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Zoomorphology

The architecture of the canal systems of *Petrosia ficiformis* and *Chondrosia reniformis* studied by corrosion casts (Porifera, Demospongiae)

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Summary. The three-dimensional organization of the canal system in two sponge species, *Petrosia ficiformis* and *Chondrosia reniformis*, was studied using corrosion casts. Casts were made of live animals, in situ, and canal replicas were analzyed by scanning electron microscopy (SEM). In *P. ficiformis* the incurrent system consists of a superficial canal network giving rise to large radial canals, which ramify and anastomosize forming an internal web. Excurrent canals are arranged into modular ramified systems radiating from atrial cavities opening to the exterior. Main excurrent canals run at various depths within the sponge, even through the superficial incurrent network. Both incurrent and excurrent canal replicas show smooth, blind-ending capillaries. Some large incurrent canals merge with excurrent ones, thus bypassing choanocyte chambers. In *C. reniformis* there is a cortical collagen layer crossed by tree-like incurrent canals, the "twigs" of which communicate with groups of inhalant pores. The stems of tree-like canals penetrate into the sponge medulla where they ramify and anastomosize to form a web. Main excurrent canals arise from large cloacal ducts leading to the oscular openings. They give rise to a sequence of branches intersecting the incurrent web. Both incurrent and excurrent canals have sharp, blind-ending capillaries. Morphometric data functions show that diameter scaling in canal branches is exponential in *Petrosia* and linear in *Chondrosia*. Structural differences and homologies between the two species are discussed.

A. Introduction

Sponges are permeated by a very complex aquiferous system, which is essential for feeding, respiration, elimination and reproduction (see review in Simpson 1984).

Three structural types occur in sponges: ascon, sycon and leucon. Ascon and sycon organizations are the most primitive, showing a radial arrangement. Leuconoid sponges are more complex, and a comprehensive description of their internal structure is still lacking. In these sponges the aquiferous system, consisting of canal system and choanocyte chambers, can be divided into three parts: (1) incurrent canals, which carry water from the environment to choanocyte chambers; (2) choanocyte chambers, which are small alveolar structures lined by choanocytes, cells capable of pumping water by flagellar beating; and (3) excurrent canals, which conduct water from choanocyte chambers back to the environment.

Anatomical reconstruction of sponge canal systems was attempted in the past with light-microscopic analysis of sponge sections (Schulze 1877). More recently, the examination of serial sections led to a gross characterization of some morphological patterns (Reiswig 1975) and the recognition of three kinds of leuconoid sponges, namely eurypylous, aphodal and diplodal, based on the connections between choanocyte chambers and canals (Brien 1973).

Sponge tissue fracturing and scanning electron microscopy (SEM) analysis allowed partial three-dimensional views of the internal structure (De Vos 1979; Weissenfels 1975, 1976, 1982). In some cases, species-specific anatomical patterns of choanocyte chambers and related canals were demonstrated by ultrastructural studies (Langenbruch 1983a, b; Langenbruch et al. 1985; Langenbruch and Scalera Liaci 1986; Langenbruch and Weissenfels 1987).

Our study is aimed at obtaining a full-scale reconstruction of the sponge canal system, which has never been achieved by sectioning and fracturing methods. We apply the technique of corrosion casts, commonly used in comparative anatomical studies of vertebrate vascular organs (Ditrich and Splechtna 1987), to sponges. The casting of the demosponges *Petrosia ficiformis* and *Chondrosia reniformis* allows, for the first time, reconstruction of the architecture of the canal system and comparison of the internal anatomy of two sponge species. In *P. ficiformis* we also show canal arrangements which prompt a reconsideration of sponge hydrodynamics.

B. Materials and methods

Specimens of *Petrosia ficiformis* (Poiret, 1789) and *Chondrosia reniformis* Nardo, 1847, from the Ligurian Sea were perfused with a pigmented plastic fluid (Batson's 17 Plastic Replica and Corrosion Kit, Polysciences Inc., Warnington, Pa.). Perfusions were carried out in situ to minimize tissue contraction. Using a polyethylene syringe, the fluid was injected through the oscule upstream of the water flow until it leaked out through the inhalant pores at the sponge surface. The fluid was allowed to cure overnight in situ. Injected specimens were macerated in daily changes of 30% KOH. Casts were treated with 5% hydrofluoric acid for

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2 h to dissolve siliceous spicules. Low-magnification pictures of casts were taken using a 35 mm camera. For SEM analyses fragments of casts were mounted on stubs, sputtered with gold, and observed in a Philips 515 SEM.

