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Pogonophora (Annelida): form and function

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Abstract

Pogonophora, also known as Siboglinidae, are tube-dwelling marine annelids. They rely on endosymbiotic chemoautotrophic bacteria for nutrition and their anatomy and physiology are adapted to their need to obtain both oxygen and reduced sulphur compounds. Frenulate pogonophores are generally long and slender, sediment-living tubeworms; vestimentiferans are stouter, inhabitants of hydrothermal vents and cool seeps; and moniliferans or sclerolinids are very slender inhabitants of decaying wood and sulphidic sediments. The anatomy and ultrastructure of the three groups are compared and recent publications are reviewed. Annelid characters are the presence of chaetae and septa, concentrated at the hind end. The adaptations to a specialised way of life include, in particular, the chitinous tube; the anterior appendages that function as gills; the internal tissue called the trophosome, where the endosymbiotic bacteria live; and the blood vascular system that transports oxygen, sulphide and carbon dioxide to the trophosome.

Introduction

Pogonophora are tube-dwelling marine worms that depend on internal symbiotic bacteria for nutrition. Their systematic position has been argued over for years. Because the adults have no mouth or functional gut, their dorsoventral orientation has been controversial. Furthermore, the segmented posterior end was unknown for a long time. Their habitats range from reducing sediments to decaying wood, cool sulphidic seeps and warm hydrothermal vents.

Within the Pogonophora, three major groups are recognized today: the Frenulata, the Vestimentifera and the Monilifera, the latter represented by the single genus *Sclerolinum*. Caullery (1914) described the first pogonophoran genus, *Siboglinum,* and erected the family Siboglinidae with unknown affinity to any existing phylum. Uschakov (1933), describing *Lamellisabella zachsi*, assigned his species to the sabellid polychaetes. The name Pogonophora has been in use since Johansson (1937, 1939) designated a class Pogonofora in the 'Vermes Oligomera', to include *Lamellisabella zachsi*. Ivanov (1951) united the previously described frenulates when he included Siboglinidae in the class Pogonophora. His monograph (Ivanov, 1963) in which he elevated Pogonophora to phylum level has formed the foundation for subsequent work on the group. He still rejected annelidan affinities (Ivanov, 1975, 1988) after the segmented posterior end was described (Webb 1964). The enterocoelic origin of the coelom (Ivanov, 1957, 1975, 1988) was a critical character in his opinion and that of his successors, who place Pogonophora close to Archicoelomata and Deuterostomia (Malakhov et al., 1996c). When the Vestimentifera were discovered, Ivanov (1994) accepted them as Pogonophora, though Jones (1985a) proposed a separate phylum. A review of the Vestimentifera (Malakhov & Galkin, 1998) divides the phylum Pogonophora into two

subphyla, Perviata and Obturata, using the names proposed by Jones (1981a); Perviata contains two classes, Frenulata and Monilifera (Ivanov, 1991); Obturata contains only one class, Vestimentifera.

The alternative hypothesis, of a close relationship to the Annelida, developed gradually, following the discovery of the multisegmented opisthosoma, with septa between the segments and serially arranged chaetae (Webb, 1964; Southward, 1975a). The original dorso-ventral orientation of the animal was reversed. Protostomian affinity was supported by discovery of the larval mouth and interim gut in both vestimentiferans and frenulates, which confirmed the change of dorsoventral orientation (Jones & Gardiner, 1988, 1989; Southward, 1988; Callsen-Cencic & Flügel, 1995). Both molecular and morphological cladistic analyses indicate that Pogonophora are annelids (e.g., Rouse & Fauchald, 1995, 1997; McHugh, 1997; Schulze, 2003; Halanych, 2005). The name Siboglinidae has since been used to include Vestimentifera, Frenulata and *Sclerolinum* (see also Rouse, 2001). Specialists on the molecular evolution of extracellular haemoglobins consider vestimentiferans and frenulates to form a class of Annelida for which they coined the name Opisthochaeta (Zal et al., 1997; Negrisolo et al., 2001). On the other hand, Salvini-Plawen (2000) argues that the present state of knowledge is insufficient and that we need more detailed investigation before reaching any conclusion about the origin of Pogonophora. Almeida et al. (2003) place Pogonophora in the Enterocoela, which they regard as a transitional taxon between protostomes and deuterostomes.

The taxonomists among us (ECS and SLG) prefer to retain the name Pogonophora for the group and to use a Linnean system of classification, with three subclasses Frenulata, Vestimentifera and Monilifera – and the accepted families within them (Table 1). We need to keep the freedom to move genera between families, to merge families, and to propose new ones. As we continue to describe more new species, some revision of the families is certainly going to be necessary, since there are now about 135 named species of frenulates, 13 vestimentiferans and 8 moniliferans.

Our aim in this paper is to review recent work, our own and other authors', emphasizing function as well as structure. More has been published lately on vestimentiferans than frenulates because of the great geological and biogeographical interest in hydrothermal vents and cold seeps (Tunnicliffe et al., 1998), where vestimentiferans are important members of the fauna. They are large and easily collected with the aid of submersibles and experimental physiology has flourished, through use of new pressurized aquaria.

Table 1. Classification

Annelida				
Pogonophora:	Subclasses	Families	Genera	Species
	Frenulata	Oligobrachiidae	6	20
		Siboglinidae	2	73
		Polybrachiidae	8	29
		Lamellisabellidae	2	9
		Spirobrachiidae	2	5
	Vestimentifera			
		Lamellibrachiidae	1	5
		Escarpiidae	3	4
		Riftiidae	1	1
		Ridgeiidae	1	1
		Tevniidae	2	2
		Arcovestiidae	1	1
		Alaysiidae	1	1
	Monilifera			
		Sclerolinidae	1	7

Morphology

The anatomy and fine structure of Pogonophora have been documented in comprehensive publications including Ivanov's (1963) monograph, Gupta & Little (1969, 1970, 1975) and two chapters in 'Microscopic Anatomy of Invertebrates' (Gardiner & Jones, 1993; Southward, 1993).

The body plan of *Siboglinum* is typical of frenulates. Figure 1 compares the regions of the adult body with those of the larva at the time of settlement and shows the position of the first septum. The diaphragm develops later, as a muscular partition. The frenulum, or bridle, is a pair of hardened cuticular ridges around the forepart, close behind the tentacle bases. *Siboglinum* species have a single tentacle but most other genera have more (from two to over 200). The trunk is very long and the postannular region of the trunk

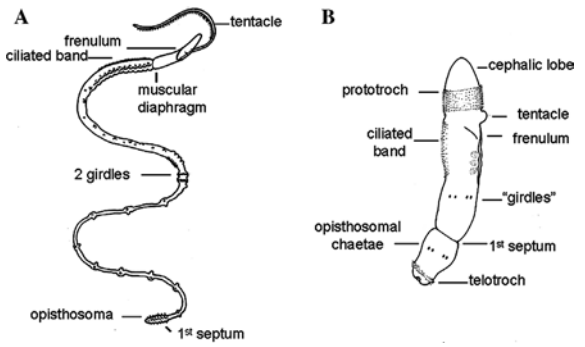

Figure 1. Frenulate body regions. (A) *Siboglinum* adult, diagrammatic. (B) Late larva of *Siboglinum fiordicum*.

contains the trophosome, visible in life as a dark streak between the red blood vessels.

Comparison of the body plans of a frenulate, a vestimentiferan and a moniliferan (Fig. 2) indicates an anterior region, a diaphragm, a trunk region and a segmented opisthosoma in all three. Whereas the anterior region of frenulates and moniliferans includes a cephalic lobe and dorsal tentacles, vestimentiferans possess a unique structure called the obturaculum which supports an extensively developed branchial plume (Fig. 3A) and which acts as an operculum to block the entrance to the tube when the animal withdraws (Jones, 1981a; Gardiner & Jones, 1993). Together, the obturaculum and associated branchial plume comprise the obturacular region of vestimentiferans.

The vestimental region is very muscular and has wide vestimental folds that normally curl over the dorsal side of the body, enclosing a space into which the gonopores open posteriorly. The outer side of the vestimental folds is covered with small papillae bearing cuticular plaques. Among them are the openings of numerous tube-secreting glands. Moniliferans have a muscular anterior region, also rich in tube-secreting glands. A ring of papillae, carrying plaques, lies just behind the tentacle bases (Fig. 3B). The trunk carries more papillae in all three groups. A ventral ciliated band is always present, but in frenulates it is on the anterior trunk, in vestimentiferans on the vestimental region and in moniliferans on the anterior region. The papillae of frenulates are varied in size and structure on different regions of the trunk. The girdle region lies at mid-trunk level and consists of four half-hoops of numerous chaetae, developed from four groups of chaetae on the second larval segment. In moniliferans trunk chaetae are just in front of the opisthosoma (absent in some species), and they are not present in adult vestimentiferans.

The opisthosoma is divided by septa into coelomate segments, with regularly arranged chaetae. Most of the features shared with annelids are

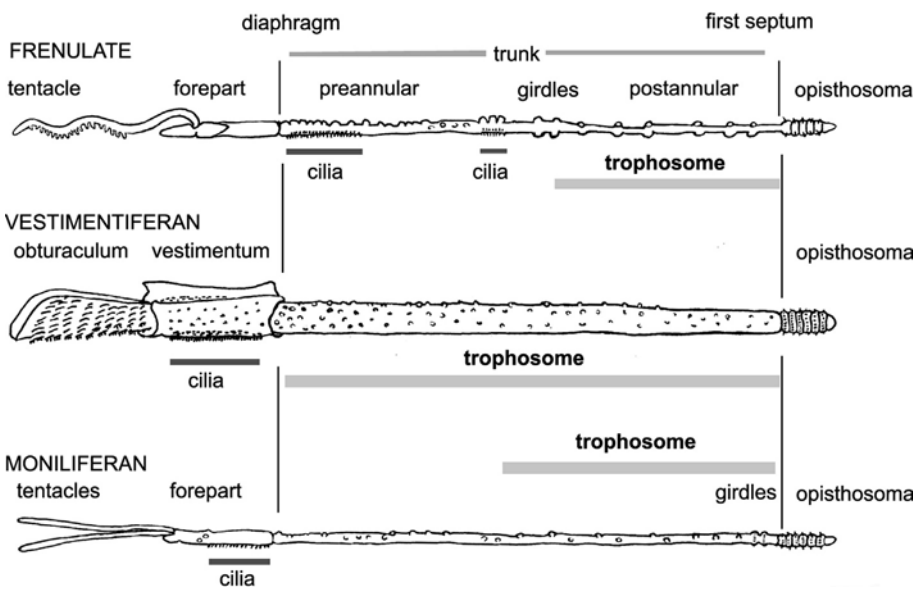

Figure 2. Comparison of body regions of frenulate, vestimentiferan and moniliferan, diagrammatic. Shading shows the extent of the trunk, trophosome and ventral ciliated bands.

