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Porifera

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2.1 Introduction

Sponges (Porifera) belong to an ancient metazoan lineage that represents one of the earliest branches of the animal tree (Simion et al. 2017). The Porifera represent one of the most diverse taxa of sessile invertebrates with over 9000 extant species. Phylum Porifera comprises classes Demospongiae, Calcarea, Homoscleromorpha, and Hexactinellida. Sponges form a monophyletic group with two clades: Demospongiae + Hexactinellida and Calcarea + Homoscleromorpha.

Sponges are aquatic, mostly marine, sedentary multicellular animals, with filtration feeding and respiration. The body shape of sponges is very diverse; they may be film-like, encrusting, lumpy or spherical, tubular, branching, flabellate, etc. The body size of sponges varies as much as their body shapes, from 3–10 mm to 1.5–2 m. Their organization is particular; they have no distinct gut, muscles, gonads, nervous system, or respiratory system; however, sponges have a complex system of canals and chambers for water pumping – the aquiferous system (Table 2.1).

Age approximations of sponge species range from several months in freshwater sponges to 100 years in some marine sponges. However, research on the Caribbean giant barrel sponge *Xestospongia muta* suggests that this species might be capable of living more than 2000 years (McMurray et al. 2008).

For sponges, both asexual and sexual reproductions are characteristic. Sexual reproduction is fundamentally no different from similar processes in other multicellular animals. Sponges can be oviparous and viviparous. In the first case, sponges are usually dioecious, while in the second, often hermaphrodites. In many viviparous sponges embryonic development is accompanied by deep destruction of aquiferous system (see section 2.4.4.3). Asexual reproduction occurs in all poriferan clades. It may proceed by fragmentation, gemmulogenesis, and budding (for review see Fell 1974, 1993; Simpson 1984; Ereskovsky 2010). With few exceptions, sponges have a biphasic pelagobenthic life cycle with a tiny, planktonic ciliated larva that metamorphoses and grows into a large, benthic adult that is sexually reproductive (Ereskovsky 2010).

Sponges are mostly filter-feeding animals. They are the only type of Metazoa, with the exception of Placozoa, that lack phagocytoblasts in the form of intestinal epithelium. Practically all of the covering cells and many cells of the internal space participate in the capture of food particles (microbes, microalgae, organic particles, dissolved organic matter) in sponges (Hahn-Keser and Stockem 1997). However, sponges can be "carnivorous." These sponges feed almost exclusively on small crustaceans, which are entangled in a kind of "trapping network" formed by long thread-like outgrowths covered by a pinacoderm with microscleres in the form of anchors on the surface (Vacelet & Boury-Esnault 1995). Digestion, lasting for several days, is carried out both extracellularly and intracellularly in the mesohyl (Vacelet and Duport 2004).

Presently sponges are gaining increased scientific attention because of their secondary metabolites and biotechnological applications. Unique and innovative structural leads have been discovered with cytotoxic, antifouling, antitumoral, antibiotic, antiviral or cytoprotective, enzyme-inhibitory, antiinflammatory and anti-Alzheimer activities. Sponges could also be promising, highly biocompatible biomaterial for stem cell-based tissue engineering applications.

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Table 2.1 Organs for histologic evaluation in Porifera.[a]

Organ system		Organs
Body wall – ectosome		Glycocalyx, cuticle, exopinacoderm, dermal membrane, cortex
Digestive		No special system
	Alimentary canal	No special organs; aquiferous canals perform these functions
	Digestive organs	No special organs; choanocyte chambers perform these functions
Excretory		No special organs or structures; these functions are realized at the cellular level
Circulatory		No special system; aquiferous system perform these functions
Aquiferous system		Ostia, subdermal (*vestibular*) cavities, inhalant canals, prosodus, choanocyte chambers/tubes, aphodus, exhalant canals, atrium, oscula
Immune		No special organs or structures; functions are realized at the cellular level
Respiratory		No special organs or structures; functions are realized at the cellular level
Nervous		No special system; some functions are realized at the cellular level
Reproductive		Only temporary structures
	Male	Temporary spermatocysts
	Female	Temporary incubate chambers and follicles
Special senses/ organs		No special system or organs; this function is realized at the cellular level

[a] Alternative names for organs are provided parenthetically, in italics.

2.2 Gross Anatomy

The superficial region of the sponge body is devoid of choanocyte chambers (which are a component of the aquiferous system) and referred to as the ectosome (Figure 2.1a). The main component of the body, which occupies the middle part of the body wall and includes choanocyte chambers, is referred as the endosome or choanosome (Figure 2.1a). The hypophare is located in the basal part of the sponge and delimited by the endopinacoderm (an internal epithelial layer) from the endosome and by the basopinacoderm (an external epithelial layer) from the external milieu. The hypophare consists of the mesohyl (which is the mesenchyme) devoid of any elements of the aquiferous system (Figure 2.1a). The aquiferous system is a continuous water-conducting system of variably branching tubes between the ostia and the oscules, which comprises the inhalant system, choanocyte chambers or tubes and the exhalant system.

The rigidity of the sponge body is ensured by the collagen and spongin fibrils (in some Demospongiae orders) of the mesohyl and by the inorganic skeleton, consisting of either calcium carbonate ($CaCO_3$) (Calcarea, some Demospongiae) or silica (SiO_2) (Hexactinellida, Demospongiae, Homoscleromorpha). Inorganic skeleton may be represented by separate small elements (spicules), connected or fused spicules, or monolithic mineral skeleton (see section 2.4.3.1). All skeleton structures are secreted or assembled by special cells.

The classes of sponges differ by the type of their organization. Sponges from classes Calcarea, Demospongiae, and Homoscleromorpha have a cellular level of organization and are combined into the nonsystematic group Cellularia. In contrast, the body of sponges from class Hexactinellida is mainly built by a voluminous network of syncytial formations. In this chapter we describe mostly the histology of Cellularia; for Hexactinellida see Leys et al. (2007).

Representatives of the class Calcarea Bowerbank, 1864, the calcareous sponges (Figure 2.1b), are characterized by a calcium carbonate mineral skeleton in the form of free diactines (i.e., spicules with one axis), triactines (i.e., spicules with three rays), tetractines (i.e., spicules with four rays), and/or multiradiate spicules. This class includes approximately 770 species. A dense basal skeleton, with the main spicules cemented together, is sometimes present. The aquiferous system may have various organizational shapes, termed asconoid, solenoid, syconoid, sylleibid, or leuconoid (see section 2.4.2.1 for definitions of these shapes). Calcareous sponges are viviparous, with hollow larvae (calciblastula and amphiblastula). All Calcarea are marine sponges.

The class Homoscleromorpha Bergquist, 1978 includes 120 species (Figure 2.1c). The inorganic skeleton, if present, consists of small siliceous calthrops (equiangular tetraxon with equal rays) and/or their derivatives. The aquiferous system is sylleibid or leuconoid, often with vast basal exhalant cavities. Choanocyte chambers are large.

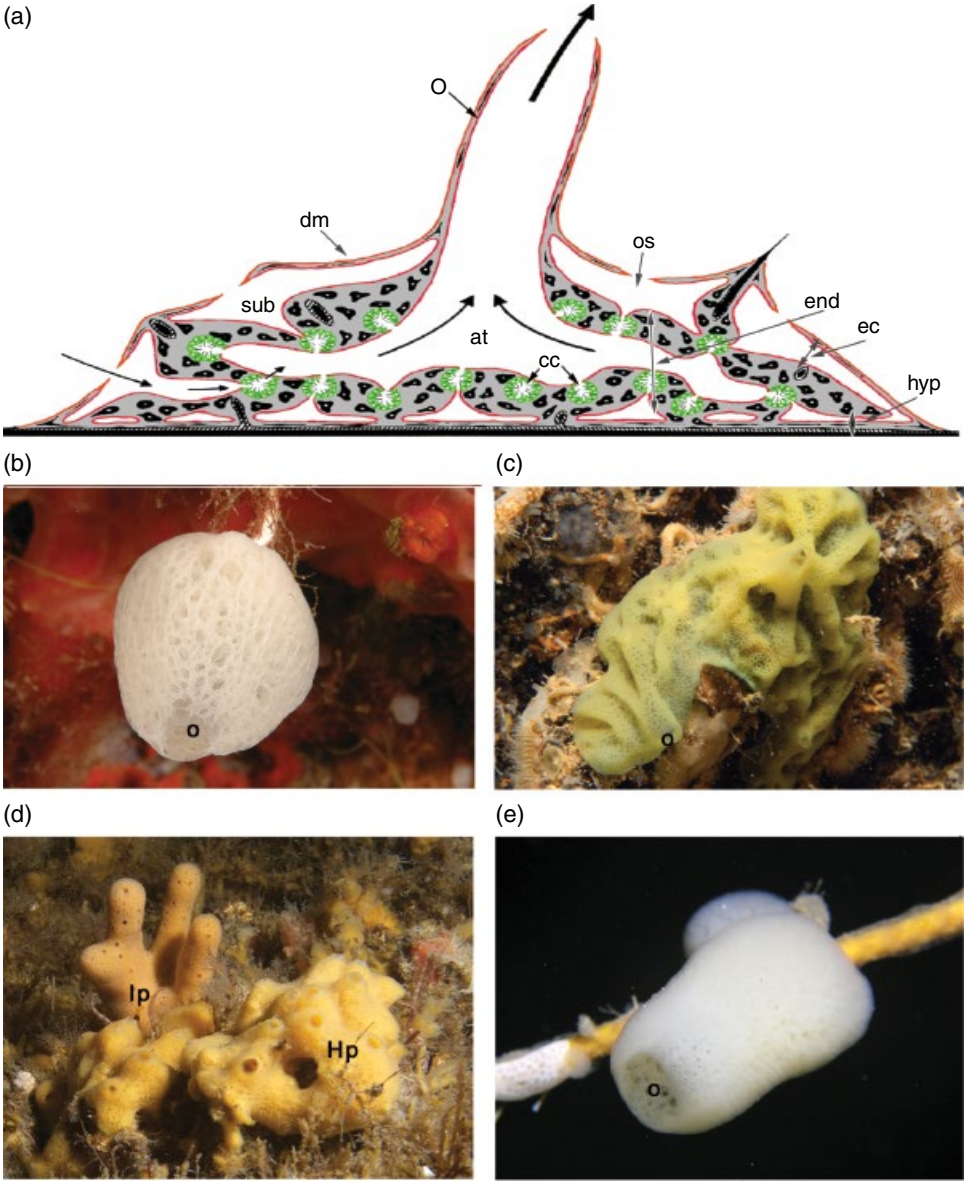

Figure 2.1 Gross anatomy. (a) Scheme of sponge organization; black arrows: water currents. (b) *Clathrina arnesenae* (Calcarea, Calcinea) *in vivo*. (c) *Oscarella viridis* (Homoscleromorpha) *in vivo*. (d) *Isodictya palmata* (Ip) and *Halichondria panicea* (Hp) (Demospongiae) *in vivo*. (e) *Oopsacas minuta* (Hexactinellida) *in vivo*.

True basement membrane underlies the choanoderm (which is the epithelium lining choanocyte chambers) and the pinacoderm (which is the epithelium lining the body cavities, external surfaces, and all portions of the aquiferous system except the choanocyte chambers); pinacocytes are flagellated. All the Homoscleromorpha are viviparous with hollow cinctoblastula larvae (entirely hollow flagellated larva, with a belt of cells with intranuclear paracrystalline bodies in the region of the posterior pole). They can be both gonochoric and simultaneous hermaphrodites. All homoscleromorphs are marine sponges.

The class Demospongiae Sollas, 1885 (about 8850 species) comprises sponges whose skeleton consists either of spongin fibers only or of spongin fibers in combination with siliceous spicules (usually, mega- and microscleres) (Figure 2.1d). Megascleres are larger than microscleres, and are mostly monoaxial and tetraxial. In some groups, the reduced spicular skeleton is compensated by a complex organic one (see section 2.4.3.2); in some other groups, there are no special skeletal elements at all. In several groups, a hypercalcified basal skeleton develops in addition to other skeletal elements. The aquiferous system is

leuconoid. Some sponges from the order Poecilosclerida lost the aquiferous system and became carnivorous. Some species are boring sponges and live in the midst of various calcareous substrate, contributing to its bioerosion. The larvae are mostly parenchymellae or, in some groups, single-layer larvae. Reproductive strategies within the class are oviparity and viviparity. Demosponges inhabit marine and fresh waters.

Representatives of the class Hexactinellida Schmidt, 1870 (about 670 species), commonly called glass sponges, are very variable in shape (Figure 2.1e). Typically, spicules are represented by hexactins (six rays), with three axes. Spicules are divided into micro- and megascleres, the latter, often fused together, forming rigid skeletal lattices. Dense spongin or nonspicular skeletons are absent. Tissues of glass sponges are syncytial and consist of the dermal and atrial membranes, the internal trabecular reticulum enclosing cellular components of the sponge and flagellated chambers. Separate nucleated cells are located in syncytial pockets. Large flagellated chambers are organized according to leuconoid type. All glass sponges are viviparous, with the trichimella larva (larva with median zone of multiciliated mononucleate cells, with syncytial structures and special larval stauractin skeleton). Hexactinellida are marine, mainly deep-sea, sponges.

2.2.1 Keys for Dissection/Processing for Histology

In general, standard protocols of tissue processing for histology are suitable for sponges. However, sponge tissues are highly sensitive to fixation procedure and subsequent manipulations. Sponges should be fixed and processed as soon as possible after collection. Contact with air should be avoided at any stage of processing, especially during manipulations with live sponges, when air could cause severe damage to fine structures in aquiferous systems. The majority of sponges possess porous tissues, highly permeable for solutions, thus requiring a shorter time for each step of the protocol. The fixation for histology should be done at 6 °C within 2–12 hours preferably in Bouin fixative or in 4% formaldehyde on sea water for marine sponges. For sponges with a mineral skeleton, its elements (spicules) should be removed after the fixation procedure by applying 5% hydrofluoric acid (for silica spicules) or 5% solution of ethylenediaminetetraacetic acid, disodium salt (EDTA) (for calcareous spicules) for two hours at room temperature. Then fixed tissues should be dehydrated through an ethanol series, placed in toluene or xylene and, finally, embedded in paraffin. Sections, 5–7 μm in thick, are mounted on glass slides and stained, with hematoxylin and/or eosin.

2.3 Histology

2.3.1 Particularity of Sponge Tissues

A characteristic feature of the Porifera, distinguishing them from the other Metazoa, is a high plasticity of cellular differentiation, anatomic and tissue structures throughout the life cycle. Various differentiated cells of the sponge can move, transdifferentiate, and switch functions. The direction of the differentiation depends on the current needs of the organism. Thus, the sponge is constantly in the state of rearrangement of all its structures (Gaino and Burlando 1990; Bond 1992; Gaino et al. 1995; Maldonado and Uriz 1999; Galera et al. 2000). This "chronic morphogenesis" contributes to the growth of the animal, reconstructing of somatic tissue after degradation during sexual and asexual reproduction, and during movements of the animal (Pavans de Ceccatty 1979; Bond 1992; Gaino et al. 1995; Bonasoro et al. 2001; Lavrov and Kosevich 2018).

In many Demospongiae, some stages of ontogenesis are accompanied by profound reconstructions of all the anatomic and histologic systems (Ereskovsky 2000), which can result in the destruction of all or most of the aquiferous system. These reconstructions may be caused by adaptation to adverse conditions (Simpson 1968; van de Vyver and Willenz 1975), regeneration processes (Borisenko et al. 2015; Ereskovsky et al. 2015), formation of reduction bodies (as delimited by a pinacoderm multicellular mass, consisting primarily of archaeocytes and presumably capable of reorganizing into a new functional sponge; reduction bodies result from a tissue disorganization of freshwater and estuarine demosponges) (Simpson 1984), gemmulogenesis (formation of gemmules, resistant asexual reproductive bodies) or sexual reproduction (Simpson 1984; Ereskovsky 2000; Ereskovsky et al. 2013). In general, these massive rearrangements do not interfere with the normal transport of water through the sponge and occur continuously along with the active pumping of water.

Histologically, the sponge body is divided into three parts: the outer epithelial layer (exopinacoderm and basopinacoderm), the inner epithelial layers (choanoderm and endopinacoderm), and mesohyl, the inner space of the sponge body that is enclosed by the epithelial layers.

