CHAPTER II

LITERATURE REVIEW

1. Sponge Biology

Sponges (phylum Porifera: Latin *porus* = "pore", *ferre* = "to bear") contain estimated 15,000 species in the three taxonomic classes: Calcarea (calcareous sponges), Hexactinellida (glass sponges), and Demospongiae (demosponges) (Hooper, 2000; Thiel *et al.*, 2006). Less than 2% of all sponges species are found in fresh water, the remaining 98% of the species being marine (Belarbi *et al.*, 2003; Pechenik, 2005). The growth habitats of sponges encompass various shapes (e.g. encrusting, rope, ball, tube, barrel, vase), colors (e.g. red, orange, yellow, green, blue, purple, brown, black, white), and sizes (a few millimeters to nearly two meters), and can be quite variable in response to environmental conditions (Hentschel *et al.*, 2006; Karleskint *et al.*, 2006; Wulff, 2006).

1.1. Sponge structure and function

Sponges are the structurally simplest multicellular animals, lacking true tissues and organs. All sponges are asymmetric, sessile animals (meaning they are permanently attached to a variety of substrates, such as bottom sediments, sand, rocks, coral reefs) (Hooper, 2000; Ruppert *et al.*, 2004; Castro and Huber, 2005; Karleskint *et al.*, 2006).

The structure of a sponge's body is unique in that it is built around a system of water canals. Numerous tiny pores, or **ostia** (singular, **ostium**), on the outer surface allow large amounts of water to enter and circulate through a series of canals. The water is a source of nutrients and oxygen, and it carries away the animal's wastes (Ruppert *et al.*, 2004; Castro and Huber, 2005; Karleskint *et al.*, 2006). The architecture of sponges is best understood by examining the simplest kind of sponge. The outer surface of a sponge's body is covered with flat cells called **pinacocytes** and occasional tube-like pore cells, or **porocytes (pore cells)**, through which a microscopic canal allows water to enter as shown in Figure 2.1 (Castro and Huber, 2005).

Choanocytes (collar cells, or funnel cells, or flagellated cells) have a flagellum. The movement of the **choanocytes'** flagella provides the force that moves water through the

sponge's body. In addition, they capture small food particles and incoming sperms for fertilization (Pechenik, 2005; Karleskint *et al.*, 2006). Water, a source of nutrients and oxygen, enters through the **ostia** or **pore cells** and eventually flows through the sponge's body in a system of flagellated canals into a spacious cavity called the **spongocoel** (SPUN-joh-seel) or **atrium**. Water then exits the spongocoel through a large exhalant opening called the **osculum** (plural, **oscula**, or **oscules**), which is situated at the upper, free end of the body. Many species have several **spongocoel** and **oscula**. Most marine sponges show a more complex arrangement in which the choanocytes are restricted to chambers (called **choanocyte chambers**, or flagellated chambers, or radial canals) connected to the outer pores by a network of canals. The increased complexity is associated with increased size, which demands higher water flow through the sponge and therefore a large surface area of choanocytes (Brusca and Brusca, 2003; Castro and Huber, 2005; Karleskint *et al.*, 2006). As filter feeders, sponges are capable of turning over many thousands of liters of water per day through a canal system (Castro and Huber, 2005; Hentschel *et al.*, 2006). **Mesohyl** (*meso* = middle; *hyl* = stuff, matter), an interepithelial mesenchyme, is rich in intercellular collagen, **archaeocytes**, and inorganic and organic skeletal elements (Young, 2002).

Archaeocytes (wandering cells, or amoebocytes, or amoeboid cells, or progenitor cells) are large, highly motile cells that wander throughout the **mesohyl** by typical cytoplasmic streaming which involves the formations of pseudopodia as in amoeboid protozoans. They play a major role in digestion and transport food and other materials within a sponge's body. These cells possess a variety of digestion food enzymes (e.g. acid phosphatase, protease, amylase, lipase) and can accept phagocytized materials from the **choanocytes**. **Arhaeocytes** perform a number of essential functions within sponges and can develop into more specialized cell types, such as **scleroblasts** (secrete the mineral skeleton), **lophocytes** and **spongioblasts** or **spongocytes** (secrete the spongin skeleton), **collencytes** and myocytes (contract the sponge), **neuroid cells** (react to stimuli) and **granular cells** (probably produce hormones or possibly secondary metabolites). As cells of maximum totipotency, **archaeocytes** can play an important role in repair and regeneration of sponges (Brusca and Brusca, 2003; Ruppert *et al.*, 2004; Pechenik, 2005; Castro and Huber, 2005; Karleskint *et al.*, 2006).
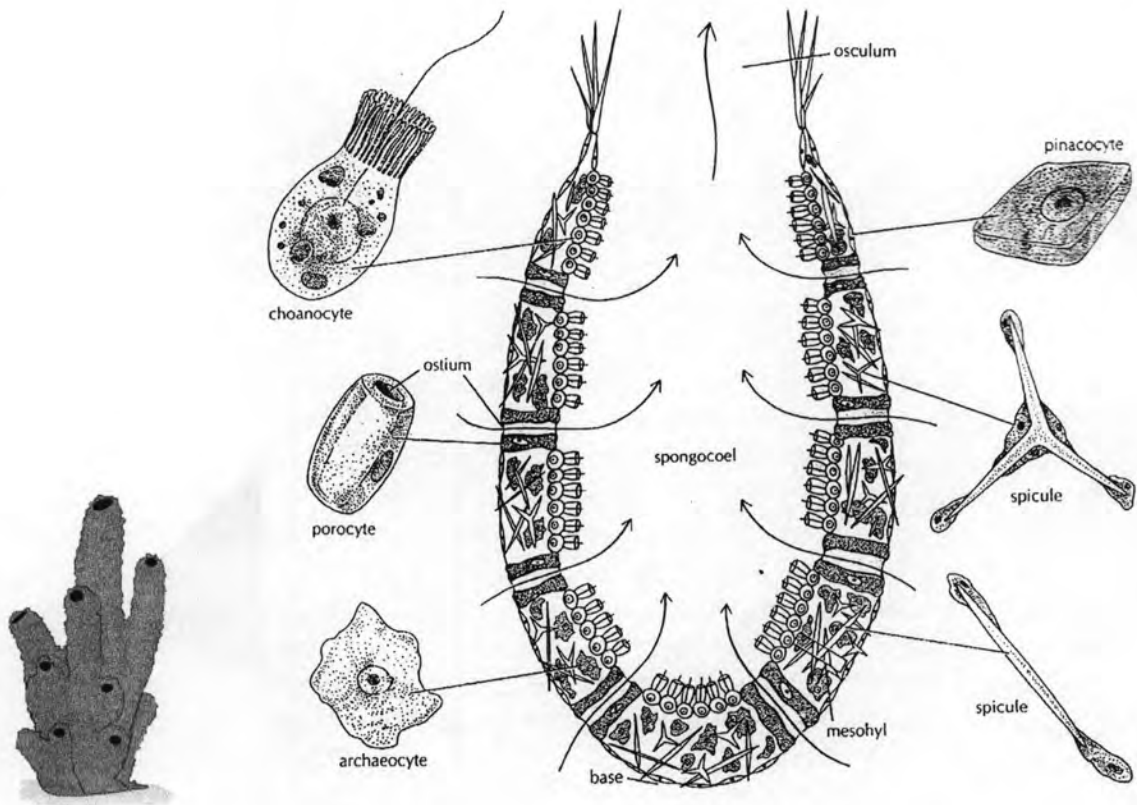
Figure 2.1. Diagrammatic representation of a simple (**asconoid**) sponge, illustrating its various cellular and structural components (Pechenik, 2005).