C. Results

1. Morphological analysis

Petrosia ficiformis. The outermost part of casts consists of a network of anastomozized canals surrounding the whole sponge except the basal portion (Fig. 1a). This superficial network is formed mostly by incurrent canals, and the density may vary with location. A system of canals distributing water to the various sponge regions develops under the superficial incurrent network. This system consists of major radial canals connected with the network by groups of branches (Fig. 1b). These canals ramify and form anastomoses, resulting in a three-dimensional web (Fig. 1b). The internal framework of canals extends through the whole sponge body, giving rise to smaller orders of branches.

The excurrent system is a series of similar modules consisting of main canals radiating from atrial cavities (about 4–10 mm in diameter) (Fig. 1a). Some of these canals run just below the sponge surface, at the level of the superficial incurrent network. They give rise to a maximum of four orders of branches which intersect the incurrent canals (Fig. 1c). The extreme capillaries of both the incurrent and excurrent canal systems are smooth and end blind (Fig. 1d).

Large incurrent canals frequently merge with excurrent ones, both deep inside the sponge body (Fig. 1e) and at the superficial level (Fig. 1f).

The excurrent canals of a single sponge are arranged into "drainage basins" corresponding to the various atrial cavities. The extent to which these systems merge with each other may vary between specimens. By contrast, the incurrent canals form a unique system.

Chondrosia reniformis. In this sponge a cortical collagen layer surrounds the medulla with choanocyte chambers. The cortical layer is poorly irrigated, with the basal part being completely devoid of canals. By contrast, the medulla is filled with a dense web of vessels (Fig. 2a).

The outermost portion of the incurrent system consists of tree-like canals spanning the sponge cortical layer. The "twigs" of these trees reach the sponge surface (Fig. 2b), and in some specimens they may merge with those of neighboring trees, forming a superficial incurrent network. The trunks of the trees are the main incurrent ducts bringing water into the sponge body (Fig. 2c). Even after penetrating into the medullar zone, these ducts are still surrounded by a sheath of cortical tissue lacking capillaries (Fig. 2a). More deeply, they ramify and anastomozize to form a three-dimensional web giving rise to smaller branches (Fig. 2d).

The excurrent system develops almost entirely in the sponge medulla. Main cloacal ducts (about 2–3 mm in diameter) run through the sponge body (Fig. 2e) and lead to the oscular openings. Modular canal systems, consisting of a maximum of five orders of branches, arise from the cloacal ducts (Fig. 2e). These canals are flattened, having an elliptical section. They interdigitate with the incurrent canal framework. Both incurrent and excurrent systems show sharp blind-ending capillaries (Fig. 2f).

2. Morphometric analysis

Whole sponge casts were used to estimate the percentage of the sponge volume occupied by the canal system (without choanocyte chambers) in the two species. Both uncorroded specimens and replicas were immersed in a graduated cylinder to estimate their volumes. In *P. ficiformis* canals occupy approximately 25% of the sponge volume, in *C. reniformis* approximately 15%.

The length and the diameter of canals were found to vary considerably from specimen to specimen. In the examined casts of *P. ficiformis* the diameter of the canals of the superficial network varies between 100 and 500 μm. The main incurrent ducts have a diameter of 370–900 μm and their density is $17 \pm 5.48\ \mathrm{cm}^{-2}$. In casts of *C. reniformis* the main incurrent ducts have a diameter of 420–660 μm and their density is $11 \pm 2\ \mathrm{cm}^{-2}$.

The sequence of branches of modular excurrent canal systems dissected from casts was divided into five stages, or "generations" (z). In *P. ficiformis* the first generation was attributed to canals arising directly from atrial cavities, and in *C. reniformis* to those arising from the cloacal ducts. Yet, in both species, branches arising from the largest canals span a wide range of sizes, and measures were taken only along branch sequences arising from main ramifications.

