ISJ 7: 211-220, 2010 ISSN 1824-307X

REVIEW

Echinoderm immunity

F Ramírez-Gómez[1], JE García-Arrarás[2]

[1]*Department of Biology, University of Massachusetts Dartmouth, 285 Old Westport Road, North Dartmouth, MA 02747, USA*
[2]*Department of Biology, University of Puerto Rico, P.O. Box 23360, UPR Station, Río Piedras, San Juan, PR 00931-3360, USA*

Accepted September 27, 2010

Abstract

Echinoderms are exclusively marine animals that, after the chordates, represent the second largest group of deuterostomes. Their diverse species composition and singular ecological niches provide at the same time challenges and rewards when studying the broad range of responses that make up their immune mechanisms. Two types of responses comprise the immune system of echinoderms: a cellular response and a humoral one. Cell-based immunity is carried by the celomocytes, a morphologically heterogeneous population of free roaming cells that are capable of recognizing and neutralizing pathogens. Celomocytes present diverse morphologies and functions, which include phagocytosis, encapsulation, clotting, cytotoxicity, wound healing among others. Humoral immunity is mediated by a wide variety of secreted compounds that can be found in the celomic fluid and play important roles in defense against infection. Compounds such as lectins, agglutinins, perforins, complement and some cytokines make up some of the humoral responses of echinoderms. Recent advances in the field of molecular biology, genomics and transcriptomics have allowed for the discovery of new immune genes and their products. These discoveries have expanded our knowledge of echinoderm immunity and are setting up the stage for future experiments to better understand the evolution of the immune mechanisms of deuterostomes.

Key Words: comparative immunology; echinoderm; immunity; celomocytes; genes

Introduction

The phylum Echinodermata is a very diverse group of marine animals that have sparked the interests of scientists for over a century. Significant discoveries have been made using echinoderms in the areas of cell biology, developmental biology and immunology. Five classes comprise the phylum: Asteroidea (sea stars or starfish), Crinoidea (crinoids or feather stars), Ophiuroidea (brittle stars), Echinoidea (sea urchins and sand dollars) and Holothuroidea (sea cucumbers or holothurians). Even though research has been done on all echinoderm classes, one group excels as the favorite of scientists: the echinoids. Thus, sea urchins have become one of the classical animal models and have been particularly exploited in studies of fertilization and developmental biology. Similarly, in the field of echinoderm immunology, sea urchins comprise the group that has been most extensively studied (Smith *et al.*, 2006). Furthermore, the availability of the genome sequence for the purple sea urchin (*Strongylocentrotus purpuratus*) has allowed for in depth studies into the genetic aspects of its immune response (Hibino *et al.*, 2006; Rast *et al.*, 2006). This trend has helped advance the field and at present, echinoderms are catching the attention of comparative immunologists. However, due to the inherent diversity of the echinoderm phylum, general assumptions cannot be easily established and what is true for one specific class may not apply to others.

Interest in echinoderm immunobiology also originates form the aquaculture field. Although little known in the western hemisphere, holothurian and echinoid cultures are an important economic activity in Asia. With an increasing demand for sea urchin roe and trepang (a generic name for sea cucumbers), commercial culture venues have increased in order to maintain the demands for these organisms. With increase in aquacultures one observes an increase in diseases, mainly infections, and therefore an increase interest in understanding how the organisms protect themselves from

Corresponding author:
Francisco J Ramirez-Gomez
University of Massachusetts Dartmouth,
285 Old Westport Road,
North Dartmouth, MA 02747-2300, USA
E-mail: framirez@umassd.edu

pathogenic threat. The present review will attempt to summarize the latest published research work on the echinoderm immune system with a special emphasis on non-echinoid groups. The review focuses on the different immune components and mechanisms present in the phyla and highlights how rich, diverse and complex this group of animals can be.

General aspects of echinoderm immunity

In terms of their immune systems, echinoderms display the same basic types of responses that most multicellular (including vertebrates) animals do. They can recognize self from non-self and, if a foreign material (e.g., microorganism/pathogen) enters the body cavity, they can readily neutralize it and dispose of it (Yui and Bayne, 1983; Dybas and Fankboner, 1986; Jans *et al.*, 1996; Glinski and Jarosz, 2000). Additionally, echinoderms possess very good wound-healing capabilities, a key feature that also plays an important role in one of the best known characteristics of the group: regeneration of lost body parts.

These defense mechanisms are mediated by cellular and humoral responses, with several homologous and analogous components found in other invertebrates and vertebrates alike. In fact, it is their key position in the evolutionary tree, being invertebrate deuterostomes (thus sharing a common evolutionary branch with vertebrates) that makes the study of their immune system a very interesting and exciting field. Therefore, this advantageous phylogenetic position allows for comparisons between immune mechanisms that have been well studied in vertebrates with those of their echinoderm counterparts. Thus, echinoderms can provide important information on the evolution of the immune response.

As in many other systems, echinoderm immune responses can be divided between cellular and humoral responses. Cellular responses are mediated by the celomocytes, which are free roaming cells that occupy the celomic cavity but can also infiltrate tissues and organs. These cells circulate in the celomic fluid and exert the vast majority of immune functions. On the other hand, humoral responses are defined by the broad variety of molecules present in the celomic fluid. These molecules are capable of recognizing and neutralizing foreign material, promoting cell migration and agglutination and also playing roles in wound healing (Ryoyama, 1973; Kanungo, 1982; Canicatti *et al.*, 1992; Smith and Davidson, 1992).

Cellular components

Celomocytes are a very abundant and diverse cell types that are present in all echinoderms. These cells are heterogeneous in morphology, size, relative abundance and function, which make a single standard classification for all echinoderms a difficult task. Extensive research has been done during the past century on the morphological aspects of celomocytes. Comprehensive reports on the celomocyte types of different echinoderms classes have also been published (Kindred, 1924; Boolootian and Giese, 1958, 1959; Boolootian, 1962; Endean, 1966). These studies clearly show the wide variety of cell morphologies present in the echinoderm celomic fluid. However, the absence of a standard reference among groups and particularly, differences in terminology and even specimen preparation, contribute to the existing heterogeneity. Nonetheless, some types of celomocytes can be found in all classes, while others have been considered to be specific to certain classes. These cell types are summarized in Table 1 along with the particular functions that have been ascribed to certain cells.

