequence has uncovered a range of fascinating but little understood sensory organs and pathways (Yankura et al. 2013; Burke et al. 2014; Elphick 2014; Wei et al. 2016; Cheatle Jarvela et al. 2016).

Germ Cell Specification: The sea urchin has emerged as an important model for the study of germ cell specification by conditional mechanisms, work that has also been spurred by the availability of genomic data (Wessel et al. 2014; Oulhen et al. 2017).

Regeneration and Ageing: The extraordinary capacity that echinoderms have for regeneration, and studies showing exceptional longevity are now being exploited by researchers. These works are currently directed at understanding the molecular and cellular mechanisms of the biological phenomena (Merino et al. 2017; Cary and Hinman 2017; Oulhen et al. 2016; Reinardy et al. 2015).

Education and Outreach: The availability of sea urchin gametes and the ease of their manipulation have made the sea urchin a popular source of educational material for many years. There are two widely used and complementary educational web sites. "Sea Urchin Embryology" (http://web.stanford.edu/group/Urchin/) provides essential information concerning animal procurement and handling, gamete collection, and fertilization, as well as detailed protocols for simple wet-lab exercises related to fertilization and early development. "Virtual Urchin" (virtualurchin.stanford.edu) supports unique, interactive web-based educational modules related to sea urchin development, including a virtual lab bench for simulating complex experimental manipulations. "Embryology Experiment" kits are commercially available from Carolina Biological Supply Company and Gulf Specimen Marine Lab, attesting to the widespread use of sea urchin gametes and embryos as educational materials.

3. CURRENT ECHINOBASE RESOURCES THAT SUPPORT COMMUNITY ACTIVITIES

(A) Available Data for Echinoderm Research at NCBI

Number of search results for echinoderm terms in PubMed for 2016	637
Number of echinoderm nuclear genome projects registered at NCBI	10
Number of echinoderm transcriptome projects in the Short Read Archive at NCBI:	292 (81 species)

(B) EchinoBase Web Information System

Number of species with assembled genomes in Echinobase	6
Number of species with skim genome sequencing in Echinobase	2
Number of bytes in the Echinobase web directories	247 Gb
Number of files in the Echinobase web directories	5,114,700
Number of bytes in 216 sequence download files	35.6 Gb

Since its creation, Echinobase (http://echinobase.org), the public portal to echinoderm genomic resources, has proved to be invaluable; for example, the remarkable progress made on developmental GRNs would not have been possible without it. Echinobase as it is now

configured will serve as the foundation for future expansion in terms of additional data and new capabilities.

(C) Genome and Transcriptome Assemblies

A total of 8 genome sequences for the echinoderms in various levels of draft assembly are housed at Echinobase (Table 1). There are assemblies from five sea urchin species (*S. purpuratus, Lytechinus variegatus, S. franciscanus, Allocentrotus fragilis* and *Eucidaris tribuloides*) posted. Genomic data from a sea star (*Patiria miniata*), a sea cucumber (*Parastichopus parvimensis*) and a brittle star (*Ophiothrix spiculata*) are also available. Thus the genomics included in the web system samples the major evolutionary diversity extant in this phylum. It might be added that much recent work has shown that the ~50 MY divergence time separating *Strongylocentrotus* and *Lytechinus* turns out to be the "sweet spot" for identification of sequence patches conserved because of their cis-regulatory function (Cameron and Davidson 2009).

Species	**Status**
Strongylocentrotus purpuratus v4.0	mature draft
Strongylocentrotus franciscanus	2x skim coverage
Allocentrotus fragilis	2x skim coverage
Lytechinus variegatus v2.2	improved draft
Patiria miniata v2.0	improved draft
Eucidaris tribuloides v1.0	first draft
Parastichopus parvimensis v1.0	first draft
Ophiothrix spiculata v1.0	first draft

Table 1- Sequencing progress for echinoderm genomes. The eight genome projects begun at the Baylor College of Medicine, Human Genome Sequencing Center and the present stage of completion of each.

Transcriptomes for many embryonic stages and adult tissues of these and other species have been assembled into gene sets and accessioned into the database. Both gene annotation and Blast databases are available for these gene sets. Echinobase also includes a number of state-of-the-art bioinformatics resources and powerful search engines. Its JBrowse genome visualization tracks include transcript maps together with gene predictions, and for *S. purpuratus* the mounted gene models have been vastly improved by cross-reference analysis based on our dense developmental transcriptome studies (Tu et al. 2012; Tu et al. 2014). A continuing effort is made in Echinobase to improve genome curation by incorporation of new literature results, and great care has also been taken to update and improve the website which serves as the portal of Echinobase to the outside world. The broad range of functionalities currently incorporated into Echinobase is indicated in Fig. 2, a snapshot from the Navigation Guide on the website.