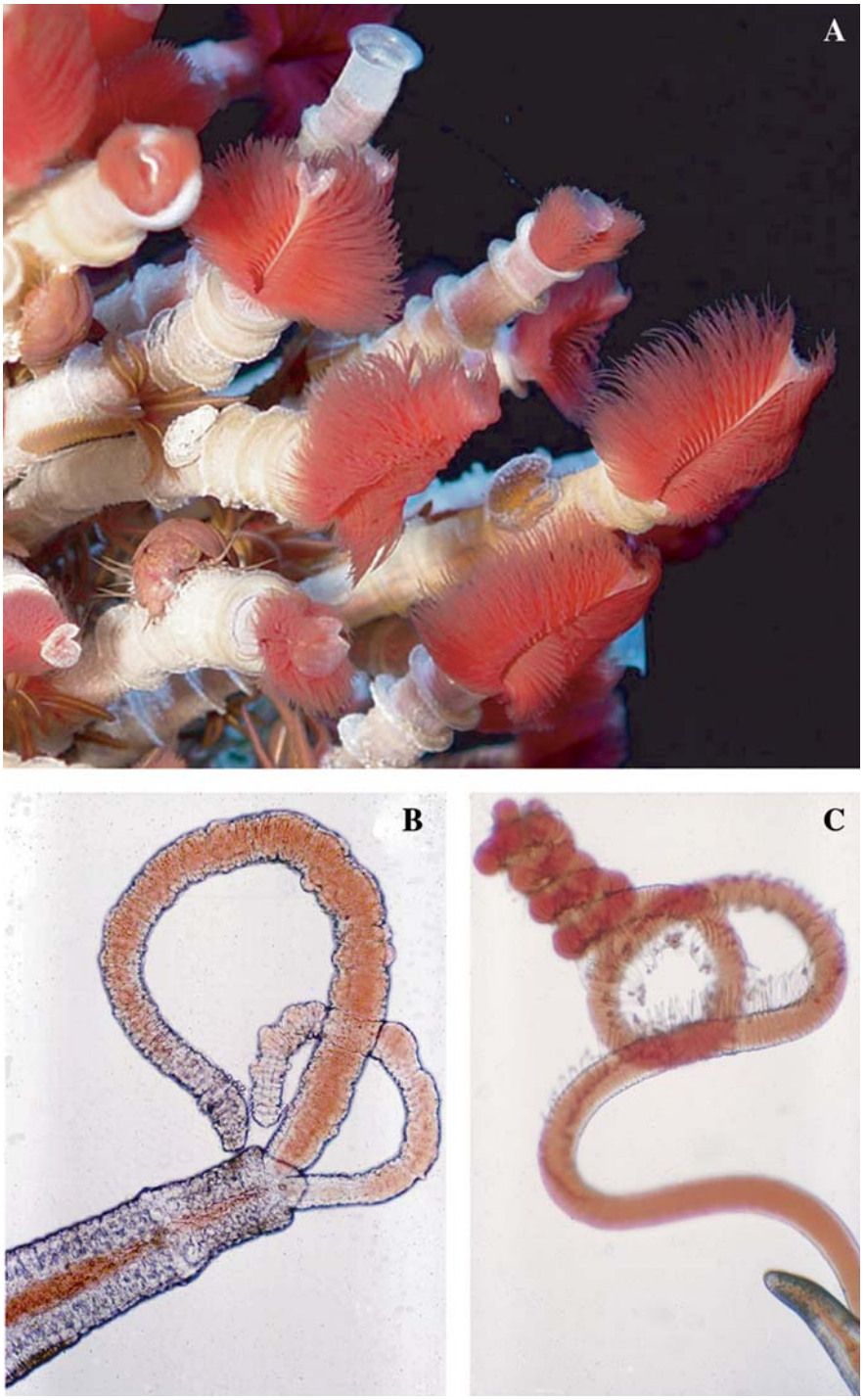

Figure 3. (A) Branchial plumes and obturacula of live vestimentiferans (*Ridgeia piscesae*). Tube diameter ca 10 mm (photo Verena Tunnicliffe). (B) Anterior end and tentacles of live moniliferan (*Sclerolinum brattstromi*), note papillae and plaques on anterior part of body. Body diameter ca. 0.1 mm. (photo A. J. Southward). (C) Tentacle and cephalic lobe of live frenulate (*Siboglinum ekmani*), note double row of pinnules. Tentacle diameter ca. 0.08 mm. (photo A. J. Southward).

concentrated in the opisthosoma, including muscular septa, segmentally arranged chitinous chaetae, ganglia and blood vessels (Southward, 1975a,b). In post-larval and juvenile vestimentiferans (up to ca. 1.7 mm long) the second segment (trunk) has chaetae of two types, capillary and claw-like (Jones & Gardiner, 1989). These stay in place while several rows of adult-type chaetae develop on the opisthosome (Fig. 4A). The claw-like larval chaetae have a group of small teeth that point anteriorly. In larger juveniles the larval chaetae are lost, and no more develop on the trunk. The adult-type chaetae of pogonophores of all three groups have long shafts and finely dentate heads, with the teeth usually in two groups, the anterior teeth pointing backward and the posterior group pointing forward (Fig. 4B, C).

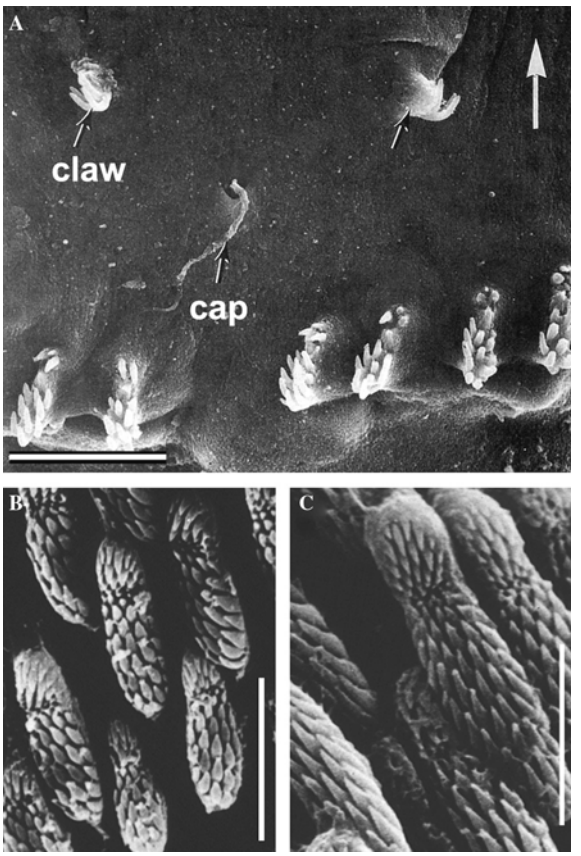

Figure 4. Chaetae. (A) Larval chaetae of trunk and adult chaetae of first opisthosomal segment of vestimentiferan post-larva (*Riftia* or *Oasisia*) (Gardiner & Jones, 1993). (B) Adult chaetae of frenulate *Siboglinum atlanticum*. (C) Adult chaetae of frenulate *Lamellisabella coronata* (B & C, From George & Southward, 1973). Scale bars = 10 μm.

Tube and tube secretion

The pogonophoran tube provides both support and protection. Webb (1971) recognized two major styles of forward growth, one continuous and flexible, the second a stiffer series of overlapping funnels, but both may be found in one species at different stages in the life history. Folding of the flexible anterior end, as found in many frenulates, can protect the animal, whereas a funnelled tube is open at the top to predators and parasites. Vestimentiferans can plug the entrance with their obturaculum, but frenulates such as *Polybrachia* and *Lamellisabella* must leave the tips of the tentacles exposed.

Chitin was originally reported from the tubes of three frenulate species (Brunet & Carlisle, 1958), and later confirmed by X-ray diffraction analysis (Blackwell et al., 1965). There are three crystallographic forms of chitin in living organisms, differing in the alignment of their chains of chitin molecules, designated α-, β- and γ-chitin. The chitin in frenulate and vestimentiferan tubes is crystalline β-chitin, which also occurs in annelid chaetae and in the 'pen' of the squid *Loligo* but is otherwise rare in the animal kingdom (Jeuniaux, 1963; Rudall & Kenchington, 1973). The use of chitin as a tube-building material is unknown in other annelids (Vovelle, 1982).

A chitin-protein complex forms a substantial proportion of the tube of the frenulate *Siboglinum fiordicum* (Foucart et al., 1965). Similarly, vestimentiferan tubes, exemplified by *Riftia pachyptila*, are composed mainly of chitin and protein (Webb, 1971; Gaill & Hunt, 1986; Shillito et al., 1995a, 1997; Chamoy et al., 2000). Chitin-containing Cambrian fossil tubes may indicate the antiquity of Pogonophora (Carlisle, 1964; Southward & Southward, 1967).

The tubes of frenulates and vestimentiferans are built up from many layers of fibrils secreted by multicellular epidermal glands (tubiparous glands or pyriform glands), with additional material from unicellular glands. A multicellular gland consists of a pear-shaped cluster of secretory cells, surrounding a central lumen, in which the fibrillar secretion of individual cells accumulates (Southward & Southward, 1966; Gupta & Little, 1970,

1975; Southward, 1984, 1993; Gardiner & Jones, 1993). Discharge is through a narrow multicellular duct. Strands of secretion emerge from the openings of such ducts in living animals and are visible in SEM preparations (Webb, 1965; Gardiner & Jones, 1993). The secretory surface of each cell forms a pocket lined with microvilli. There are simple digitate microvilli over much of the surface, but cup-shaped ones in the deeper part of the pocket or ampulla (Fig. 5B, D).