The distribution of these cell types is highly variable among species and also even at the individual level. For example, in some sea star species the vast majority (> 90 %) of celomocyte types are amebocytes, while other cell types seem to be exclusive of certain groups (e.g., holothurian crystal cells). Table 2 summarizes the general distribution of celomocytes in three echinoderm classes (echinoids, holothuroids and asteroids) and how they differ depending on the group and the species. This cell distribution is also very dynamic, changing in accordance to the physiological or immune state of the animal. For example, in the sea star *Asterias rubens* specific sub-populations of amoebocytes increase in number after injection of gram-positive bacteria while other sub-groups remain unchanged (Coteur *et al.*, 2002). Our studies with the sea cucumber *Holothuria glaberrima* have shown that the total number of celomocytes remains unchanged after challenges with diverse pathogen associated molecular patterns (PAMPs). However, the distribution of particular sub-types changes after immuno-stimulation, e.g., lymphocytes numbers diminish, while phagocytes increase (Ramirez-Gomez *et al.*, 2010).

From all the celomocyte types, probably the one that is present in all the echinoderm classes is the phagocyte/amebocyte type. This cell ranges in size from 3 to 20 μm and its main characteristic is its ability to phagocytize other cells or foreign particles (Endean, 1966). Other roles have been attributed to phagocytes, most of them immune related, demonstrating that this cell type is the main effector of the echinoderm immune system. In fact, the discovery of these cells in the sea star, back in the late 1800's by Russian zoologist Ilya Metchnikoff gave rise to the field of cellular immunity, for which he was awarded the Nobel prize in 1908 (Metchnikoff, 1891). Amebocyte roles include: graft rejection, chemotaxis, reactive oxygen species production, encapsulation, cytotoxicity, immune gene expression, agglutination and clotting reactions (Gross *et al.*, 1999, 2000; Beck *et al.*, 2001; Coteur *et al.*, 2001; Lin *et al.*, 2001; Hillier and Vacquier, 2003; Clow *et al.*, 2004; Matranga *et al.*, 2005; Sun *et al.*, 2008). Several authors sub-categorize phagocytes according to their size and morphology, but since these classifications are not the same for all echinoderms, some sub-types may overlap or on the other hand can be rendered as a different cell type altogether. Lymphocytes are another cell type that might be present in all echinoderms (Endean, 1966), but it is most frequently found in holothurians and some sea stars

Table 1 Summary of celomocyte types reported for echinoderm classes. E: Echinoidea, H: Holothuroidea, A: Asteroidea, C: Crinoidea, O: Ophiuroidea.

Cell type	Present in class	Role	Reference
Discoidal cell	E, H	Phagocytosis, clotting, encapsulation, chemotaxis, opsonisation, graft rejection	(Coteur *et al.*, 2002; de Faria and da Silva, 2008; Eliseikina and Magarlamov, 2002; Endean, 1966; Matranga *et a*l. 2005; Ramirez-Gomez *et al.*, 2010; Smith *et al.*, 2006)
Polygonal cell	E		
Small phagocyte	E, H		
Amebocytes /Phagocytes	E, H, A, C, O		
Colored spherule	E, H, C	Antibacterial activity	(de Faria and da Silva, 2008; Endean, 1966; Smith *et al.*, 2006)
Colorless spherule	E, H, A, C, O	Antibacterial, inflammation, Wound healing, ECM remodeling	(Coteur *et al.*, 2002; de Faria and da Silva, 2008; Eliseikina and Magarlamov, 2002; Endean, 1966; Garcia-Arraras *et al.*, 2006; Ramirez-Gomez *et al.*, 2010; Smith *et al.*, 2006)
Lymphocyte	E, H, A	Progenitor cells	(Coteur *et al.*, 2002; Eliseikina and Magarlamov, 2002; Endean, 1966; Ramirez-Gomez *et al.*, 2010; Xing *et al.*, 2008)
Vibratile	E, H, A, O	Celomic fluid movement, clotting	(de Faria and da Silva, 2008; Eliseikina and Magarlamov, 2002; Endean, 1966; Matranga *et al.*, 2005; Pinsino *et al.*, 2008; Ramirez-Gomez *et al.*, 2010; Smith *et al.*, 2006; Xing *et al.*, 2008)
Crystal cells	H	Osmoregulation	(Eliseikina and Magarlamov, 2002; Endean, 1966; Ramirez-Gomez *et al.*, 2010; Xing *et al.*, 2008)
Hemocytes	H, A, O	Oxygen transport	(Eliseikina and Magarlamov, 2002; Endean, 1966; Pinsino *et al.*, 2008)

(Smith, 1981). These are small cells (4-6 μm), with a large nucleus and a thin layer of cytoplasm whose only common characteristic with their vertebrate namesakes is their morphology. Lymphocytes are regarded as progenitor cells and may be the precursors of other celomocyte types (Xing *et al.*, 2008; Ramirez-Gomez *et al.*, 2010). They can show phagocytic capabilities but this may reflect an intermediate state of maturity before becoming phagocytes (Ramirez-Gomez *et al.*, 2010).

Spherule cells (spherulocytes) are present mostly in echinoids and holothuroids (Endean, 1966; Eliseikina and Magarlamov, 2002; Smith *et al.*, 2006; de Faria and da Silva, 2008; Xing *et al.*, 2008; Ramirez-Gomez *et al.*, 2010) and in at least one sea star species (Penn, 1979). They are characterized by the presence of vesicles in their cytoplasm, some containing pigment (red, yellow, green, brown) other being colorless. Spherulocytes range in sizes from 8 to 20 μm and their distribution varies substantially between species. They have been associated with antibacterial activity (Johnson, 1969; Service and Wardlaw, 1984; Haug *et al.*, 2002), inflammatory responses (Pagliara and Canicatti, 1993), extracellular matrix remodeling (Garcia-Arraras *et al.*, 2006), and wound healing (San Miguel-Ruiz and Garcia-Arraras, 2007).

Another cell type present in echinoids and holothuroids are the vibratile cells. These are cells whose size ranges from 6 to 20 μm and are highly motile due to the presence of a flagellum. Their distribution varies accordingly to the species and their function is still not completely determined. They have been associated with clotting reactions (Bertheussen and Seijelid, 1978) and are also thought to be involved in the movement of the celomic fluid (Xing *et al.*, 2008).