2.3.2 Bordering Tissues – Epithelia

2.3.2.1 Pinacoderm

The pinacoderm is represented by the exo-, baso-, and endopinacoderm. Exopinacoderm forms the external cover of the sponge. Basopinacoderm develops at the sponge base, attaching it to the substrate. Endopinacoderm forms the walls of the subdermal cavities and the aquiferous

system canals, excluding regions of choanocyte chambers (which are lined by choanoderm; see below). There are, correspondingly, several types of pinacocytes.

The **exopinacoderm** consists of the exopinacocytes – the covering cells of the sponge, that may be T-shaped or spindle shaped in cross-section (Figures 2.1a, 2.2a,b, 2.3c,d,f). The spindle-shaped exopinacocytes are described in many Demospongiae from the orders Spongillidae and Poecilosclerida (Bagby 1970; Weissenfels 1989), in all the Homoscleromorpha (Muricy et al. 1996, 1999; Ereskovsky et al. 2014) and in some Calcarea (Borojevic 1969; Eerkes-Medrano and Leys 2006). The T-shaped exopinacocytes are described in part of Demospongiae and Calcarea (see section 2.4.1) (Boury-Esnault 1973; Willenz and Hartman 1989; Ereskovsky et al. 2011; Lavrov et al. 2018).

In most sponges, exopinacocytes lack specialized cell junctions, but are united with a well-developed adhesive system (Blumbach et al. 1998; Schütze et al. 2001); for example, in *Hippospongia communis*, *Ephydatia fluviatilis*, *Sycon coactum*, *S. ciliatum*, and *Leucosolenia variabilis*, the sites of exopinacocyte contacts have electron-dense thickenings of the membranes resembling *zonula adhaerens* (Pavans de Ceccatty et al. 1970; Pottu-Boumendil 1975; Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). In Homoscleromorpha exopinacocytes have specialized cell junctions, and the exopinacoderm in general has all the structural features of eumetazoan epithelium (Ereskovsky and Tokina 2007). Unique characteristics of the homoscleromorph exopinacocytes are the flagellum (Muricy et al. 1996, 1999; Ereskovsky et al. 2014), and the ability to synthesize spicules (Maldonado and Riesgo 2007). Exopinacoderm contains ostia – numerous microscopic structures, 4–100 μm in diameter, through which water is drawn into the aquiferous system of the sponge.

Exopinacoderm exhibits many functions characteristic of the typical eumetazoan epithelia, such as absorption, secretion, transport, excretion and protection (Harrison and de Vos 1991; Meyer et al. 2006; Leys and Hill 2012). Moreover, exopinacocytes are capable of contractile responses (Pavans de Ceccatty 1986; Wachtmann and Stockem 1992a, b; Adams et al. 2010), amoeboid movement (Ereskovsky et al. 2015), incorporation of exogenous silica particles (Bavestrello et al. 1998), and phagocytizing of food particles (Willenz and van de Vyver 1982).

The **basopinacoderm** consists of the basopinacocytes – flattened cells, which are located at the basal surface of the sponge and function in attachment of the sponge to the substrate. Synthesizing basal spongin and fibronectin, basopinacocytes function as spongocytes (Garrone and Rozenfeld 1981; Labat-Robert et al. 1981). During sponge growth, marginal basopinacocytes actively secrete proteins that make up spongin (Garrone 1978).

In the demosponges with a massive calcareous skeleton, such as *Acanthochaetetes wellsi* (order Clionaida), *Ceratoporella nicholsoni* and *Stromatospongia norae* (order Agelasida), basopinacocytes participate in the formation of this skeleton (Willenz and Hartman 1989; Reitner and Gautret 1996). In calcareous sponge *Petrobiona massiliana* basopinacocytes participate in formation of the basal massive skeleton by producing an extracellular organic framework that might guide the assemblage of submicronic amorphous Ca- and Mg-bearing grains into higher structural units (Gilis et al. 2012).

Basopinacocytes of the freshwater demosponges have a well-organized cytoskeleton (Wachtmann et al. 1990; Wachtmann and Stockem 1992a, 1992b; Kirfel and Stockem 1997; Adams et al. 2005). Actin is located in the cortical layer and in the fibrils in the cytoplasmic matrix. Microtubules radiate from the perinuclear zone, finishing at the cell periphery. At the same time, intermediate filaments have not been described. In *Ephydatia muelleri*, basopinacocytes were shown to have desmosome-like junctions (Pavans de Ceccatty 1986).

The **endopinacoderm** consists of the endopinacocytes – flattened, polygonal cells, spindle shaped on cross-section (Figures 2.1a, 2.2b, 2.3c; see also Figure 2.7a,b,f). The endopinacocytes are divided into prosopinacocytes, lining the inhalant canals, and apopinacocytes, lining the exhalant canals of the aquiferous system. The external surface of the endopinacocytes is covered with a glycocalyx layer (Boury-Esnault et al. 1984; Vacelet et al. 1989; Harrison and de Vos 1991). The basal surface often forms numerous projections (pseudopodia) for anchoring in the extracellular matrix. In all Homoscleromorpha and in some Demospongiae, endopinacocytes bear flagella (Boury-Esnault et al. 1984; Vacelet et al. 1989; Ereskovsky et al. 2014). In particular, this is the case in most studied representatives of the orders Dictyoceratida and Dendroceratida (Thiney 1972; Donadey 1982; Vacelet et al. 1989; Boury-Esnault et al. 1990). The presence of flagella appears to be associated with the involvement of endopinacocytes in the generation of water currents through the aquiferous system. However, in some demosponges, the endopinacocytes lining the oscular tube (large exhalant opening) have short nonmotile cilia, presumably with sensory function, which may be involved in the coordination of simple sponge behavior (Ludeman et al. 2014).

Generally, endopinacocytes contact each other by simple overlap. However, in the oscular tubes of freshwater sponges they are united by desmosome-like junctions (Masuda et al. 1998). Endopinacocytes of Homoscleromorpha are joined by *zonula adhaerens* junctions and underlined with basement membrane (Ereskovsky and Tokina 2007; Ereskovsky et al. 2009).

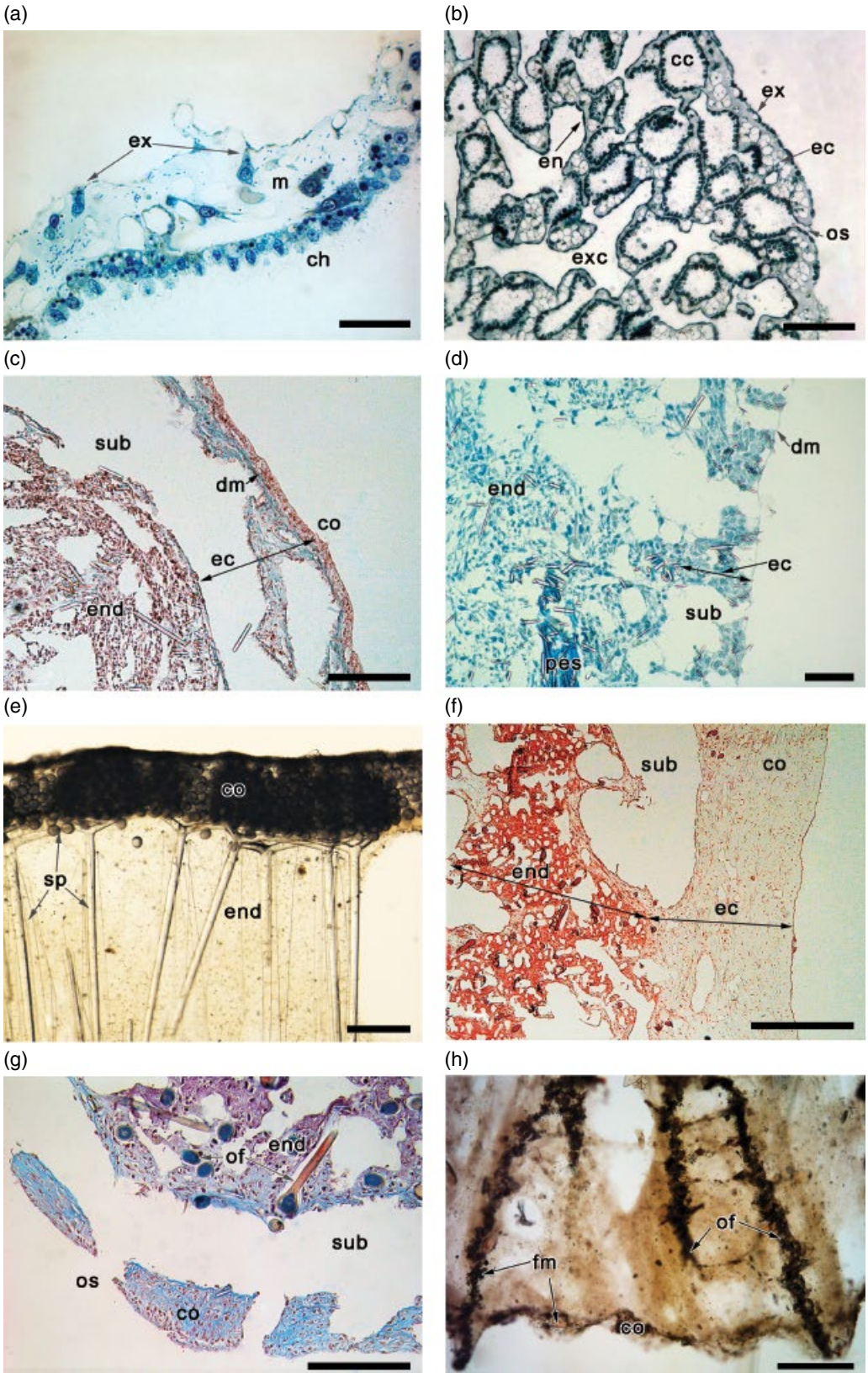

Figure 2.2 The ectosome, dermal membrane, and cortex. (a) Semithin section of a body wall of asconoid *Leucosolenia variabilis* (Calcarea, Calcaronea, Leucosolenida), showing a very thin ectosome, T-shaped exopinacocyte, mesohyl, and choanoderm. (b) Semithin section of leuconoid *Oscarella tuberculata* (Homoscleromorpha, Oscarellidae), showing a very thin ectosome, flat exopinacocytes, and endosome. (c) Histologic section of the ectosome of *Crambe crambe* (Demospongiae, Poecilosclerida). (d) Histologic section of the ectosome of *Petrosia ficiformis* (Demospongiae, Haplosclerida). (e) Histologic section of the ectosome of *Geodia atlantica* (Demospongiae, Tetractinellida) showing a cortex, reinforced with special skeletal elements – microscleres (astroscleres), and the endosome with the megascleres. *Source:* Image courtesy of Paco Cardenas. (f) Histologic section of the ectosome of *Pleraplysilla spinifera* (Demospongiae, Dictyoceratida). (g) Histologic section of the ectosome of *Spongia officinalis* (Demospongiae, Dictyoceratida). (h) Histologic section of upper part of *Dysidea incrustans* (Demospongiae, Dictyoceratida), showing the cortex with foreign material embedded into the organic skeleton. Scale bars: (a) 20 μm; (b,c,d) 100 μm; (e) 1 mm; (f,g,h) 500 μm.

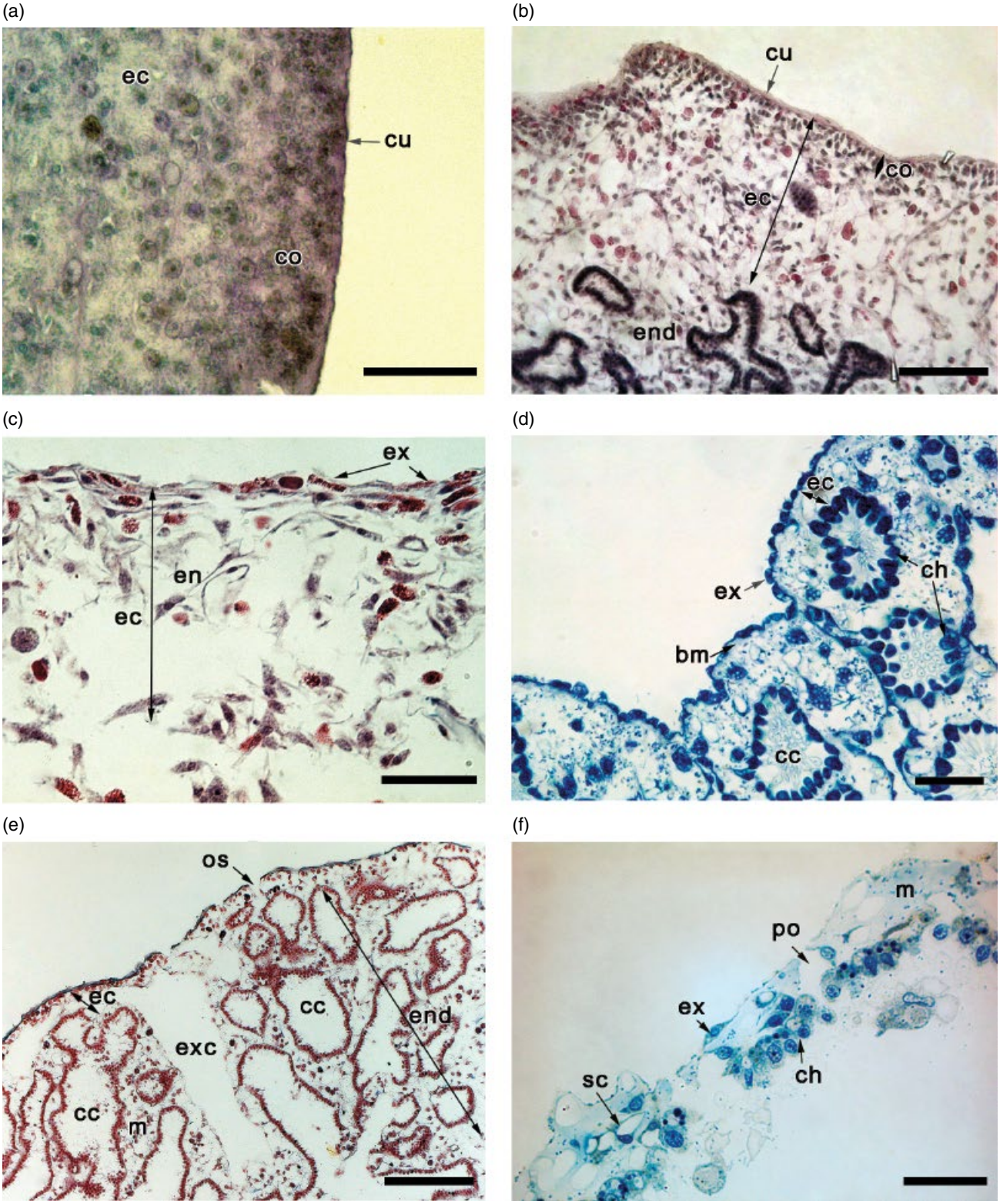

Figure 2.3 Cuticle, exopinacoderm, and pores. (a) Histologic section of *Aplysina cavernicola* (Demospongiae, Verondiida): cortex and cuticule. (b) Histologic section of *Halisarca dujardinii* (Demospongiae, Chondrillida): ectosome, noncellular, amorphous cuticle. (c) Histologic section of ectosome *of Lubomirskia baicalensis* (Demospongiae, Spongillida), showing flat exopinacocytes. (d) Semithin section of ectosome and choanosome of *Oscarella lobularis* (Homoscleromorpha, Oscarellidae), showing a basal membrane lining pinacoderm and choanocyte chambers. (e) Histologic section of upper part of *Halisarca dujardinii* (Demospongiae, Chondrillida): ectosome, choanosome, and multicellular pores. (f) Semithin section of a body wall of asconoid *Leucosolenia variabilis* (Calcarea, Calcaronea, Leucosolenida), showing porocytes. Scale bars: (a,c,d) 50 μm; (b) 100 μm; (e) 200 μm; (f) 20 μm.