Recent studies have confirmed the suggestion made by Gupta & Little (1975) that the cup-shaped

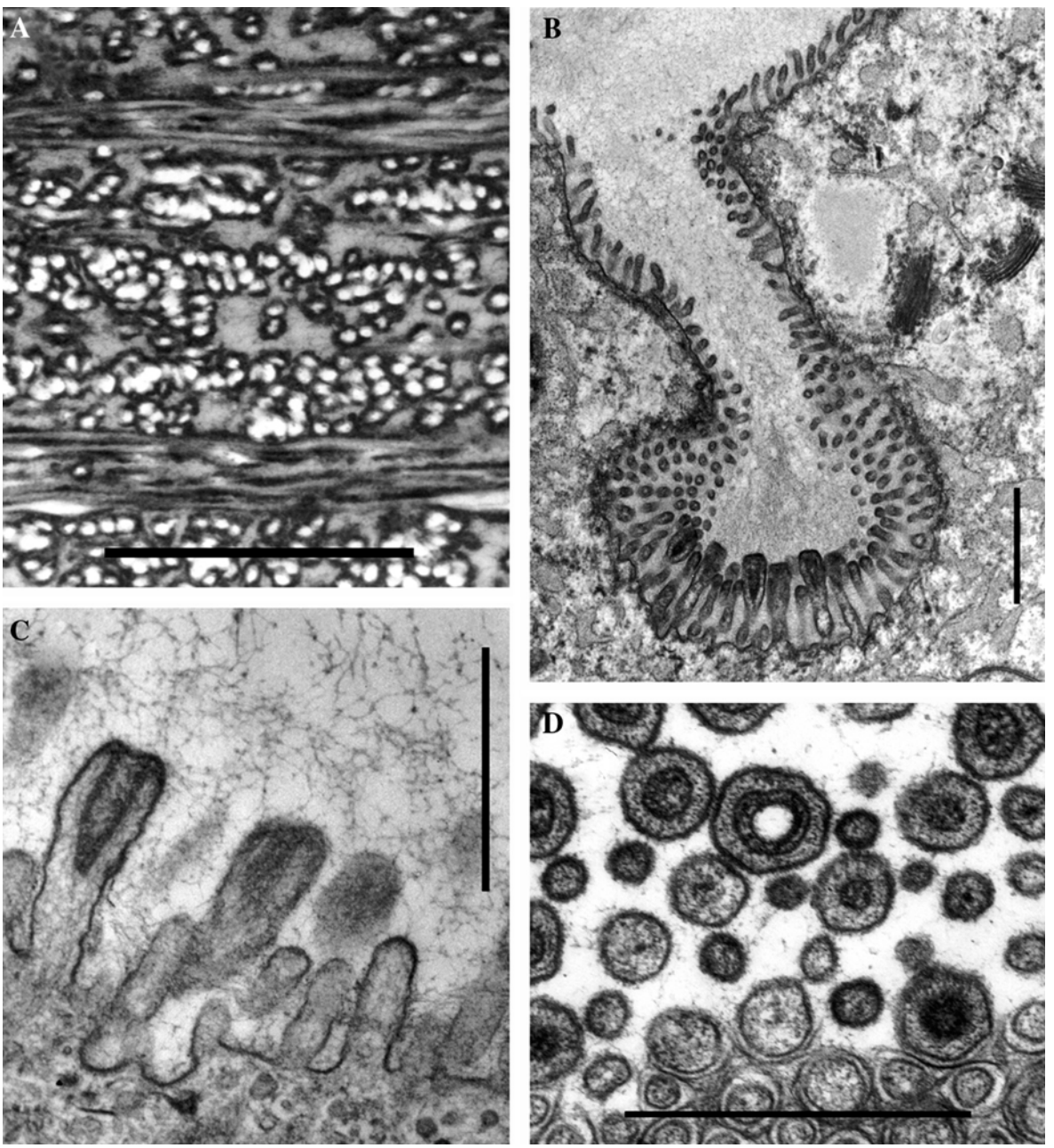

Figure 5. Tube of frenulate, *Nereilinum punctatum*. (A) Part of T S tube wall, chitin fibrils unstained, protein dark. (B) Cup-shaped microvilli in ampulla of pyriform gland. (C) Cup-shaped microvilli LS. (D) Cup-shaped microvilli TS. (TEMs courtesy of B. L. Gupta). Scale bars = 1 μm.

microvilli are the source of chitin for tube building. The site of secretion of chitin microcrystallites has been located very precisely in *Riftia pachyptila* with an immuno-gold labelling technique (Shillito et al., 1993, 1995b). There is labelling inside the hollow of the cup but not in the intracellular compartment of the gland cell, therefore the chitin molecules must be assembled in the cups. Chitin synthase activity has been identified in the tissue and the synthase is suspected to be in the cup membrane (Ravaux et al., 1998). Cells of the outer epidermis produce the major protein of the tube (Chamoy et al., 2000, 2001).

Shillito et al. (1997) describe the laying down of fibrils in plywood-like layers, 15 to 20 layers in 5 μm thickness of tube of *Riftia pachyptila*. There is a very similar fine structure in the tubes of frenulates (Fig. 5A), likened to a molecular sieve by Gupta & Little (1975). The closely packed fibrils form a physical barrier to even the smallest bacteria (0.1 μm), but water and small molecules such as sodium chloride can pass through, as shown in the tubes of the frenulates *Siboglinum atlanticum* and *S. ekmani* (Southward & Southward, 1963). The use of ^{14}C-labelled compounds showed that amino acids, glucose and fatty acids could be taken up by the animals, via their epidermis (Little & Gupta, 1968, 1969; Southward & Southward, 1970). The tube wall slowed the rate of uptake, but did not block it. After more experiments it was concluded that *Siboglinum* species could take up and metabolise dissolved organic compounds from known environmental concentrations at a rate sufficient to sustain respiration but not enough for growth and reproduction (Southward & Southward, 1981).

The symbiotic bacteria in the trophosome require oxygen and reduced sulphur compounds as a source of energy and CO_2 for carbon fixation. Dissolved gases can enter the tubes of frenulates that are buried in reducing sediments. Dissolved sulphide and other reduced sulphur compounds diffuse from the sediment through the tube wall to the trunk epidermis and thence to the trophosome and the bacteria. Oxygen from the overlying water diffuses into the top part of the tube and to the tentacles, where it is bound to haemoglobin in the blood and carried to the trophosome (Terwilliger et al., 1987). Hydrothermal vent tubeworms such as *Riftia pachyptila* are able to absorb both oxygen and sulphide from the mixed vent and ambient water bathing their branchial plumes (Childress & Fisher, 1992) but *Lamellibrachia* sp., living at cool seeps in the Gulf of Mexico, probably obtain much of their sulphide via a thin-walled basal extension of the tube that penetrates into sulphidic sediment and has been shown to be permeable to dissolved sulphide (Julian et al., 1999). Sulphide uptake by the posterior region of the body can be enough to fuel total inorganic carbon uptake via the plume (Freytag et al., 2001). Studies of *Lamellibrachia satsuma* at a shallow site in southern Japan used sulphur isotope ratios to show that the major source of sulphide to these animals was from the sediment, through a similar thin-walled extension of the tube (Miura et al., 2002).

Cuticle and body wall

Two distinct types of collagen are present in pogonophores, as shown by immuno-labelling: a cuticular and an interstitial type. Recent analysis of the two types of collagen in the vestimentiferan *Riftia pachyptila* has shown that cuticle collagen has a great fibre length (1.5 μm), is non-striated, has high thermal stability (37 °C) and high threonine content. The cuticle consists largely of layers of collagen fibrils, often arranged helically, and penetrated by microvilli from the cell surface (reviewed by Southward, 1993; Gardiner & Jones, 1993). It forms thickened ridges and plates, particularly the frenulum or bridle of frenulates, the scale-like adhesive plaques typical of all pogonophores, and the rectangular scales on the tentacles of the frenulate *Lamellisabella coronata*. Compared with this, the interstitial collagen of the extracellular matrix (ECM) is formed of shorter, striated fibres, has a melting point of 29 °C and contains less threonine (Gaill et al., 1995; Mann et al., 1996). It is present under the epidermis, between muscle layers and in the matrix of the obturaculum (Gaill et al., 1994).

The epidermis is rich in secretory cells and contains the completely intraepidermal nervous system (Gupta & Little, 1970; Southward, 1984, 1993; Gardiner & Jones, 1993). It is underlain by basal lamina, ECM of varying thickness, and the basal lamina of the body wall muscle (Gupta & Little, 1975; Jensen & Myklebust, 1975; Matsumo

& Sasayama, 2002). The myoepithelial cells of the frenulates *Siboglinum fiordicum* and *Oligobrachia mashikoi* show a relative lack of striation as compared to the tubificid *Branchiura sowerbyi*. From this observation, Matsumo & Sasayama (2002) concluded that *Oligobrachia mashikoi* could not be an annelid. However, the difference in body wall musculature is more probably the result of an inactive mode of life in pogonophores than an indication of phylogenetic affinities. Studies of muscle systems of about 25 polychaete species have shown that there is so much variation that it is difficult to draw phylogenetic conclusions from ultrastructural details (Lanzavecchia et al., 1988).

Anterior appendages

The anterior appendages of pogonophores have been termed tentacles in frenulates and moniliferans and branchial filaments in vestimentiferans. Rouse & Fauchald (1997) and Rouse (2001) coded 'peristomial grooved palps' or 'palps' as present in siboglinids/pogonophores, in their cladistic analyses of polychaetes. That is, they assume for the purpose of analysis that the anterior appendages are homologous with the peristomial grooved palps of polychaetes. The grooved palps of spionids have a longitudinal ciliated groove and a blind-ending blood vessel in a muscle-lined coelomic cavity; they receive nerves from two different parts of the forebrain (refs in Müller & Orrhage, *this volume*). They are both sensory and used for food collection. The homology with the 'tentacles' of frenulates, vestimentiferans and moniliferans is doubtful because the latter lack a ciliated groove, and are not used for food collection. Their blood system consists of two longitudinal blood vessels that are dilations of a blood sinus lying in the ECM between the epidermis and the muscle layer surrounding the central coelom. Narrow sinuses running through the pinnules where these are present (Fig. 6A, B), or under the epidermis, link the longitudinal vessels and complete the circulation (Gupta & Little, 1969; Van der Land & Nørrevang, 1977; Gardiner & Jones, 1993; Southward, 1993).