Crystal cells seem to be exclusive of holothurians, these cells display a very regular geometric morphology (rhomboidal or hexagonal) and present a crystal inclusion within their cytoplasm (Endean, 1966). Their role is still not well defined, but it is likely that they play osmoregulatory roles (Xing *et al.*, 2008).

Table 2 Summary of celomocyte distribution in the celomic fluid in three echinoderm classes and six different species. L.var: *Lytechinus variegatus*; S. purp: *Strongylocentrotus purpuratus*; E. luc: *Echinometra lucunter*; H. glab: *Holothuria glaberrima*; A. jap: *Apostichopus japonicus*; A. rub: *Asterias rubens*.

Cell type	Echinoidea			Holothuroidea		Asteroidea
	L. var (Borges *et al.*, 2005)	S. purp (Smith *et al.*, 2006)	E. luc (de Faria and da Silva, 2008)	H. glab (Ramirez-Gomez *et al.*, 2010)	A. jap (Xing *et al.*, 2008)	A. rub (Coteur *et al.*, 2002)
Lymphocytes	n.f.	.n.f	n.f.	60 %	59 %	n.f.
Phagocytes/ amebocytes	> 60 %	40-80 %	77 %	30 %	17 %	80-95 %
Colored spherules	< 40 %	7-40 %	1 %	N.F.	N.F.	N.F.
Colorless spherules	+	3-25 %	3 %	5 %	23 %	N.F.
Vibratile cells	+	11-20 %	19 %	< 1 %	N.A.	N.F.

N.F., not found; N.A., not accounted.
n.f., not found.
+ spherules and vibratile cells were accounted together.

Interestingly, echinoderm cellular immunology has escaped the classical characterization of their components by phenotyping (identification of cell-specific epitopes expressed on cell membranes) as vertebrate lymphocytes do. The lack of definite surface markers for celomocytes has helped maintain the confusion in distinguishing between specific cell types and sub-types, limiting it to just morphological observations. However, this trend is slowly changing, as demonstrated by studies with sea urchin celomocytes, in which a sub-population of phagocytes was defined by NK cell surface markers and characterized by their cytotoxic properties (Lin *et al.*, 2001). Furthermore, monoclonal antibodies were generated against these cells showing a successful identification of a specific cytotoxic phagocyte sub-type (Lin *et al.*, 2007). Our research group has also identified sub-groups of sea cucumber celomocytes using monoclonal antibodies, each sub-population showing distinct characteristics and different responses to immunostimulation (Ramirez-Gomez *et al.*, 2010). Similarly, a recent study in the sea cucumber *Apostichopus japonicus*, has also led to the development of a monoclonal antibody that specifically recognizes spherulocytes. Initial characterization of the antigen being recognized by this antibody resulted in the identification of a 136 kDa protein according to Western blotting (Li *et al.*, 2010). However, what is still missing is the full characterization of the antigens these antibodies are recognizing (protein sequences and cloning). Future experiments where cell markers are used to describe celomocyte populations and compare these populations among the different echinoderm classes should be the basis for a clear and universal classification of echinoderm celomocytes.

Celomocyte origin

The origin of celomocytes is still a matter of debate. Two theories have been proposed to address this issue: one involving specific organs or tissues as the source of celomocytes while the other points at the celomocytes themselves as self-replicating cells (Bossche and Jangoux, 1976; Matranga *et al.*, 2005). Potential cytopoietic organs include the axial organ, Tiedemann bodies, Polian vesicles, connective tissue and the celomic epithelium (Endean, 1966). The latter has received particular attention in studies involving the sea star *A. rubens*, showing the epithelial origin of sea star celomocytes (Bossche and Jangoux, 1976; Holm *et al.*, 2008). Even though no direct evidence have been proposed for a self-replicating population of celomocytes, the idea of a circulating stem cell has not been ruled out and if anything has become more attractive in view of recent findings of stem cells in other metazoans (Handberg-Thorsager *et al.*, 2008; Watanabe *et al.*, 2009; Funayama, 2010). It must be stated that the evidence to ascertain the origin of celomocytes is far from definitive, and would not be acceptable by modern scientific standards. Thus, it is necessary for scientists to use modern methodologies to verify the celomocyte origins proposed by past investigators.

Humoral components

Echinoderms present a wide rich variety of secreted immune molecules. They have been the subjects of extensive research, even to the point of potential medical applications (Kelly, 2005). As mentioned before, the humoral components present in celomic fluid of echinoderms are capable of

recognizing foreign matter, neutralizing or destroying pathogens, inducing or enhancing cellular responses (opsonization) and helping during wound healing.

A well-known group of recognition molecules are the lectins, which recognize carbohydrate moieties on the surface of host cells (self) and of bacteria and fungi (non-self). Several lectins have been identified from the celomic fluid of echinoderms, where they play important roles in opsonization, lytic cytotoxicity, clot formation and wound repair (Gross *et al.*, 1999). Different lectins with specific recognition abilities have been found in asteroids (Kamiya *et al.*, 1992), and holothuroids (Matsui *et al.*, 1994; Gowda *et al.*, 2008a, b). Echinoidin, a C-type lectin (calcium-dependent) identified in a sea urchin also possess an RGD sequence, suggesting an additional role in cell-to-cell adhesion (Giga *et al.*, 1987; Ozeki *et al.*, 1991). Moreover, the C-type lectin CEL-III from the sea cucumber *Cucumaria miniata*, which possess a strong hemolytic activity have been transgenically expressed in mosquitoes and shown to successfully impair malaria parasite development (Yoshida *et al.*, 2007).

Other humoral factors include hemolysins, that interact with plasma membranes and form holes in the membrane of cells causing lysis of target cells (Canicatti, 1990, 1991). Hemolysins have been identified in sea stars (Leonard *et al.*, 1990), sea urchins (Ryoyama, 1973; Stabili *et al.*, 1992) and sea cucumbers (Canicatti and Parrinello, 1985). Agglutinins are another type of humoral factor that play roles in cell aggregation, encapsulation and clotting and have also been studied in wound repair. They have been found in echinoids (Ryoyama, 1973; Canicatti *et al.*, 1992), the sea star *Asteria pectinifera* (Kamiya *et al.*, 1992) and in the sea cucumber *Holothuria polii* (Canicatti and Parrinello, 1985).