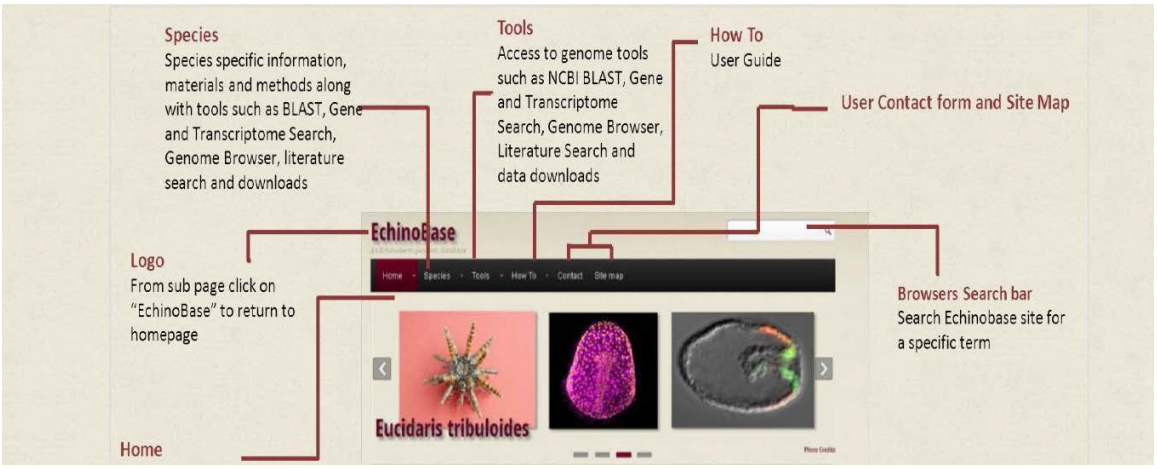

Fig. 2- Portals to various publicly accessible resources mounted in Echinobase.

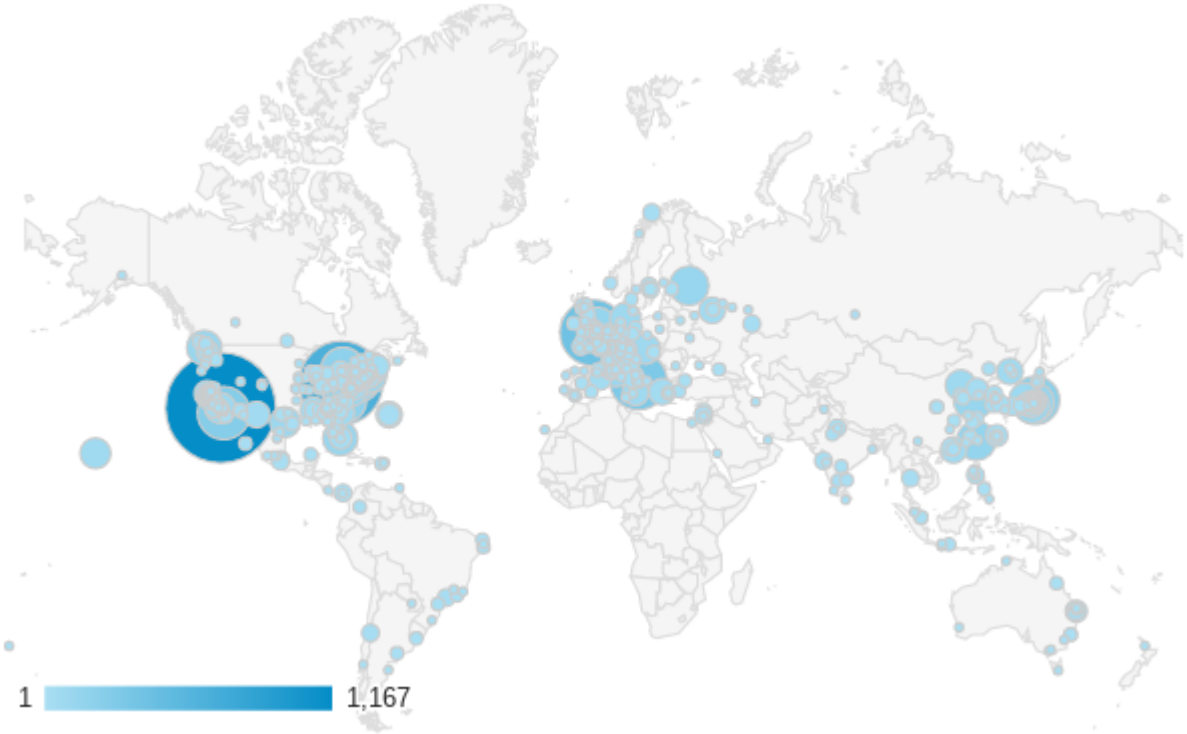

Fig. 3- Current world-wide use of Echinobase website per annum; map shows hits in Taiwan, Japan, India, Australia, Canada, S. America, in addition to Europe and the U.S. (Data from Google Analytics.)