Endopinacocytes and choanocytes of some demosponges, homoscleromorphs, and calcareans are thought to be functionally and ontogenetically interrelated. For example, during reparative regeneration the choanocytes of different species from these taxa can differentiate into endopinacocytes by reduction of the flagellum and the microvilli and the subsequent flattening of the cell (Diaz 1974; Borisenko et al. 2015; Ereskovsky et al. 2015; Lavrov et al. 2018).

The apopylar cell is a particular cell type forming the boundary between apopinacoderm (epithelium lining the exhalant canal) and choanoderm (see section 2.3.2.2) and linking choanocytes and apopinacocytes (de Vos et al. 1990). Apopylar cells display morphologic characteristics intermediate between those of choanocytes and pinacocytes. In Homoscleromorpha, these cells possess a flagellum and an unfolded collar of microvilli (Boury-Esnault et al. 1984). In demosponges, the apopylar cells were observed in all investigated species of Dictyoceratida and Dendroceratida, in some Chondrosida (*Halisarca dujardinii*, *Thymosia guernei*) and Haplosclerida, in the freshwater sponge *E. fluviatilis* and in *Tethya wilhelma* (Tethyida) (Langenbruch et al. 1985; de Vos et al. 1990; Hammel and Nickel 2014). Earlier, these cells were referenced as "cone cells" and "cell-ring" (de Vos et al. 1990). In *T. wilhelma*, the apopyle (the exit from the choanocyte chamber) has also a reticuloapopylocyte, a modified apopylar cell, which has numerous small intracellular pores, which give them a mesh or grid-like morphology (Hammel and Nickel 2014).

2.3.2.2 Choanoderm

The choanoderm consists only of choanocytes, which form the choanocyte chambers (or tubes in asconoid and solenoid sponges) (see section 2.4.2) (Figure 2.2b; see also Figure 2.6a–e). Contrary to the pinacoderm, the choanoderm has a cubic or palisade epithelium. Choanocytes can be cylindrical, cubic, trapezoid, or slightly flattened. These cells bear a flagellum surrounded with a collar of cytoplasmic microvilli interconnected by glycocalyx bridges. In some demosponges choanocytes have a periflagellar sleeve, such as in Suberitidae, Polymastiidae, Acanthochaetetidae, and Halisarcidae (Connes et al. 1971; Boury-Esnault et al. 1990, 1994; Ereskovsky et al. 2011).

Another cell type associated with the choanocyte chambers of some demosponges is the central cell (Reiswig and Brown 1977; Diaz 1979; Langenbruch and Scalera-Liaci 1986; Langenbruch and Jones 1989; Sciscioli et al. 1997; Ereskovsky et al. 2017a). Central cells have an irregular, branched shape with numerous projections and holes. The cell is perforated with a vast canal, into which flagella of the choanocytes enter. The central cells participate in the regulation of beating of the choanocyte flagella within a chamber and thus in the regulation of water currents through the aquiferous system.

2.3.3 Tissues of the Internal Environment

Diverse cells, which compose the tissue of the internal environment of sponges, are located in the mesohyl – a highly complex system occupying the internal parts of the animal's body, between its surface and elements of the aquiferous system. Besides cells, the mesohyl includes a skeleton (both organic and mineral) (see section 2.4.3), organic ground substance, which encompasses all cellular and skeletal elements, dissolved macromolecules and often bacteria, archaea and cyanobacteria. The mesohyl has a parenchymal structure with various cell types intermixed with each other and with noncellular elements. No structural compartments can be defined in the mesohyl. Moreover, the majority of cells demonstrate apparent motility and the ability to transdifferentiate, making the structure of the mesohyl highly unstable.

However, the sponge cell populations of the internal environment can be subdivided according to their functions: supportive-connecting tissue, protective-secretory tissue, and contractile cells, which occur in some demosponges (Ereskovsky 2010). Although these tissues are not structurally delimited from each other, they comprise groups of cells with a specific function.

In addition, occasionally during various stages of sexual and asexual reproduction the gametes, embryos, and specific somatic cells participating in gamete formation (trophoblasts, nurse cells, histoblasts, thesocytes, etc.) develop in the mesohyl, significantly changing its overall structure (see section 2.4.4).

2.3.3.1 Supportive-Connective Tissue

This tissue comprises a variety of cells participating in the formation of organic and mineral skeleton and ground substance of the mesohyl.

Collencytes (lophocytes) are mobile cells participating in the secretion of collagen and formation of its fibrils (Figure 2.4a). These cells are frequently characterized by a nucleus without nucleolus, a well-developed rough endoplasmic reticulum (RER), few nonspecific inclusions, and specific vacuoles containing collagen, which can be dense and homogenous or contain clear fibrillar material. Collencytes are devoid of phagosomes. These cells can be found all over the mesohyl. The secretory activity of these cells is evident from the deposition of oriented collagen fibrils near the cells, sometimes attached to the cell membrane (Borojevic 1966; Bonasoro et al. 2001).

Two names are used for this type of cell (Lévi 1970; Simpson 1984; Boury-Esnault and Rützler 1997): *collencyte*

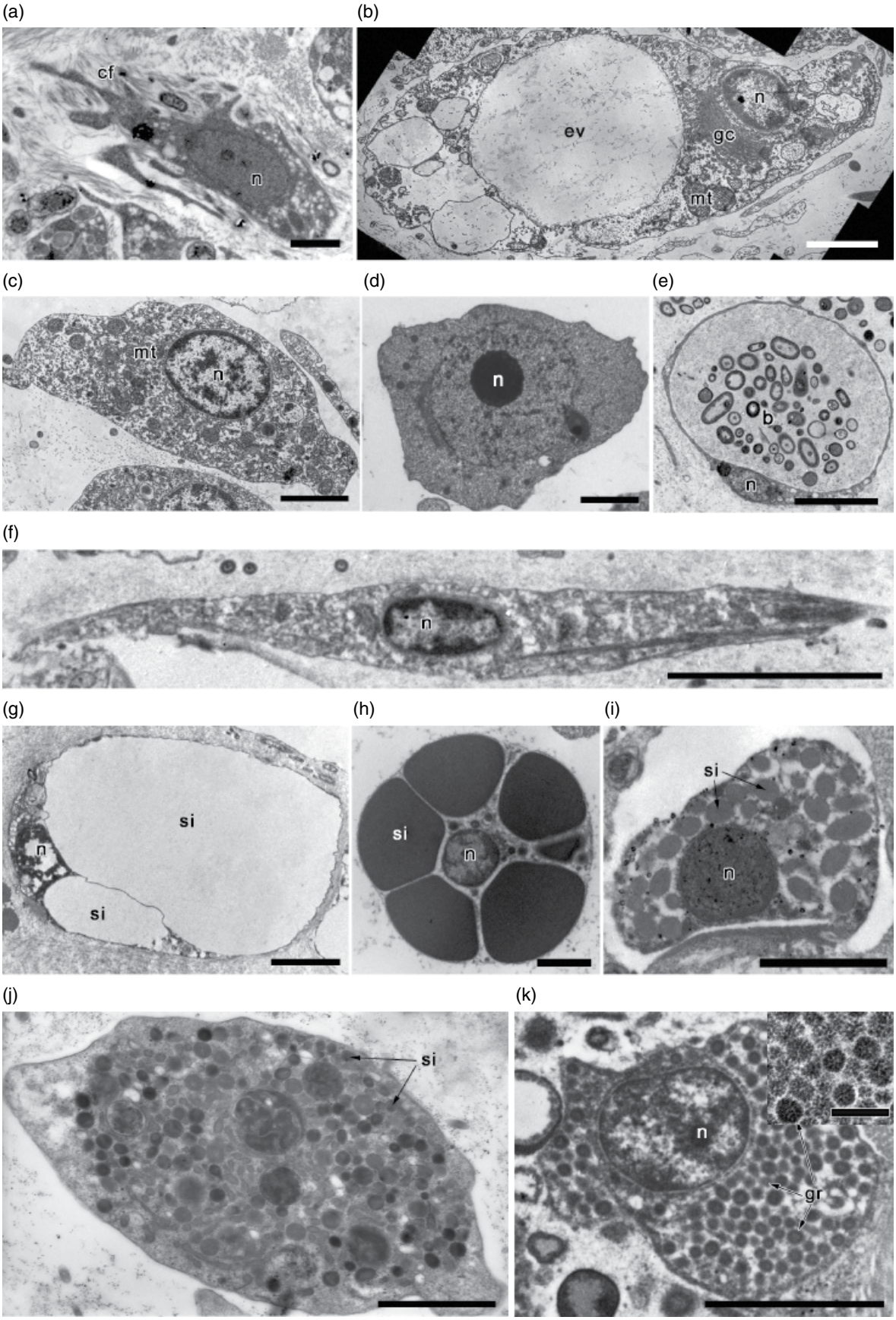

Figure 2.4 Cells of the internal environment. (a) Lophocyte of *Chondrilla* sp. (b) Sclerocyte of *Leucosolenia variabilis*. (c) Amoebocyte of *Leucosolenia variabilis*. (d) Archaeocyte of *Crellomima imparidens*. (e) Bacteriocyte of *Aplysina cavernicola*. (f) Myocyte of *Leucosolenia* sp. (g) Vacuolar cell of *Oscarella tuberculata*. (h) Spherulous cell of *Halisarca caerulea*. (i) Granular cell of *Chondrilla* sp. (j) Microgranular cell of *Halisarca dujardinii*. (k) Gray cell of *Chondrilla* sp. Inset – glycogen rosettes. Scale bars: (a,c,d,g,h,i,j,k) 2 µm; (b) 1 µm; (e,f) 5 µm; (inset) 0.25 µm.

usually refers to a stellar-like or spindle-like cell, while *lophocyte* refers to an obviously motile cell with anterior–posterior polarity and often forming a collagen bundle, which is associated with its posterior pole (Garrone 1978; Bonasoro et al. 2001).

Spongocytes are amoeboid cells responsible for the secretion of spongin in different forms (see section 2.4.3.2). Spongocytes always form groups of several cells during spongin secretion. They are characterized by a nucleus with nucleolus, well-developed RER, perinuclear cisterns of Golgi complex and vesicular cytoplasm, containing numerous homogenous dense inclusions with spongin precursor (Garrone 1978).

Special types of spongocytes participate in the development of gemmules (a resistant asexual reproductive body, composed of internal mass of archaeocytes [thesocytes] charged with reserves and enclosed in a noncellular protective envelope) in freshwater sponges from the family Spongillidae. These spongocytes form a palisading epithelium around the developing gemmule and secrete collagenous shell and chitin for its coat (de Vos 1971, 1977; Langenbruch 1981, 1982; Ehrlich et al. 2013).

Sclerocytes are mobile cells, secreting elements of the mineral skeleton – spicules. Depending on the size and hence type of spicule produced (megasclere or microsclere) (see section 2.4.3.1), the sclerocytes are divided into megasclerocytes and microsclerocytes. Megasclerocytes have a nucleus with nucleolus, prominent Golgi complex, free ribosomes, mitochondria, few phagosomes and cisterns of RER (Figure 2.4b). Microsclerocytes are characterized by smaller size and a nucleus without nucleolus (Wilkinson and Garrone 1980; Garrone et al. 1981; Custodio et al. 2002).

Microscleres and microsclerocytes are characteristic only for Demospongiae and Hexactinellida. The mineral skeleton of Homoscleromorpha and Calcarea consists of megascleres of different size, so representatives of these classes have only megasclerocytes.

Silica spicules of Demospongiae, Hexactinellida, and Homoscleromorpha are synthesized intracellularly in vacuoles around an organic axial filament (Uriz et al. 2003; Uriz 2006; Leys et al. 2007; Maldonado and Riesgo 2007). During synthesis of large spicules, which are bigger than sclerocytes, several cells join (Uriz et al. 2003). In Demospongiae and Hexactinellida the membrane forming the spicule vacuole has a specific structure and is called a silicalemma (Uriz et al. 2003; Uriz 2006; Leys et al. 2007). It pumps silica inside the vacuole, producing a higher concentration inside for effective deposition around the organic axial filament (Uriz 2006). No information exists about the structure of analogous membranes in Homoscleromorpha.

In contrast, in calcareous sponges spicules are always synthesized extracellularly by the group (2–6 cells) of sclerocytes, which are joined by septate junctions and form an extracellular vacuole, where increased concentration of calcium ions is produced (Jones 1970; Ledger and Jones 1977; Uriz 2006).

Transport cells are peculiar amoeboid cells of the mesohyl described from the freshwater sponge *E. fluviatilis* (Nakayama et al. 2015). Transport cells are attached to the newly synthesized megascleres and transport a spicule from its place of synthesis to the final position in skeletal framework. No specific structural features of the transport cells are yet known. This cell type is defined only by its location on the newly synthesized megascleres, motile behavior, and specific expression of the gene EflSoxB1 (Nakayama et al. 2015).

2.3.3.2 Protective-Secretory Tissue

Various amoeboid cells and cells with specific inclusions compose this tissue. The functions of protective-secretory tissue include transfer and distribution of nutrition and oxygen, excretion, immune protection, and secretion of specific substances.

Amoebocytes *sensu lato* are common motile cells of the mesohyl. There is no clear definition of these cells and at various times they were called thesocytes (Sollas 1888), spherulous cells (Topsent 1892), polyblast or hyaline cells (Tuzet and Pavans de Ceccatty 1958), amoebocytes (Müller 1911), or nucleolated amoebocytes (Wilson and Penney 1930; Faure-Fremiet 1931; Efremova 1972). They occur in all regions of the mesohyl and often are the main cell type in it. The amoebocytes have a large nucleus with nucleolus associated with Golgi complex, numerous RER cisterns and unspecific inclusions, especially phagosomes, and symbiotic zoochlorellae in the cytoplasm of freshwater sponges (Figure 2.4c) (Gilbert and Allen 1973; Williamson 1979). Amoebocytes are traditionally considered to execute several functions including digestion and distribution of nutrition, immune response in the form of phagocytes, elimination of refractory leftovers, and functioning as stem cells. Considering the broad variety of executed functions and absence of obvious structural features, amoebocytes could represent a highly heterogenous cell group and should be further researched. Currently, one subpopulation of amoebocytes can be distinguished – archaeocytes.

Archaeocytes are amoebocytes with a high nuclear/cytoplasmic ratio, with cytoplasm reach in RER and ribosomes and devoid of special cytoplasm inclusions (Figure 2.4d) (Smith and Hildemann 1990; Harrison and de Vos 1991). They occur in Demospongiae and Hexactinellida (in which they are the only cellular elements independent from the main syncytial tissues) and represent one of the stem lines in sponges of these classes (Lévi 1970; Korotkova, 1981, 1997; Simpson 1984; Harrison and de Vos 1991; Funayama, 2008, 2018).

In addition, participation of some amoebocytes in various immune reactions is well known (Smith and Hildemann 1986, 1990), and some attempts to distinguish this subpopulation have been made, using molecular markers (Funayama et al. 2005).

Bacteriocytes represent mobile cells with specific vacuoles, containing various symbiotic prokaryotes. This cell type is known only in demosponges. Bacteriocytes can contain single large or several small vacuoles with symbionts (Figure 2.4e) (Vacelet 1970; Vacelet and Donadey 1977; Bigliardi et al. 1993; Vacelet and Boury-Esnault 1996; Maldonado 2007). Bacteriocytes participate in food digestion in carnivorous sponges (Vacelet and Duport 2004) and are responsible for vertical transmission of symbionts in some demosponges (Ereskovsky et al. 2005; Maldonado 2007), as they penetrate the embryos during their development and remain intact until the larval settlement and metamorphosis (Lévi and Lévi 1976).

Cells with specific inclusions are an important element in sponge mesohyl. This heterogenous cell group includes various cells types, which are united by the presence of specific inclusions in the cytoplasm but differ by structure of these inclusions. Currently, the function of most cells with specific inclusions is unknown. Some evidence indicates that these cells can realize the content of their vacuoles in the mesohyl, participate in metabolism of glycogen, excrete metabolic by-products, and produce metabolites with antibiotic functions, which may be involved in the regulation of symbiotic bacteria or defense against foreign bacteria. Cells with inclusions can be found in most demosponges and homoscleromorphs but are rare in calcareous sponges and hexactinellids. According to Simpson (1984), cells with specific inclusions are subdivided into two major categories: cells with larger inclusions and cells with smaller inclusions.