In addition, archaeocytes can become a specialized to secrete the supporting elements located in the **mesohyl** layer. These supporting elements may be calcareous or siliceous **spicules**, or they may be fibers composed of a collagenous protein called **spongin**. The cells secreting spicules are termed sclerocytes as shown in Figure 2.2 (Castro *et al.*, 2005; Pechenik, 2005). Figure 2.3 shows diversity of spicule morphologies from sponges that give support to a sponge's body.
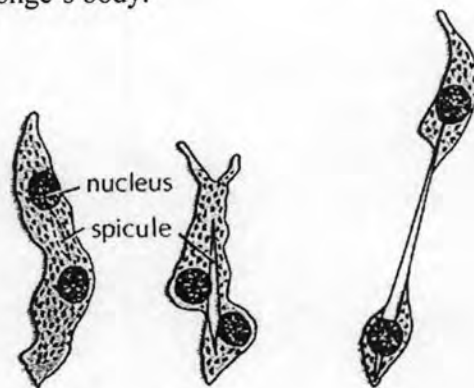

Figure 2.2. The production of a sponge spicule by a binucleate sclerocyte (Pechenik, 2005).
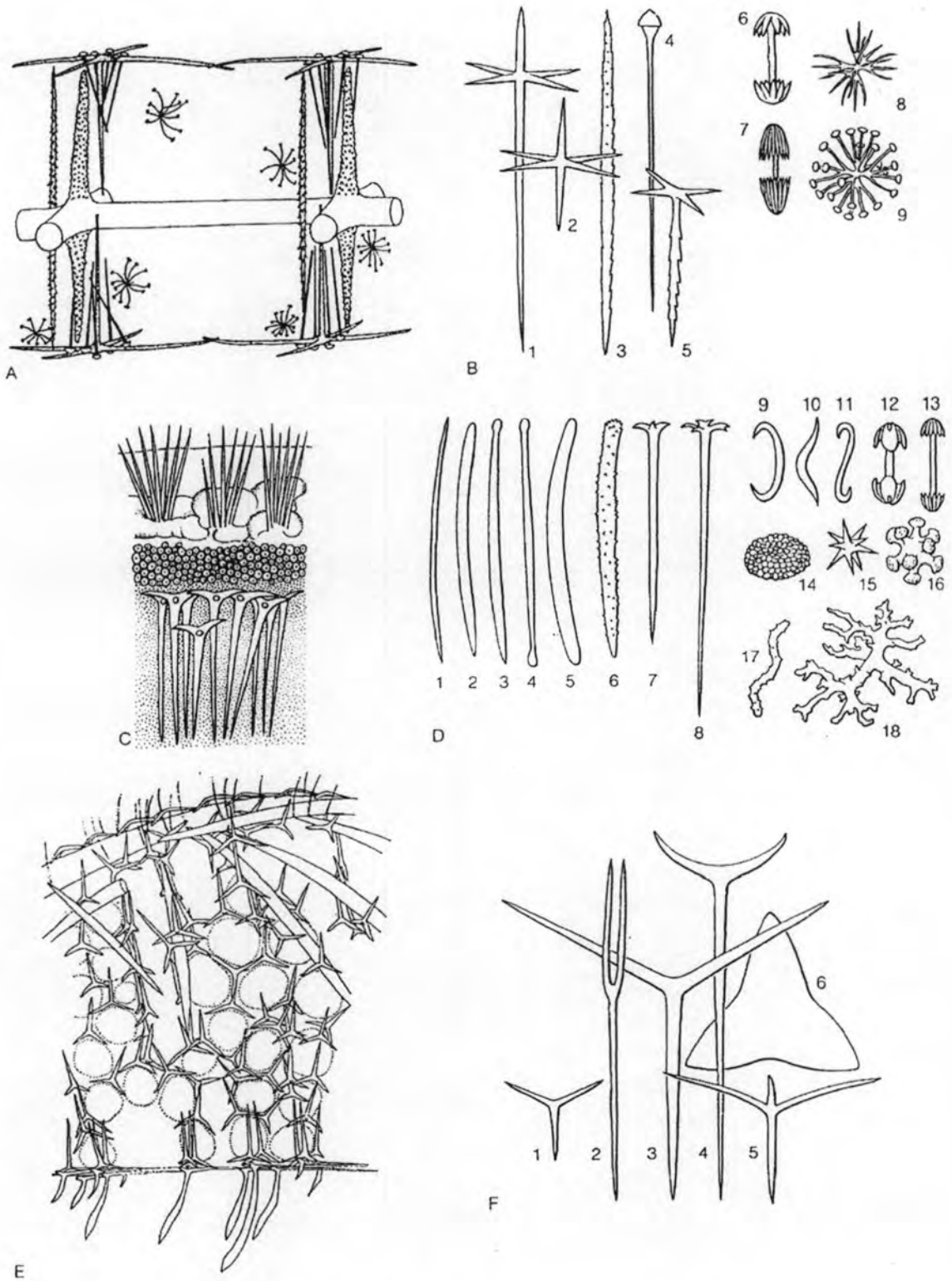
Figure 2.3. Spicular skeletons and spicules from sponges (Ruppert *et al.,* 2004)
A) *Farrea sollasii* (Hexactinellida); B) Hexactinellid spicules; C) *Geodia* sp.
(Demospongiae); D) Demosponge spicules; E) *Afroceras ensata* (Calcarea); and
F) Calcarean spicules.

Figure 2.4 illustrates sponge body forms including, **asconoid, synconoid**, and **leuconoid** which are limited by water circulation through sponge body. The highest degree of folding takes place in leuconoid sponges. These modifications are accompanied by many chambers lined with collar cells called **choanocyte chambers** or radial canals. Areas occupied by collar cells are shown in black. **Leuconoid** organization is typical of most calcareous sponges and all members of the Demospongiae.
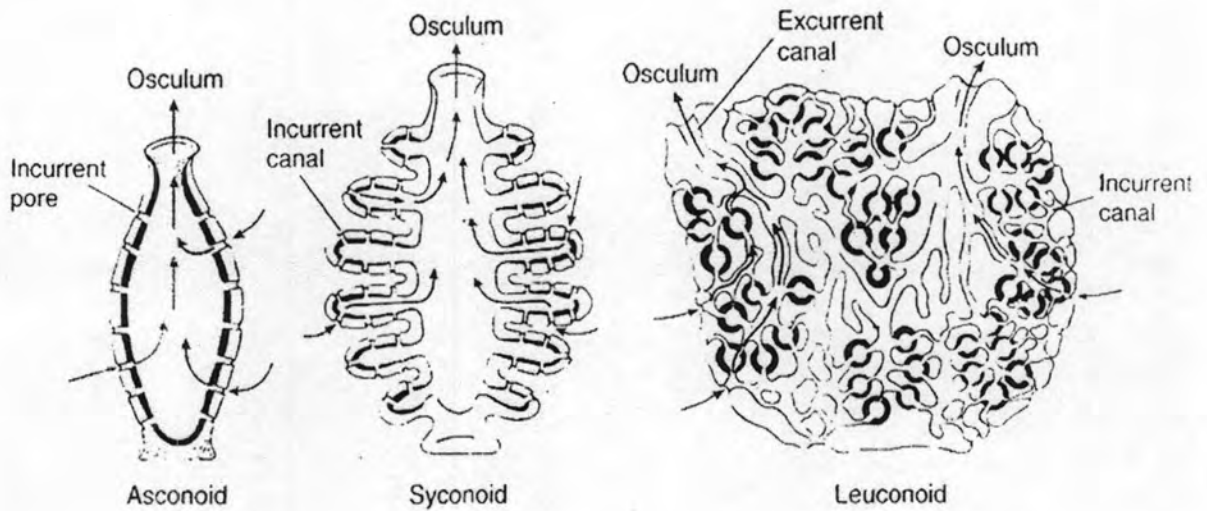
Figure 2.4. Sponge body forms, **asconoid, synconoid**, and **leuconoid**. The arrows represent the direction of water flow (Karleskint *et al.*, 2006).