The pinnules are unicellular in frenulates and multicellular in vestimentiferans, but the cuticle is always extremely thin over the pinnules and the

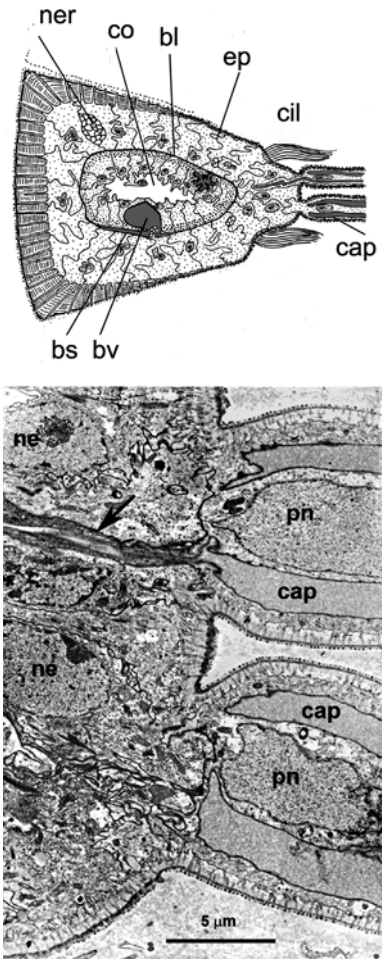

Figure 6. Frenulate tentacles, *Siphonobrachia ilyophora*. (A) TS tentacle and base of pinnules; bl – basal lamina; bs – blood sinus; bv – blood vessel; cap – capillary in pinnule; cil – cilia; co – coelom; ep – epidermis; ner – nerve. (Modified from Little & Gupta, 1969). (B) TEM of pinnule bases; cap – capillary: ne – nucleus of epidermal cell; pn – nucleus of pinnule cell; arrow – linking vessel. (Courtesy of B. L. Gupta).

blood circulates close to the surface. If ciliated cells are present, they form two narrow tracts along the tentacle or filament, on either side of the row of pinnules, but the pinnules themselves are not ciliated. Pinnules are not present in *Sclerolinum* species or some of the smaller frenulates, where the tentacles are extremely slender and their epidermal cuticle is thin. In all species the 'tentacles' seem to function as gills, and their structure is similar to that of many polychaete gills (e.g., Storch & Alberti, 1978). They have some sensory function, being provided with nerves from the sides of the intraepidermal brain.

Sensory cells are generally rather few, but *Riftia pachyptila* has specialised sensory filaments scattered among the respiratory ones (Gardiner & Jones, 1993).

Frenulates may possess over 200 tentacles/filaments, emerging from the body immediately behind the cephalic lobe (Southward, 1993). In their more complex branchial plume, vestimentiferans typically possess from several hundred to many thousands of filaments (Webb, 1969; Jones, 1981a).

In the branchial plume of vestimentiferan, the filaments are organized into right and left groups of lamellae (Fig. 3A). Depending on the species and, to some degree, where the lamellae occur along the length of the obturaculum, the filaments within a lamella are fused by their adherent cuticles along some portion of their length (Gardiner & Jones, 1993). In most vestimentiferans, the lamellae emerge directly from the anterior vestimental region and there is a basibranchial system of blood vessels connecting to the main dorsal and ventral vessels; each lamella is fused to its more medial lamella, with the two most medial lamellae (=earliest lamellae formed) fused to the lateral surface of the obturaculum (see Jones, 1985a; Gardiner & Jones, 1993; Miura et al., 1997).

In *Riftia pachyptila*, axial extensions of the main dorsal and ventral vessels run in an extended layer of vestimental tissue adhering closely to each side of the lower end of the obturaculum proper; from these axial blood vessels transverse vessels arise to supply the branchial vessels in the overlapping lamellae, from which the distal ends of separate filaments project at right angles (Jones, 1981a, Gardiner & Jones, 1993; Andersen et al., 2002; ECS, personal observation). The total branchial surface area per unit wet mass averages 22 $cm^2\ g^{-1}$ (Andersen et al., 2002), which is second among aquatic organisms only to another hydrothermal vent inhabitant, the polychaete *Paralvinella grasslei* (Jouin & Gaill, 1990). This average value translates to nine times the surface area of the remainder of the body (Andersen et al., 2002). Diffusion distances are very short, ranging from slightly more than 25 μm from the filament surface to the central coelomic cavity and its paired blood vessels to as short as 1 μm in certain regions of pinnules associated with the filaments. Their observations led Andersen et al. (2002) to conclude that the branchial plume is the predominant exchange organ in *Riftia pachyptila*.

Obturaculum

In *Riftia pachyptila*, the obturacular region occupies 11–35% of the length of the body (Jones 1981a). In other species where complete specimens are known, it comprises about 3.5% (juvenile specimen of *Alaysia spiralis*; Southward, 1991) and 2.8% (*Paraescarpia echinospica*; Southward et al., 2002) of the length of the body.

The anterior face of the obturaculum may be lined by a simple cuticle, as in *Alaysia spiralis* and *Lamellibrachia* species (see Southward, 1991, among others) and *Arcovestia ivanovi* (Southward & Galkin, 1997), or be covered by a crust-like material as observed for species of *Escarpia* (Jones, 1985a), *Seepiophila jonesi* (Gardiner et al., 2001) and *Paraescarpia echinospica* (Southward et al., 2002). In addition, some type of medial structure, which is secreted by the obturacular epithelium, may project from between the apical halves of the obturaculum. This medial structure takes the form of a small fin in *Tevnia jerichonana* and *Escarpia laminata*, an elongated spike (Jones, 1985a) in *Escarpia spicat*a, a longer spike in *Paraescarpia echinospica* (Southward et al., 2002), but a series of saucer-like structures in *Oasisia alvinae* and *Ridgeia piscesae* (Jones, 1985a).

The obturaculum consists of paired obturacular halves, each surrounded by an epithelium with an overlying cuticle (see Webb, 1969; Van der Land & Nørrevang, 1977; Jones, 1981a,b, 1985b; Gardiner & Jones, 1993; Malakhov et al., 1996a). Numerous bundles of longitudinal muscle fibres are situated immediately internal to the epithelium. Most of the internal volume of the obturacular halves is occupied by an extensively developed extracellular matrix (ECM), consisting mainly of collagen fibres, among which are spindle-shaped cells believed to be involved in collagen secretion (Andersen et al., 2001). In the centre of each obturacular half is a slender perivascular coelom that surrounds a blind-ending obturacular vessel.

Andersen et al. (2001) demonstrated that the muscle bundles consist of smooth muscle fibres, which is an uncommon feature of muscles in annelids, and that the ECM displays an organization

different from a typical annelid cartilage. Based on an assessment of the nature of the muscle fibres and the relationship between the muscle bundles and the ECM, Andersen et al. (2001) suggested that the muscle fibres produce a tension that allows the system to act as a catch connective tissue capable of changing its softness and stiffness. The large volume of cartilaginous matrix provides a force opposing the contraction of the surrounding muscle bands and thus maintains the shape and solidity of the obturaculum. The obturaculum supports the filaments when outside the tube (Fig. 3A) and acts as a plug when the animal retreats.

The development of the obturaculum begins later than the filaments. In a juvenile 1.8 mm long of a species of *Ridgeia*, Southward (1988) documented the first appearance of the obturaculum as a pair of D-shaped bulges of cuticle-covered epithelium projecting above the anterior end of the brain, but buried in a depression between two semicircles of seven filaments. Each bulge contained a mesodermal core with a central blood vessel. This observation suggests that the obturaculum may be an outgrowth of the vestimental region, rather than the first body segment (Jones, 1985b) or the first pair of filaments (Ivanov, 1989).

In the light of this possibility, one of us (SLG) examined serial sections of the obturaculum of a juvenile specimen (45-filament stage) of *Oasisia alvinae*. The obturaculum displays the typical organization discussed above (Fig. 7A) with the exception of the most apical region. Here the obturacular vessel is absent and each obturacular half is completely filled with cells, i.e., a layer of ECM is not apparent (Fig. 7B). Near the base of the obturaculum, the branchial lamellae lose their structural integrity, and the branchial epithelium becomes continuous with the epithelium apical to the brain. Also, a layer of epithelium, which is derived from the branchial lamellae, encircles the obturaculum providing it with a double layer of epithelium (Fig. 7C). This second epithelial layer is bounded externally by a cuticle. At the region where the obturaculum merges with the vestimentum, both epithelial layers become continuous with the epithelium surrounding the brain (Fig. 7D). These observations support Southward's (1988) observations and strongly suggest that the obturaculum is an apical outgrowth of the vestimental region. Such a developmental pattern may have significance for future discussions of patterns of segmentation in vestimentiferans.

Anatomy of the blood vascular system

The major blood vessels in Pogonophora, as in other annelids, are the dorsal and ventral vessels (Webb, 1969; Van der Land & Nørrevang, 1977; Gardiner & Jones, 1993; Malakhov et al., 1996b). The dorsal vessel is contractile throughout most of its length, but especially in the heart region, located in the anterior vestimental region in Vestimentifera (Fig. 8A) (Gardiner & Jones, 1993) and in the forepart in Frenulata (Southward, 1993). The dorsal vessel pumps blood forward into the branchiae. The flow is reversed at the tips of the branchiae and continues from there into the ventral vessel. More posteriorly, the dorsal and ventral vessels are connected by trophosomal vessels and by the septal vessels in the opisthosome.

Throughout most of its length, the dorsal vessel is suspended in paired coelomic cavities by a dorsal and a ventral mesentery. The extracellular matrix between the two cell layers of the mesenteries is continuous with the vascular lamina (Fig. 8B, C). There is no cellular endothelium. In the heart region, the perivascular cavities disappear and the lumen of the blood vessel is constricted by a thick musculature. Behind the heart, an intravasal body occupies the lumen of the blood vessel in Vestimentifera (Schulze, 2002) and Frenulata (Ivanov, 1963; Southward, 1993). An intravasal or heart-body is a strand of tissue located inside the dorsal contractile blood vessel. It is separated from the blood by a basal lamina. An intravasal body is also present in several taxa in the Terebellida (Dales & Pell, 1970; Spies, 1973; Jouin-Toulmond et al., 1996). In Vestimentifera, it extends through the trunk and into the opisthosome and adheres to the ventral side of the lumen of the dorsal vessel where the mesenteries split (Schulze, 2002) (Fig. 8B). The basal lamina of the intravasal body is thicker than the vascular lamina but constructed more loosely (Fig. 8C). The cytoplasm of the epithelial cells contains numerous mitochondria, rough endoplasmic reticulum, some Golgi vesicles and electron-dense inclusions. The electron-dense inclusions resemble haematin-bodies with their heterogeneous, lamellar substructure (Fig. 8C).

