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THE BIOLOGY OF VESTIMENTIFERAN TUBEWORMS

MONIKA BRIGHT[1] & FRANÇOIS H. LALLIER[2,3]

[1]*Department of Marine Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*
E-mail: monika.bright@univie.ac.at

[2]*UPMC—Paris 6, Laboratoire Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France*

[3]*CNRS UMR 7144, Station Biologique de Roscoff, 29680 Roscoff, France*
E-mail: lallier@sb-roscoff.fr

Abstract Vestimentiferan tubeworms, once erected at a phylum level, are now known to comprise a part of the specialised deep-sea polychaete family Siboglinidae. Their widespread and abundant occurrence at hydrothermal vents and hydrocarbon seeps has fostered numerous studies of their evolution and biogeography, ecology and physiology. Harbouring autotrophic, sulphide-oxidising, intracellular bacterial symbionts, they form large populations of 'primary' producers with contrasting characteristics, from fast-growing, short-living species at vents, to slow-growing, long-living species at seeps. These different life strategies and the ways they modify the biogeochemistry of their respective environments have consequences on the macro- and meiofaunal assemblages that develop within vestimentiferan bushes. New findings indicate that postlarval recruits get infected through the skin by free-living bacteria for which growth is rapidly and specifically limited by the host to mesoderm cells around the gut that further transform into the characteristic trophosome. The resulting internal location of symbionts prompts specific adaptations of the hosts to fulfil their metabolic requirements, including unusual sulphide and carbon dioxide assimilation and transport mechanisms. Symbiont genome sequencing has improved our knowledge of potential bacterial metabolism and should rapidly open the way for new research approaches to resolve the intricate physiological relationships between a eukaryotic host and its chemoautotrophic bacterial symbionts.

Introduction

Since the discovery of *Riftia pachyptila* around deep-sea hydrothermal vents on the Galapagos Spreading Centre (GSC) in 1977 (Corliss et al. 1979), a considerable amount of work has been devoted to the biology of vestimentiferan tubeworms, a group of animals with rather low diversity and less than 30 recognised species mostly living in the deep sea. Yet, the conspicuous aggregations they form around deep-sea hot vents or cold seeps and their strict reliance on endosymbiont chemoautotrophic metabolism have granted vestimentiferans, and among them *R. pachyptila*, the rank of model organisms. However, since the reviews by Tunnicliffe (1991) and Childress & Fisher (1992), there has not been a comprehensive review of the biology of these tubeworms despite the publication of more than 500 papers on the topic to date (444 record count with "Riftia" in topic on the Web of Science as of July 2009; 358 with "vestimentifer*" and 628 with "Riftia OR vestimentifer*"). Some recent reviews have included vestimentiferan tubeworms in a broader coverage of symbiosis in annelids (Bright & Giere 2005) or chemosynthetic symbiosis (Dubilier et al. 2008, Vrijenhoek 2010) or have dealt with phylogeny and biogeography (McMullin et al. 2003, Halanych 2005), palaeontology (Little & Vrijenhoek 2003, Campbell 2006), morphology and anatomy (Southward

et al. 2005), development (Southward 1999, Southward et al. 2005), reproduction and dispersal (Tyler & Young 1999), physiology and biochemistry (Minic & Herve 2004), while others have focused on the bacterial symbionts (Stewart et al. 2005, Vrijenhoek 2010).

The aim of the present review is to summarise recent work on the host biology in a broad sense and on host–symbiont relationships. The review briefly considers the extant species known to date and their phylogeny, mostly inferred from recent molecular studies, and introduces the present hypothesis regarding the phylogeography of Vestimentifera. The two differing habitats occupied by vestimentiferan tubeworms are described (i.e., hydrothermal vents and cold seeps), and the different life strategies associated with them are considered, including the abiotic conditions to which they are exposed, the biotic interactions they develop and the role of tubeworms as foundation species and their associated fauna. An important part of the review is devoted to the description of the life cycle of vestimentiferans, focusing on *R. pachyptila*, from the aposymbiotic larval stage to the symbiotic adult stage, embracing the brief but crucial symbiont transmission phase. The last part of the review focuses on the progress made in understanding the physiology and biochemistry of the chemoautotrophic host–symbiont association. The general use of the term *tubeworm* in this review refers to vestimentiferans and not to other taxa.