1.2. Reproduction and development of sponges

Sponges can reproduce both asexually and sexually as shown in Figure 2.5. Sponges reproduce asexually by fragmentation, the production of gemmules, reduction bodies, and budding. The sexual reproduction is through the production of eggs and sperms (Brusca and Brusca, 2003; Ruppert *et al.*, 2004; Castro and Huber, 2005; Pechenik, 2005; Karleskint *et al.*, 2006). In many cases, embryonic development occurs inside the sponge, leading to a larval stage (the free-swimming stage) that leaves the parent for dispersal. Gametes are produced by choanocytes and totipotent archaeocytes; there are no gonads or reproductive ducts. Oocytes, embryos, and larvae usually leave the parent sponge in the outgoing water flow, via excurrent canals, but the larvae of *Tedania ignis* (Poecilosclerida) and *Callyspongia diffusa* (Haplosclerida) leave the parent by creeping through the ectosome (Young, 2002). The embryos of most demosponges develop directly into a solid mass of cells and differentiate to form extensively flagellated **parenchymella larvae** or **parenchymula larvae** as shown in Table 2.1 and Figure 2.6 (Young, 2002; Ruppert *et al.*, 2004; Castro and Huber, 2005).
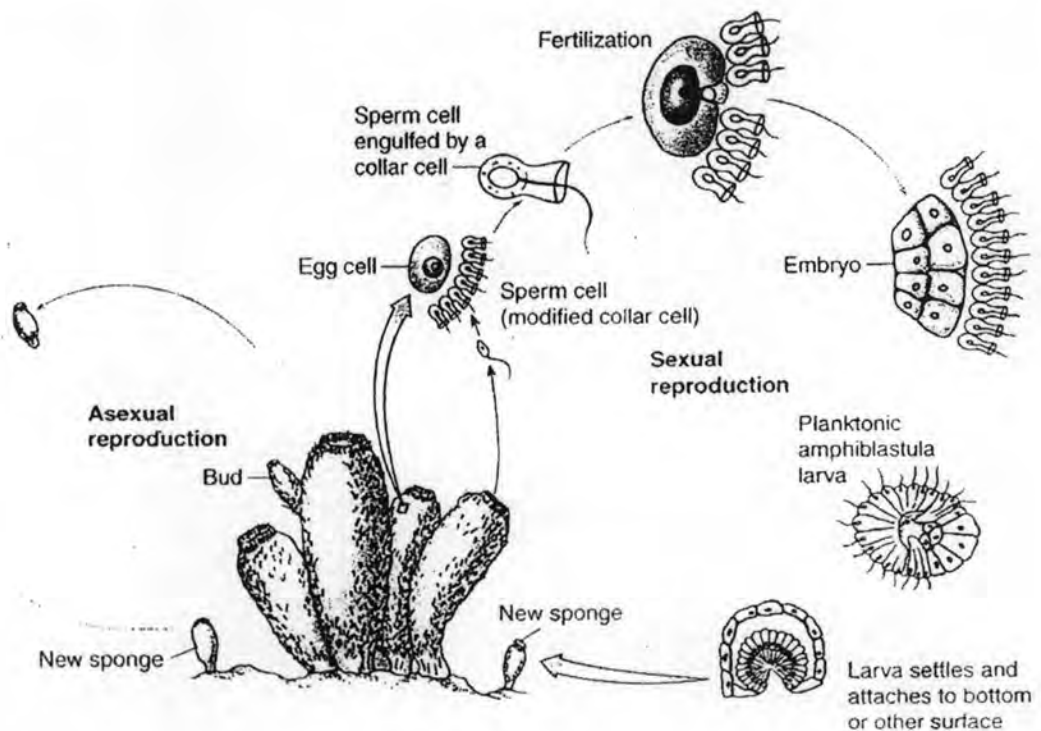

Figure 2.5. Schematic of asexual and sexual reproduction of sponges (class Calcarea) (Karleskint *et al.*, 2006).

Table 2.1. Larval type in the major taxonomic categories of Porifera (Young, 2002).

Taxon	*Laval type*
Subphylum Cellularia	
Class Calcarea	
Subclass Calcinea	
Order Clathrinida	Calciblastula (viviparous)
Order Murrayonida	Calciblastula? (viviparous)
Subclass Calcaronea	
Order Leucosoleniida	Amphiblastula (viviparous)
Order Lithonida	Amphiblastula (viviparous)
Subclass Calcinea	
Class Demospongiae	
Subclass Homoscleromorpha	
Order Homosclerophorida	Cinctoblastula (viviparous)
Subclass Tetractinomorpha	
Order Astrophorida	? (oviparous)
Alectona and *Thoosa*	Hoplitomella
Order Spirophorida	Direct development
Superorder Clavaxinellida	
Order Chondrosida	Clavablastula (oviparous)
Order Hadromerida	Clavablastula (oviparous)
Order Axinellida	Clavablastula (oviparous)
Order Agelasida	Clavablastula? (oviparous)
Subclass Ceractinomorpha	
Order Verticillitida	Parenchymella-like? (viviparous)
Order Halichondrida	Parenchymella (viviparous)
Order Poecilosclerida	Parenchymella (viviparous)
Order Haplosclerida	Parenchymella (viviparous)
Order Petrosiida	Parenchymella-like? (oviparous)
Order Dendroceratida	Parenchymella-like (viviparous)
Order Dictyoceratida	Parenchymella-like (viviparous)
Order Verongida	? (oviparous)
Order Halisarcida	Dispherula (viviparous)

?, taxon in which the larva remains elusive.
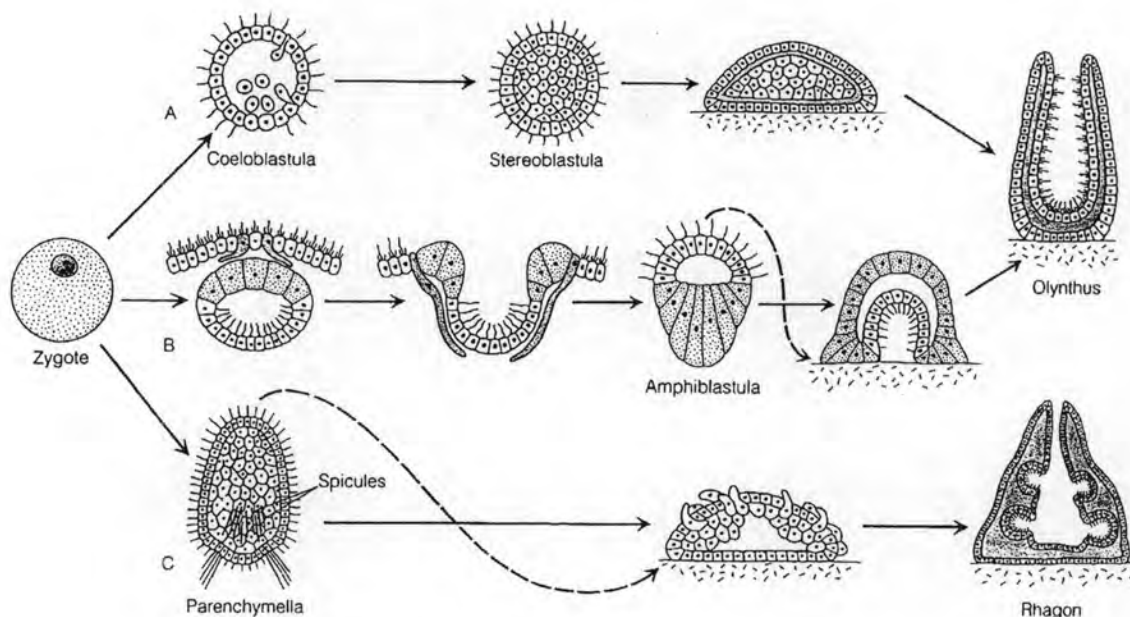
Figure 2.6. Porifera sexual reproduction: larval development and metamorphosis
A) *Clathrina* (Calcarea: Calcinia); B) *Sycon* (Calcarea: Caalcaronea); and C) *Haliclona*
(Demospongiae) (Ruppert *et al.*, 2004).