In vertebrates a well-known group of effector molecules are the cytokines, which play a wide variety of roles in the immune response. In echinoderms, homologues of cytokines have also been identified. The first glance at an echinoderm cytokine came from the sea star *A. forbesi*, in which a humoral factor named the sea star factor was isolated and found to possess cytokine-like properties (Prendergast and Suzuki, 1970; Prendergast and Liu, 1976; Kerlin *et al.*, 1994). Furthermore, interleukin-like molecules were identified in the sea star, e.g., a protein with IL-1 activity and an IL-6 like molecule (Beck *et al.*, 1989, 1993; Beck and Habicht, 1991a, 1991b, 1996). However, none of these findings resulted in a definite identification of the cytokine factor or the cloning of the corresponding gene(s). The issue appears to be complicated since no IL-1 homologues were found in the sea urchin genome. However, other members of the cytokine network (mostly pro-inflammatory) have indeed been found, e.g., TNF and IL-17 (Hibino *et al.*, 2006).

Another important humoral factor present in echinoderms is the complement protein family. Most of the components of the alternative and lectin pathways have been identified in the sea urchins, being the purple sea urchin the first invertebrate in which a complement system was identified (Smith *et al.*, 1996; Smith, 2001). Initial evidence gathered from sea urchins, sea cucumbers and sea stars hinted at the presence of a complement system in echinoderms (Kaplan and Bertheussen, 1977; Parrinello *et al.*, 1979; Bertheussen 1981a, 1981b, 1982, 1983; Bertheussen and Seljelid, 1982), but they were mostly from complement-derived or -dependent activity and no definite identification of a complement protein was achieved. Smith and colleagues (1996, 1998) successfully identified the first echinoderm (and invertebrate) homologue of the C3 component and later another complement protein was found (Factor B) (Smith *et al.*, 1998). A recent publication reported the finding of a C3 complement homologue in the sea star *A. rubens*, whose expression is also induced by LPS stimulation (Mogilenko *et al.*, 2010). Additional components of the system have been identified from the sea urchin genome, suggesting that echinoderms possess a complement pathway mostly directed towards opsonization, since the components of canonical terminal pathway could not be found (Hibino *et al.*, 2006).

Molecular studies and the genomic era

The vast majority of molecular studies have been done in echinoids, particularly the purple sea urchin *S. purpuratus*. A broad number of immune genes have been identified from the sea urchin since the early 1990's and pinnacled with the publication of the *S. purpuratus* genome (Sodergren *et al.*, 2006). An in depth analysis of the immune repertoire contained within the sea urchin genome can be found in the publications of Hibino *et al.* (2006) and Rast *et al.* (2006).

However, other species of echinoderms have also been the subject of molecular studies in order to better understand their immune responses. These studies altogether benefit greatly the advancement of the field, providing further insights into the genetic and molecular aspects of echinoderm immunity.

Echinoderm molecular immunogenetics has evolved in parallel with the technologies available for its study. Starting with gene-by-gene approaches, in which single genes were analyzed at a time and their immune roles determined. An example of this is the case of the Profilin gene, an actin binding and cytoskeletal modification protein, expressed in celomocytes and up-regulated after injury and LPS injections (Smith *et al.*, 1992, 994, 1995). Then, when sequencing technologies became accessible, high-throughput sequencing projects were launched, mostly screening cDNA libraries. In the late 1990's a survey of a cDNA library from LPS-activated celomocytes provided the first glimpses of the immune repertoire of an echinoderm (Smith *et al.*, 1996). Several interesting findings were made in this study on sea urchins, beginning with the discovery of an echinoderm complement and a collection of putative immune effector genes that set the basis for future comparative studies between echinoderm species.

Our research group has been dwelling into the molecular immune aspects of holothurians since the

year 2000, when a homologue of the acute phase response protein serum amyloid A (SAA) was identified for the first time in an invertebrate (Santiago *et al.*, 2000). Its expression was found mostly in intestinal tissues during the regeneration process but also after immune stimulation with LPS. SAA mRNA was found to be overexpressed following an immune challenge not only in the intestine (Santiago-Cardona *et al.*, 2003) but also in celomocytes (Ramirez-Gomez *et al.*, 2008). Additionally, a series of immune-related genes were identified in the holothurian from intestinal cDNA libraries. This identification was mostly done by sequence comparison with other immune genes present in other organisms and whose immune role was clearly defined. The expression of these holothurian immune genes was corroborated in celomocytes to determine if they were part of the gene repertoire of these cells. In addition their expression was analized after an LPS challenge (Ramirez-Gomez *et al.*, 2008). Among these genes we found a C-type lectin, ferritin, cathepsin, toposome and an alpha 2 macroglobulin domain (A2M)-containing protein. One sequence of particular interest was a homologue of the DD104 protein from the sea urchin, which is up-regulated in celomocytes after injury and infection (Rast *et al.*, 2000) with the holothurian DD104 following a similar pattern but with higher expression levels in celomocytes after LPS injection. Recently, analysis of the expression of immune-related genes has also been done in embryos and larvae of the sea cucumber, *A. japonicus*. Nine genes were studied: six of them (heat shock proteins -70, -90 and -gp96; thymosin-beta, ferritin and DD104) showed no changes upon LPS challenge while the remaining three (mannan-binding C-type lectin, lysozyme and serine proteinase inhibitor) were found to be up-regulated upon challenge (Yang *et al.*, 2010).

The advent of array technologies that allow for studies on the expression of multiple genes at the same time, have opened the door for the identification of potential novel genes. Many of these genes were missed in previous approaches probably due to their lack of homology to known genes. This approach was first carried out with the sea urchin, comparing immune-stimulated and immunoquiescent animals. An unexpected diversity of genes was found to be differentially expressed and, more interestingly, a set of novel genes, the 185/333 family of proteins, were then identified (Nair *et al.*, 2005). This family of genes represents a highly variable set of proteins that are involved in the immune response of the sea urchin (reviewed in Ghosh *et al.*, 2010).

We have also used immune activation and microarray technologies to compare LPS-injected sea cucumbers with seawater-injected controls and thus, identify immune-responsive genes in the holothurians. We have found 50 unique sequences differentially expressed after LPS stimulation. The vast majority of these sequences showed no known homologies in the databases (Ramirez-Gomez *et al.*, 2009). Ongoing efforts are being done to further characterize these unknown genes. By complete sequencing of the mRNAs we expect to find similarities and/or conserved domains that will provide either proper identification, or the characterization of novel holothurian LPS-responsive genes.