Cells with larger inclusions. Vacuolar cells (cystencytes) are characterized by the presence of one or several large transparent vacuoles, occupying almost all cytoplasm of these cells (Figure 2.4g; see also Figure 2.7b,f). The free cytoplasm is reduced to a thin film around inclusions and nucleus. The nucleus may be displaced to the cell periphery by the inclusions. In addition, cytoplasm may contain Golgi complex, some RER and smooth endoplasmic reticulum, few mitochondria, and small phagosomes. Cystencytes are vacuolar cells of freshwater sponges, having one large inclusion with amorphous material of a polysaccharide nature (Tessenow 1969; Pottu-Boumendil 1975; Ereskovsky et al. 2016). Vacuolar cells can be used as a diagnostic characteristic in closely related species of sponges without a skeleton, for example *Oscarella* and *Halisarca* (Muricy et al. 1996; Ereskovsky 2006, 2007).

Spherulous cells have several large membrane-bounded inclusions (0.8–8 μm diameter). Free cytoplasm is reduced to narrow strands between the inclusions and on the cell periphery. The cytoplasm contains few mitochondria and rare cisterns of RER (Figure 2.4h; see also Figure 2.8f). The nucleus is usually small, anucleolated, and deformed by the inclusions. Inclusion content is usually homogenous but can be paracrystalline, fibrillar, or lamellar (Diaz 1979; Thompson et al. 1983; Bonasoro et al. 2001; Ereskovsky et al. 2017b). In some species, spherulous cells are localized near the sponge surface or aquiferous system canals, although they can lie diffusely in the mesohyl (Uriz et al. 1996; Bonasoro et al. 2001; Ereskovsky 2007; Maldonado 2016). The presumable function of the spherulous cells varies in different species, indicating possible heterogeneity of this cell type. The spherulous cells were reported to participate in storage of various metabolites (including toxic ones) (Thompson et al. 1983; Uriz et al. 1996; Becerro et al. 1997), immune response against nonsymbiotic bacteria, defense against fouling, predation by release of metabolites (Thompson et al. 1983; Ternon et al. 2016), excretion (Vacelet 1967; Donadey 1978; Maldonado 2016; Ereskovsky et al. 2020), and mesohyl extracellular matrix synthesis and maintenance (Donadey and Vacelet 1977; Donadey 1982; Bretting et al. 1983; Smith and Hildemann 1990).

Granular cells have numerous membrane-bound inclusions (0.5–2 μm diameter) in their cytoplasm. The inclusions of the granular cells are smaller, and their number is higher in comparison with spherulous cells (Figure 2.4i). The shape of inclusions varies from round to irregularly ovoid. Using electron microscopy, the inclusions are usually homogenous, but they also can be fine-grained or have a fine-grained periphery with a homogenous central region. The content of the inclusions is often separated from the surrounding membrane by the transparent space. The nucleus is round with or without a nucleolus. A few phagosomes, unspecific inclusions and vacuoles can appear in the cell cytoplasm (Pomponi 1976; Ereskovsky et al. 2011, 2017a, 2017b; Willenz et al. 2016). The exact functions of granular cells are unknown, but they may be involved in the immune response, as the inclusion contents show antimicrobial activity (Krylova et al. 2003). In addition, in some demosponges maternal granular cells penetrate into the forming larvae (Ereskovsky and Gonobobleva 2000; Rützler et al. 2003) and can be retained there until the beginning of metamorphosis (Gonobobleva and Ereskovsky 2004).

Granular and spherulous cells can be used as a diagnostic characteristic in closely related species (Pomponi 1976; Boury-Esnault et al. 1994; Bergquist 1996; Muricy et al. 1996; Reveillaud et al. 2012; Gazave et al. 2013; Willenz et al. 2016).

Microgranular cells are characterized by cytoplasm filled with minute dense granules (~0.09–0.3 μm diameter). The nucleus is often anucleolated and cytoplasm contains few mitochondria and RER cisterns (Figure 2.4j) (Sciscioli et al. 2000; Pinheiro et al. 2004). These cells could contribute to the synthesis of glycoprotein components of the extracellular matrix. Others functions of the microgranular cells remain unknown.

Cells with smaller inclusions. Gray cells (glycocytes) contain numerous small ovoid membrane-bounded inclusions (~0.2–0.8 μm diameter). The inclusions are acidophilic and osmiophilic. Another characteristic feature of these cells is glycogen rosettes in the cytoplasm (Figure 2.4k). Besides the glycogen rosettes, the cytoplasm of gray cells contains well-developed RER and Golgi complex (Boury-Esnault 1977). The nucleus usually contains a small nucleolus. Presumably these cells participate in glycogen metabolism (Boury-Esnault 1977) and also have been considered as immunocytes, responsible for the allogeneic response (Humphreys 1994; Yin and Humphreys 1996; Sabella et al. 2007).

Rare type of cells with inclusions. The types of cells with inclusions described above are widespread and found in many sponge species. In addition to these, several rarer types of cells with inclusions occur in some sponges: rhabdiferous cells (Simpson 1968; Smith 1968; Smith and Lauritis 1969; Ereskovsky et al. 2011), sacculiferous cells (Smith 1968; Smith and Lauritis 1969), spumeuse cells (Donadey and Vacelet 1977; Donadey 1982), globoferous cells (Borojevic and Lévi 1964; Simpson 1968) and styllocytes (Harrison et al. 1974). These rare cell types have been found in one or several sponge species, consequently additional studies of their structure and functions are required. Moreover, these rare cells with inclusions could possibly be species-specific modifications of common types of cells with inclusions.

Contractile cells of the mesohyl, myocytes, are found in the mesohyl of many demosponges and calcareous sponges. In some sponges, the myocytes can occur in large concentric multilayered structures (sphincters) around large exhalant canals and oscula, while in other species they lie sparsely in the mesohyl near the aquiferous system canals and/or dermal membrane (Bagby 1966). The myocytes are fusiform cells with an ovoid nucleus, lying in the central part of the cell (Figure 2.4f). Other organelles (Golgi complex, mitochondria, unspecific inclusions) are usually located near the poles of the nucleus. The bundles of myofilaments are concentrated at the cell periphery. In some sponges, the myocytes have two types of filaments, which are spatially organized, forming regular patterns (e.g., *Tedania ignis*) (Bagby 1966; Thiney 1972). Considering their position and ultrastructural features, the myocytes are thought to be contractile cells, which regulate the diameter of large canals of the aquiferous system, thus participating in the regulation of water flow.

2.3.4 Loose Connective Tissues (Mesohyl)

The mesohyl occupies the internal spaces of the sponge body and is delimited by the pinacoderm and choanoderm. The degree of mesohyl development varies greatly according to the type of sponge body organization: in asconoid sponges the mesohyl has a thickness of only dozens of micrometers (see Figure 2.6a), while in leuconoid sponges it comprises the main volume of a sponge (see Figure 2.6e).

The mesohyl is a complex compartment, comprising numerous cells of different types, organic and inorganic skeletal components, collagen fibers, unstructured extracellular ground substance, and symbiotic organisms. It does not have a permanent structure or structural units, appearing as a highly dynamic and variable system, although the mesohyl of the specialized parts of the sponge body (e.g., cortex, dermal membrane, etc.) can have permanent structural features like extracellular matrix arrangement and/or cell type composition (see section 2.4.1).

All mesohyl cells are in constant movement (Bond 1992; Gaino et al. 1995). However, semipermanent structures can appear in the mesohyl. Mesohyl cells have a tendency to form small transient groups of 2–10 cells. Sometimes such groups are united in a single accumulation of cells, moving in the same direction, so-called cell tracts. Cell tracts can be formed in various regions of the sponge mesohyl, but usually are characteristic for regions of growth and leading edge of moving sponge (Bond and Harris 1988; Bond 1992). The only known permanent structures in sponge mesohyl are peculiar cellular strands, described in the genus *Aplysina* (Leys and Reiswig 1998). These strands run through the endosome of the sponge and are composed of elongate cells tightly aligned along bundles of collagen (Figure 2.5). The cells have a permanent position in the strands and do not actively move. According to experiments, the strands are involved in nutrition transport and thus may represent a primitive nutrient transport pathway (Leys and Reiswig 1998).

The mesohyl always comprises well-developed extracellular matrix. A common fibrillar component in mesohyl of all sponges is collagen. The collagen fibrils may be dispersed through the mesohyl or form bundles and tracts. In some species collagen fibrils are the only skeletal elements and are greatly elaborated (see section 2.4.3.2). The mesohyl ground substance is rich in glycoproteins, but also contains fibronectin, various glycosaminoglycans, mucopolysaccharides, proteoglycans, sugars (including the unusual arabinose), amino acids, etc. (Gross et al. 1956; Katzman

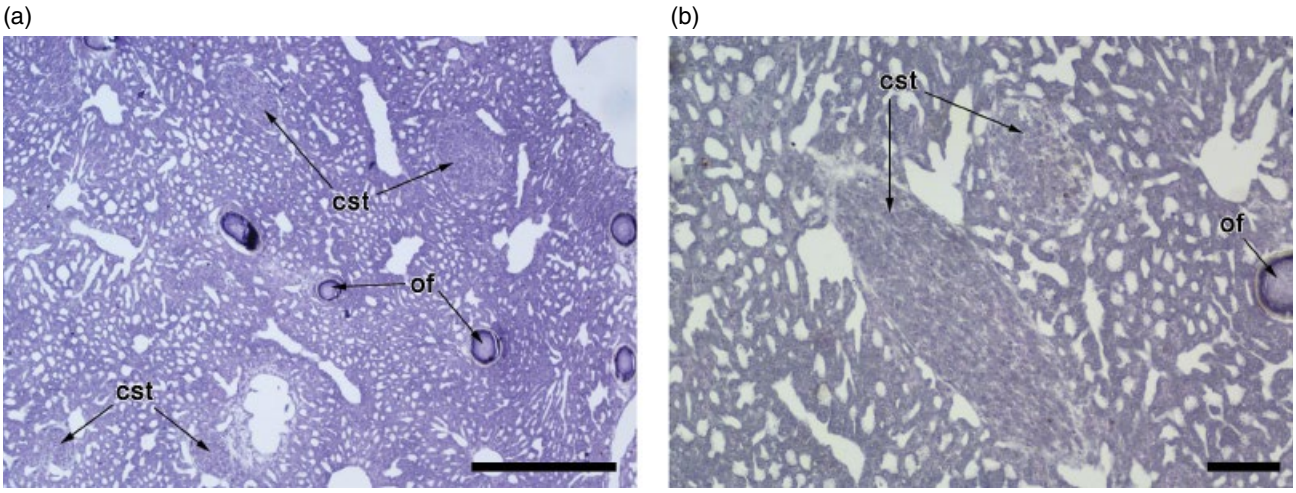

Figure 2.5 Mesohyl cellular strands of *Aplysina cavernicola*. (a) General view of endosome with several cellular strands. (b) Structure of cellular strands. Scale bars: (a) 500 µm; (b) 100 µm.

et al. 1970; Evans 1975; Junqua et al. 1975; Garrone 1978). The composition of ground substance varies by species and even from individual to individual within a single species. It appears likely that a considerable proportion of the components of the mesohyl ground substance is synthesized and released by various cells with specific inclusions (see section 2.3.3.2). The mesohyl ground substance and collagen fibrils represent a basic scaffold for mesohyl cells and may play a crucial role in cell–cell or cell–matrix interactions, immune reaction, self/nonself recognition, cytodifferentiation, cell aggregation (e.g., during formation of gemmules), and possibly other physiologic processes.

The mesohyl varies in cell type and composition, both between and within species. For instance, the mesohyl of calcareous sponges contains few cells, most of which are sclerocytes, while amebocytes and cells with specific inclusions are rare (Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). In homoscleromorphs, the mesohyl contains numerous cells with inclusions, especially vacuolar cells, which can be represented by several types (see Figure 2.7b) (Gazave et al. 2013). Both calcareous and homoscleromorph sponges could lack archaeocytes in their mesohyl. Demosponges usually have highly cellular mesohyl amoebocytes, archaeocytes, skeleton-secreting cells and several types of cells with inclusions, which vary from species to species (Simpson 1984). The intraspecies variations in mesohyl cell composition occur due to different physiologic states of sponge tissues, mainly during the reproduction and life cycles (see section 2.4.4.3).

All sponges are associated with microbial communities, with representatives of 41 different prokaryotic phyla (Thomas et al. 2016; Moitinho-Silva et al. 2017), which are located in the mesohyl, extracellularly, in the ground substance, or in the special cells, bacteriocytes (Lee et al. 2001). Sponge species were observed to harbor dense communities of symbiotic microorganisms in their tissues, while others were almost devoid of microorganisms. The former were termed "high microbial abundance" (HMA) and the latter "low microbial abundance" (LMA) sponges (Hentschel 2003). In HMA sponges, microbial biomass can comprise up to one-third of the total biomass (Vacelet 1975), and bacterial densities are 2–4 orders of magnitude higher than in LMA sponges. Moreover, HMA microbiomes are highly complex, while LMA microbiomes are mainly represented by Proteobacteria and Cyanobacteria (Moitinho-Silva et al. 2017). The sponge-associated microorganisms participate in nutrient cycling, vitamin and secondary metabolism, and chemical defense (Taylor et al. 2007; Webster and Taylor 2012).

2.4 Organ Systems

2.4.1 Body Wall – Ectosome

Sponges lack a body wall, homologous to eumetazoans (because sponges lack embryonic anlages homologous of these animals) (Ereskovsky and Dondua 2006). External surfaces of sponges have an important role in the exchange of particles and gases between the animal and the environment, and may help maintain the constancy of the sponge's internal milieu and separate it from the surrounding water. The exopinacocytes are the main cells of the dermal structures of sponges.

The ectosome is the peripheral zone of a sponge, devoid of choanocyte chambers. This region is directly in contact with the external environment. The internal surface of the ectosome is often separated from the endosome by aquiferous system cavities or vestibules, coated with endopinacocytes.

The ectosome has a very variable thickness. It is reduced to a single exopinacoderm layer with external glycocalyx and thin layer of extracellular matrix in *Oscarella* and asconoid Calcinea (Figure 2.2a,b); it can reach 2–5 mm in some Demospongiae where it is reinforced by a very important spicular or collagenic skeleton (Figure 2.2e,f).

The number of symbiotic microbes may be different in the ectosome and endosome. For example, the remarkable scarcity of bacteria in the ectosome of *Ceratoporella nicholsoni* and *Stromatospongia norae* (Willenz and Hartman 1989), relative to the choanosome, can be compared to the great reduction of bacterial density observed in superficial regions of *Aplysina aerophoba* (Vacelet 1975). In contrast, in sponges with photosynthetic symbionts (unicellular algae or cyanobacteria), the number of symbionts in the superficial regions of the sponge body is much higher in comparison to deeper parts (Sarà and Liaci 1964; Oren et al. 2005).

The ectosome is the general term for describing the peripheral zone of the sponge body. In different sponges, the ectosome shows various modifications, having specific names – for example, in the cortex, the ectosome is reinforced with specific skeletal elements.

The ectosome could include the following structures:

- glycocalyx layer
- cuticle (if present)
- exopinacoderm
- pores or ostia
- dermal membrane
- cortex (if present)
- inhalant canals
- lacunae and subdermal cavity.

The exopinacocytes of all sponges produce an external layer of mucopolysaccharides – the **glycocalyx**, which is continuous along the surface of the pinacoderm. The glycocalyx can have a variable thickness in different seasons and in different species and be modified into a cuticle. It plays a role in the adhesion of external particles to cell surfaces, prior to their phagocytosis (Willenz 1982).

The **cuticle** is a noncellular, amorphous, sometimes fibrillar covering present in some sponges (Figure 2.3a,b). An external cuticle has been recorded in Dictyoceratida (Garrone 1975; Donadey 1982; Teragawa 1986), Verongida (Vacelet 1971), Chondrosiida (Vacelet and Perez 1998), Chondrillida (Ereskovsky et al. 2011; Willenz et al. 2016), Poecilosclerida (Bagby 1970; Turon et al. 1999), and Tetractinellida (Simpson et al. 1985). The cuticle has been recognized as a structure allowing isolation of the sponge tissues from the environment for cell repair, reorganization, or survival during adverse environmental conditions (Vacelet 1971; Diaz 1979). Another possible function of the cuticle is defense against harmful epibionts since it is periodically shed (Connes et al. 1971; Donadey 1982).