## 2. Taxonomical characteristic of the blue sponge *Xestospongia* sp.

The sponge genus *Xestospongia* belongs to phylum Porifera, subphylum
Cellularia, class Demospongiae, order Haplosclerida, and family Petrosiidae. According to
"Sponguide" (Hooper, 2000), the characters of genus *Xestospongia* de Laubenfels, 1932
(syn. *Neopetrosia* de Laubenfels, 1949; *Prianos* Gray, 1867) was described as following:

"Ectosomal skeleton indistinct; choanosomal skeleton confused isotropic
reticulation of multispicular tracts, generally lacking spongin and sometimes with single
spicules scattered throughout mesohyl between major spicule tracts, stony texture; oxeote
spicules in one size category only".

A bluish sponge sample was collected from Sichang Island, Cholburi Province,
Thailand. The sponge was identified by Dr. John N. A. Hooper as *Xestospongia* sp. #2133
(family Petrosiidae). The voucher specimens have been deposited at the Queensland
Museum, South Brisbane, Australia (sample code QMG306998) and at the Department of
Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok,
Thailand. The sponge *Xestospongia* sp. exhibits thick, encrusted, lobate growth. Its texture

is hard, brittle, and easily crumbled. It is light bluish-gray when alive and pinkish in ethanol. Oscules are numerous and of moderate size and are found, on apexes of surface lobes, with slightly raised lips. The surface has prominent bulbous surface lobes with some that are nearly digitate in size. The surface is translucent, membranous, optically smooth, macroscopically bulbous, and microscopically even, with choanosomal drainage canals that are slightly visible below the surface. Ectosomal skeleton membranes have no specialized speculation or structure. Choanosomal skeleton with isotropic reticulation of paucispicular tracts of to moderately sized subdermal cavities are observed throughout skeleton. There are no visible fibers, and only small amounts of collagen in the mesohyl. The oxeas are robust, straight or slightly curved at center, sharply pointed, hastate (190-210 x 12-18 µm). This species is probably a new species as shown in Figure 2.7 (Hooper, 2000).
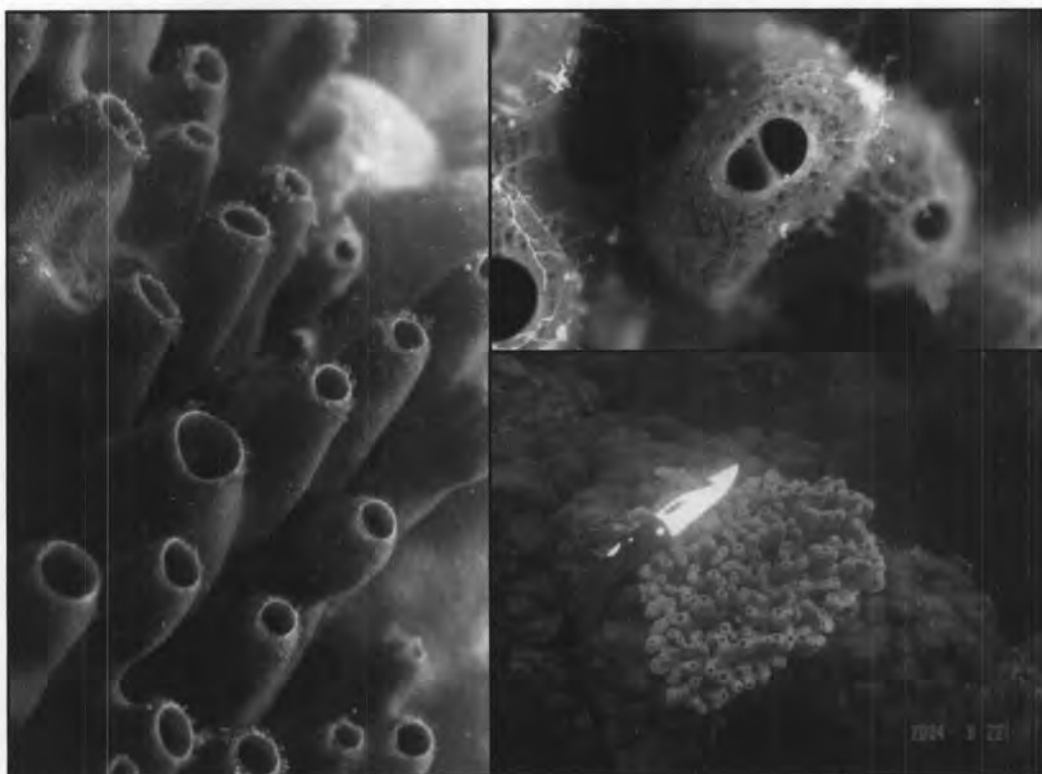
Figure 2.7. Pictures of the blue sponge *Xestospongia* sp.
(photographs by Khanit Suwanborirux and Udomsak Darumas).

3. Chemistry of renieramycins

Renieramycins from the Thai blue sponge *Xestospongia* sp. are bistetrahydroisoquinoline alkaloids, of which the chemical structures are closely related to other tetrahydroisoquinoline natural products from different sources such as marine organisms and actinomycetes (Table 2.2). These structures are classified into two groups, the bisquinones and the quinone-hydroquinones, depending on whether ring E is quinone or hydroquinone (Amnuoypol *et al.*, 2004; Saito *et al.*, 2004). The structures of renieramycins and other tetrahydroisoquinoline marine natural products are shown in Figures 2.8 and 2.9, respectively.

The Thai blue sponge *Xestospongia* sp. was pretreated with potassium cyanide to increase the mass-production of renieramycin M, a major renieramycin, and four minor compounds; renieramycins O **(2o)**, Q **(2q)**, R **(2r)** and S **(2s)**. Addition of potassium cyanide converted renieramycin E **[1e]** into renieramycin M **[1m]**. This strategy has provided more stable renieramycins from marine sources for further chemical and biological investigations (Suwanborirux *et al.*, 2003; Amnuoypol *et al.*, 2004).

He and Faulkner (1989) isolated renieramycins E **[1e]** and F **[1f]** from the marine sponge *Reniera* sp. They suggested that unstable renieramycin E **[1e]** undergoes oxidative cleavage to give the "monomeric" isoquinolines; mimosamycin and renierone. Several other isoquinoline quinone alkaloids have been previously reported from marine sponges as shown in Figure 2.10 (Frincke and Faulkner, 1982; He and Faulkner, 1989; Edrada, *et al.*, 1996; Petit, *et al.*, 2000; Rashid, *et al.*, 2001; Saito, *et al.*, 2004; Sung, *et al.*, 2006).

Table 2.2. Sources of bis-tetrahydroisoquinoline alkaloids.