An interesting case derived from our microarray study, is the echinoderm mayor yolk protein (MYP) and its closely related protein, toposome. In our microarray, the holothurian MYP gene was one of the top genes that showed differentially expression following LPS injection. Echinoderm MYP was initially identified as an unconventional iron-binding vitellogenic protein making up to 50 % of the protein content of the sea urchin celomic fluid (Brooks and Wessel, 2002). It is synthesized in the digestive tract and also binds zinc ions (Unuma *et al.*, 2007, 2009). A possible immune role for this protein had been suggested due to its affinity for iron, making it an excellent bacteriostatic agent. Our results from the holothurian microarray, have shown that MYP mRNA is up-regulated after LPS stimulation in the digestive tract but its expression remains unchanged in celomocytes (Ramirez-Gomez *et al.*, 2009). Nonetheless, anti-MYP labeling is found in phagocytic lymphocytes (Ramirez-Gomez *et al.*, 2010). The toposome protein (which is closely related to MYP), functions as an adhesion protein in the sea urchin embryo (Cervello and Matranga, 1989; Scaturro *et al.*, 1998; Noll *et al.*, 2007), but is also related to stress and injury respones (Cervello *et al.*, 1994; Matranga *et al.*, 2005; Pinsino *et al.*, 2007). We have found toposome mRNA to be expressed in *H. glaberrima* celomocytes at relatively high levels that remain unchanged after LPS stimulation (Ramirez-Gomez *et al.*, 2008) as well as in intestinal tissues, in which its levels remained unchanged also (Ramirez-Gomez *et al.*, 2009). These results show that both MYP and toposome are indeed associated with the immune response but also suggest additional roles that might not be part of the traditional functions associated with celomocytes but might be associated with the immune functions of the digestive tract.

Now that we have entered the genomic era, further advances are expected as genome sequencing technologies become faster and more economically accessible. The *S. purpuratus* genome represents a cornerstone in echinoderm research that can be used to compare findings from other echinoderm species. However, as presented here, the great diversity of the animals within the echinoderm phylum suggest that having the genome of only one member of only one echinoderm class will not be enough to understand the echinoderm immune system. Take for example one of the most diverse set of genes found in the sea urchin genome: the NLR gene family (nucleotide-binding domain, leucine-rich repeat containing proteins). These genes encode cytoplasmic pattern recognition proteins, which in humans are represented by about 20 genes (Inohara and Nunez, 2003). However, in the sea urchin 203 NLR predicted genes can be found. Similar to the vertebrate counterparts, the major site of expression of the sea urchin NLRs is the gut (Hibino *et al.*, 2006). Nonetheless, we were not able to identify sequences for this gene family in any of our holothurian intestinal cDNA libraries nor in our intestinal microarray studies. This may reflect key

differences in gene repertoires between these two species related to their phylogenetic divergence. This variety of gene repertoires may also be attributed to differences in habitat and developmental history, and to differences in the microbe flora that challenges the organisms. These differences will eventually shape the type of immune responses that organisms react to.

Therefore, we still need more information on immune-related genes present in other species from as many different groups as possible in order to have a better understanding of the molecular events that are involved with the echinoderm immune response.

Concluding remarks

Echinoderm immunity is a challenging yet promising field to study. The large diversity of echinoderm species, with different internal organs (most of them with little physiological information as to their functions) and different lifestyles make it difficult to identify those tissues or cells that might be playing an immune role. Moreover, different species might be responding to different immune challenges not usually associated with other animal groups (Think about the fact that echinoderms occupy large number of niches in the benthic zone). An additional complication is the difficulty in establishing the immune status of the animals used in experimentation. For example, in studies by Smith and colleagues, sea urchins were kept in aquaria in what appeared to be an immunoquiescent status. In this scenario it is difficult to compare the LPS response of these animals to that of animals that have been directly collected from the wild. Nonetheless, overcoming these difficulties can provide exciting and rewarding goals. Among these, are the identification of novel immune associated genes and proteins and the characterization of new immune signaling pathways. Moreover, the key phylogenetic position of echinoderms in the tree of life assures that whatever we learn about echinoderm immunity will help us understand the evolution of metazoan immune systems.

References

Beck G, Ellis T, Zhang H, Lin W, Beauregard K, Habicht GS, Truong N. Nitric oxide production by coelomocytes of *Asterias forbesi*. Dev. Comp. Immunol. 25: 1-10, 2001.

Beck G, Habicht GS. Primitive cytokines: harbingers of vertebrate defense. Immunol. Today 12: 180-183, 1991a.

Beck G, Habicht GS. Purification and biochemical characterization of an invertebrate interleukin 1. Mol. Immunol. 28: 577-584, 1991b.

Beck G, Habicht GS. Characterization of an IL-6-like molecule from an echinoderm (*Asterias forbesi*). Cytokine 8: 507-512, 1996.

Beck G, O'Brien RF, Habicht GS. Invertebrate cytokines: the phylogenetic emergence of interleukin-1. BioEssays 11: 62-67, 1989.

Beck G, O'Brien RF, Habicht GS, Stillman DL, Cooper EL, Raftos DA. Invertebrate cytokines. III: Invertebrate interleukin-1-like molecules stimulate phagocytosis by tunicate and echinoderm cells. Cell. Immunol. 146: 284-299, 1993.

Bertheussen K. Endocytosis by echinoid phagocytes in vitro. II. Mechanisms of endocytosis. Dev. Comp. Immunol. 5: 557-564, 1981a.

Bertheussen K. Endocytosis by echinoid phagocytosis in vitro. I. Recognition of foreign matter. Dev. Comp. Immunol. 5: 241-250, 1981b.

Bertheussen K. Receptors for complement on echinoid phagocytes. II. Purified human complement mediates echinoid phagocytosis. Dev. Comp. Immunol. 6: 635-642, 1982.

Bertheussen K. Complement-like activity in sea urchin coelomic fluid. Dev. Comp. Immunol. 7: 21-31, 1983.

Bertheussen K, Seijelid R. Echinoid phagocytes in vitro. Exp. Cell Res. 111: 401-12, 1978

Bertheussen K, Seljelid R. Receptors for complement on echinoid phagocytes. I. The opsonic effect of vertebrae sera on echinoid phagocytosis. Dev. Comp. Immunol. 6: 423-431, 1982.