The **exopinacoderm** forms the external cover of the sponge (Figure 2.3c,d,f). The surface part of an exopinacocyte is polygonal in shape and covered with a self-secreted glycocalyx. The exopinacocytes can secrete components of the extracellular matrix and synthesize collagen (Garrone 1978; Simpson 1984; Gaino et al. 1986). The exopinacocytes of Homoscleromorpha are closely associated with the underlining dense fibrillar layer, the basal membrane, comprising collagen IV, laminin, and tenascin (Boute et al. 1996). This basal membrane is identical to the lamina reticulata in the basal lamina of the vertebrate epithelia (Figure 2.3d) (Humbert-David and Garrone 1993; Boute et al. 1996).

The exopinacoderm contains **ostia** or pores – numerous microscopic structures 4–100 μm in diameter, through which the water is drawn into the aquiferous system of the sponge. In most Demospongiae and in all Homoscleromorpha, ostia are intercellular (Figures 2.1a, 2.2b,g, 2.3e; see also Figure 2.7a,b,e). In the Calcarea and many Demospongiae, the ostia are formed inside special cylindrical tubular cells, the porocytes (Figure 2.3f) (Jones 1966; Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). The porocytes contact both exopinacocytes and choanocytes or endopinacocytes by their lateral surfaces. In *Sycon coactum*, porocytes can contract in response to mechanical stimulation and treatment with anesthetics (Eerkes-Medrano and Leys 2006). The porocytes of some Demospongiae from order Haplosclerida are flattened cells with a central or peripheral opening, which can open and close like a sphincter (Harrison 1972a; Weissenfels 1980; Willenz and van de Vyver 1982; Langenbruch and Scalera-Liaci 1986; Harrison et al. 1990). Thus, they may constrict or dilate the pore (Harrison 1972b) and influence the rate of flow of environmental water into the sponge.

The **dermal membrane** is a part of the ectosome, which comprises the exopinacoderm and endopinacoderm, lining subdermal spaces (subdermal cavities) and forming the inner surface of the dermal membrane, and thin mesohyl in between these pinacoderms (Figures 2.1a, 2.2c,d) (Bagby 1970; Willenz and Hartman 1989). The mesohyl layer includes a dense fibrillar component, consisting of collagen fibrils scattered between pinacocyte layers (Garrone and Pottu 1973; Garrone and Rozenfeld 1981; Willenz and van de Vyver 1982; Teragawa 1986). The dermal membrane can have a special "dermal skeleton" that differs from the ectosomal skeleton and serves as the diagnostic characteristic in taxonomy. This skeleton is often formed by special categories of spicules (e.g. in Poecilosclerida).

The **cortex** is the superficial specialized reinforced part of the ectosome. It is characteristic for syconoid and leuconoid sponges. The cortex is not an obligatory structure for all

Porifera; if present, it occupies the area immediately below the exopinacoderm and consists of a specialized portion of the mesohyl. The cortex can be highly structured and contains (i) layers or tracts of cells (Figure 2.2c), (ii) special skeletal elements (could include special spicules that are absent in other body parts) (Figure 2.2e), (iii) spongin fibers or exceptionally dense fibrils (Figure 2.2f,g), (iv) a combination of these, and (v) occasionally foreign material embedded into the tissue (Figure 2.2h) (Teragawa 1986). In some species the cortex has variable thickness (10–100 μm).

The cortex can comprise particular cell types, including lophocytes (Paris 1961), degenerating spongocytes (Connes et al. 1972), spherulous cells, and granular cells (Simpson 1968). The particular skeletal elements of the cortex may be represented by specific types of inorganic microscleres (e.g. Tetractinellida, Poecilosclerida) or organic fibrils (e.g. Ircinia, Dysidea – Dictyoceratoda) in a special arrangement.

The cortex presumably acts as a special supportive device for openings of the canal system and as a protective layer (Vacelet 1971) with the result that superficial injury does not involve the choanocytes.

2.4.2 Aquiferous System

2.4.2.1 Types of Aquiferous System

The circulatory aquiferous system is the most characteristic feature of the poriferan organization (Figure 2.1a). Water drawn into the inhalant canals via ostia moves through inhalant canals to choanocyte chambers and then, via the system of exhalant canals, to the large exhalant opening – the osculum. The aquiferous system brings water through the sponge to the cells responsible for food gathering and gas exchange. At the same time, excretory and digestive wastes are expelled by way of the water currents. The unidirectional flow of water is ensured by the coordinated beating of the choanocytes' flagella. The aquiferous system is a modular, easily rearranged system (Gaino et al. 1995; Plotkin et al. 1999; Ereskovsky 2003).

The aquiferous system is situated, mainly, in the endosome (choanosome) – the internal region of a sponge, comprising the choanocyte chambers. The aquiferous system consists of three main parts: (i) inhalant system – canal system between ostia and prosopyle (entrance of choanocyte chamber); (ii) choanocyte chambers; and (iii) exhalant system – canal system between the apopyle (exit from choanocyte chamber) and osculum.

However, some representatives of the families Cladorhizidae and Esperiopsidae (Poecilosclerida, Demospongiae) lack all the elements of the aquiferous system (Vacelet 2006, 2007; Ereskovsky and Willenz 2007) due to the change of their nutrition strategy from water pumping to predation.

Five types of aquiferous system occur in sponges:

1) asconoid (Greek, skin bag) – ostia lead directly to the internal cavities completely lined with choanoderm, which open via an osculum (Figure 2.6a)
2) solenoid (Greek, tube) – ostia open to a network of anastomosed tubes completely lined by choanoderm (i.e., choanocyte tubes), which lead into an atrium lined with endopinacoderm, opening via an osculum (Figure 2.6b)
3) syconoid (Greek, fig) – ostia open directly to radially elongated choanocyte chambers or to short inhalant canals, which are connected to elongated choanocyte chambers via prosopyles; the choanocyte chambers are connected to a single atrium via apopyles; the atrium, lined with endopinacoderm, opens via an osculum (Figure 2.6c)
4) sylleibid (Greek, collect + ibi) – ostia lead to short inhalant canals which connect to prosopyles of choanocyte chambers, arranged radially around large exhalant canals; chambers are connected to exhalant canals via apopyles; the exhalant canals lead to the atrium, opened via oscula (Figure 2.6d)
5) leuconoid (Greek, a disease like elephantiasis) – ostia open to short inhalant canals or to large subdermal cavities, leading to inhalant canals; inhalant canals are connected via prosopyles to numerous small choanocyte chambers, scattered in the mesohyl; choanocyte chambers lead to exhalant canals via apopyles; exhalant canals open to atria which lead to oscula (Figure 2.6e).

2.4.2.2 Histology, Cell Types, Arrangement, Extracellular Structures

2.4.2.2.1 Canals of Aquiferous System: Inhalant System

Incurrent openings – ostia, pores. These structures could be intracellular, formed by special cells, porocytes (Figure 2.3f), or intercellular, formed as an opening between adjacent pinacocyte margins (Figure 2.7a,b,e). However, in some demosponges, such as *Eunapius fragilis* (Spongillida), both types of ostia occur (Harrison and de Vos 1991). The ostia have the ability to open and close within a relatively short period of time for flow-regulating purposes (Harrison 1972a; Weissenfels 1980). Hence number and diameter of ostia within specimens appear highly variable at any given time.

In some demosponges (e.g., order Poecilosclerida), the ectosome forms specialized inhalant structures: (i) pore sieve (fr. crible) – a contractile cluster of ostia, located on the sponge surface; (ii) pore groove – a furrow on the sponge surface, where the ostia are located (Boury-Esnault 1972); (iii) poral face – a specific surface on the sponge body, where all ostia are located (e.g., family

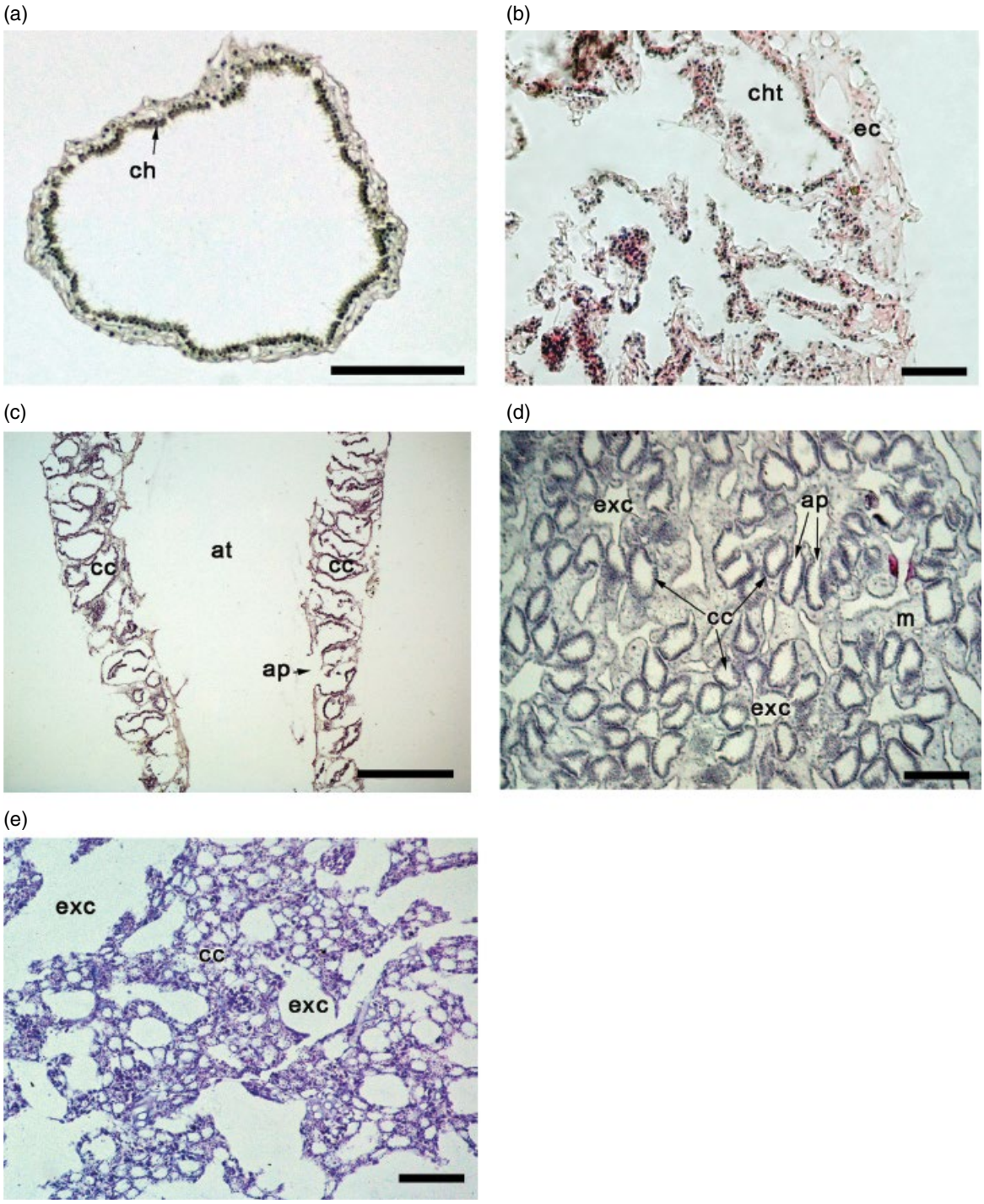

Figure 2.6 Types of aquiferous systems in sponges. (a) Asconoid aquiferous system in *Clathrina clathrus*. (b) Solenoid aquiferous system in *Leucascus* sp. *Source:* Image courtesy of M. Klautau. (c) Syconoid aquiferous system in *Sycon ciliatum*. (d) Sylleibid aquiferous system in *Oscarella tuberculata*. (e) Leuconoid aquiferous system in *Myxilla incrustans*. Scale bars: (a,d,e) 100 μm; (b) 500 μm; (c) 50 μm;

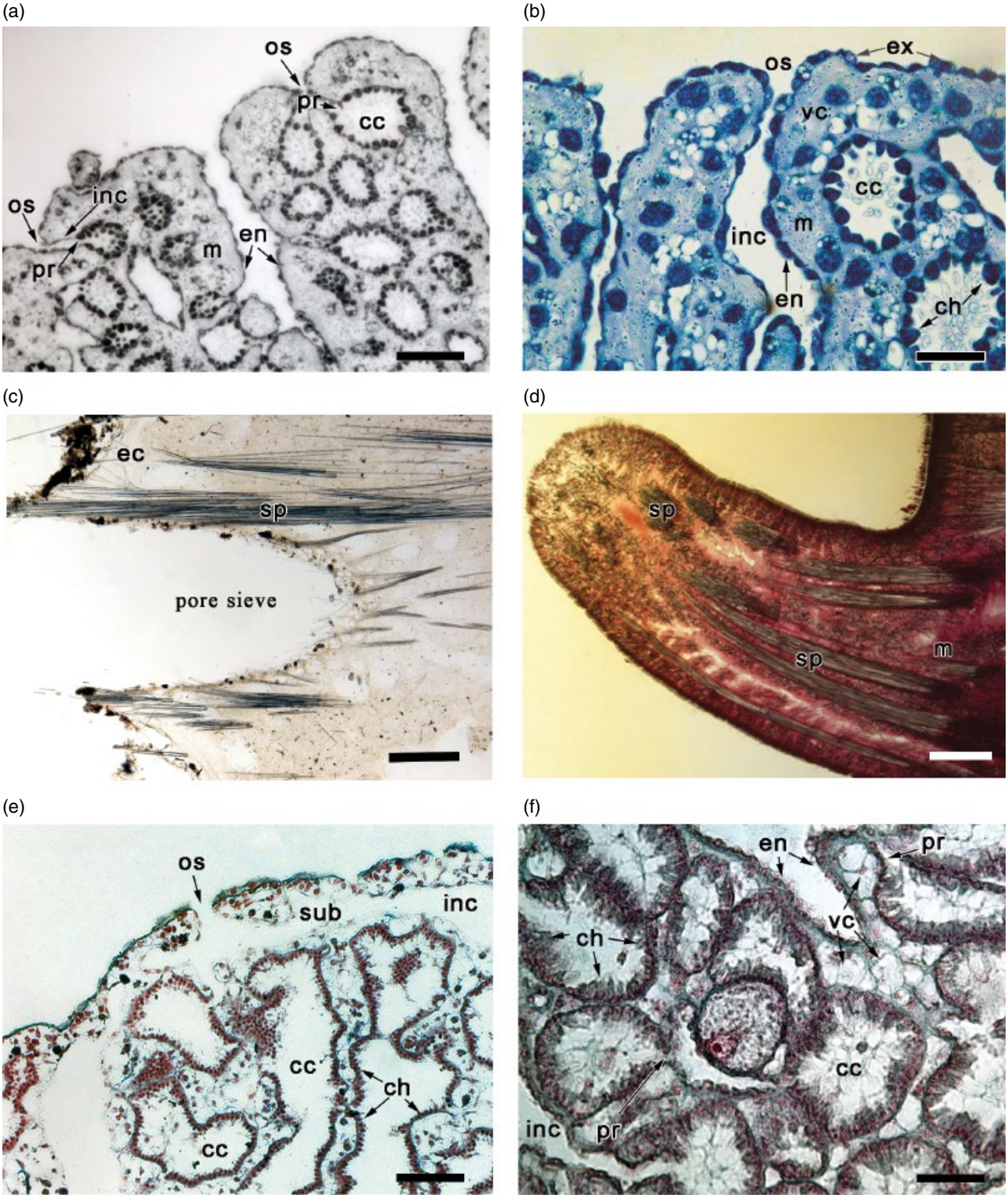

Figure 2.7 Inhalant aquiferous system. (a,b) Ostia and inhalant canals lined with prosendopinacocytes in *Oscarella lobularis*. (c) Porocalyx in *Cinachyrella apion*. *Source:* Image courtesy of Paco Cardenas. (d) Papilla of *Proteleia sollasi*. *Source:* Reproduced with permission from Plotkin et al. (2016). (e) Pores, inhalant canals and subdermal (vestibular) cavities in *Halisarca dujardinii*. (f) Prosopyles in *Halisarca dujardinii*. Scale bars: (a) 100 μm; (b,f) 50 μm; (c) 20 μm; (d) 1 mm; (e) 200 μm.