(D) Usage

Echinobase is heavily used by many laboratories. Use can be measured by visits to the website, where a meaningful visit might be one in which the visitor stays to consult multiple website pages. By this criterion Echinobase receives about 4000 visits annually. Echinobase is used in many parts of the world (though mainly in the US); Fig. 3 is based on data retrieved from Google Analytics and filtered for users as stated above. But numbers of visits do not tell the whole story: for anyone who is working on genes or genomes of these model organisms, **the genomic database is an absolutely essential resource, and much of the most important research in the last few years in this field could not have been carried out without this resource**.

4. RESOURCES ASSOCIATED WITH ECHINOBASE

An important source of new information requiring curation by Echinobase will be the Resource for Developmental Regulatory Genomics (RDRG), a community research resource for which NIH R24 funding is currently pending. This resource will produce ATAC-seq chromatin accessibility profiles of several purified embryonic cell types at different developmental stages, for the purpose of enhancing CRM identification and GRN analysis. The utility of these data will require public access to the peak tracks and tools for comparing peaks across samples; the latter will allow users to identify candidate, cell type-specific or temporally regulated CRMs. RDRG will also develop a large number of new reagents for the research community, including fluorescent BAC reporters, NanoString codesets, and photoactivatible morpholinos. For researchers to benefit from these resources it will be critically important that Echinobase maintain up-to-date lists of these reagents. We anticipate that the activities of Echinobase and RDRG will be highly synergistic.

5. PROPOSAL FOR ENHANCEMENT OF ECHINOBASE

A scientific advisory board meeting was held at the most recent Developmental Biology of the Sea Urchin meeting (XXIV; April, 2017), along with a public forum to discuss the needs of the research community with respect to Echinobase and other resources. Approximately 60 people attended the public meeting. The group unanimously supported the continuation and expansion of Echinobase.

Based on these meetings, we established a survey to assess the needs and priorities of the community, which was emailed to the Echinobase email list and posted on the website. We received 105 responses, including 47 identified as faculty and lab PIs (https://www.surveymonkey.com/results/SM-P363JRL6/). 96% of respondents stated that echinobase was essential for their research, or that it would be difficult to do their work without it. This survey provided a clear set of priorities for the Echinobase user community. In order of priority, these are:

(A) To improve genome assemblies of species of Echinoderms used as model organisms in community labs. The genomes of echinoderms are large and polymorphic. Indeed, efforts to sequence and assemble them have often served as experiments for this kind of effort in general

(English et al. 2012). A summary shown in Table 2 below indicates the state of the various genomes in our resource and the efforts needed to improve them to a state where they are useful for comparative genomics, and to adequately serve the work of the echinoderm research community. A first priority must therefore be to improve assembly completeness and accuracy of the Echinoderm genomes most frequently used as model organisms by the community.

	Current Status			***Proposed Status***		
Genome	**Contig N50 (kb)**	**Scaffold N50 (kb)**	**Sequencing**	**Additional Sequencing**	**Target Assembly Level**	**Functionality**
S. purpuratus (v4.0)	17.6	431	40x Illumina 11x PacBio 18x SOLiD 8.3x Sanger	40x PacBio	MB Scaffold N50	Comparative genomics; Improved ID of regulatory ncDNA; Improved gene prediction; Facilitate generation of accurate molecular reagents
P. miniata (v2.0)	18	76	70x Illumina 15x Roche 454 19x PacBio	30x PacBio	MB Scaffold N50	Comparative genomics; Improved ID of regulatory ncDNA; Improved gene prediction; Facilitate generation of accurate molecular reagents
L. variegatus (v2.2)	9.7	46	21x Illumina 23x Roche 454 13x PacBio	40x PacBio	MB Scaffold N50	Comparative genomics; Improved ID of regulatory ncDNA; Improved gene prediction; Facilitate generation of accurate molecular reagents
E. tribuloides	2.8	28.2	23x Illumina 23x Roche 454	10x PacBio	10kb contig N50	Improved gene prediction; Facilitate generation of accurate molecular reagents
P. parvimensis	7.1	40	~140x Illumina	10x PacBio	10kb contig N50	Improved gene prediction; Facilitate generation of accurate molecular reagents
O. spiculata	4.5	43	~160x Illumina	10x PacBio	10kb contig N50	Improved gene prediction; Facilitate generation of accurate molecular reagents

Table 2- Current and proposed minimum genome assembly and annotation statistics for flagship species of Echinoderms.