Boolootian RA. The perivisceral elements of echinoderm body fluids. Amer. Zool. 2: 275-284, 1962.

Boolootian RA, Giese AC. Coelomic corpuscles of echinoderms. Biol. Bull. 115: 53-63, 1958.

Boolootian RA, Giese AC. Clotting of echinoderm coelomic fluid. J. Exp. Zool. 140: 207-229, 1959.

Borges JC, Jensch-Junior BE, Garrido PA, Mangiaterra MB, Silva JR. Phagocytic amoebocyte sub populations in the perivisceral coelom of the sea urchin *Lytechinus variegatus* (Lamarck, 1816). J. Exp. Zool. A Comp. Exp. Biol. 303: 241-248, 2005.

Bossche JP, Jangoux M. Epithelial origin of starfish coelomocytes. Nature 261: 227-228, 1976.

Brooks JM, Wessel GM. The major yolk protein in sea urchins is a transferrin-like, iron binding protein. Dev. Biol. 245: 1-12, 2002.

Canicatti C. Hemolysins: pore-forming proteins in invertebrates. Experientia 46: 239-244, 1990.

Canicatti C. Binding properties of *Paracentrotus lividus* (Echinoidea) hemolysins. Comp. Biochem. Physiol. 98A: 463-468, 1991.

Canicatti C, Pagliara P, Stabili L. Sea urchin coelomic fluid agglutinin mediates coelomocyte adhesion. Eur. J. Cell Biol. 58: 291-295, 1992.

Canicatti C, Parrinello N. Hemaglutinin and hemolysin level in coelomic fluid from *Holothuria polii* (Echinodermata) following sheep erythrocyte injection. Biol. Bull. 168: 175-182, 1985.

Cervello M, Arizza V, Lattuca G, Parrinello N, Matranga V. Detection of vitellogenin in a subpopulation of sea urchin coelomocytes. Eur. J. Cell Biol. 64: 314-319, 1994.

Cervello M, Matranga V. Evidence of a precursor-product relationship between vitellogenin and toposome, a glycoprotein complex mediating cell adhesion. Cell. Differ. Dev. 26: 67-76, 1989.

Clow LA, Raftos DA, Gross PS, Smith LC. The sea urchin complement homologue, SpC3,

functions as an opsonin. J. Exp. Biol. 207: 2147-255, 2004.
Coteur G, Danis B, Fowler SW, Teyssie JL, Dubois P, Warnau M. Effects of PCBs on reactive oxygen species (ROS) production by the immune cells of *Paracentrotus lividus* (Echinodermata). Mar. Pollut. Bull. 42: 667-672, 2001.
Coteur G, DeBecker G, Warnau M, Jangoux M, Dubois P. Differentiation of immune cells challenged by bacteria in the common European starfish, *Asterias rubens* (Echinodermata). Eur. J. Cell Biol. 81: 413-418, 2002.
de Faria MT, da Silva JR. Innate immune response in the sea urchin *Echinometra lucunter* (Echinodermata). J. Invertebr. Pathol. 98: 58-62, 2008.
Dybas L, Fankboner PV. Holothurian survival strategies: mechanisms for the maintenance of a bacteriostatic environment in the coelomic cavity of the sea cucumber, *Parastichopus californicus*. Dev. Comp. Immunol. 10: 311-330, 1986.
Eliseikina MG, Magarlamov TY. Coelomocyte morphology in the holothurians *Apostichopus japonicus* (Aspidochirota: Stichopodidae) and *Cucumaria japonica* (Dendrochirota: Cucumariidae). Russian J. Mar. Biol. 28: 197-202, 2002.
Endean R. The coelomocytes and coelomic fluids. In: Boolootian RA (ed), Physiology of Echinodermata, Intersciences, New York, 1966.
Funayama N. The stem cell system in demosponges: insights into the origin of somatic stem cells. Dev. Growth Differ. 52: 1-14, 2010.
Garcia-Arraras JE, Schenk C, Rodrigues-Ramirez R, Torres II, Valentin G, Candelaria AG. Spherulocytes in the echinoderm Holothuria glaberrima and their involvement in intestinal regeneration. Dev. Dyn. 235: 3259-3267, 2006.
Ghosh J, Buckley KM, Nair SV, Raftos DA, Miller C, Majeske AJ, *et al*. Sp185/333: a novel family of genes and proteins involved in the purple sea urchin immune response. Dev. Comp. Immunol. 34: 235-245, 2010.
Giga Y, Ikai A, Takahashi K. The complete amino acid sequence of echinoidin, a lectin from the coelomic fluid of the sea urchin *Anthocidaris crassispina*. Homologies with mammalian and insect lectins. J. Biol. Chem. 262: 6197-6203, 1987.
Glinski Z, Jarosz J. Immune phenomena in echinoderms. Arch. Immunol. Ther. Exp. (Warsz.) 48: 189-193, 2000.
Gowda NM, Goswami U, Khan MI. Purification and characterization of a T-antigen specific lectin from the coelomic fluid of a marine invertebrate, sea cucumber (*Holothuria scabra*). Fish Shellfish Immunol. 24: 450-458, 2008a.
Gowda NM, Goswami U, Khan MI. T-antigen binding lectin with antibacterial activity from marine invertebrate, sea cucumber (*Holothuria scabra*): possible involvement in differential recognition of bacteria. J. Invertebr. Pathol. 99: 141-145, 2008b.
Gross PS, Al-Sharif WZ, Clow LA, Smith LC. Echinoderm immunity and the evolution of the complement system. Dev. Comp. Immunol. 23: 429-442, 1999.
Gross PS, Clow LA, Smith LC. SpC3, the complement homologue from the purple sea urchin, *Strongylocentrotus purpuratus*, is expressed in two subpopulations of the phagocytic coelomocytes. Immunogenetics 51: 1034-1044, 2000.
Handberg-Thorsager M, Fernandez E, Salo E. Stem cells and regeneration in planarians. Front. Biosci. 13: 6374-6394, 2008.
Haug T, Kjuul AK, Styrvold OB, Sandsdalen E, Olsen OM, Stensvag K. Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea), and *Asterias rubens* (Asteroidea). J. Invertebr. Pathol. 81: 94-102, 2002.
Hibino T, Loza-Coll M, Messier C, Majeske AJ, Cohen AH, Terwilliger DP, *et a*l. The immune gene repertoire encoded in the purple sea urchin genome. Dev. Biol. 300: 349-365, 2006.
Hillier BJ, Vacquier VD. Amassin, an olfactomedin protein, mediates the massive intercellular adhesion of sea urchin coelomocytes. J. Cell. Biol. 160: 597-604, 2003.
Holm K, Dupont S, Skold H, Stenius A, Thorndyke M, Hernroth B. Induced cell proliferation in putative haematopoietic tissues of the sea star, *Asterias rubens* (L.). J. Exp. Biol. 211: 2551-2558, 2008.
Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. Nat. Rev. Immunol. 3: 371-382, 2003.
Jans D, Dubois P, Jangoux M. Defensive mechanisms of holothuroids (Echinodermata): Formation, role and fate of intracoelomic brown bodies in the sea cucumber *Holothuria tubulosa*. Cell Tissue Res. 283: 99-106, 1996.
Johnson PT. The coelomic elements of sea urchins (*Strongylocentrotus*). 3. *In vitro* reaction to bacteria. J. Invertebr. Pathol. 13: 42-62, 1969.
Kamiya H, Muramoto K, Goto, R, Sakai M. Lectins in the hemolymph of a starfish, *Asterina pectinifera*: purification and characterization. Dev. Comp. Immunol. 16: 243-250, 1992.
Kanungo K. In vitro studies on the effect of cell-free coelomic fluid calcium, and/or magnesium on clumping of coelomocytes of the sea star *Asteria forbesi*. Biol. Bull. 163: 438-452, 1982.
Kaplan G, Bertheussen K. The morphology of echinoid phagocytes and mouse peritoneal macrophages during phagocytosis *in vitro*. Scand. J. Immunol. 6: 1289-1296, 1977.
Kelly MS. Echinoderms: their culture and bioactive compounds. Prog. Mol. Subcell. Biol. 39: 139-165, 2005.
Kerlin RL, Cebra JJ, Weinstein PD, Prendergast RA. Sea star factor blocks development of T-dependent antibody secreting clones by preventing lymphokine secretion. Cell. Immunol. 156: 62-76, 1994.
Kindred JE. The cellular elements in the perivisceral fluid of echinoderms. Biol. Bull. 46: 228-251, 1924.