Thorectidae, order Dictyoceratida) (Cook and Bergquist 2002). All mentioned inhalant structures are underlaid by a large inhalant cavity, the vestibule (Boury-Esnault 1972).

Another type of specialized inhalant structure is the porocalyx which is a circular, poriferous depression in the ectosome, disturbing the cortex structure and appearing as a distinctive oval or flask-shaped pit. The porocalyx bottom contains the inhalant and, occasionally, also exhalant orifices. Porocalices may be contractile. These structures are typical for some demosponges from family Tetillidae (order Tetractinellida) (Figure 2.7c) (Rutzler 1987).

In some demosponges there are special structures associated with the aquiferous system – papillae which are nipple-like protuberances projecting from the sponge surface and bearing either ostia, oscula, or both, discussed in detail by Simpson (1984) (Figure 2.7d).

Inhalant canals lead from ostia to choanocyte chambers (Figures 2.7a,b, 2.8b,f). In most sponges, ostia open directly into the inhalant canals. However, in many cases smaller canals, called canalicules, connect ostia with the larger inhalant canal. The inhalant canals and canalicules are lined by special endopinacocytes called prosopinacocytes. The prosopinacoderm often has intercellular gaps, allowing water flow between the canal lumen and mesohyl.

Subdermal (vestibular) cavities. Most demosponges possess a leuconoid canal system, in which inhalant water, passing through ostia, enters a large subepithelial space, the incurrent vestibule or subdermal cavity. These structures are located just below the exopinacoderm or dermal membrane and are lined with prosopinacocytes (Figures 2.2c,d,f,g, 2.7e).

The **prosodus** (Greek *prosodos*, procession) is a tiny canal connecting the larger inhalant canal with the entrance to the choanocyte chamber, a prosopyle. Prosodus occurs only in sponges with leuconoid aquiferous systems.

The **prosopyle** (Greek *prósō*, forward + *pýlē*, gate) is the opening through which water enters a choanocyte chamber (Figures 2.7a,f, 2.8b). Prosopyles can be formed by prosopinacocytes, that contact the choanocytes and form a structure like a pore (pinacocytic prosopyle), as in *Tethya wilhelmia* (Hammel and Nickel 2014), or by pseudopodial extensions between adjacent choanocytes, forming small gaps (choanocytic prosopyles), as in *Petrosia ficiformis* (Langenbruch and Scalera-Liaci 1990).

2.4.2.2.2 Choanocyte Chambers According to Boury-Esnault and Rützler (1997), a choanocyte (= flagellated) chamber is any cavity lined by choanocytes and located between the inhalant and exhalant systems. The structure of choanocyte chambers and the way they are attached to each canal are characteristic of sponge families and genera (Boury-Esnault et al. 1984, 1990).

In sponges with asconoid and solenoid aquiferous systems, it is possible to consider all the internal cavities, lined by choanocytes as one branching choanocyte tube (Figure 2.6a,b).

In sponges with syconoid aquiferous systems, the large choanocyte chambers are ovoid and lie perpendicular (radially) to the central atrial cavity (Figures 2.6c, 2.8a).

The choanocyte chambers of the sponges with sylleibid and leuconoid aquiferous systems are categorized according to the type of their connection to the inhalant and exhalant canal systems (Sollas 1888). There are three main types of chamber organization.

1) In the aphodal choanocyte chamber, water enters directly from inhalant canals through prosopyles and leaves through a narrow canal, the aphodus, which lies between the chamber and exhalant canal (*Plakina trilopha*) (Figure 2.8d).
2) In the diplodal chamber, water enters by a prosodus and leaves through the aphodus, connecting the chamber with the exhalant canal (Figure 2.8e). The aphodal and diplodal choanocyte chambers are delimited externally by the mesohyl (*Pseudocotricium jarrei*, *Chondrosia reniformis*, *Tethya wilhelma*).
3) In the eurypylous choanocyte chamber, water enters from the inhalant canal directly through prosopyles (typically, these are spaces between choanocytes and termed choanocytic prosopyles) and leaves through a large apopyle, that opens directly to the large exhalant canal (*Spongilla lacustris*, *Oscarella* spp., *Aplysina*) (Figure 2.8b,c,f). Two types of eurypylous choanocyte chambers are known.
   - The first type, which we propose to call a mesohylar eurypylous choanocyte chamber, is characterized by direct contact of choanocytes with the mesohyl (e.g., Homoscleromorpha, Verongida) (Figure 2.8b,c).
   - In the second type, which we propose to call a canal eurypylous choanocyte chamber, described in many Haplosclerida, the choanocyte chambers lie free in the lumen of inhalant canals and are separated from the mesohyl by endopinacocytes of these canals (Langenbruch 1988, 1991; Langenbruch and Jones 1990) (Figure 2.8f). In these chambers choanocytes are arranged in a regular hexagonal pattern with uniform interstitial spaces around each cell – choanocytic prosopyles. In this case two modes of organization are observed: (i) the choanocyte chamber is completely enveloped by pinacocytes (e.g., *Haliclona elegans*, *Haliclona mediterranea*, *P. ficiformis*), and (ii) choanocyte epithelia partially enveloped by pinacocytes (e.g., *Haliclona fulva*, *Niphates digitalis*) (Langenbruch 1991). In the Homoscleromorpha,

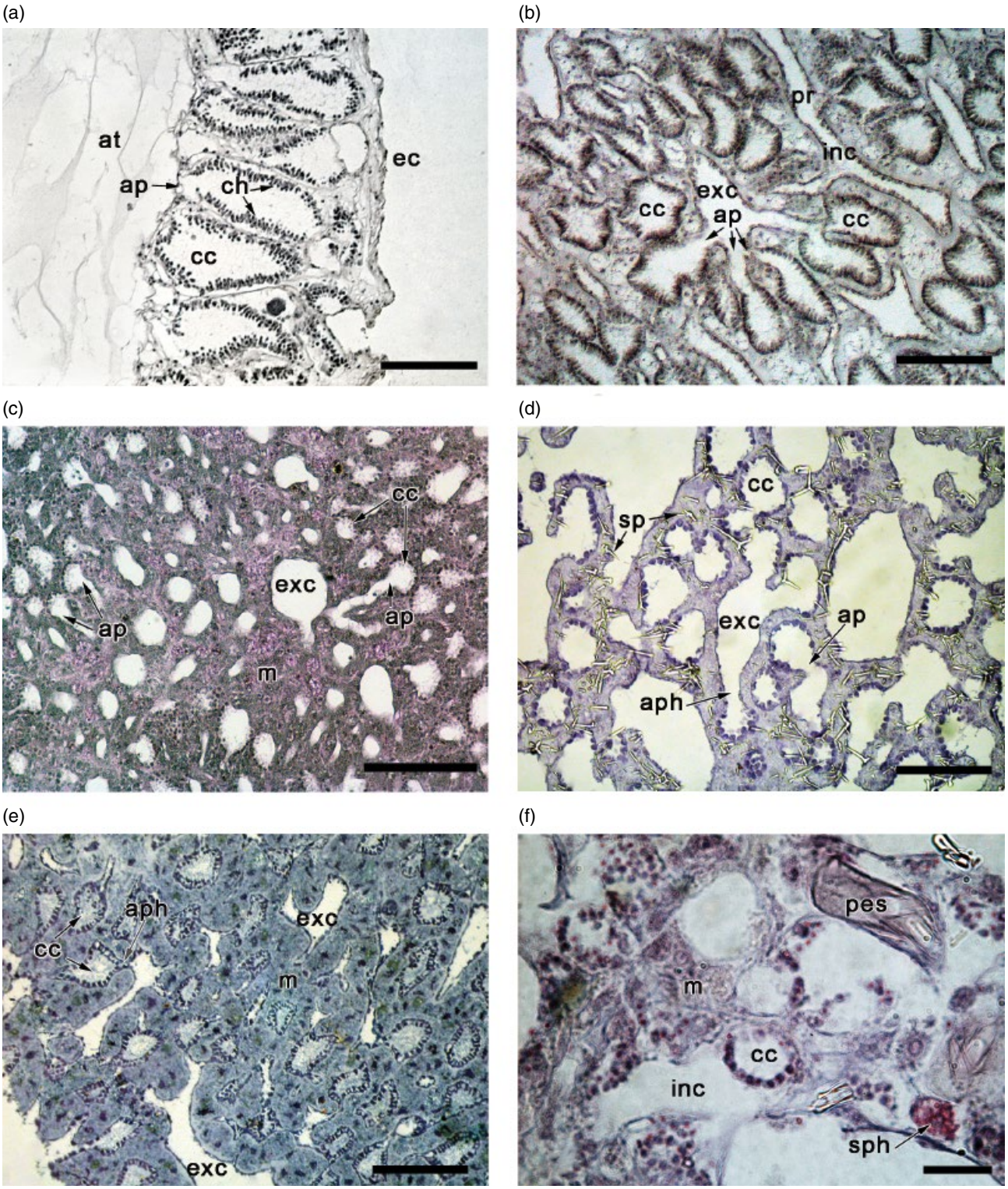

Figure 2.8 Choanocyte chambers. (a) Syconoid choanocyte chambers in *Sycettusa murmanensis*. (b) Mesohylar eurypylous choanocyte chambers in *Oscarella tuberculata*. (c) Mesohylar eurypylous choanocyte chambers in *Aplysina cavernicola*. (d) Aphodal choanocyte chambers in *Plakina trilopha* with the spicules dispersed in the mesohyl. (e) Diplodal choanocyte chambers in *Pseudocorticium jarrei*. (f) Canal eurypylous choanocyte chambers in *Haliclona fulva*. Scale bars: (a,b,c,d) 100 μm; (e) 50 μm; (f) 20 μm.

choanocytes are connected to each other by close junctions along their lateral surfaces, and there is a basement membrane and collagenous mat covering the chamber (Figure 2.7b).

Choanocyte chambers of leuconoid sponges can have different shapes and sizes, from small and spherical or ovoid (Figures 2.6e, 2.8f) to long and tubular (Figure 2.7e). The volume of chambers within different species varies between 350 (Agelasida) and 480 000 μm^3 (Halisarcidae) (Boury-Esnault et al. 1990). Halisarcidae have tubular branched choanocyte chambers, which are the largest known chambers in Demospongiae (Figure 2.7e; see also Figure 2.12a) (Ereskovsky et al. 2011). Larger chambers are found in the sponges with a less extensive mesohyl. Small chambers occur mostly in sponges with a dense mesohyl. Differences also exist in choanocyte size and shape, collar and flagellar length and ornamentation, and anchorage of the choanocytes in the mesohyl (Boury-Esnault et al. 1984, 1990). The number of choanocytes per chamber varies from 5 (Agelasida) to 2800 (Halisarcidae) (Boury-Esnault et al. 1990).

2.4.2.2.3 Canals of Aquiferous System: Exhalant System The **apopyle** (Greek *apo*, from + *pylē*, gate) is an opening in a choanocyte chamber, connecting it with an exhalant canal. The apopyles are larger than prosopyles. De Vos et al. (1990) described three types of apopyle organization. The first is characterized by the absence of apopylar cells. In this case the choanocytes and apopinacocytes directly contact each other. This type of apopyle is found in demosponges from the orders Suberitida, Clionaida, Tetractinellida, and Axinellida. In the second type, a structure of apopylar cells connects choanocytes and apopinacocytes and each has a flagellum directed toward the exhalant canal. This type was described in Homoscleromorpha, Dysideidae, Aplysillidae, and Halisarcidae (Figure 2.8b–e). The third type of apopyle is found in *Ephydatia fluviatilis* (Spongillida) and *Petrosia ficiformis* (Haplosclerida). In this case, the apopylar cells are completely immersed inward, so that they are invisible from the outside and give a specific cone shape to the apopylar opening from the inside (Langenbruch et al. 1985). The localization of these cells suggests that they play a role in controlling water current. In addition, in *Tethya wilhelma* the apopyle also has a reticuloapopylocyte (see above) (Hammel and Nickel 2014).

The **aphodus** (plural **aphodi**; Greek *aphodos*, departure) is the short canal leading from an apopyle of a choanocyte chamber to an exhalant canal (Figure 2.8d,e).

Exhalant canals gather water from the choanocyte chambers and lead it to larger canals, which finally merge in a large atrial cavity, underlying an osculum (Figure 2.8b–e). Exhalant canals are lined by apopinacocytes, which in some sponges may bear a flagellum (Donadey 1979; Boury-Esnault et al. 1984; Hammel and Nickel 2014). The lining of the exhalant canals structurally appears more homogenous than the lining of inhalant canals and may contain porocytes, connecting the canal lumina with the mesohyl.

The **atrium** is the central exhalant, preoscular cavity. It is not characteristic of sponges with asconoid aquiferous systems. The atrium is lined with apopinacocytes and is roofed by the exhalant dermal membrane (Figures 2.1a, 2.6c, 2.9c). In some cases, mostly in encrusting sponges, the large exhalant canals merging at the atrium run parallel to the sponge surface, thus forming radiating "astrorhizae."

The **osculum** is a large opening through which the water leaves a sponge. Oscula are bounded externally by exopinacocytes, while their inner surface is formed by endopinacocytes (Figure 2.1a–c,e). The oscula of some sponges are lined internally by ciliated cells as in Hexactinellida (Leys et al. 2007), Homosclermorpha (Boury-Esnault et al. 1984), and freshwater sponges (Saller 1990; Ludeman et al. 2014). It is supposed that these ciliated cells have sensory functions (Ludeman et al. 2014).

In the oscular rim of *Microciona prolifera*, *Tedania ignis*, and some other sponges, contractile cells – myocytes – are present (Bagby 1966) (see section 2.3.3). These cells can change the diameter of the oscular opening, thus controlling water currents through the aquiferous system. The freshwater sponge *E. fluviatilis* has no myocytes but does have contractile pinacocytes with actin bundles in its oscular diaphragm (Masuda et al. 1998).

2.4.3 Skeleton

The typical sponge skeleton is a composite of organic and inorganic materials that form a scaffold-like framework supporting the sponge body. The organic material is composed of various types of fibrillar collagen, which can form fibers up to several millimeters in thickness. This proteinaceous material provides a flexible but sturdy matrix for the sponge skeleton. The inorganic material is composed of either silica (SiO_2) or calcium carbonate ($CaCO_3$) in the form of calcite or aragonite. Sponges are the only animals that use hydrated silica as a skeletal material.