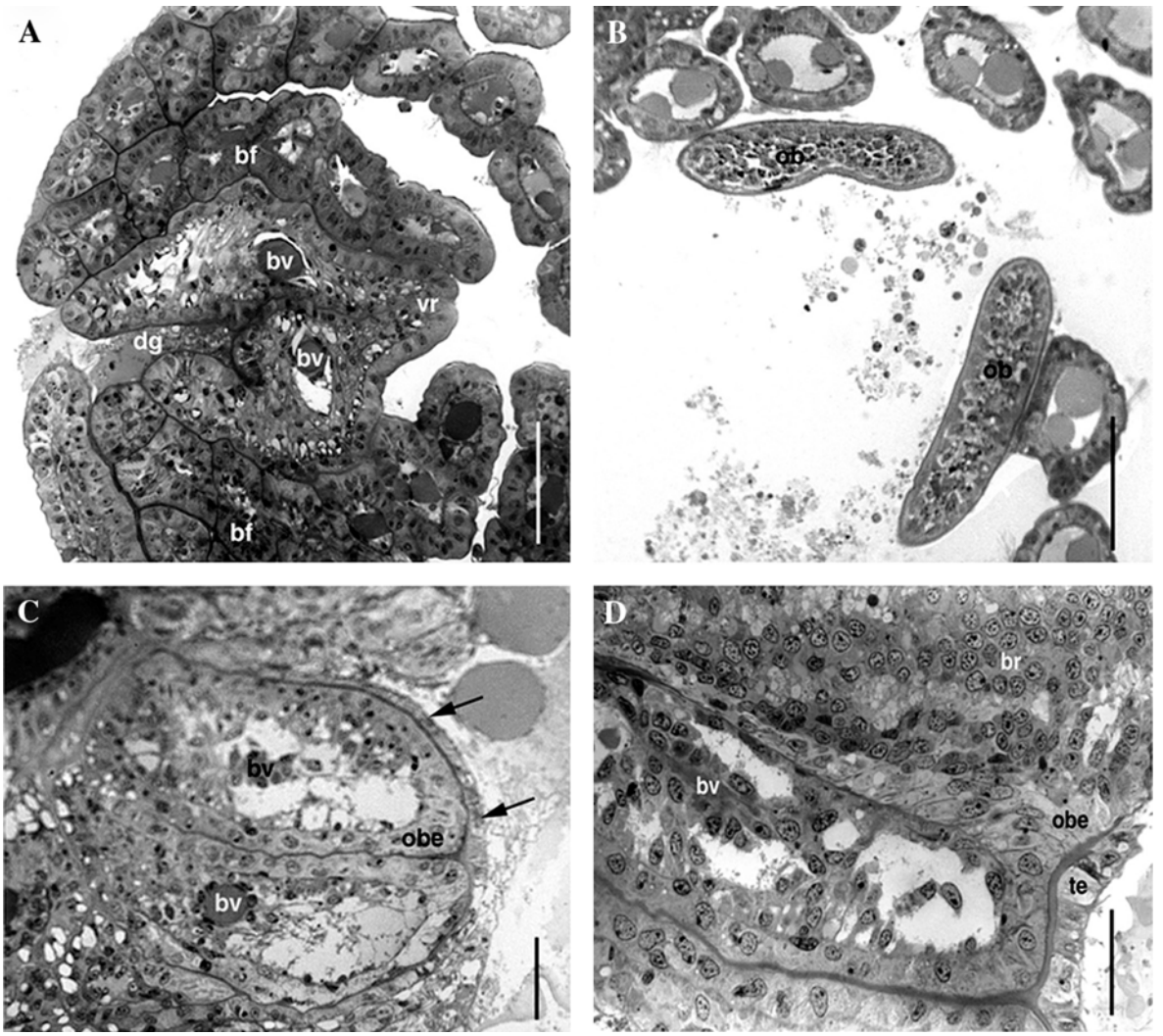

Figure 7. Obturaculum of juvenile *Oasisia alvinae*. Light microscopy. (A) cross section in mid-region showing typical shape of obturaculum with dorsal groove (dg) and prominent ventral ridge (vr). (B) cross section in apical region showing obturacular halves (ob) separated and lacking obturacular associated blood vessels. (C) cross section near base of obturaculum. Note tentacular epithelium (arrows) surrounding obturacular epithelium (obe). (D) cross section at region of merging of obturaculum with vestimental region. Note obturacular epithelium (obe) continuous with epithelium surrounding brain (br). bf – branchial filaments; te – tentacular epithelium. Scale bars: A–C = 75 μm; D = 25 μm.

Haemoglobin function and synthesis

An essential characteristic of pogonophoran blood is extracellular haemoglobin (Hb). Three types of Hb have been identified in the vestimentiferan *Riftia pachyptila* (Zal et al., 1996): V1 (~3500 kDa), V2 (~400 kDa) and C1 (~400 kDa). Whereas V1 and V2 occur in the blood, C1 is restricted to the coelom. V1 is a hexagonal bilayer hemoglobin (HBL), common in many annelid groups (Green et al., 2001). It consists of two monomeric globin chains, four linker units and one disulphide bonded dimer, whereas V2 and C1 have one monomeric globin chain and one disulphide bonded dimer, but no linker units (Zal et al., 1996). Two types of HBL exist in annelids (Jouan et al., 2001): type I occurs in oligochaete, hirudinean and vestimentiferan Hbs as well as sabellid chlorocruorins; type II occurs in other polychaetes. HBL appears to be absent in frenulates; they have V2

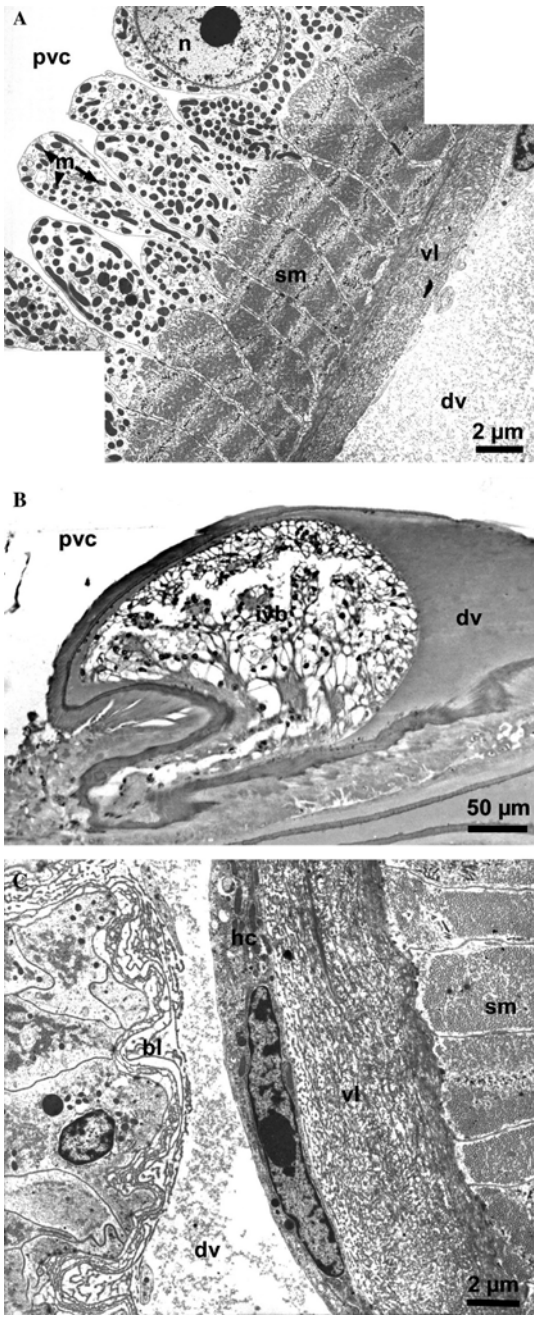

Figure 8. Dorsal vessel and intravasal body in Vestimentifera. (A) *Ridgeia piscesae*, TEM, wall of dorsal vessel in vestimental region, posterior to the heart. (B) *Paraescarpia echinospica,* LM, intravasal body in vestimentum. (C) Intravasal body (left), blood haemocyte and wall of dorsal blood vessel in vestimentum. Density and abundance of striated collagen fibres is greater in vascular lamina of dorsal vessel than in basal lamina of intravasal body. dv – dorsal vessel; hc – haemocyte; ivb – intravasal body; m – mitochondria; n – nucleus; pvc – perivascular cavity; sm – striated muscle of dorsal vessel wall; vl – vascular lamina.

(light haemoglobin) only (Terwilliger et al., 1987; Yuasa et al., 1996).

Haemoglobin synthesis may take place in the intravasal body in pogonophores as in other polychaetes (see Kennedy & Dales, 1958; Breton-Gorius, 1963; Mangum & Dales, 1965; Dales & Pell, 1970). In Pogonophora, more direct evidence for haemoglobin synthesis, such as the presence of globin mRNA, haemoglobin molecules or activity of enzymes for haeme synthesis is required. On the other hand, based on *in situ* hybridizations of juvenile *Riftia pachyptila*, Andersen et al. (2001) showed that globin A2, a haemoglobin component common to all three haemoglobin types, is present in the branchial plume, the vestimental region and trunk, and particularly in the afferent trophosomal vessels. Ultrastructural studies of the afferent trophosomal vessels showed a well-developed rough endoplasmic reticulum, making them another likely site for haemoglobin synthesis. The juveniles examined by Andersen et al. (2001) did not yet have a distinct intravasal body. It is possible that the site of haemoglobin production changes during ontogeny or that different types of haemoglobin are produced at different sites.

Arp et al. (1987) showed that vestimentiferan haemoglobin is capable of binding oxygen and sulphide simultaneously and reversibly, providing a very effective mechanism to satisfy the high demands for both dissolved gases in symbiont metabolism (Childress & Fisher, 1992) (see next section). Vestimentiferan haemoglobins vary in their affinities for oxygen and sulphide, with C1 having the highest oxygen affinity and V1 the highest sulphide affinity. Zal et al. (1997, 1998) identified two mechanisms of sulphide binding in *Riftia pachyptila*:

1. All three Hbs can form *S*-sulphohaemoglobin by binding HS^- to cysteine residues according to the following equilibrium:

$$R\text{—}SH + HS^- + H^+ \leftrightarrow R\text{—}SSH$$

2. Only V1 Hb is able to form persulphide bonds:

$$R\text{—}SS\text{—}R + 2HS^- + 2H^+ \leftrightarrow 2R\text{—}SSH$$

Persulphide bonds are formed near neutral pH but with an optimum at pH 7.5. The lower pH in the trophosome might trigger sulphide release to the symbionts.