Measurements of canal diameter were taken at each branch generation (Table 1). *t*-Test comparisons show that each z generation of *C. reniformis* is much smaller than that of *P. ficiformis* ($P << 0.01$). Shifted comparisons between z and z+1 generations also show significant differences ($P < 0.01$), except between *Chondrosia* $z = 4$ and *Petrosia* $z = 5$, which have almost the same size.

Average canal diameters of *P. ficiformis* plotted against generation in a semilogarithmic diagram suggest an exponential diameter scaling, since they fit a straight regression line ($r = 0.978$) with a slight harmonic variation about this straight line (Fig. 3a). By contrast, the corresponding values of *C. reniformis* fit a straight line in a linear diagram ($r = 0.983$), suggesting a linear scaling of canal diameter (Fig. 3b). In this case also, there is a slight harmonic variation about the regression line.

D. Discussion

Sponge casting allows a direct analysis of the three-dimensional arrangement of the canal systems, even though some sponge properties may influence microscopical details of casts, such as the shape of capillaries. In *P. ficiformis*, the size of the smallest excurrent replicas corresponds to that found in vessels draining the aphodal canals, which exit from choanocyte chambers (Langenbruch et al. 1985). In *C. reniformis*, the size of the smallest vessels suggests that they connect directly with choanocyte chambers.

The arrangement of canals in the two species shows similar patterns and suggests anatomical homologies. The incurrent and excurrent canals are highly intersected with each other, but show different architectures (Fig. 4). The incurrent system consists of a superficial arrangement of canals connected by radial ducts to a diffuse internal web of anastomozized vessels. By contrast, the excurrent system is constructed of modular sequences of branches radiating from main excurrent cavities.

The vessels of the superficial incurrent network and the main incurrent radial ducts of *P. ficiformis* are homologous

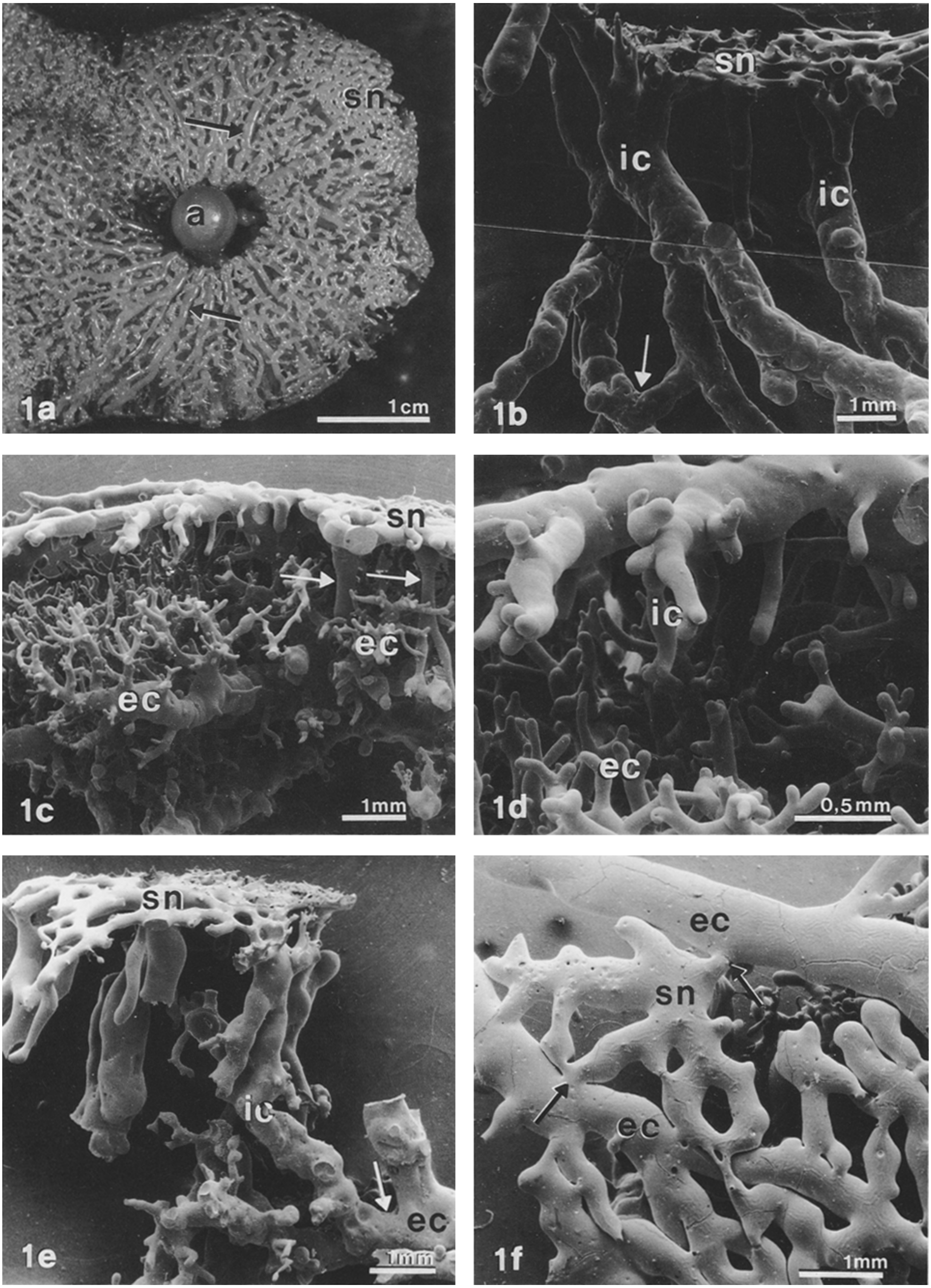
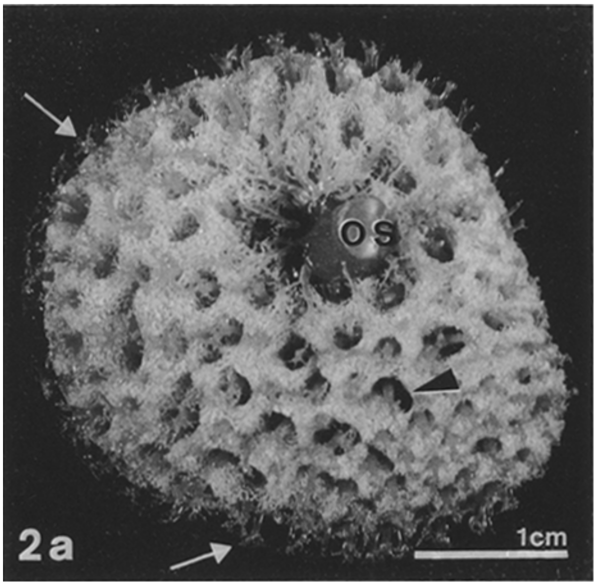