The distinction of inorganic material has important taxonomic and phylogenetic value. About 92% of all living sponge species are siliceous. Sponges belonging to the classes Demospongiae, Hexactinellida, and Homoscleromorpha produce siliceous spicules although some demosponges (*Chondrosia, Halisarca, Hexadella, Myxospongia*) and homoscleromorphs (*Oscarella, Pseudocorticium, Aspiculophora*) have lost the inorganic component of the skeleton. All representatives of the class Calcarea

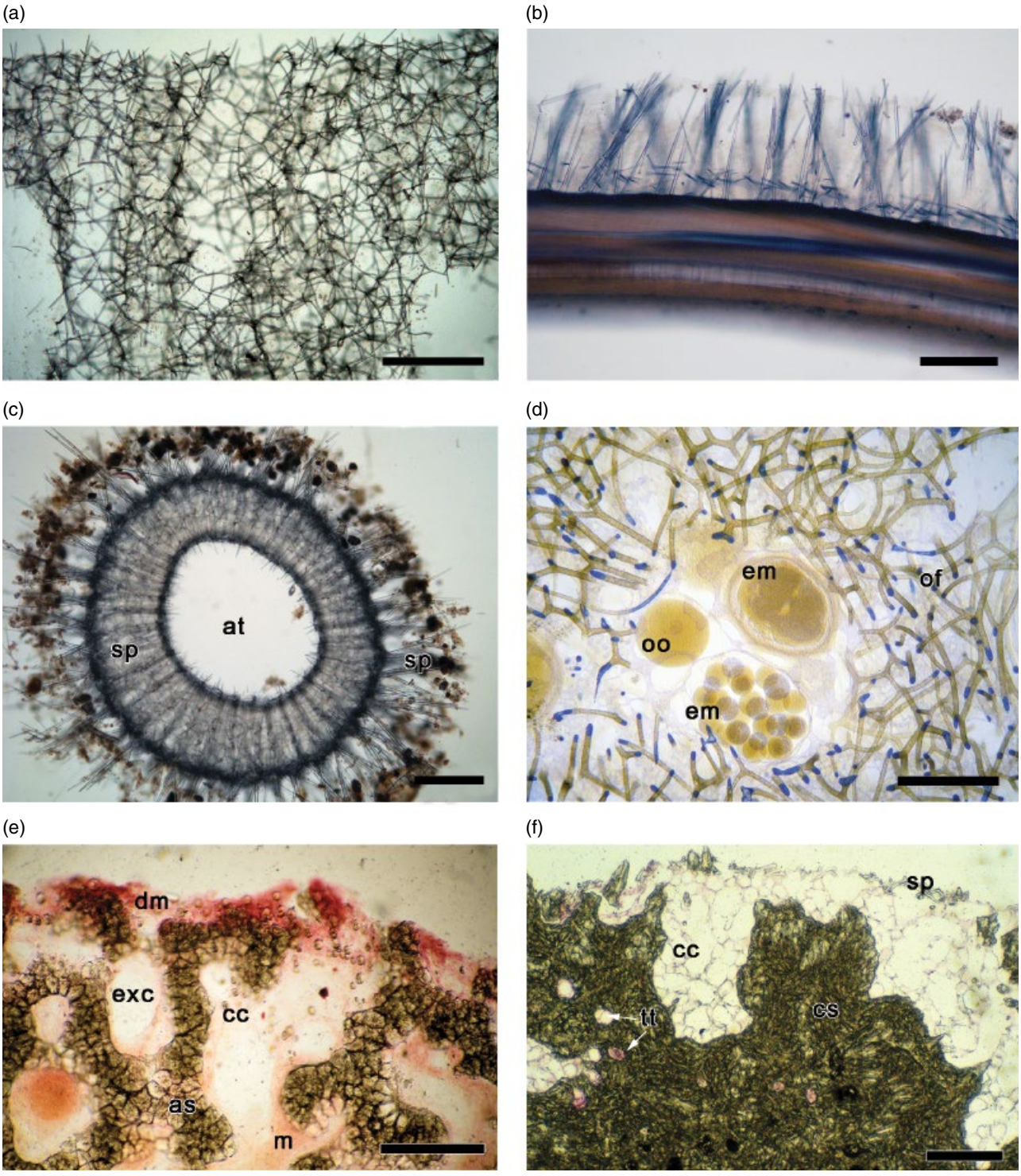

Figure 2.9 Skeleton. (a) Inorganic skeleton (SiO_2) of *Haliclona* sp. with regular reticulation of multispicular tracts of megascleres. (b) Inorganic skeleton (SiO_2) of *Protosuberites mereui* with choanosomal skeleton of brushes of tylostyles, erected from the substrate. (c) Inorganic skeleton ($CaCO_2$) of *Sycon vigilans* with radial organization around a central atrium (at). (d) Organic skeleton of *Spongia officinalis* with homogeneous skeletal fibers. (e) Hypercalcified *Ceratoporella nicholsoni* (Demospongiae) with aragonitic skeleton. *Source:* Image courtesy of J. Vacelet. (f) Hypercalcified *Petrobiona massiliana* (Calcarea). *Source:* Image courtesy of J. Vacelet. Scale bars: (a) 500 µm; (b,c) 200 µm; (d,e,f) 300 µm.

produce calcitic spicules. Spicules may be either dispersed in the mesohyl (Figure 2.8d) or assembled into a defined three-dimensional framework structuring the soft tissue (Boury-Esnault and Rützler 1997; Uriz 2006). Spicules are divided into megascleres and microscleres, according to their size, morphology, and role in the skeletal framework. Megascleres are usually assembled into tracts (Figure 2.9a,b) and maintain the gross form of the sponge, while microscleres are dispersed throughout the sponge body and support various microanatomical structures (Figure 2.2e). In addition to a spicular skeleton, some demosponges and calcareous sponges develop a massive basal skeleton, composed of calcite or aragonite.

2.4.3.1 Inorganic Skeleton

The inorganic skeleton in the form of siliceous spicules in Demospongiae includes about 12 basic types of megascleres and 28 types of microscleres; in Homoscleromorpha, about four megascleres; in Hexactinellida, 20 basic types of megasclere and 24 types of microscleres (Boury-Esnault and Rützler 1997; Tabachnick and Reiswig 2002). Megascleres in both demosponges and hexactinellids usually form the main sponge skeleton (Figure 2.9a,b). Spicules can be joined by spongin (demosponges), fuse (some hexactinellids), or articulate with each other (Lithistidsa). Microscleres may be widespread in the sponge body or are concentrated in the ectosome-forming crusts of the cortex (Figure 2.2e) or spread in the choanosome. In demosponges there are six elemental types of spicule frameworks, with intermediate forms, that can be differentiated: hymedesmoid, plumose, axial, radiate, reticulated, and disarranged (Boury-Esnault and Rützler 1997; Uriz 2006). In some demosponges (e.g., Lithistida) and many Hexactinellida, the spicules may be linked or fused into such a rigid framework that it is capable of fossilizing.

Skeletons made up of calcium carbonate usually appear in the form of networks of spicules or rarely can be massive, occurring in combination with spicular elements. Calcareous spicules are made of calcium carbonate, mainly crystallized as magnesium-rich calcite (Jones and Jenkins 1970). The majority of Calcarea have a skeleton composed of free spicules, without calcified nonspicular reinforcements. Frameworks in most calcareous sponges are simple and delicate but well organized, with several spicule types localized in particular regions of the sponge body. The skeleton in general has radial organization around a central atrium (Figure 2.9c).

In addition to a spicular skeleton, some representatives of the Demospongiae and Calcarea secrete a massive basal skeleton, composed of calcite or aragonite. All such species are referred as hypercalcified sponges. Living hypercalcified sponges are restricted to deep or cryptic habitats like bathyal cliffs, sublittoral dark caves, and coral reef tunnels (Vacelet et al. 2010).

In living hypercalcified Demospongiae, several morphologic types or grades of organization are represented (Vacelet et al. 2010). The chaetetid type corresponds to laminar or domical sponges in which the superficial parts of the skeleton display a honeycomb structure, with more or less hexagonal tubes, somewhat resembling the corallites of scleractinian corals but smaller. The living tissue occurs as a thin veneer at the surface and within the outer parts of the tubes (Figure 2.9e). This type is known in the Ceratoporellidae, Merliidae, and Acanthochaetetidae.

The stromatoporoid type is found in domical to flattened, laminar sponges with a calcified skeleton consisting of a meshwork of tubes, pillars, and laminae. This type is known in Calcifibrospongia and Astrosclera.

In the sphinctozoid type found in Vaceletia (Dictyoceratida), the skeleton is external, resulting in a discontinuous growth, with separate chambers linked by a central siphon.

Only a few living calcareous sponges of the orders Murrayonida (Calcinea), Lithonida, and Baerida (Calcaronea) secrete massive or reinforced calcareous skeletons (Vacelet et al. 2002a, b). In Murrayonida, the basal skeleton reticulates with a meandering structure made up of fused, irregularly shaped calcitic sclerodermites, generally without entrapped spicules. In Baerida, the basal skeleton is composed of a solid mass of calcite, consisting of spiny elongated or irregular sclerodermites that form a series of crests between which lies the living tissue (Figure 2.9f).

2.4.3.2 Organic Skeleton

In addition to an inorganic skeleton, all sponges have a collagenous organic skeleton. This collagenous skeleton may include two types of the fibrils: (i) "classic" collagen fibrils of 20–25 nm in diameter, which are cross-striated ultrastructurally (striations are not visible by light microscopy) and (ii) thin (10 nm) spongin fibrils.

The fibrillar collagen is located throughout the mesohyl of the sponge and is the only form of organic skeleton characteristic for all sponge classes (Garrone 1985). Such fibrils are always presented in the sponge mesohyl, while the extent to which fibrillar collagen reinforces the sponge varies in different classes and species. In Calcarea, the fibrillar collagen is always lightly dispersed through the mesohyl, never occurring in dense concentration, except for the formation of sheaths around spicules (Jones 1967; Ledger 1974). In contrast, in some demosponges (e.g., *Halisarca*) and homoscleromorphs (e.g., *Oscarella*), which are devoid of inorganic and spongin skeletons, the fibrillar collagen plays a central role as a skeleton component and is greatly elaborated (Bergquist 1996). In such cases, collagen

fibrils are usually organized into bundles, which are interlaced and form complex three-dimensional supporting structures (Garrone et al. 1975).

In contrast to fibrillar collagen, which shows more or less the same structure in all sponges, the spongin skeletal structures are diverse, but occur only in demosponges. Spongin is a collagenous protein (Exposito et al. 2002), which was called "spongin B" by Gross et al. (1956). According to Garrone (1978), the spongin microfibers can appear in demosponges in five different states.

1) Spongin microfibrils form an adhesive layer, which attaches sponges to their substrate. In this form, spongin appears in all demosponges (Borojevic and Lévi 1967; Garrone 1985). However, the participation of chitin in the formation of a sponge holdfast has been proposed in Lubomirskiidae freshwater sponges (Ehrlich et al. 2013).
2) Another widespread form of spongin is perispicular spongin, characteristic of demosponges with inorganic skeletons, composed of spicules (Figure 2.8f). Usually, the perispicular spongin is deposited at the points of intersection of spicules, incorporating their ends, thus uniting single spicules to integrate the skeleton (Weissenfels 1978; Willenz and Hartman 1989; Galera et al. 2000). In some cases, perispicular spongin can be highly developed and form spiculated fibers. The spiculated fibers are macroscopic spongin structures, which envelop whole spicule tracts. Occasionally, the spiculated fibers can be very wide with only a thin row of spicules in the center (Garrone 1969, 1978; Garrone and Pottu 1973).
3) In some demosponges (e.g., orders Verongiida, Dendroceratida, Dictyoceratida) spongin appears as macroscopic fibers, reaching a thickness of several millimeters. These fibers begin from the basal adhesive layer of spongin but are submersed into the sponge body (Figures 2.2g, 2.9d). In some species (e.g., freshwater sponges), all spongin fibers inside the body are covered with a continuous epithelium, connected with the basopinacoderm. Thus, in these species the whole spongin skeleton is an exoskeleton (Weissenfels 1978; Garrone 1985). The spongin fibers form a complex skeleton with hierarchic structure and of either a dendritic or an anastomosing pattern. The internal structure of the fibers varies: they can have fine fibrillar pith (central area of a fiber, made up of more or less diffuse wisps of collagen or of a coarsely granular collagenous material), surrounded by the laminar bark (the dense area of compacted spongin, in which concentric layers are visible) (e.g., Dendroceratida, Verongiida) (Figure 2.2g), or be homogenous (e.g., Dictyoceratida) (Garrone 1978). In Verongiida, the outermost layer of the fiber is composed of the chitin, making them more rigid and chemically resistant (Ehrlich et al. 2007). The content of chitin in the fibers varies between 10% and 60% depending on the species (Ehrlich et al. 2018). Occasionally, cellular (degenerate spongocytes) elements or exogenous particles (sand grains) (Cerrano et al. 2007) are incorporated into the fibers.
4) In contrast to fibers, which are organized into a continuous skeleton, spongin can appear in the form of individual macroscopic skeletal elements. In the genus Ircinia, in addition to fibers, spongin appears as macroscopic filaments, which are up to several millimeters in length and dozens of micrometers thick and terminate with a knob at each end (Garrone et al. 1973; Junqua et al. 1974). Another form of individual spongin macrostructure is spiculoids of the genus Darwinella, which are regular diactinal, triactinal, and tetractinal structures, resembling the siliceous spicules (Bergquist 1996).
5) The last spongin formation is a deposition of its fibrils in the gemmular coat. In this case spongin microfibers are densely packed and form thick layers around the forming gemmule (see section 2.3.3.1).

2.4.4 Reproductive System

For sponges, both asexual and sexual reproductions are characteristic. Sexual reproduction is fundamentally the same as similar processes in other multicellular animals. No sexual dimorphism exists in sponges. Sponges can be oviparous (Figure 2.10a) and viviparous (brooding) (Figures 2.10 c,d, 2.11c,d, 2.12b). In the first case, the sponges are usually gonochoric (Figure 2.10a), while in the second, they are often hermaphrodites (Figure 2.10b) (Ereskovsky 2010, 2018). Viviparous sponges release larvae and oviparous sponges release zygotes or unfertilized eggs. Embryonic development in the oviparous sponges is always external, leading to free-swimming larvae. Viviparous or ovoviviparous sponges are characterized by brooding of embryos in the mesohyl or inside a special temporary structure – follicles (Ostrovsky et al. 2016). The resulting free-swimming larvae exit through exhalant canals of the aquiferous system. Direct development without a larval stage exists in some demosponges (Sarà et al. 2002).

The only common feature for all sponge species is the absence of organized gonads; gametogenesis is usually diffuse. A characteristic feature of this process in sponges is the origin of gametes by direct transformation from the somatic cells – choanocytes or, rarely, archaeocytes. Otherwise, the stages and cytologic features of gamete development in sponges are similar to those in other animals (Boury-Esnault and Jamieson 1999; Maldonado and Riesgo 2008; Ereskovsky 2010).

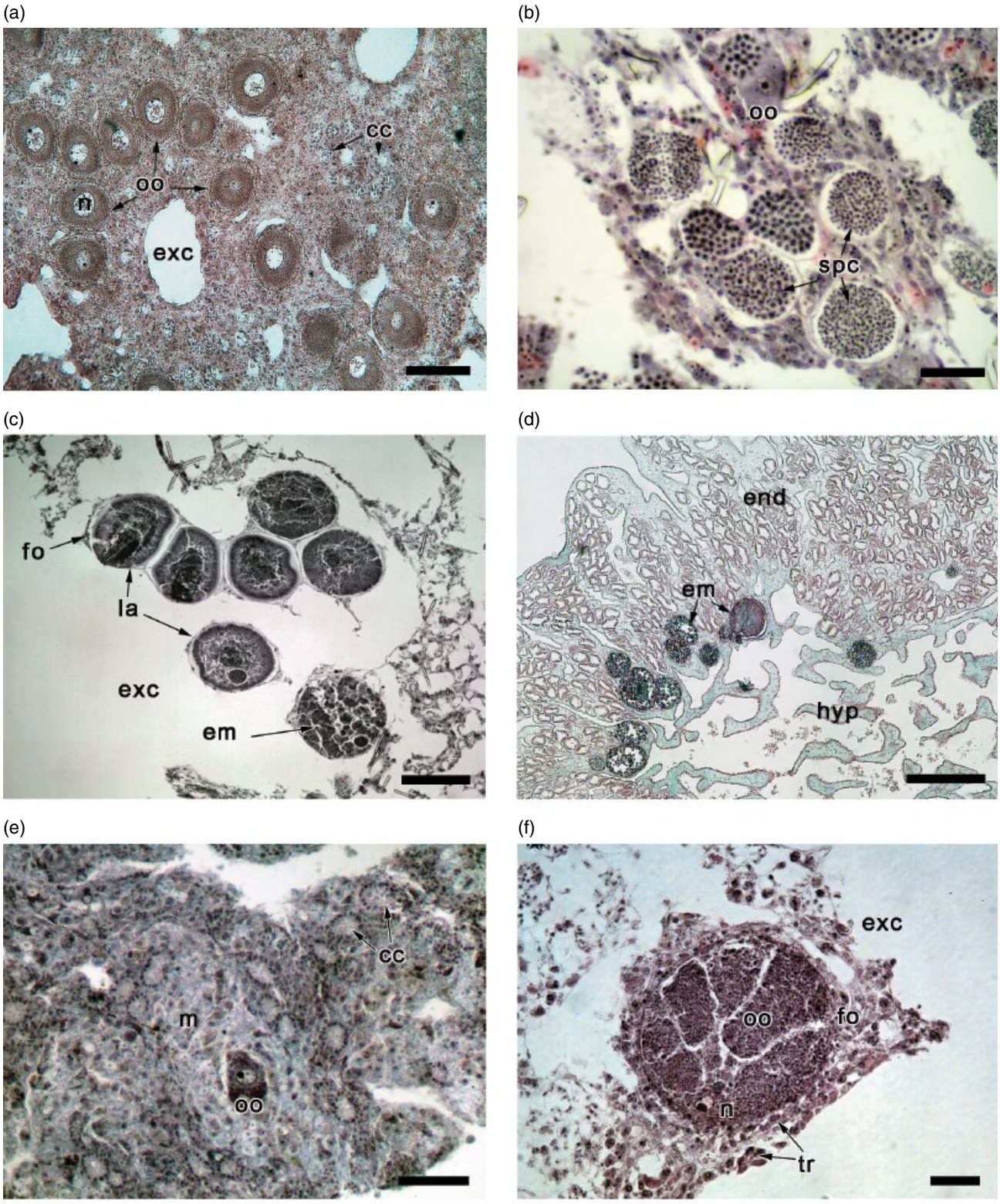

Figure 2.10 Reproduction, female. (a) Oviparous gonochoric sponge *Aplysina cavernicola* with oocytes diffusely distributed in the endosome. Note the aquiferous system degradation. (b) Viviparous hermaphrodite sponge *Esperiopsis koltuni* with oocyte and spermatocysts diffusely distributed in the endosome. (c) Viviparous *Haliclona aquaeductus* with the clusters of embryos and larvae in brooding chamber. (d) Basal position of embryos in the endosome of *Oscarella tuberculata*. (e) Amoeboid-like oocytes in *Crellomima imparidens*. (f) Amoeboid cells and trophocytes, concentrating around the egg of *Haliclona aquaeductus*. Scale bars: (a) 100 μm; (b,e,f) 50 μm; (c) 250 μm; (d) 300 μm.