Frenulates appear to have only a lower molecular weight vascular Hb. In *Siboglinum fiordicum* and *Galathealinum* sp. the Hb has a very high affinity for oxygen, and apparently a very low ability to bind sulphide (Terwilliger et al., 1987; Childress & Fisher, 1992) but the amino acid sequence of the globin-like chains of *Oligobrachia mashikoi* Hb indicates a sulphide-binding potential (Zal et al., 1997). The very long slender bodies of frenulates penetrate deeply into sediments containing low concentrations of reduced sulphur compounds. Sulphide may reach the trophosome mainly by diffusion and the carrying capacity of the haemoglobin may be less important than in vestimentiferans.

Sulphide binding might have evolved in vestimentiferans as a mechanism to detoxify sulphide, as well as to transport and store large quantities to fuel symbiont metabolism (Schulze & Halanych, 2003). On the other hand, Bailly et al. (2003) suggest that sulphide binding is a plesiomorphic feature in annelids and that secondary loss occurred repeatedly as a result of constraint relaxation.

Trophosome

The tissue that hosts symbiotic bacteria in pogonophores lies between the dorsal and ventral longitudinal blood vessels in the trunk region. It occupies most of the length of the trunk in vestimentiferans (Fig. 2) but is restricted to the posterior two thirds of the trunk in frenulates and moniliferans (Southward, 1993; Gardiner & Jones, 1993). The intracellular bacteria in the trophosome of *Riftia pachyptila* were discovered by Cavanaugh et al. (1981), who suggested that the large, globular bacteria were sulphur-oxidizing chemoautotrophs, a new concept of symbiosis at the time, but one which solved the long-standing problem of how these 'gutless' animals feed. Felbeck et al. (1981) and Rau (1981) found evidence of autotrophy. A similar type of symbiosis was found in frenulates and moniliferans, with thin rod-shaped bacteria in the trophosome of frenulates, and thick rods in *Sclerolinum brattstromi* (Southward, 1982). One frenulate, *Siboglinum poseidoni*, contains methanotrophic symbionts (Schmaljohann & Flügel, 1987), but other species examined appear to be sulphide oxidizers (Southward et al., 1986). The position and structure of the frenulate trophosome suggest that it replaces the alimentary canal. Outer peritoneal cells surround a core of bacteriocytes, with a central lumen in some species. Blood-filled sinuses between the basal laminae of the two epithelia are connected to the longitudinal blood vessels. Ultrastructural studies of juvenile *S. poseidoni* indicate that the bacteriocytes are endodermal in origin (Callsen-Cencic & Flügel, 1995).

The bulky vestimentiferan trophosome has a more complicated blood vascular system than that of frenulates, but it seems to have basically the same two-layered structure. This can be seen during the post-larval development as described by Jones & Gardiner (1988, 1989), Southward (1988) and Gardiner & Jones (1993). In the juveniles of *Ridgeia piscesae* and *Riftia pachyptila* it appears that the initial symbiotic bacteria are engulfed from the ciliated gut lumen by the endodermal cells. These bacteria remain undigested in vacuoles, while other types of bacteria are digested in separate phagocytotic vacuoles. Later, the multiplication of the symbiotic bacteria and the bacteriocytes that contain them occurs in the central region of each lobule, around an axial blood vessel. Growing bacteriocytes with larger bacteria are in the median layer while cells with degenerating bacteria lie towards the periphery, where there is a network of afferent blood vessels (Bosch & Grassé, 1984a,b; Gardiner & Jones, 1993; Bright et al., 2000; Bright & Sorgo, 2003). Blood circulates through sinuses among the bacteriocytes, from the peripheral vessels to the axial vessels.

Experimental work on uptake, transport and metabolism of sulphide and oxygen and fixation of inorganic carbon in *Riftia pachyptila* has been reviewed by Childress & Fisher (1992) and Goffredi et al. (1998). The nitrogen requirements of the symbiosis appear to be met by nitrate, absorbed by the host, reduced to ammonia by the symbionts and transferred to the host as amino acids (Girguis et al., 2000). Bright et al. (2000) used ^{14}C bicarbonate and autoradiography to show that carbon fixation and release of organic carbon from the symbionts to the host occur rapidly (within 15 min

of exposure) in small *Riftia pachyptila*. The released carbon is incorporated into fast-growing tissues and into the tube-secreting glands, as might be expected from the speed of tube secretion observed by Gaill et al. (1997). The trophosome of *Riftia* contains much glycogen, both in the bacteria and the host cells (Sorgo et al., 2002). Before the trophosome of frenulates was known, glycogen was found to be abundant in the central core of the postannular trunk of the frenulate *Siboglinum atlanticum* (Southward, 1973). Later studies of the fine structure of the trophosome of *Siboglinum* and *Oligobrachia* species found that the outer, peritoneal epithelium was the chief storage region for glycogen particles and large droplets, probably of lipid and protein, (Southward, 1982, 1993). The trophosome also accumulates metals. In older animals it contains so many dark granules that it becomes brown or greenish black. In frenulates these mineral granules are multilayered and present mainly in the peritoneal cells (Southward, 1982). Granules in the peritoneal cells of *Riftia pachyptila* contain S, Fe, Cu and Ag, while granules in bacteriocytes contain Mg, P, S, Ca, Fe, Cu and Ag (Truchet et al., 1998).

Excretory organs

The role of nephridia is the removal of waste products, in particular nitrogenous wastes, by filtration of body fluids and subsequent modification of the primary urine by secretion and/or selective re-absorption. In Pogonophora, the trophosome probably partially takes over these functions; mineral particles are stored as insoluble granules and bacterial symbionts might recycle nitrogen compounds (Southward, 1993). In addition, excretory organs are present in the anterior forepart in frenulates (Ivanov, 1963; Southward, 1993) and the anterior vestimentum in vestimentiferans (Van der Land & Nørrevang, 1977; Gardiner & Jones, 1993; Schulze, 2001b). To date, no observations have been made on excretory organs of *Sclerolinum*.

Ivanov (1963) distinguished two orders within the Frenulata, based on the anatomy of excretory organs: the Thecanephria and the Athecanephria. The major difference is that nephridial ducts are longer in Thecanephria and enclosed in a 'renal sac'. Southward (1993), however, using ultrastructural methods found the distinction unclear and could not observe a renal sac in any of the species she examined.

In general, excretory organs occur in two basic designs: metanephridia and protonephridia. In metanephridia, filtration is driven by muscular contraction of blood vessels and is accomplished through the basal lamina of podocytes, representing a barrier between a blood vessel and a coelomic compartment. According to the functional model of nephridial design by Ruppert & Smith (1988) Pogonophora are expected to have metanephridia because their blood vascular system is well developed (see previous section). However, in no pogonophoran species have all three components of a metanephridial design been observed:

1. A blood vessel surrounded by a coelomic compartment: The dorsal vessel in all Pogonophora and the obturacular vessels in Vestimentifera are suspended in perivascular cavities.
2. Podocytes: not detected in any pogonophoran. In Vestimentifera, the dorsal vessel is thickly muscularized, not only in the heart region, but also along the entire length (Fig. 8A, B), making it unlikely that podocytes are present.
3. Nephrostome: Ivanov (1963) presents anatomical diagrams of the thecanephrian *Lamellisabella zachsi* and the athecanephrians *Oligobrachia dogieli* and *Siboglinum caulleryi* and shows coelomoducts in all of them. However, Southward (1993) found them only in one representative of the Thecanephria, *Siphonobrachia lauensis*, but not in several *Siboglinum* species or *Oligobrachia gracilis*. Gardiner & Jones (1993) observed nephrostomes in the vestimentiferans *Tevnia jerichonana* and *Oasisia alvinae*. Despite careful examination of serial sections of eight vestimentiferan species, including the species examined by Gardiner & Jones, Schulze (2001b) did not observe nephrostomes in any vestimentiferans.

In protonephridia, fluids are filtered through a filtration weir of a terminal cell driven by ciliary action. The only species of pogonophoran in

which terminal cells have been observed are *Oligobrachia gracilis* and *Siboglinum ekmani* (Southward, 1993: Fig. 20A, B). They seem to filter blood from the ventral blood vessel (Fig. 9A). Primary urine is then transported into a median section, consisting of convoluted ducts that eventually connect to ectodermal ducts leading to the nephropores. A median section of convoluted ducts is also present in Vestimentifera (Schulze, 2001b: Figs 1–3). It is located behind the brain and in the area of the sinus valvatus, where the efferent plume vessels join to form the ventral vessel. The sinus valvatus is a valve-like structure that seems to prevent backflow of blood into the tentacles. It is possible that the blood pressure in this area is increased due to the small opening to the ventral vessel. The convoluted, ciliated ducts of the excretory organ are closely apposed to the blood vessels in this region (Fig. 9B). Although no terminal cells have been observed, it is the most likely site for their presence.

The apparent absence of nephrostomes, at least in some species, might be correlated with the presence of extracellular coelomic hemoglobin. Coelomic proteins might be lost through coelomoducts if these were present, a case parallel to nephtyid polychaetes (Smith & Ruppert, 1988).

In conclusion: No uniform picture of the nature of nephridia in pogonophores is emerging. Considering that Pogonophora show close affinities to polychaetes that clearly have a metanephridial design (Rouse & Fauchald, 1997), it is possible that more basal groups have metanephridia, whereas more derived groups, such as vent-inhabiting vestimentiferans might have a protonephridial design and show only rudimentary nephrostomes if any.

Opisthosome

The opisthosome is the segmented posterior section of the pogonophoran body. As in other annelids, the segmentation arises from a posterior growth zone (Webb, 1964; Nørrevang, 1970; Southward, 1975a). The septa between adjacent segments consist of double mesodermal layers with a thin layer of extracellular matrix between them.

In general, the vestimentiferan opisthosome has more segments than the frenulate and moniliferan opisthosome. In frenulates, four to eight peg-like chaetae are present in each segment, arranged dorsolaterally and ventrolaterally on both sides. In vestimentiferans, the anterior opisthosomal segments bear rows of uncini (Schulze, 2001a) similar to uncini in the frenulate girdles and parapodial uncini in some Sabellidae (Knight-Jones & Fordy, 1979; Knight-Jones, 1981), Arenicolidae (Bartolomaeus & Meyer, 1997) and Oweniidae (Meyer & Bartolomaeus, 1996). Rows of uncini are also present in the opisthosomal segments of *Sclerolinum* (Southward, 1972, 2000; Smirnov, 2000). The different designs in the three groups may be correlated with different lifestyles: whereas the opisthosome of Vestimentifera and Monilifera is enclosed inside the tube and probably serves as an anchor, the opisthosome in the Frenulata extends outside the tube and is mainly used as a digging organ.