Systematics, phylogeny and biogeography

What are vestimentiferan tubeworms?

Vestimentifera is a taxon of marine deep-sea worm-like animals living in chitinous tubes and lacking a digestive tract. Instead, they harbour chemoautotrophic bacteria in an internal organ, the trophosome, and derive their metabolic needs from these bacteria. They share these characteristics with some other animals, namely, the Monilifera (*Sclerolinum*) and Frenulata (Figure 1), forming together the taxon Siboglinidae (Caullery 1914), previously referred to as Pogonophora, and nested within the Annelida. From a cladistic point of view, as stated by Rouse (2001), "Vestimentifera can be defined as the first siboglinid and all its descendants to have a vestimentum as seen in the

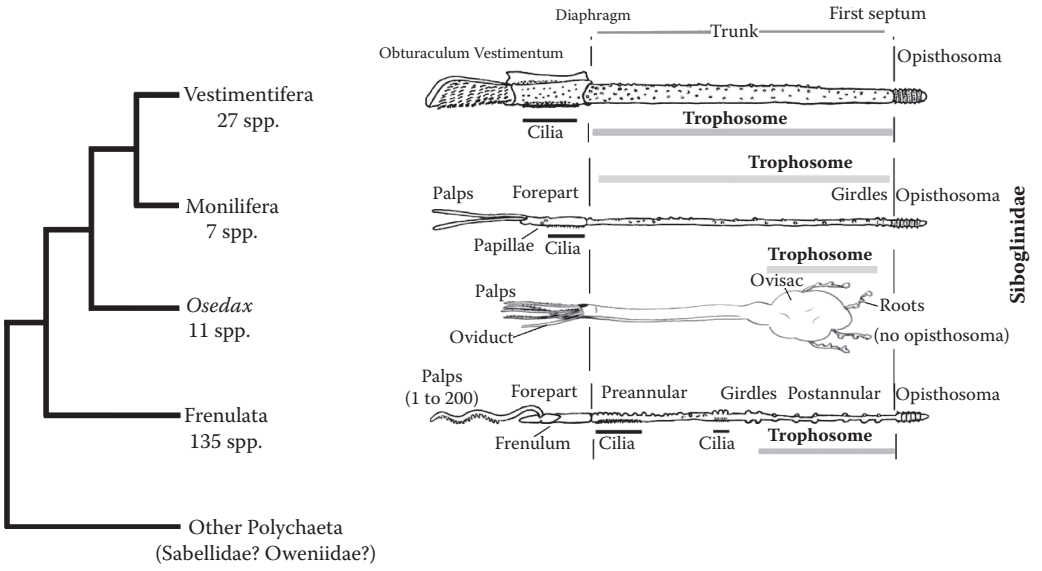

Figure 1 Schematic tree depicting the position of Vestimentifera within the polychaete family Siboglinidae and comparing the main morphological features of the different taxa of the group. (Drawings for Vestimentifera, Monilifera and Frenulata were modified from Southward et al. 2005.) See text for details.

holotype of *Riftia pachyptila*", the vestimentum being a "waistcoat-like" body region "with lateral flaps that enfold the anterior part of the body, behind the plume" (Southward 2000).