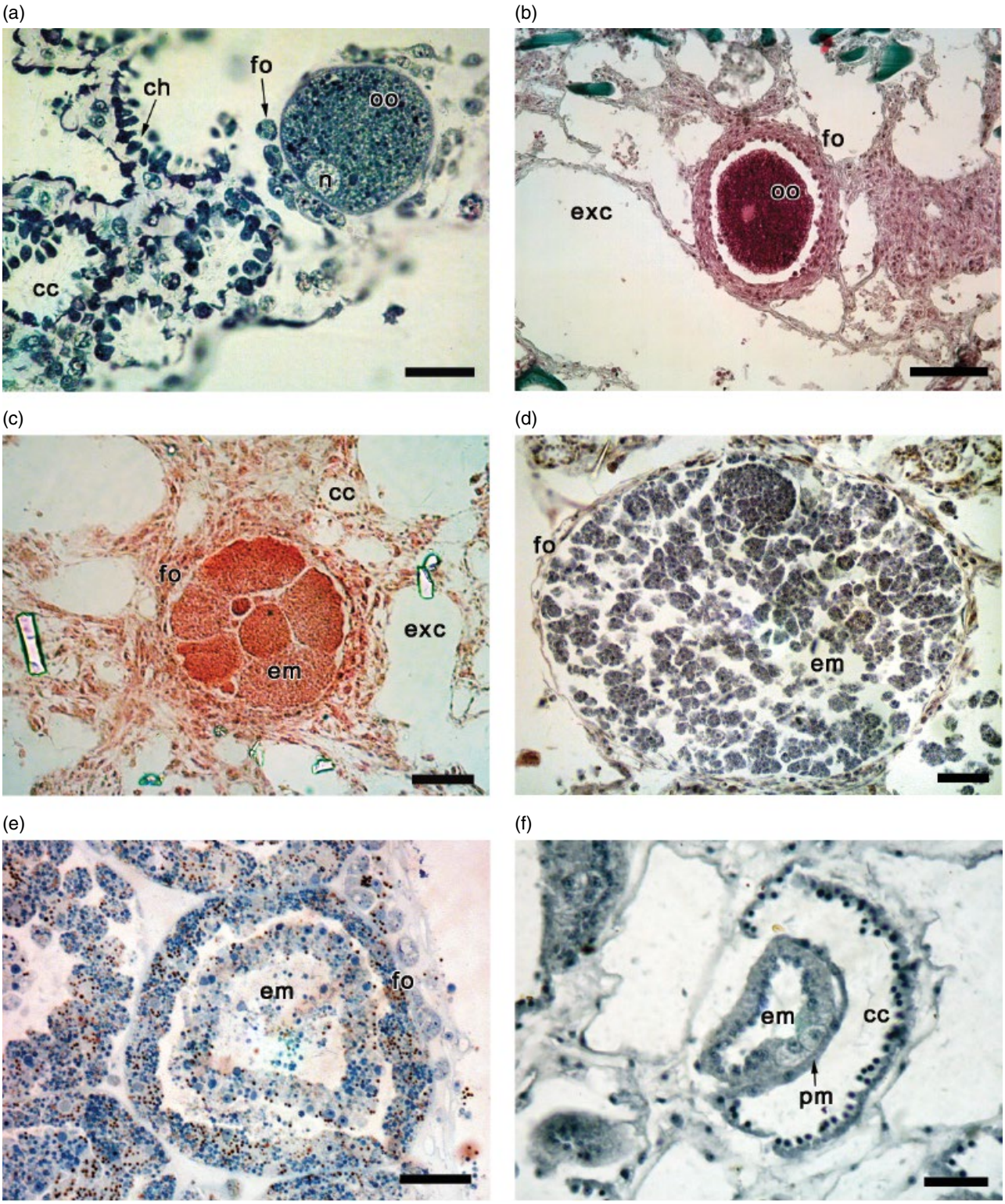

Figure 2.11 Follicle. (a) Beginning of follicle development in *Oscarella nicolai*. (b) Multilayer follicle in *Spongia officinalis*. (c) Multilayered follicle around cleaving embryo in *Iophon piceum*. (d) Monolayered follicle around a morula in *Haliclona aquaeductus*. (e) Complex two-layered follicle in *Clathrina arnesenae*. (f) "Placental membrane" in the embryo of *Sycon raphanus*. Scale bars: (a,e,f) 25 μm; (b) 200 μm; (c) 100 μm; (d) 50 μm.

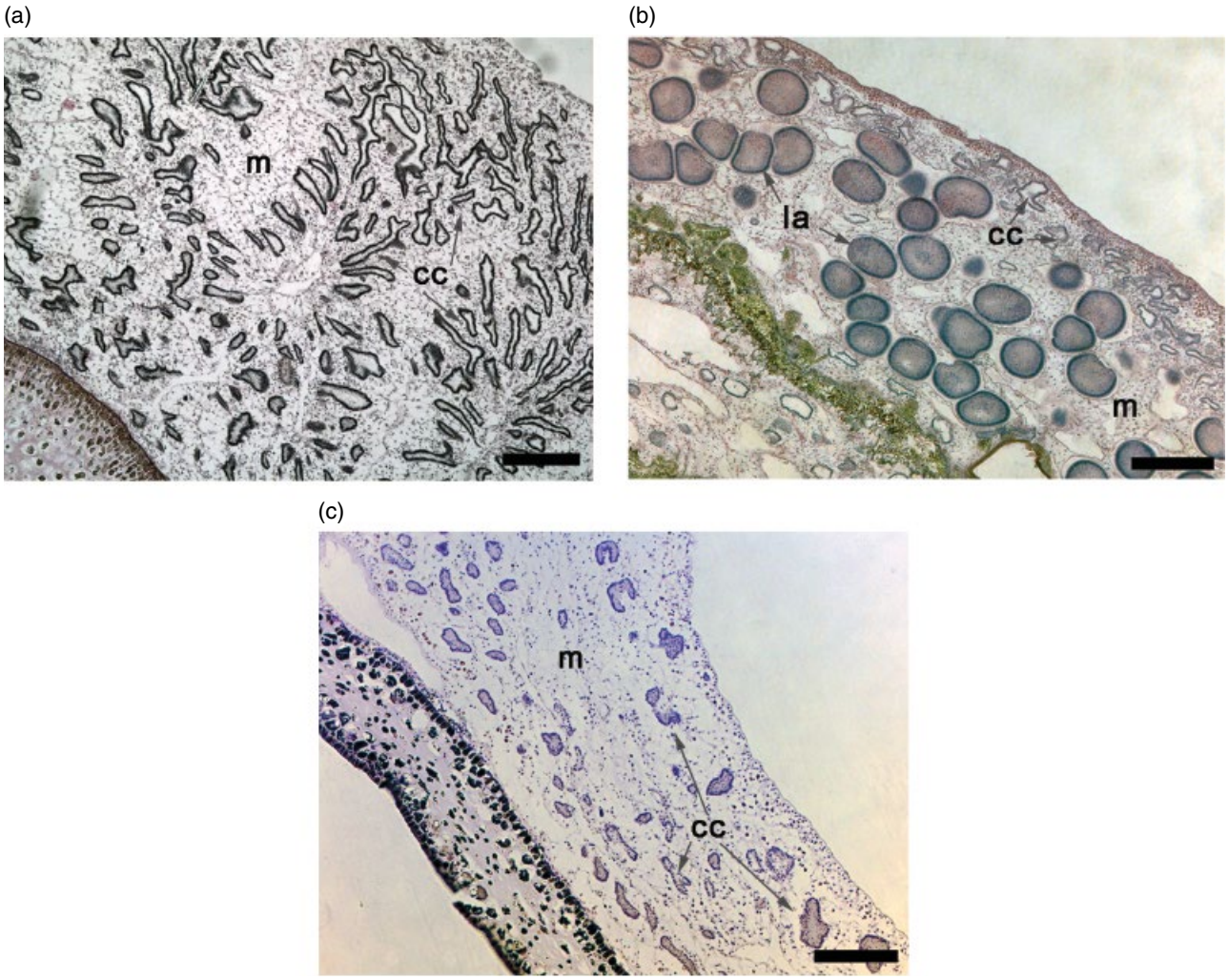

Figure 2.12 Tissue modification during sexual reproduction in *Halisarca dujardinii*. (a) Tissues in nonbreeding sponge. (b) Tissues of sponge with prelarvae, showing reduction of aquiferous system in the endosome. (c) Postreproduction rehabilitation of parental sponge tissue. Scale bars: 250 μm.

2.4.4.1 Female

In both oviparous and viviparous sponges, female gametes develop in small clusters (Figure 2.10c), located diffusely in the endosome (Figure 2.10a,b), or in the basal part of the body (Figure 2.10d) in encrusting sponges.

At the early stages of oogenesis, oocytes have an amoeboid-like shape and migrate through the mesohyl (Figure 2.10e). As the oocyte grows, it takes an oval form and proceeds to vitellogenesis, which passes via autosynthesis, heterosynthesis or both processes simultaneously. Heterosynthesis involves participation of different somatic cells and is typical for most viviparous sponges. These somatic cells (choanocytes, different mesohyl cells) are often referred to as trophocytes or "nurse cells." However, strictly speaking, sponges do not possess true trophocytes (Ereskovsky 2010). In some demosponges (orders Haplosclerida, Spongillida, and Suberitida), vitellogenesis is accompanied by the emergence of a population of specialized phagosome-rich amoebocytes that migrate toward the oocyte and are phagocytosed by it (Figure 2.10f).

In most sponges, at the end of vitellogenesis the oocyte stops near the exhalant canal and is surrounded by a temporary follicle, where embryos develop (Figure 2.11a–e). Embryonic development of ovoviviparous and viviparous sponges proceeds in these temporary follicles. In many viviparous demosponges during vitellogenesis, amoeboid cells, concentrating around the growing oocyte, form a well-developed multilayered capsule that makes up the follicle (Figures 2.10f, 2.11b,c). The phagocytosed cells of this capsule become yolk granules in the oocyte (Diaz 1973; Fell and Jacob 1979; Witte and Barthel 1994; Gerasimova and Ereskovsky 2007). As its cells are phagocytosed, the capsule gets thinner, and all that is left around the mature

egg is a single-layer follicle (Figures 2.10f, 2.11d). The follicle consists of flat pinacocyte-like cells, originating from the choanocytes, amoebocytes, or endopinacocytes, and an external layer of collagen fibers parallel to the cell surface that are synthesized by mesohyl cells (Figure 2.11d) (Fell 1983; Ereskovsky 2010).

In some calcareous sponges from subclass Calcinea, a two-layered follicle forms (Figure 2.11e). The external layer consists of dense extracellular matrix, the internal layer is made up of large cells, which may be cubic, prismatic, or flattened. Follicular cells are close to the embryo; they produce projections on the side, opposite to the embryo. These projections anchor the follicle cells in the extracellular matrix (Ereskovsky and Willenz 2008).

In many viviparous demosponges from orders Haplosclerida, Dendroceratida, Dictyoceratida, and Suberitida, groups of 6–20 oocytes of different stages concentrate into a common collagenous brood chamber (Figure 2.10c) (Ereskovsky 2010; Degnan et al. 2015). Inside the brood chambers, embryos are enveloped by follicles.

In some calcareous sponges from subclass Calcaronea, a special structure made up of flattened cells, derived from choanocytes, is formed during embryonic development. This structure is referred to as the placental membrane (Figure 2.11f). The placental membrane ensures the embryo's nutrition and participates in inversion (Duboscq and Tuzet 1937; Lufty 1957; Lanna and Klautau 2012). This structure is formed from parent choanocytes that gradually spread around the embryo (Gallissian 1983; Gallissian and Vacelet 1992; Lanna and Klautau 2012).

2.4.4.2 Male

In Hexactinellida, Demospongiae, and Homoscleromorpha, spermatogenesis proceeds in spermatocysts – temporary spherical structures bounded by flattened somatic cells diffusely distributed in the endosome (Figure 2.10b). Calcaronea have no spermatocysts (Boury-Esnault and Jamieson 1999; Maldonado and Riesgo 2008; Ereskovsky 2010, 2018). The spermatocysts are surrounded by the follicle cells derived from the transformation of pinacocytes or archaeocytes. In demosponges, cell junctions between the follicle cells are simple (apposition) with no detectable membrane specialization (Riesgo et al. 2008). The follicular cells of the spermatocysts of Homoscleromorpha possess specialized cell junctions and basement membrane (Ereskovsky 2010; Riesgo et al. 2007). Development of male gametes within a cyst is usually synchronous. In Homoscleromorpha, there is a gradient of male gamete maturation within a cyst, a feature they have in common with the Eumetazoa (Gaino et al. 1986; Riesgo et al. 2007; Ereskovsky 2010).

2.4.4.3 Reproduction and Tissue

During sexual reproduction, sponge tissue and the elements of the aquiferous system may be completely or partially destroyed, depending on the intensity of gametogenesis and embryogenesis. It occurs in both oviparous and viviparous sponges, gonochoric and hermaphroditic species. This period is marked by the complete disorder of central and basal parts of the choanosome. Normal tissue organization persists only in the narrow marginal zone of the sponge. For example, in *Halichondria panicea* and *H. dujardinii* after intensive gametogenesis and embryogenesis, the endosome transforms almost completely into a "gonad" filled with brood chambers containing larvae (Figure 2.12a,b) (Barthel 1986; Witte and Barthel 1994; Ereskovsky 2000; Gerasimova and Ereskovsky 2007). Slow postreproduction rehabilitation of parental sponge tissue continues after the end of reproduction (Figure 2.12c).

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Abbreviations for Figures

ap	apopyle
aph	aphodus
as	aragonitic skeleton
at	atrium
b	bacteria
bm	basal membrane
cc	choanocyte chamber
cf	collagen fibrils
ch	choanocytes
cht	choanocyte tube
co	cortex
cs	calcareous skeleton

cst	cellular strands
cu	cuticle
dm	dermal membrane
ec	ectosome
em	embryos
en	endopinacocytes
end	endosome
ev	extracellular vacuole for spicule synthesis
ex	exopinacocytes
exc	exhalant canal
fm	foreign material
fo	follicle
gc	Golgi complex
gr	glycogen rosettes
hyp	hypophare
inc	inhalant canal
la	larva
m	mesohyl
mt	mitochondria
n	nucleus
o	osculum
of	organic fibers
oo	oocyte
os	ostia
pes	perispicular spongin
pm	placental membrane
po	porocyte
pr	prosopyle
poc	porocalyx
sc	sclerocyte
si	special inclusions
sp	spicules
sph	spherulous cell
spc	spermatocyst
sub	subdermal cavity
tr	trophocytes
tt	trabecular tract
vc	vacuolar cells

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