Leonard LA, Strandberg JD, Winkelstein JA. Complement-like activity in the sea star, *Asterias forbesi*. Dev. Comp. Immunol. 14: 19-30, 1990.

Li Q, Li Y, Li H, Wang Y, Xu D. Production, characterization and application of monoclonal antibody to spherulocytes: A subpopulation of coelomocytes of *Apostichopus japonicus*. Fish Shellfish Immunol. 29: 832-838, 2010.

Lin W, Grant S, Beck G. Generation of monoclonal antibodies to coelomocytes of the purple sea urchin *Arbacia punctulata*: characterization and phenotyping. Dev. Comp. Immunol. 31: 465-475, 2007.

Lin W, Zhang H, Beck G. Phylogeny of natural cytotoxicity: cytotoxic activity of coelomocytes of the purple sea urchin, *Arbacia punctulata*. J. Exp. Zool. 290: 741-750, 2001.

Matranga V, Pinsino A, Celi M, Natoli A, Bonaventura R, Schroder HC, *et al.* Monitoring chemical and physical stress using sea urchin immune cells. Prog. Mol. Subcell. Biol. 39: 85-110, 2005.

Matsui T, Ozeki Y, Suzuki M, Hino A, Titani K. Purification and characterization of two $Ca(2^+)$-dependent lectins from coelomic plasma of sea cucumber, *Stichopus japonicus*. J. Biochem. 116: 1127-1133, 1994.

Metchnikoff I. Lectures on the comparative pathology of inflammation delivered at the Pasteur Institute in 1891, Dover, New York, 1891.

Mogilenko DA, Kudriavtsev IV, Orlov SV, Kharazova AD, Polevshchikov AV. [Expression of the starfish complement component C3 gene homologue under the influence of bacterial lipopolysaccharide]. Mol. Biol. (Mosk.) 44: 74-84, 2010.

Nair SV, Del Valle H, Gross PS, Terwilliger DP, Smith LC. Macroarray analysis of coelomocyte gene expression in response to LPS in the sea urchin. Identification of unexpected immune diversity in an invertebrate. Physiol. Genomics 22: 33-47, 2005.

Noll H, Alcedo J, Daube M, Frei E, Schiltz E, Hunt J, *et al*. The toposome, essential for sea urchin cell adhesion and development, is a modified iron-less calcium-binding transferrin. Dev. Biol. 310: 54-70, 2007.

Ozeki Y, Matsui T, Titani K. Cell adhesive activity of two animal lectins through different recognition mechanisms. FEBS Lett. 289: 145-147, 1991.

Pagliara P, Canicatti C. Isolation of cytolytic granules from sea urchin amoebocytes. Eur. J. Cell Biol. 60: 179-184, 1993.

Parrinello N, Rindone D, Canicatti C. Naturally occurring hemolysins in the coelomic fluid of *Holothuria polii delle chiaie* (Echinodermata). Dev. Comp. Immunol. 3: 45-54, 1979.

Penn PE. Wound healing in the tropical intertidal asteroid, *Napanthia belcheri* (Perrier). Amer. Zool. 19: 1006, 1979.

Pinsino A, Thorndyke MC, Matranga V. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. Cell Stress Chaperones 12: 331-341, 2007.

Pinsino A, Thorndyke MC, Matranga V. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. Cell Stress Chaperones 12: 331-341, 2008.

Prendergast RA, Liu SH. Isolation and characterization of sea star factor. Scand. J. Immunol. 5: 873-880, 1976.

Prendergast RA, Suzuki M. Invertebrate protein simulating mediators of delayed hypersensitivity. Nature 227: 277-279, 1970.

Ramirez-Gomez, F, Aponte-Rivera F, Mendez-Castaner L, Garcia-Arraras JE. Changes in holothurian coelomocyte populations following immune stimulation with different molecular patterns. Fish Shellfish Immunol. 29: 175-185, 2010.

Ramirez-Gomez F, Ortiz-Pineda PA, Rivera-Cardona G, Garcia-Arraras JE. LPS-induced genes in intestinal tissue of the sea cucumber *Holothuria glaberrima*. PLoS ONE 4: e6178, 2009.