No.	Compounds	Sources	Origin	Biological activities	References
1	**Renieramycins:** Renieramycins A-P	**Sponges:** *Reniera* sp. *Haliclona* sp. *Xestospongia* sp. *Cribochalina* sp. *Neopetrosia* sp. **Nudibranch mollusc:** *Jorunna funebris*	Mexico Indian ocean Fijian Thailand Australian Japan Thailand	Antimicrobial; Antileishmania; Antitumor	Frincke and Faulkner, 1982; He and Faulkner, 1989; Parameswaran *et al.*, 1998; Pettit *et al.*, 2000; Suwanborirux *et al.*, 2003; Saito *et al.*, 2004, 2005; Amnuoypol *et al.*, 2004; Nakao *et al.*, 2004
2	**Saframycins**	**Bacteria:** *Streptomyces lavendulae* No. 314	-	Antibacterial; Antitumor	Arai *et al.*, 1977, 1980a; Lown *et al.*, 1981; Yazawa *et al.*, 1986
3	**Jorumycins** and **Jorunamycins**	**Nudibranch mollusc:** *Jorunna funebris*	Thailand Pacific Ocean	Antitumor	Fontana *et al.*, 2000; Saito *et al.*, 2004
4	**Ecteinascidins**	**Tunicates:** *Ecteinascidia turbinata* *Ecteinascidia thurstoni*	Caribbean Mediterranean Thailand	Antibacterial; Antitumor	Rinehart *et al.*, 1990b; Wright *et al.*, 1990; Sakai *et al.*, 1992,1996; Manzanares *et al.*, 2001; Suwanborirux *et al.*, 2002; Kijjoa and Sawangwong, 2004; Fayette *et al.*, 2005; Mayer and Gustafson, 2006
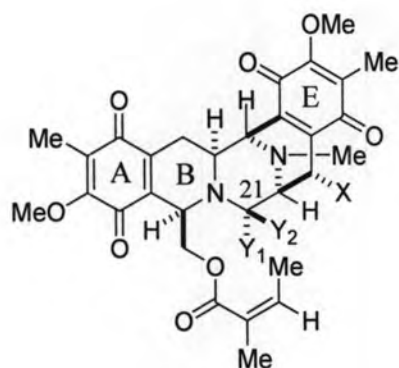
renieramycins

A [1a]: X = OH, Y$_1$ = Y$_2$ = H

B [1b]: X = OC$_2$H$_5$, Y$_1$ = Y$_2$ = H

C [1c]: X = OH, Y$_1$, Y$_2$ = O

D [1d]: X = OC$_2$H$_5$, Y$_1$, Y$_2$ = O

E [1e]: X = H, Y$_1$ = OH, Y$_2$ = H

F [1f]: X = OMe, Y$_1$ = OH, Y$_2$ = H

G [1g]: X = H, Y$_1$, Y$_2$ = O

J [1j]: X = Y$_2$ = O, Y$_1$ = CH$_2$COCH$_3$

K [1k]: X = OCH$_3$, Y$_1$ = CH$_2$COCH$_3$, Y$_2$ = H

M [1m]: X = Y$_2$ = H, Y$_1$ = CN

O [1o]: X = OH, Y$_1$ = CN, Y$_2$ = H

R [1r]: X = OMe, Y$_1$ = CN, Y$_2$ = H
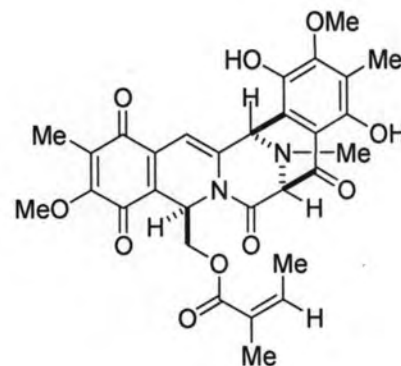
renieramycin H [1h]

(= cribrostatin 4)
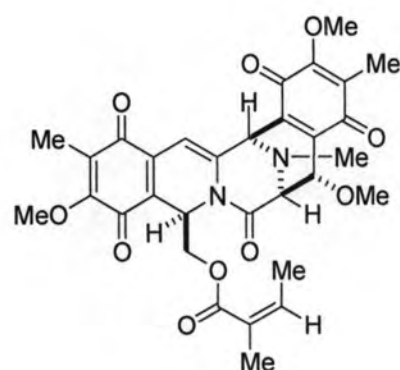
renieramycin I [1i]

Figure 2.8. The chemical structures of renieramycins from marine sponges.
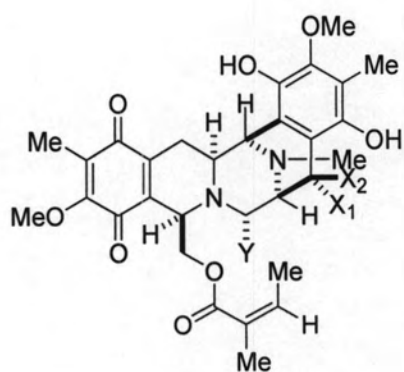
renieramycins L [1l]: X_1, X_2 = O, Y = CH$_2$COCH$_3$

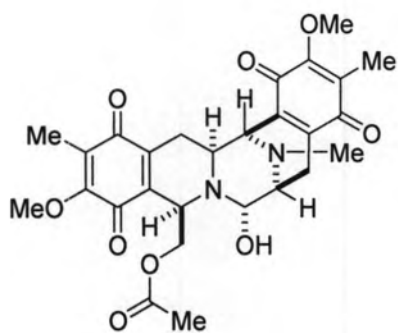P [1p]: X_1 = OH, X_2 = OH, Y = OH

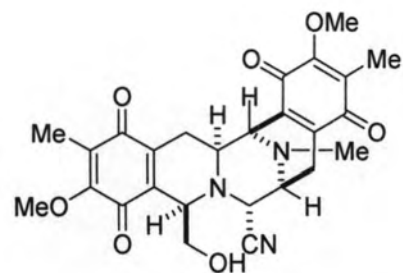Q [1q]: X_1, X_2 = O, Y = CN
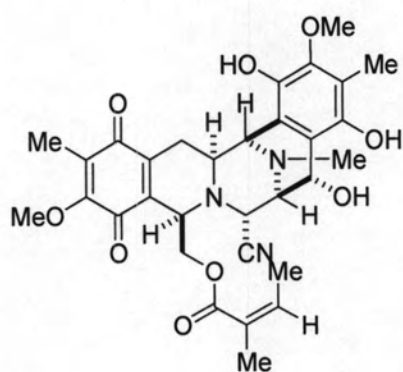
renieramycin N [1n]
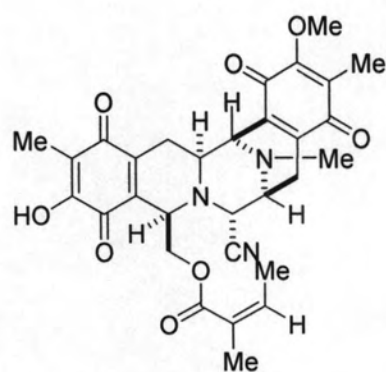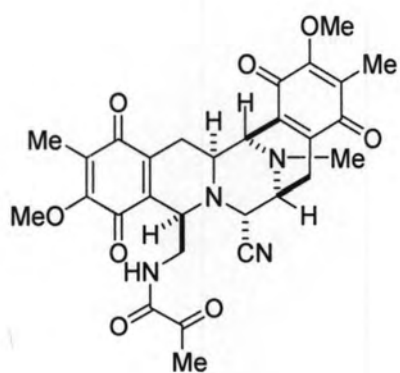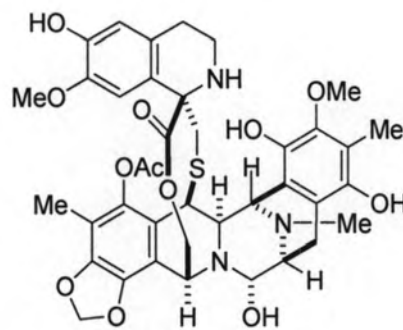

renieramycin S [1s]
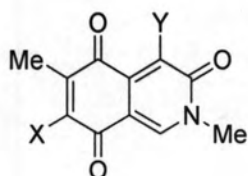
Figure 2.8. (*continued*).