Fig. 1. **a** General view of a cast of *Petrosia ficiformis* showing the superficial network of incurrent canals (*sn*) and main excurrent canals (*arrows*) radiating from the atrium (**a**). **b** Scanning electron micrograph showing the superficial network of incurrent canals (*sn*) connected with main incurrent ducts (*ic*) forming anastomoses (*arrow*). **c** Ramified excurrent canals (*ec*) intersecting incurrent ones (*arrows*) arising from the superficial incurrent network (*sn*). **d** Detail showing smooth, blind-ending replicas of incurrent (*ic*) and excurrent (*ec*) capillaries. **e** Part of a cast showing merging (*arrow*) of an incurrent canal (*ic*) and an excurrent one (*ec*). *sn* superficial incurrent network. **f** Detail showing mergings (*arrows*) of the superficial incurrent network (*sn*) and superficial excurrent canals (*ec*)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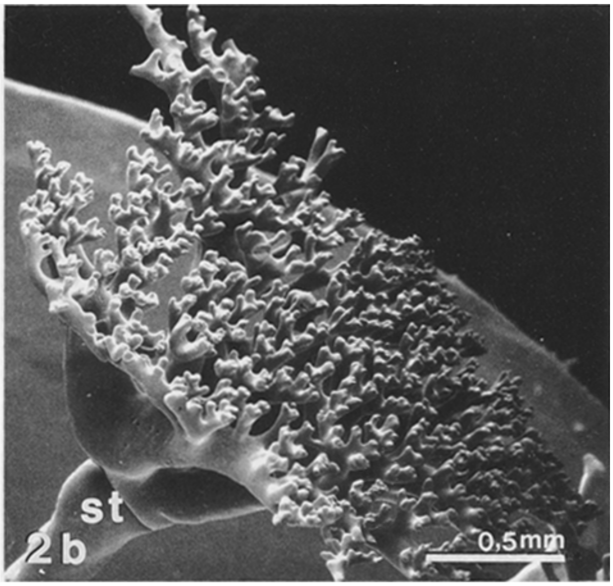
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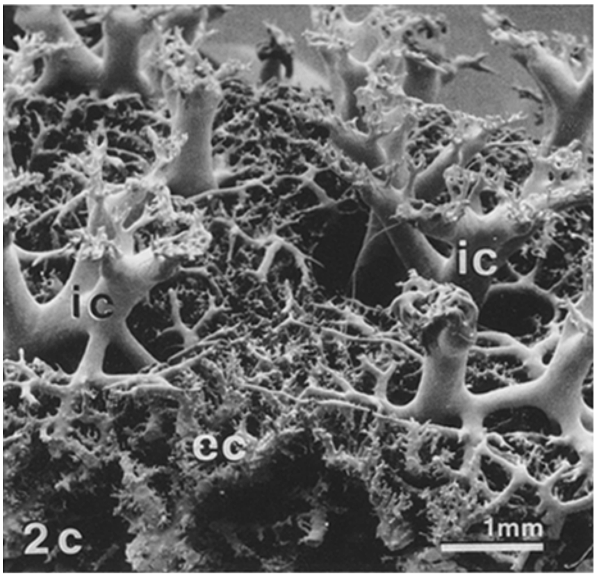
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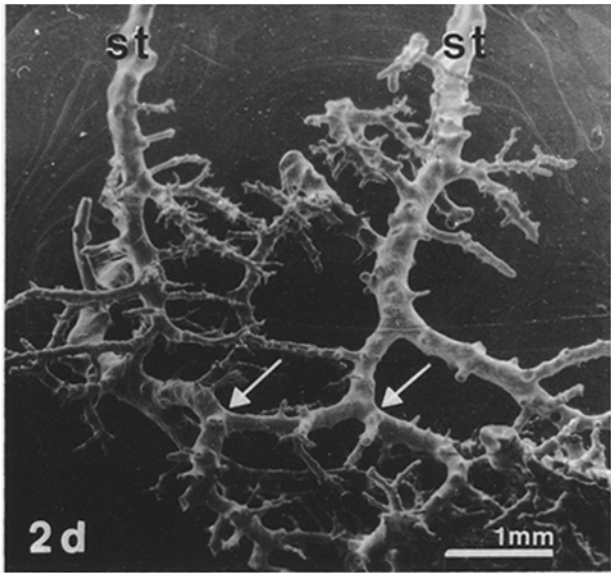
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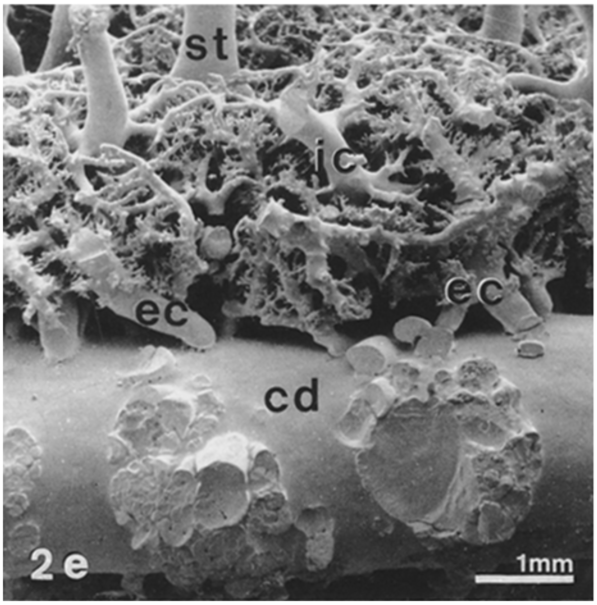
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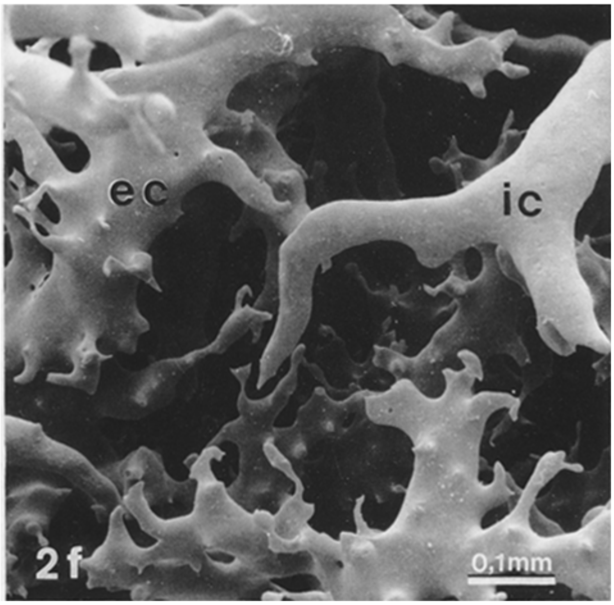
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Table 1. Summary statistics of excurrent canal diameters (µm)