On the cellular level, chaetogenesis in Pogonophora is similar to chaetogenesis in related polychaetes (Gupta & Little, 1970; George & Southward, 1973; Bartolomaeus, 1995; Schulze, 2001a). The chaetoblast and follicle cells produce chitinous chaetal material that accumulates around the apical microvilli of the chaetoblast. As the chaeta grows, the microvilli retract and the chaetal material forms hollow cylinders. In *Ridgeia piscesae*, chaetae are formed in chaetal follicles, consisting of a chaetoblast, a follicle cell and an epidermis cell. Sometimes two follicle cells may be present. There are two follicle cells in the frenulate *Siboglinum ekmani* (Gupta & Little, 1970). No particular formative zone has been detected for chaetal formation in any pogonophoran.

When Jones (1985a) erected Obturata (= Vestimentifera) and Perviata (= Frenulata) as separate phyla he based his decision partly on the difference in opisthosomal segmentation between the two groups. While in Vestimentifera each opisthosomal segment comprises two symmetrical coelomic cavities, the opisthosomal coeloms in adult Frenulata are unpaired. However, studying early juveniles, Southward (1975a) showed that in frenulates the opisthosomal segments arise from paired mesodermal blocks and that the mesenteries are lost in later development. Furthermore, contrary to Jones (1985a), we found that in the vestimentiferan *Ridgeia piscesae* only the posterior

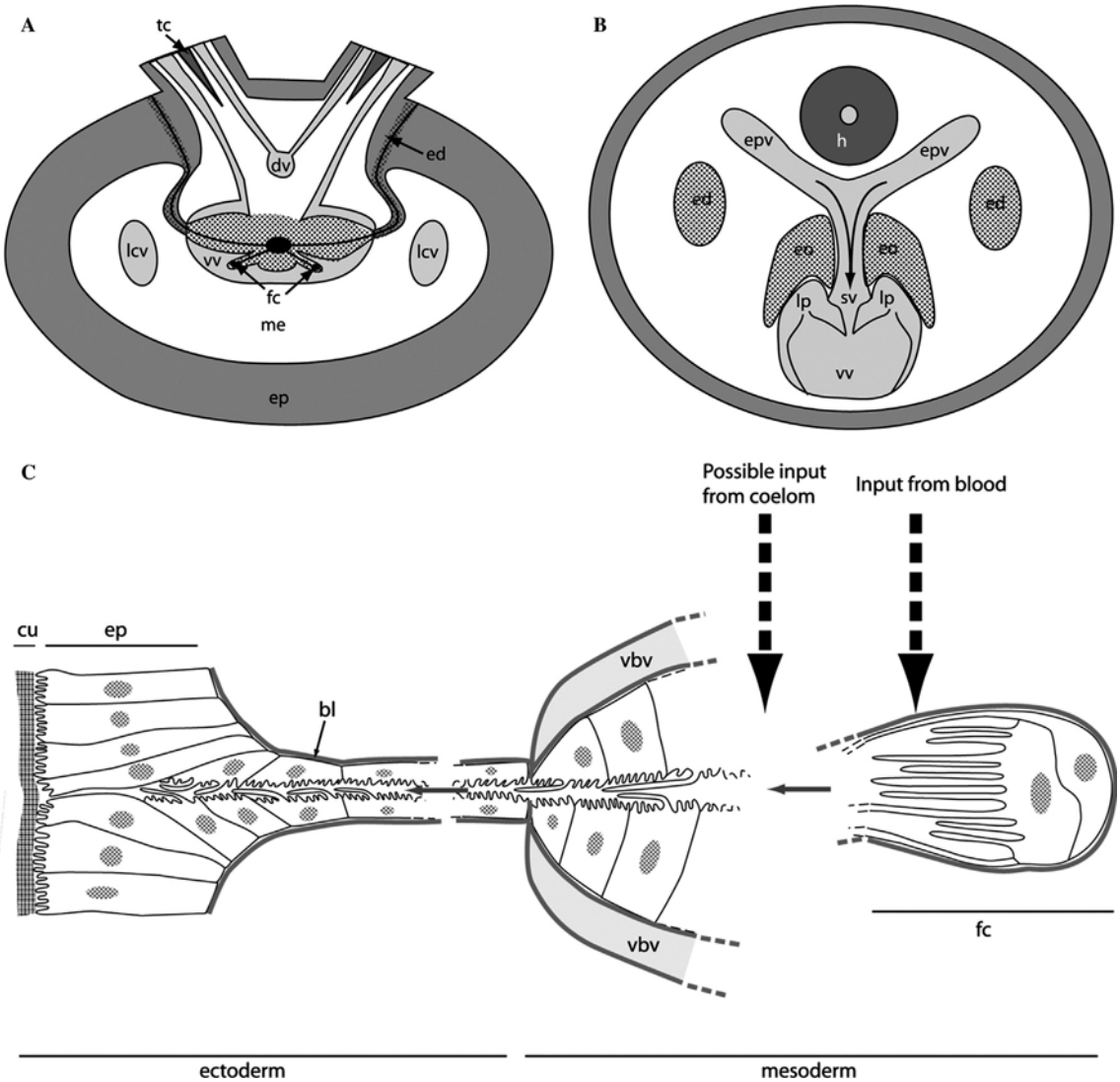

Figure 9. Association of excretory organs with blood vessels in Frenulata and Vestimentifera. (A) *Oligobrachia gracilis*, cross section in region of tentacle origin. The convoluted ducts of the medial part of the excretory organ are completely enclosed in the ventral vessel. (B) *Ridgeia piscesae*, cross section in region of heart. The convoluted ducts in the excretory organ surround the junction of the efferent plume vessels and the sinus valvatus. Arrows indicate direction of blood flow. (C) Cellular arrangement in excretory organs of *Oligobrachia gracilis*. Gray arrows indicate direction of flow of excretory fluids. Whether additional influx through coelomoducts happens in addition to filtration through filtration cells could not be detected. bl – basal lamina; cu – cuticle; dv – dorsal vessel; ed – excretory duct; eo – excretory organ; ep – epidermis; epv – efferent plume vessels; fc – filtration cell; h – heart; lcv – lateral cephalic vessels; lp – lateral pockets of sinus valvatus; sv – sinus valvatus; tc – tentacular coelom; vv – ventral vessel.

faces of the septa are muscular instead of both faces (Fig. 10). This is also the case in frenulates, suggesting that the opisthosomes are more similar in the two groups than previously assumed.

In both Vestimentifera and Frenulata, the septa are vascularised by septal vessels. These originate from the ventral vessel and run between the two mesodermal layers of the septa without a cellular lining (Fig. 10B). Glands that resemble the tube-secreting pyriform glands in the vestimentum and trunk are associated with the septa. Gardiner & Jones (1993) distinguished in *Riftia pachyptila* long glands that are linked to the blood vascular system and short glands that are not. This distinction was

not clear in histological sections of *Ridgeia piscesae* or *Tevnia jerichonana*. In *Ridgeia piscesae*, the peripheral glands have a pore to the exterior and resemble the pyriform glands of the vestimentum and trunk whereas the more central glands have no direct connection to the exterior. They are formed by one of the mesodermal layers of the septa and are usually surrounded by blood spaces (Fig. 10A).

Unlike the nerve cord in the trunk, the ventral nerve cord in the opisthosome has no giant axons in either Frenulata or Vestimentifera. Southward (1975a) describes three nerve trunks in the opisthosome of the frenulate *Siboglinum fiordicum* and 'nerve bulges' that may be ganglia, probably associated with chaetal movement. No 'nerve bulges' are present in Vestimentifera but nerve tracts are often present between the chaetal sacs.

Although adult pogonophores have no continuous gut, a residual gut can be detected in the opisthosomes even in adult vestimentiferans. An anus was detected in a *Tevnia jerichonana* specimen with an opisthosome of 7×4 mm size (Fig. 11B). The gut tissue is closely associated with the dorsal vessel and enclosed in musculature. In a small *Ridgeia piscesae* specimen (5 mm) the gut wall in cross section consists of approximately seven multiciliated cells and is completely enclosed in a basal lamina (Fig. 11A). In another small specimen bacterial cells with diameters up to 4.3 μm were observed around the gut (Fig. 11C). Bacterial cells were absent from the lumen of the ciliated duct. Previously, a rudimentary gut has only been demonstrated in early developmental stages (Jones & Gardiner, 1988; Southward, 1988) of a maximum length of 2.6 mm. As it is not continuous in adult specimens, it is obviously non-functional as a digestive tract. Whether it has taken over another function in the adult needs to be examined.

Reproduction, embryos and larvae

In pogonophores the sexes are separate, except for the hermaphrodite *Siboglinum poseidoni*. The reproductive organs are normally paired and when mature, occupy quite a lot of space in the trunk, often severely restricting the trophosome. The male gonopores are always at the anterior end of the trunk, as are the female gonopores of vestimentiferans, but in frenulates the oviducts open more posteriorly (Ivanov, 1963). In *Sclerolinum* species paired sperm ducts open at the anterior end of the trunk, but female gonopores have not yet been found (ECS, personal observation). The long-headed sperm of frenulates and vestimentiferans have mitochondria wrapped around the nucleus (Gardiner & Jones, 1985, 1993; Southward, 1993). Frenulates produce spermatophores wrapped in a mucopolysaccharide coat (Flügel, 1978), secreted in a special region of the sperm duct. No spermatophores have been observed in *Sclerolinum* species. The early report of spermatophores in *Sclerolinum sibogae* (Southward, 1961) was erroneous; recent re-examination of the type material (ECS) found sperm in the gonoducts but no spermatophores, while examination of living *Sclerolinum brattstromi* found small clusters of long-headed sperm able to swim in concert (personal observation ECS). In vestimentiferans, sticky sperm masses are transferred between males and females of *Ridgeia piscesae* (Southward & Coates, 1989; MacDonald et al., 2003) and *Tevnia jerichonana* (personal observation ECS). *Riftia pachyptila* in natural populations release puffs of eggs and sperm into the surrounding water (Van Dover, 1994), but internal fertilization remains a strong possibility in this species because sperm have been detected inside the oviducts (Gardiner & Jones, 1985; P. A. Tyler, personal communication).

Some frenulates incubate large eggs in their tubes until the settlement stage, whereas vestimentiferans disperse their eggs and have lecithotrophic planktonic development. Frenulates with small eggs possibly have planktonic larvae but these have never been reared (Southward, 1999). The known larvae have been described as trochophore-like.