Historically, it took almost a century and a rather complicated path to reach this present status. A more detailed account may be found in Rouse (2001) and Pleijel et al. (2009), but the story may be summarised as follows. The first *Siboglinum* species described by Caullery (1914) was not assigned to any phylum, but Uschakov (1933) described *Lamellisabella* as a polychaete. Revision by Johansson (1939) resulted in the erection of the class Pogonophora in 1939. Pogonophora reached phylum status in 1944, and Ivanov (1951) united *Siboglinum* and *Lamellisabella* within this phylum. Pogonophora were often considered as deuterostomes at that time with a tripartite body plan. The description of the segmented, chaetae-bearing terminal part of *Siboglinum fiordicum* (Webb 1964) progressively brought the Pogonophora back to the protostomes. The description of *Lamellibrachia barhami* by Webb (1969) introduced a new class, Vestimentifera, in the phylum Pogonophora. The discovery of *Riftia pachyptila* and other vent species, however, prompted Jones (1985) to place Vestimentifera at phylum rank besides Pogonophora. This view was challenged by many authors, and finally Rouse & Fauchald (1997) reunited the two phyla within the annelids as the family Siboglinidae Caullery, 1914.

Siboglinids are now firmly anchored within the polychaete annelids, even though their sister taxon within polychaetes is still debated, with some favouring Sabellida (Schulze & Halanych 2003) and others Oweniidae (Rousset et al. 2004, Struck et al. 2007). As can be seen in Figure 1, Frenulata are basal to the Siboglinidae, and Vestimentifera form the crown group with Monilifera as a sister taxon. This schematic phylogenetic tree is a summary based on molecular and morphological sources (Boore & Brown 2000, Halanych et al. 2001, Schulze 2003, Halanych 2005, Jennings & Halanych 2005). Frenulata typically inhabit anoxic sediments, Monilifera live on decaying organic matter or reduced sediments, and Vestimentifera are mostly found at hydrocarbon seeps and hydrothermal vents. Other siboglinids can be found on whale falls; these belong to the recently discovered genus *Osedax* with two species described initially (Rouse et al. 2004), and now the number of species described has increased to nine (Glover et al. 2005, Fujikura et al. 2006, Goffredi et al. 2007, Rouse et al. 2008) with several other species awaiting description (R. C. Vrijenhoek, F. Pradillon, personal communications). According to molecular data, these species fall between frenulates and moniliferan/vestimentiferan tubeworms (Rouse et al. 2004), and although they are undoubtedly siboglinid polychaetes, they have aposymbiotic dwarf males. The females lack an opisthosome, and their trophosome harbours heterotrophic symbionts and is located in the most posterior body regions, the ovisac and the so-called roots, which penetrate deeply into whale bones (Goffredi et al. 2007).

Morphology and anatomy of the adult vestimentiferan tubeworm

This part briefly introduces the external morphology and general anatomy of adult Vestimentifera. More detailed descriptions may be found elsewhere (e.g., Gardiner & Jones 1993, Southward 2000, Southward et al. 2005), with a noteworthy list of useful characters provided in Appendix 1 of Schulze (2003). Further details are given if appropriate in the context of this review.

The body of adult tubeworms is enclosed in a chitinous tube that is closed at the posterior end, a character in contrast to the open-ended tube of Monilifera and Frenulata. In *Riftia pachyptila*, the tube is cylindrical, virtually straight, and rather flexible; it can reach a length of more than 2 m and a diameter of 5 cm at the apex, with a tube wall thickness of 2–3 mm (Gaill & Hunt 1986, Gardiner & Jones 1993). The constant diameter of the tube of *R. pachyptila* and the existence of basal partitions, or clumps, not occupied by the worm (Gaill et al. 1997) contrast with the tubes of other vent-endemic species (e.g., within genera *Tevnia*, *Oasisia*, *Ridgeia*), which are conical, tapering at the basal end, more or less twisted, harder and often reinforced with annular extensions (collar). Seep genera (e.g., *Lamellibrachia*, *Escarpia*) have long tapering tubes, generally

hard and thick at their anterior, water-immersed part, but much thinner and fragile at their posterior, sediment-buried part (see, e.g., Andersen et al. 2004); the functional rationale for this is explained in the section 'Acquisition of inorganic substrata from the environment', p. 247.