Branch generation (z)	*P. ficiformis*		*C. reniformis*	
	Average diameter	*n*	Average diameter	*n*
1	1302±13	12	452±82	17
2	682±21	35	339±71	35
3	389±56	35	174±33	35
4	217±28	35	55±16	22
5	53±11	35	10± 3	20

to the twigs and the stems, respectively, of the *C. reniformis* tree-like cortical canals. This would be difficult to show in tissue sections, since the superficial vessels of *P. ficiformis* appear as subdermal spaces (Simpson 1984), whereas in *Chondrosia* the tree-like canals are seen as distinct inhalant organs (Schulze 1877).

A distinctive feature of *Petrosia* is the high incidence of large mergings between incurrent and excurrent canals. This has never been described in sponges before. Such connections allow water to flow freely from the incurrent openings directly to the excurrent ones, bypassing choanocyte chambers. Passive flows induced in sponges by ambient water currents (Vogel 1974) may vent through these mergings, thus avoiding both interference with choanocyte water pumping and pressure damage to fine structures. According to this scheme, water circulation in *P. ficiformis* could depend principally on passive water transport in large vessels, whereas choanocyte pumping would be effective mainly in small canals. Regulation of induced water flows in *C. reniformis* may occur through variation of canal diameter or closure of the incurrent openings, because this sponge is much more contractile than *P. ficiformis* and shows no large incurrent-excurrent mergings.

Morphometric data show that the canals of *P. ficiformis* are larger on average than those of *C. reniformis*. However,

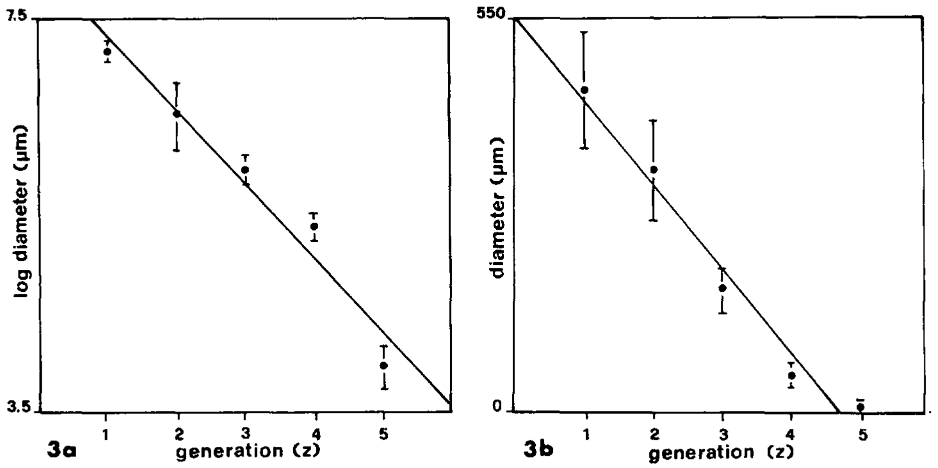

Fig. 3. **a** Log-linear plot and regression line of canal diameter vs branch generation of *P. ficiformis* casts. **b** Linear plot and regression line of canal diameter vs branch generation of *C. reniformis* casts