Marsh et al. (2001) reared *Riftia pachyptila* at *in situ* temperature and pressure (2 °C and 250 atm). Cleavage was slow but a swimming ciliated larva was observed after 34 days, when it had two equatorial bands of simple cilia, but no mouth, apical tuft or telotroch. The larval metabolic lifespan was estimated to be 34–44 days, based on respiration rate and energy reserves. Study of the current regime showed that there is a high probability that larvae will remain within a few tens of km of their source but the potential along-ridge dispersal could be as much as 100 km. Cool seep vestimentiferans studied

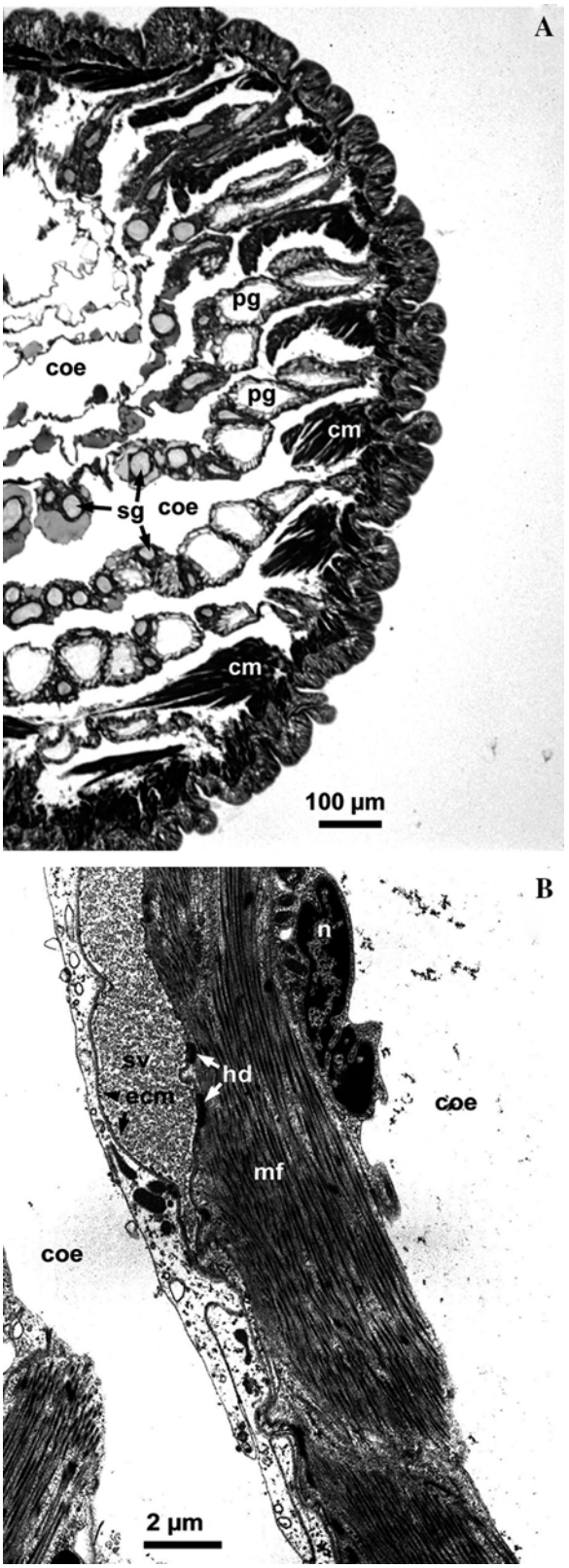
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Figure 10. Opisthosomal septa in the vestimentiferan *Ridgeia piscesae*, associated glands and septal vessels. (A) horizontal section, LM, showing pyriform and septal glands. (B) TEM, opisthosomal septum, anterior is to the left. cm – circular muscle; coe – coelom; ecm – extracellular matrix; hd – hemidesmosomes; mf – myofilaments; n – nucleus; pg – pyriform glands; sg – septal glands; sv – septal vessel.

earlier, took 3 days to develop from cleavage to a larva with prototroch (at 9 °C, 1, 50 and 100 atm.) and survived at least 3 weeks (Young et al., 1996). *Lamellibrachia satsuma* larvae studied by Miura et al. (1997) had both prototroch and telotroch. Eggs removed directly from the oviduct of this species began cleavage without insemination and developed to a trochophore-like larva in three days (Fukunaga et al., 2000) suggesting that they had already been fertilized in the oviduct. A similar observation of cleavage starting *in vitro* without the addition of sperm was noted in the frenulate *Siboglinum fiordicum* (Bakke, 1976).

The larvae of vestimentiferans may have to survive for some weeks, without feeding, in fully oxygenated conditions until they can settle in a suitable environment for the adult to develop. The question of whether the symbionts are transmitted via the egg, or whether they are definitely acquired from the environment is still debatable. Cary et al. (1993) could not detect any symbionts in the oocytes of *Riftia pachyptila*, and none have been found in early settlement stages of vestimentiferans or frenulates, but slightly later stages, having a temporary mouth and alimentary canal, do have bacteria in some of their endodermal cells and this seems a logical way to take them up from the environment (Southward, 1988; Jones & Gardiner, 1988, 1989; Callsen-Cencic & Flügel, 1995). Schmaljohann & Flügel (1987) found that the symbionts in the trophosome of *Siboglinum poseidoni* and methanotrophic bacteria isolated from its environment were similar in ultrastructure and size. Other evidence for environmental origin of the bacteria rests on the discovery that vestimentiferans of different genera, but from the same locality, host identical symbionts, demonstrated by molecular techniques (Feldman et al., 1997). Further detailed analysis and review (McMullin et al., 2003) supports environmental acquisition of symbionts and absence of co-speciation between vestimentiferans and their symbionts. However, free-living bacteria identifiable by new molecular

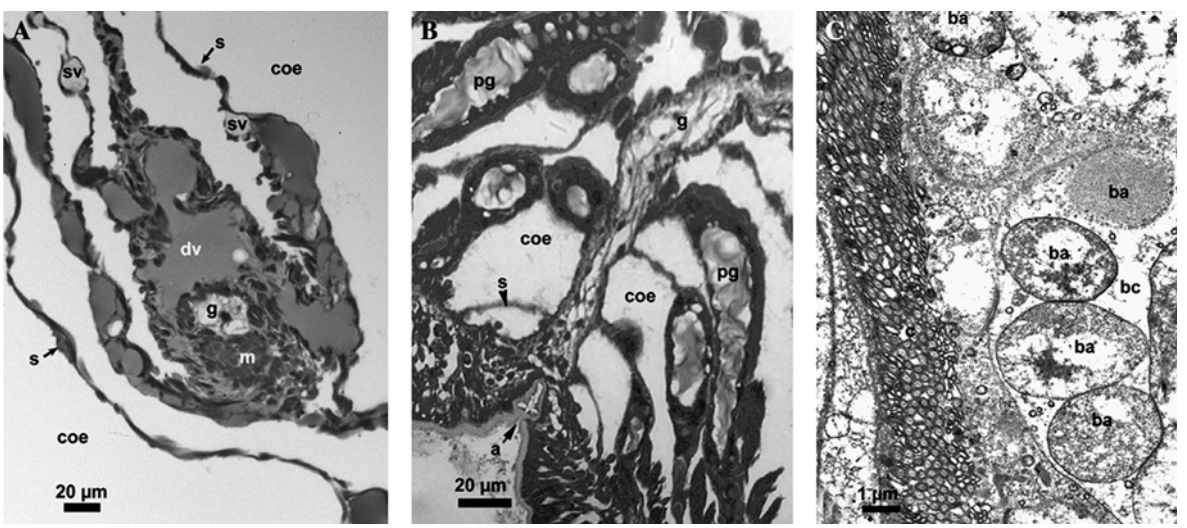

Figure 11. Rudimentary gut and anus in opisthosome. (A) *Ridgeia piscesae*, LM, the rudimentary gut is located on the ventral side of the dorsal vessel, enclosed in muscular tissue, ciliated gut lumen appears dark. (B) *Tevnia jerichonana*, LM, hindgut and anus, lumen of gut lies in a different plane. (C) *Ridgeia piscesae*, TEM, ciliated lumen of rudimentary gut and bacteriocytes with endosymbionts in close proximity; a – anus; ba – bacteria; bc – bacteriocyte; coe – coelom; dv – dorsal vessel; g – gut; m – muscle; pg – pyriform glands; s – septa; sv – septal vessel.

techniques as the vestimentiferan symbionts have not yet been reported, to our knowledge, from the natural environment near vents or seeps.

Systematic implications

Aspects of the anatomy of pogonophores have been discussed here with the aim of understanding their way of life, and understanding the differences between frenulates, vestimentiferans and moniliferans/sclerolinids. Halanych (2005) has reviewed publications on their molecular phylogeny, concluding that vestimentiferans are monophyletic, *Sclerolinum* is sister to the vestimentiferan clade and frenulates form a monophyletic clade sister to the vestimentiferan/moniliferan clade. This fits the anatomical differences discussed here, though the position of *Sclerolinum* seems a little uncertain.

Relationships within the groups have not been discussed in this paper, but we agree with Halanych that a new investigation of frenulate phylogeny would be worthwhile. Ivanov's anatomical work resulted in a division into two orders, Athecanephria and Thecanephria, on the basis of differences in the excretory anatomy, the arrangement of papillae and glands on the postannular trunk, and the shape of spermatophores, none of which seem as clear cut now (Southward, 1993). It would be best to abandon this subdivision. The division of vestimentiferans into the orders Axonobranchia and Basibranchia (Jones, 1985a) may also be abandoned. The distinction was based on the anatomy of the branchial plume, separating Riftiidae from the rest of the families because of the difference in the pattern of blood supply to the branchial plume of *Riftia*. This, however, is an autapomorphy for *Riftia pachyptila*, which might have arisen as a consequence of the large size of the species and the unusual length of its plume and obturaculum. Molecular phylogenies clearly indicate that *R. pachyptila* is closely related to other vent-inhabiting species from the Eastern Pacific. We then have seven vestimentiferan and five frenulate families to consider (see Table 1, p. 226). This is not the place to revise them, but the phylogenetic trees of genera produced by Halanych (2005) and Rouse (2001) should provide guidance, and have already indicated that some of the original descriptions are inadequate, so there is scope for new descriptive and anatomical work, as well as new molecular work if suitable material can be collected.

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