jorumycin

jorunnamycin A

(deangeloylrenieramycin M)

saframycin A

ecteinascidin 743

(ET-743)

Figure 2.9. The chemical structures of bistetrahydroisoquinolines from other natural sources
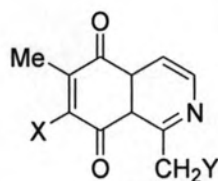
mimosamycin: X = OMe, Y = H

4-amino-mimosamycin: X = OMe, Y = NH_2

7-amino-7-demethoxy mimosamycin: X = NH_2, Y = H

renierone: X = OMe, Y =
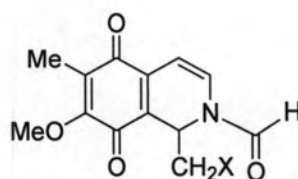
O-demethylrenierone: X = OH, Y =

renierol: X = OMe, Y = OH

renierol acetate: X = OMe, Y = $OCOCH_3$

renierol propionate: X = OMe, Y = $OCOCH_2CH_3$

N-formyl-1,2-dihydrorenierone: X =
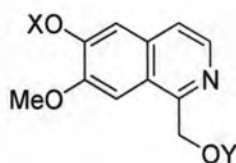
N-formyl-1,2-dihydrorenierol acetate: X = $OCOCH_3$

N-formyl-1,2-dihydrorenierol propionate: X = $OCOCH_2CH_3$

Figure 2.10. The chemical structures of monomeric isoquinolinequinones.

1-hydroxymethyl-7-methoxyisoquinolin-6-ol   X = H, Y = H

1-hydroxymethyl-6,7-dimethoxyisoquinoline   X = Me, Y = H

1-hydroxymethyl-7-methoxyisoquinolin-6-ol acetate X = Ac, Y = Ac

Figure 2.10. (*continued*).

4. Biological Activity

Renieramycins showed cytotoxic activity against three human cell lines, HCT116 (colon carcinoma), QG56 (lung carcinoma), and DU145 (prostate cancer) as summarized in Table 2.3 (Suwanborirux *et al.*, 2003; Amnuoypol *et al.*, 2004; Saito *et al.*, 2004).

Saito *et al.*, 2004 revealed that the leaving group at C-21 position, a cyano or a hydroxyl group, is essential for potent cytotoxic activity under physiological conditions and suggested that the elimination of this functional group resulted in the formation of a reactive iminium species which was responsible for covalent bond formation with the target.

Renieramycins A-D (Frincke and Faulkner, 1982) and renieramycins H and I (Parameswaran *et al.*, 1998) showed moderate antimicrobial activities.

Renieramycin G **[1g]** was isolated from the sponge *Xestospongia caycedoi* by Davidson in 1992 and exhibited cytotoxicity against KB (human epidermoid carcinoma of nasopharynx) and LoVo cell lines (human colon adenocarcinoma cell) with MIC values of 0.5 and 1.0 µg/mL, respectively. These results were surprising that virtually all biologically active members of tetrahydroisoquinoline alkaloids possessed a cabinolamine or cyano function at C-21 and permitted the formation of a potent, electrophilic iminium ion species to form covalent bonds to ds-DNA and possibly, other biomacromolecules at this position (Lane *et al.*, 2006). Furthermore, renieramycin G and analogue, 3-*epi*-renieramycin G

exhibited mild cytotoxic activity against both human colon (HCT116) and human lung (A549) cancer cell lines at GI_{50} in nM as shown in Figure 2.11 and Table 2.4 (Lane *et al.*, 2006).

Two related alkaloids, reinerol and mimosamycin were reported from the marine sponge *Xestospongia calycedoi* and exhibited mild antimicrobial activity against gram positive bacteria (Fontana *et al.*, 2000). In 2001, Rashid *et al.* also described mimosamycin from the cytotoxic fractions of an aqueous extract of the marine sponge *Haliclona* sp., which was the principle cytotoxin with an IC_{50} approximately 10 µg/mL against melanoma and ovarian human tumor cell lines.

Table 2.3. Cytotoxicity of renieramycins.

Compound	IC_{50} (µM)		
	HCT116	QG56	DU145
renieramycin E [1e]	0.00038>	0.001	0.00038>
renieramycin J [1j]	0.73	0.51	0.37
renieramycin M [1m]	0.0079	0.019	NT[a]
renieramycin N [1n]	0.0056	0.011	NT[a]
renieramycin O [1o]	0.028	0.040	NT[a]
renieramycin Q [1q]	0.059	0.071	NT[a]
renieramycin R [1r]	0.023	0.029	NT[a]
renieramycin S [1s]	0.015	0.026	NT[a]

[a] NT : not tested;

HCT116 = human colon carcinoma;

QG56 = human lung carcinoma;

DU145 = human prostate cancer

renieramycin G [1g]   3-*epi*-renieramycin G
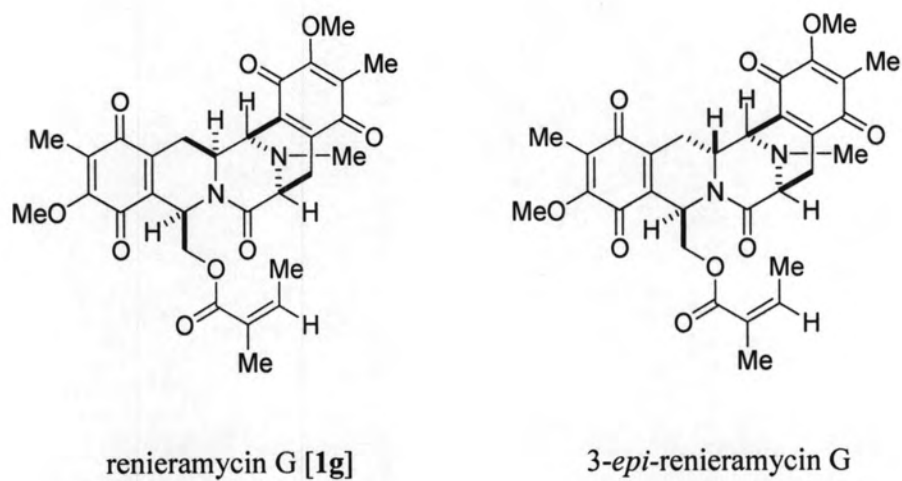
Figure 2.11. The chemical structures of renieramycin G and 3-*epi* analogue.

Table 2.4. The bioactivities of the renieramycin G and analogue, 3-*epi*-renieramycin G.