(B) To improve gene annotations and ortholog identities. A central objective in many research programs in our community is to assay gene expression and function. Efforts should be made to generally improve gene annotations (e.g. splicing isoforms, non-coding RNAs, translation starts sites, and UTRs). This will globally improve the utility of Echinobase for all researchers. This will also facilitate CRISPR guide RNA design (Lin and Su 2016). Providing a way for users to efficiently modify gene annotations was also viewed as an important goal (e.g. implementation of a wiki). In order to facilitate comparison across Echinoderms, and also with vertebrate animals, it will be important to provide a rigorous analysis of gene orthologies.

The number of user-generated echinoderm transcriptomes is ballooning. There are 292 echinoderm transcriptomes in the NCBI sequence read archive (SRA) representing 81 species. Most of these were collected for an explicit experimental purpose and no consolidation has been undertaken. Thus a huge amount of data is lost to the experimentalist. A further goal therefore is to collate transcriptomes from these many sources to provide high quality reference transcriptomes from multiple species and, where possible, provide details of time points and tissue types.

(C) To provide a resource for sharing protocols, reagents and community news. Research progress can be enhanced by ensuring that protocols and validated reagents (e.g. morpholino sequences, CRISPR guide RNA sequences, PCR primers, etc.) are readily available and searchable for the community. Echinobase should serve as a platform for such information sharing. Current efforts should be maintained and extended.

(D) To provide options to include genome-wide scans of features of a regulatory nature (including ATAC-seq data) for all species. Echinoderms are famous for the ease with which synchronous embryo cultures can be obtained, suiting them perfectly for developmental profiling of chromatin architecture. Most importantly, echinoderm embryos are unusually well suited for functional cis-regulatory analyses of gene expression, an essential component of GRN studies. Such data are emerging from many labs and it will be crucial to provide genome browser tracks or other portals to these data on Echinobase. This will greatly facilitate improved annotations of functional noncoding DNA and the use of echinoderms for regulatory functional genomics. Such data are also routinely needed by researchers from other models systems and, in particular, the growing body of researchers performing comparative functional genomics that would like to use this major phylum in their analyses.

(E) To further incorporate expression data into Echinobase. New endeavors to include spatial and quantitative expression should be included for *S. purpuratus* and other important experimental species. Significant individual lab efforts are directed at identifying spatial and quantitative gene expression profiles that can benefit the community as a whole. Providing these data in a format that can be readily accessed and cross-referenced from multiple species will aid comprehensive syntheses of gene regulatory network analyses, including for researchers from other communities. These should, as much as possible, also follow standards for other models systems outside of the echinoderms, to facilitate broader accessibility. Controlled vocabularies for developmental anatomy, and developmental stages, should be developed with the intent of coordinating with other taxa.

(F) To improve accessibility and ease of use of the web resource. As the types (e.g. annotation and expression) and the quantity of data expand, it becomes imperative to remodel the Echinobase web information system to ensure that it remains easily accessible to researchers, regardless of their experience with echinoderms. This will include use of uniform nomenclature and searching tools, as well as intuitive links to external resources and databases. Efforts will be made to seek input from researchers in other systems, and in particular other genomic web resource developers, to stimulate outreach efforts to service a broader community. The goal is to increase the impact of Echinobase and Echinoderm research, and to ensure that researchers from other communities can take advantage of the work done in echinoderms.

These recommendations address critically important needs identified by the community, seek to make best use of current resources, and are directed at enhancing the unique strengths of the echinoderm model system for the coming decade.

Echinobase is a critically important source of genomic information for the Echinoderm Phylum. Without it, most of the important research resources that have been developed over the past decade (including the genome sequence itself) would be almost useless. **The continual improvement of this vital resource is therefore of the highest priority.**

Echinobase also **leads the way in working with highly polymorphic genomes,** which are typical for many species with large population sizes. Echinobase therefore serves as a

technical resource to scientists establishing new organisms for genomic studies. As a medium sized coopetitive community we are also well placed to trial new approaches for data sharing. Echinobase therefore serves as an important resource for a wide community beyond echinoderm-specific researchers.

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