Ramirez-Gomez F, Ortiz-Pineda PA, Rojas-Cartagena C, Suarez-Castillo EC, Garcia-Arraras JE. Immune-related genes associated with intestinal tissue in the sea cucumber *Holothuria glaberrima*. Immunogenetics 60: 57-71, 2008.

Rast JP, Pancer Z, Davidson EH. New approaches towards an understanding of deuterostome immunity. Curr. Top. Microbiol. Immunol. 248: 3-16, 2000.

Rast JP, Smith LC, Loza-Coll M, Hibino T, Litman GW. Genomic insights into the immune system of the sea urchin. Science 314: 952-956, 2006.

Ryoyama K. Studies on the biological properties of coelomic fluid of sea urchin. I. Naturally occurring hemolysin in sea urchin. Biochim. Biophys. Acta 320: 157-165, 1973.

San Miguel-Ruiz, J. E. and Garcia-Arraras, J. E. Common cellular events occur during wound healing and organ regeneration in the sea cucumber Holothuria glaberrima. BMC Dev. Biol. 7: 115, 2007.

Santiago P, Roig-Lopez JL, Santiago C, Garcia-Arraras JE. Serum amyloid A protein in an echinoderm: its primary structure and expression during intestinal regeneration in the sea cucumber *Holothuria glaberrima*. J. Exp. Zool. 288: 335-344, 2000.

Santiago-Cardona PG, Berrios CA, Ramirez F, Garcia-Arraras JE. Lipopolysaccharides induce intestinal serum amyloid A expression in the sea cucumber *Holothuria glaberrima*. Dev. Comp. Immunol. 27: 105-110, 2003.

Scaturro G, Zito F, Matranga V. The oligomeric integrity of toposome is essential for its morphogenetic function. Cell Biol. Int. 22: 321-326, 1998.

Service M, Wardlaw AC. Echinochrome-a as a bactericidal substance in the coelomic fluid of *Echinus esculentus* (l). Comp. Biochem. Physiol. 79B: 161-165, 1984.

Smith LC. The complement system in sea urchins. In: Beck G, M. Sugumaran M, Cooper C (eds), Phylogenetic perspectives on the vertebrate immune systems: Adv. Exp. Med. Biol, pp 363-

372, Kluwer Academic/Plenum Publishing Co., New York, 2001.

Smith LC, Britten RJ, Davidson EH. SpCoel1: a sea urchin profilin gene expressed specifically in coelomocytes in response to injury. Mol. Biol. Cell 3: 403-414, 1992.

Smith LC, Britten RJ, Davidson EH. Lipopolysaccharide activates the sea urchin immune system. Dev. Comp. Immunol. 19: 217-224, 1995.

Smith LC, Chang L, Britten RJ, Davidson EH. Sea urchin genes expressed in activated coelomocytes are identified by expressed sequence tags. Complement homologues and other putative immune response genes suggest immune system homology within the deuterostomes. J. Immunol. 156: 593-602, 1996.

Smith LC, Davidson EH. The echinoid immune system and the phylogenetic occurrence of immune mechanisms in deuterostomes. Immunol. Today 13: 356-362, 1992.

Smith LC, Harrington MG, Britten RJ, Davidson EH. The sea urchin profilin gene is specifically expressed in mesenchyme cells during gastrulation. Dev. Biol. 164: 463-474, 1994.

Smith LC, Rast JP, Brockton V, Terwilliger DP, Nair SV, Buckley K, *et al*. The sea urchin immune system. Inv. Surv. J. 3: 25-39, 2006.

Smith LC, Shih CS, Dachenhausen SG. Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. J. Immunol. 161: 6784-6793, 1998.

Smith VJ. Invertebrate blood cells. In: Ratcliffe NA, Riowley AF (eds), The echinoderms, Academic Press, New York, pp 513-562, 1981.

Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC, *et al*. The genome of the sea urchin *Strongylocentrotus purpuratus*. Science 314: 941-952, 2006.

Stabili L, Pagliara P, Metrangolo M, Canicatti C. Comparative aspects of Echinoidea cytolysins: the cytolytic activity of Spherechinus granularis (Echinoidea) coelomic fluid. Comp. Biochem. Physiol. 101A: 553-556, 1992.

Sun Y, Jin L, Wang T, Xue J, Liu G, Li X, *et al*. Polysaccharides from *Astragalus membranaceus* promote phagocytosis and superoxide anion (O^{2-}) production by coelomocytes from sea cucumber *Apostichopus japonicus in vitro*. Comp. Biochem. Physiol. 147C: 293-298, 2008.

Unuma T, Ikeda K, Yamano K, Moriyama A, Ohta H. Zinc-binding property of the major yolk protein in the sea urchin - implications of its role as a zinc transporter for gametogenesis. FEBS J. 274: 4985-4998, 2007.

Unuma T, Konishi K, Kiyomoto M, Matranga V, Yamano K, Ohta H, *et al*. The major yolk protein is synthesized in the digestive tract and secreted into the body cavities in sea urchin larvae. Mol. Reprod. Dev. 76: 142-150, 2009.

Watanabe H, Hoang VT, Mattner R, Holstein TW. Immortality and the base of multicellular life: Lessons from cnidarian stem cells. Semin. Cell Dev. Biol. 20: 1114-1125, 2009.

Xing K, Yang HS, Chen MY. Morphological and ultrastructural characterization of the coelomocytes in *Apostichopus japonicus*. Aquat. Biol. 2: 85-92, 2008.

Yang A, Zhou Z, Dong Y, Jian B, Wang X, Chen Z, *et al*. Expression of immune-related genes in embryos and larvae of sea cucumber *Apostichopus japonicus*. Fish Shellfish Immunol. 29: 839-845, 2010.

Yoshida S, Shimada Y, Kondoh D, Kouzuma Y, Ghosh AK, Jacobs-Lorena M., *et al*. Hemolytic C-type lectin CEL-III from sea cucumber expressed in transgenic mosquitoes impairs malaria parasite development. PLoS Pathog 3: e192, 2007.

Yui MA, Bayne CJ. Echinoderm immunology: bacterial clearance by the sea urchin *Strongylocentrotur purpuratus*. Biol. Bull. 165: 473-486, 1983.