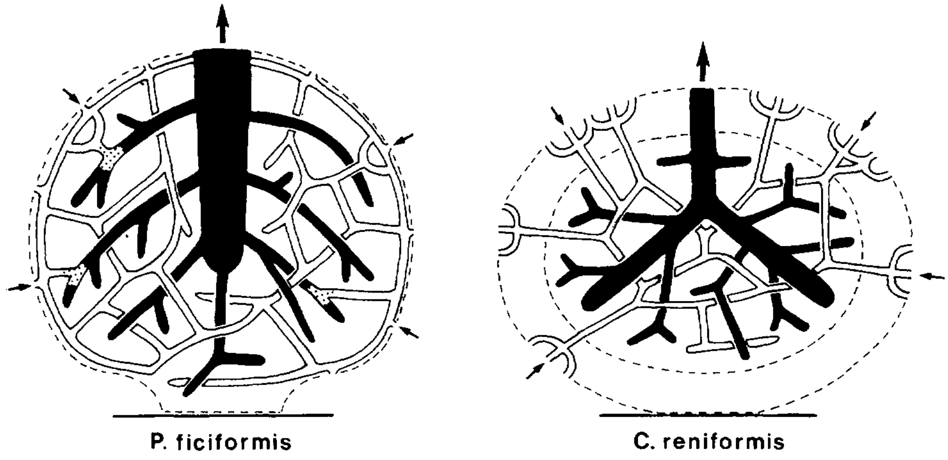

Fig. 4. Diagram showing the architecture of main canals in the two species. *White canals*, incurrent; *black canals*, excurrent; *dotted areas*, mergings of incurrent and excurrent canals. *Arrows* indicate the direction of water flow. See text for further explanation

Fig. 2. **a** General view of a cast of *Chondrosia reniformis*, showing tree-like incurrent canals (*arrows*) of the cortex penetrating into the medulla. The medulla shows a dense web of incurrent and excurrent canals and empty areas surrounding the bases of tree-like canals (*arrowheads*). *os* oscule. **b** Scanning electron micrograph of the outermost portion of a tree-like incurrent canal. *st* stem of tree-like canal. **c** Cortical tree-like incurrent canals (*ic*) penetrating into the medulla where they give rise to ramifications which intersect excurrent canals (*ec*). **d** Part of the incurrent canal system showing the "trunks" of cortical tree-like canals (*st*) forming ramifications and anastomoses (*arrows*) in the medulla. **e** Portion of a cast showing an excurrent cloacal duct (*cd*) giving rise to ramified excurrent canals (*ec*) intersecting incurrent ones (*ic*). *st* Stems of cortical tree-like canals. **f** Detail showing sharp, blind-ending replicas of incurrent (*ic*) and excurrent (*ec*) capillaries

measures of canal size only are scarcely informative, since they vary considerably from specimen to specimen and may also be biased by sponge tissue contraction during casting.

Numerical functions depending on the sequential order of excurrent canals allow a formal description of these complex structures and show differences between the two species. The exponential diameter scaling of *P. ficiformis* is more similar to the scaling law of the vertebrate bronchial tree (Weibel and Gomez 1962) than the linear one of *C. reniformis*. The harmonic variation observed in both sponges was also found in bronchial trees (West et al. 1986), and may be related to some structural property of branched vascular systems.

According to Simpson's (1984) report on types of exhalant openings, the excurrent openings at the surface of *P. ficiformis* are secondary oscules and excurrent canals open into the atrial spaces via primary oscules. *C. reniformis* has cloacal ducts and no atrial spaces, which more closely resembles a situation with true oscules at the surface. The analysis of casts, however, suggests that the atrial spaces of *P. ficiformis* are structurally equivalent to the cloacal ducts in *C. reniformis*. Each of these structures drains a number of modular branched systems of excurrent canals, and their average diameter markedly diverges from the scaling laws of these systems.

Our three-dimensional analysis of canal arrangements integrates information from cell and tissue research to give a description of sponge internal structures. In particular, our data concerning *P. ficiformis* are complementary to those recorded during recent transmission electron microscopy investigations by Langenbruch et al. (1985). Sponge casting may also be advantageous in the following research applications:

1. Reconstruction of the internal anatomy may contribute to an understanding of growth mechanisms.
2. Morphovolumetric analyses of canal replicas could contribute to physiological studies on pumping rate and hydrodynamics.
3. Specific features of casts may be used in taxonomy or to identify different ecotypes in a single species.
4. A comparative analysis of casts may help to clarify phylogenetic relationships among Porifera.

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