Compound	GI$_{50}$ (μM)	
	HCT116	A549
renieramycin G [1e]	3.87 ($\pm$0.28)	12.9
3-*epi*-renieramycin G	1.40 ($\pm$0.46)	10.1 ($\pm$1.6)

HCT116 = human colon carcinoma; A549 = human lung cancer

5. Cellular origins of bioactive compounds in sponges

Sponges are a source of a wide diversity of secondary metabolites (Garson, 1994; Faulkner, 2000; Belarbi, *et al.,* 2003; Richelle-Maurer *et al.,* 2001, 2003), presumably used in defense mechanisms against predators, overgrowing space competitors, and invading microorganisms, etc. (Pawlik, 1993; Belarbi, *et al.,* 2003; Richelle-Maurer *et al.,* 2001, 2003). Many sponges are hosts to other organisms. Some of these relationships are mutually beneficial for both sponges and their symbionts (**mutualism**), whereas others seem to favor symbionts without harming sponges (**commentalism**) (Karleskint *et al.,* 2006). Some of these natural products may be useful for novel source-derived drugs. However, sponges do not in general occur in large enough populations to allow harvesting for mass production. The chemical synthesis of many compounds involves generally many steps and provides low yields. Therefore, cell culture may be a promising alternative way for increasing production of bioactive compounds in the sponges (Garson *et al.,* 1998; Richelle-Maurer *et al.,* 2001, 2003). Chemical analyses of the compounds in the sponges are the subjects of an impressive body of literature but biological and cellular studies are rare (Richelle-Maurer *et al.,* 2001, 2003). Several research groups have attempted to clarify the true producers of these natural products in sponges.

In 1998, Garson *et al.,* revealed that cytotoxic alkaloids, haliclonacyclamines A and B, isolated from the tropical marine sponge *Haliclona* sp., were localized within the sponge cells rather than the dinoflagellates.

In 2001, Richelle-Maurer *et al.,* presented the localization of the secondary metabolites, (2R,3R,7Z)-2-aminotetradec-7-ene-1,3-diol from the Caribbean sponge *Haliclona vansoesti* associating with the sponge cells, archaeocytes (2 μg/10^5 archaeocytes, concentration). In 2003, they reported that chemical analyses of the bromopyrrole alkaloids, oroidin and sceptrin, isolated from the Caribbean sponge *Agelas conifera*, were located within the sponge origin, spherulous cells. Figures 2.12 and 2.13 illustrate the cell types of the sponges *Haliclona vansoesti* and *Agelas conifera*, respectively. These sponges also haboured the photosynthetic symbionts (cyanobacteria) and heterotrophic bacteria. In contrary results, HPLC analyses of the sponge *Xestospongia muta* revealed that straight-chain acetylenic compounds were associated with heterotrophic bacteria (Richelle-Maurer *et al.,* 2003). The cellular sources of bioactive compounds in the marine sponges are summarized in Table 2.5.

However, there are some reports, which suggested that the bioactive compounds might be produced by microsymbionts rather than directly by the sponge themselves (Brantley *et al.*, 1995; Van Soest *et al.*, 1998; Belarbi *et al.*, 2003; Richelle-Maurer *et al.*, 2003).
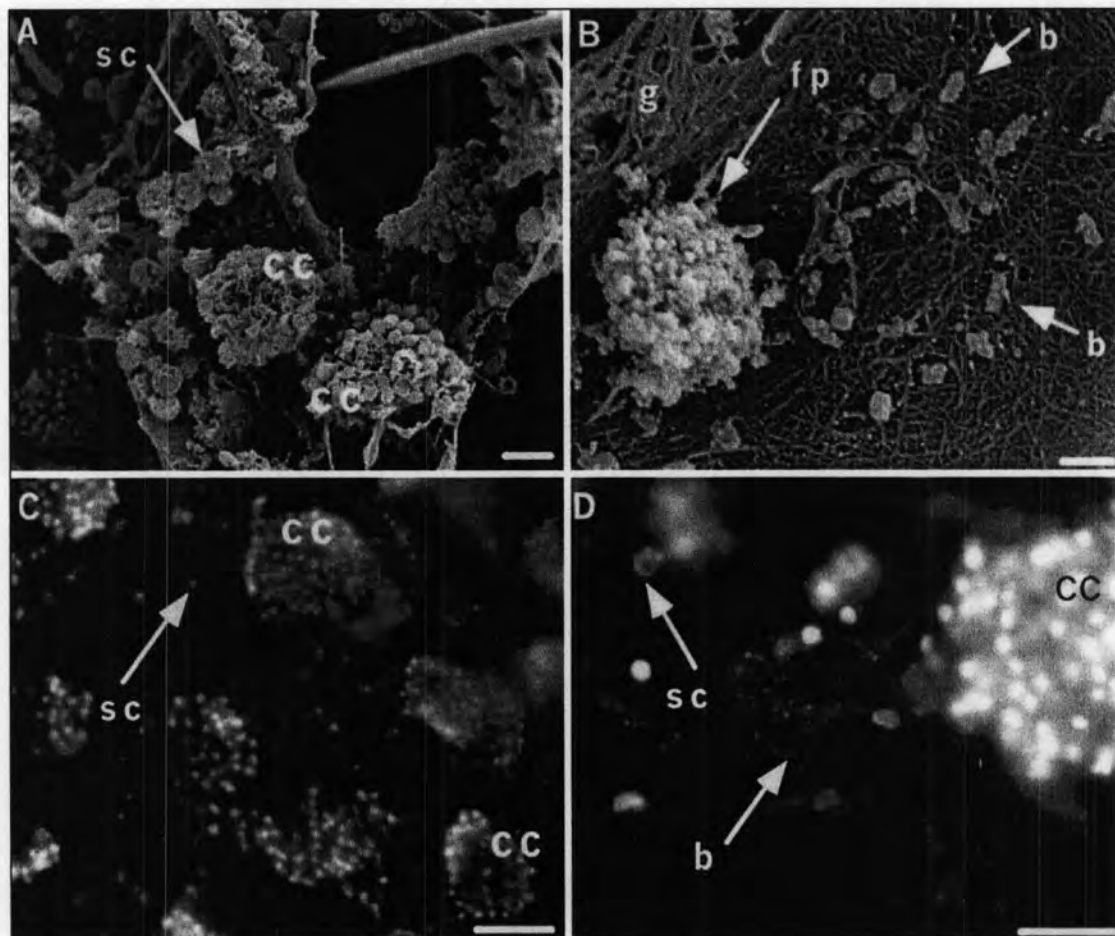

Figure 2.12. Scanning electron micrographs of *Haliclona vansoesti*: **(A)** choanosome with numerous choanocyte chambers (cc) in contact with large sponge cells, probably archaeocytes (sc), scale bar 10 μm; **(B)** mesohyl showing individual bacteria (b) embedded in the glycocalyx matrix (g) of the sponge and possible faecal pellet (fp), scale bar 1 μm. DAPI- and DIOC-stained paraffin sections; **(C)** choanosome, scale bar 20 μm; and **(D)** mesohyl, scale bar 10 μm. (Richell-Maurer *et al.*, 2001).
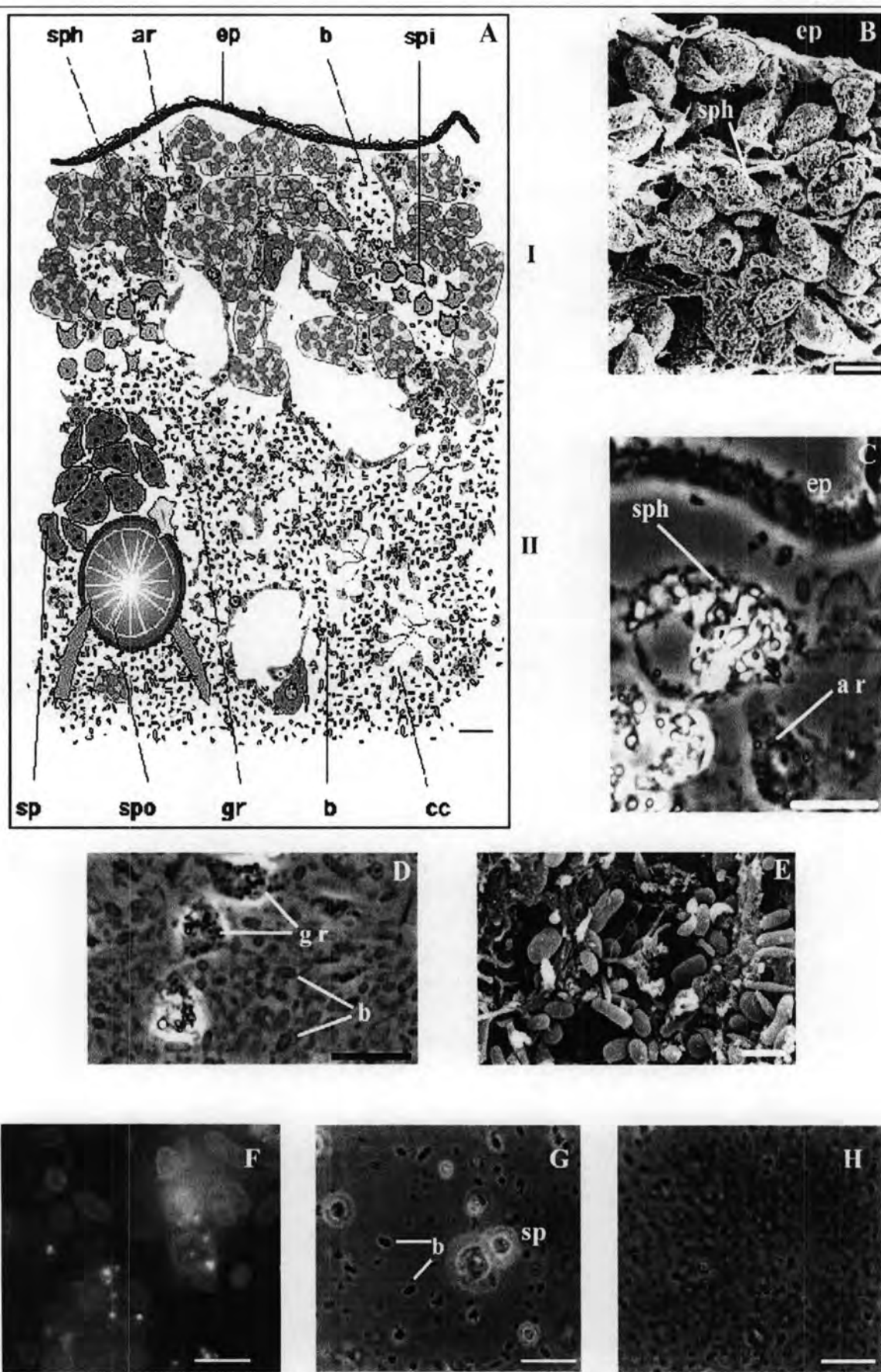
Figure 2.13. Microscopy of *Agelas conifera*: (A) schematic drawing of a cross section through the sponge tissue; I: ectosome, II: mesohyl, ar: archaeocytes, b: bacteria, cc: choanocyte chamber, ep: sponge exopinacoderm, gr: granular cell, sp: large sponge cell, sph: spherulous cell, spi: spicule, spo: sponging, scale bar 10 μm; (B) SEM image of densely packed spherulous cells (sph) beneath the exopinacoderm (ep), scale bar 20 μm. (C) phase-contrast image of a cross section through the ectosome showing a spherulous cell (sph) with fluorescent inclusions and an archaeocytes (ar) with aprominent nucleolated nucleus, scale bar 10 μm; (D) phase-contrast image of a crosssection through the mesohyl showing granular cells (gr) and numerous bacteria (b), scale bar 10 μm. (E) SEM image of heterogeneous populations of free-living bacteria in the mesohyl, scale bar 2 μm; (F) DAPI staining of a cell suspension showing large bacteria with nostained nucloid, scale bar 5 μm; (G) light micrograph of C1 heavy cell fraction showing sponge cells (sp) and large bacteria (b), scale bar 20 μm; (H) light micrograph of C2 light cell fraction showing small and large bacteria; scale bar 20 μm. (Richelle-Maurer *et al.*, 2003).
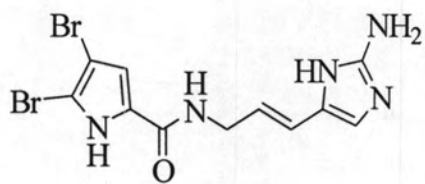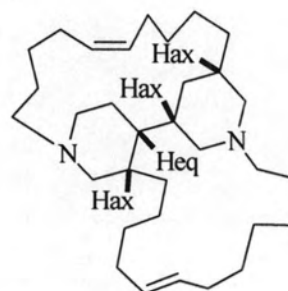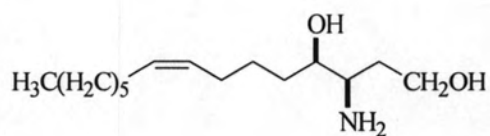
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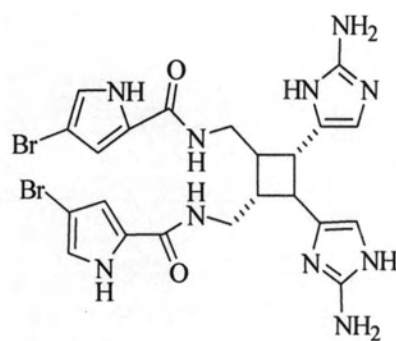
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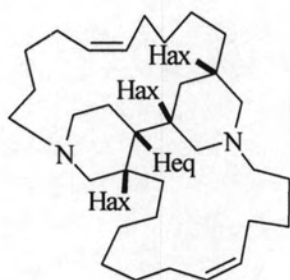
haliclonacyclamine-A

haliclonacyclamine-B

(2R,3R,7Z)-2-aminotetradec-7-ene-1,3-diol

Table 2.5. Cellular origins of bioactive compounds in the marine sponges.

Source of sponge	Compound	Type	Bioactivity	Cellular origin	References
Haliclona sp., class Demospongiae collected at Coral Gardens, in Heron Island, Australia, at 10-15 m. depth	Haliclonacyclamines A and B	Alkaloids	Cytotoxic	Sponge cells (spongocytes, choanocytes, and archaeocytes)	Garson *et al.*, 1998
Haliclona vansoesti, class Demospongiae collected from the Caribbean Sea off Curaçao (in Slangebaai at 45-55 m. depth)	(2R,3R,7Z)-2-amino tetradec-7-ene-1,3-diol	Sphingosines (aminodiol)	Antibacterial Antiyeast Toxic against nauplii of the brine shrimp *Artemia salina* with LD_{50} 9 mg/L	Archaeocytes	Richelle-Maurer *et al.*, 2001
Agelas conifera, class Demospongiae collected from the Caribbean Sea off Curaçao (in Slangebaai at 20-30 m. depth)	Oroidin and screptin	Bromopyrrole alkaloids	Antiviral Anti-histaminic Anti-muscarinic Anti-predation function (fish predators)	Spherulous cells	Richelle-Maurer *et al.*, 2003
Xestospongia muta, class Demospongiae collected from the Caribbean Sea off Curaçao (Holiday Beach at 20 and 50 m depth and from the Florida Keys National Marine Sanctuary (Pickles and Sombrero Reef at 20 m depth)	Straight-chain bromoacetylenic acids	Acetylenic compounds	Antimicrobial Cytotoxic	Heterotrophic bacteria	Richelle-Muarer *et al.*, 2003