The anterior part of the body, the obturacular region, consists of a branchial plume that can be extended from the tube and is composed of filaments, partly fused into lamellae and supported by a rigid, collagenous extension, the obturaculum (Figure 1) (Andersen et al. 2001). When the animal withdraws into its tube, the terminal part of the obturaculum blocks the entrance of the tube; it may be flat or may bear saucer-like or rod-like collagenous extensions in some species or even among some individuals within the same species (Southward et al. 1995, Andersen et al. 2004). Insertion of branchial lamellae is basal relative to the obturaculum in most species (the "Basibranchia" in Jones 1988) with the exception of *Riftia pachyptila* (the only "Axonobranchia" in Jones 1988), for which lamellae are inserted along the whole length of the obturaculum. In lamellibrachids and alaysiids the outer lamellae fuse to form a sheath surrounding the other lamellae.

Following the obturacular region, the vestimentum is a muscular region with two ventral wings that fold dorsally. When the animal extends its branchial plume from the tube, the vestimentum comes up to the rim of the tube and contracts, blocking the animal in this position. A ciliated area is located ventrally on the vestimentum, and gonopores open dorsally, extended by external paired ciliated grooves in males only. Conspicuous pores on the epidermis are connected to pyriform, chitin-producing glands (Gaill et al. 1992, Shillito et al. 1993) involved in tube biosynthesis. The vestimentum also encloses the heart, which is an extended and muscular portion of the dorsal vessel in the anterior part of the vestimentum; the brain, which is anteroventral; and the excretory organ, which is posterodorsal and adjacent to the brain (Gardiner & Jones 1993, Schulze 2001a, Gardiner & Hourdez 2003).

The trunk forms the most extended part of the adult body, although its relative length varies greatly during growth and between individuals in most species (see, e.g., Fisher et al. 1988, Andersen et al. 2004). Externally, the epidermis is relatively smooth and covered by a collagenous cuticle, with scattered pores connected to chitin-producing glands similar to those of the vestimentum. Internally, a muscular layer of circular and longitudinal muscles surrounds a vast coelomic cavity mostly filled with coelomic fluid (CF) and the conspicuous trophosome, a soft and highly vascularised tissue harbouring the symbiotic bacteria. The ontogeny and anatomy of the trophosome is described in detail in the life-cycle section of this review. Gonads and blood vessels run along the length of the trunk; their description can be found in the life-cycle and physiology sections of the review.

The opisthosome forms the posterior end of the body; it is a short, multisegmented region bearing rows of uncini that function to anchor the animal within its tube. The uncini of Vestimentifera, and Siboglinidae in general, are considered homologous to those of Terebellida and Sabellida, implying a monophyletic origin of these three taxa (Bartolomaeus 1995, 1997), although the phylogenetic value of the uncini has been questioned (Schulze 2001b) as their similar design may be the result of functional convergence.

Present state of vestimentiferan tubeworm systematics and phylogeny

Table 1 presents a comprehensive list of the 19 species of vestimentiferan tubeworms described to date, beginning with *Lamellibrachia barhami* Webb, 1969 and ending with *L. juni* Miura & Kojima, 2006 and *Oasisia fujikurai* Miura & Kojima, 2006. It also includes eight unnamed species recognised through their CO1 gene sequence (Kojima et al. 2001, Kojima et al. 2002, Kojima et al. 2003) but not yet described. A number of additional unnamed species have been reported in various cruise reports but without formal description or even gene sequence comparison, therefore preventing a confident assignation at the generic level. For example, species of vestimentiferan have been found recently in Mediterranean seep sites and North Sea mud volcanoes and await formal description.

Table 1 Taxonomic list of described[a] species of Vestimentifera

Species	Habitat[c]	Distribution[b]	References
Alaysia spiralis	HV	Lau	Southward 1991
Alaysia sp. *A1*	CS	Nankai	Kojima et al. 2003
Alaysia sp. *A2*	HV	Okinawa	Kojima et al. 2003
Alaysia sp. *A3*	HV	Manus	Kojima et al. 2003
Alaysia sp. *A4*	HV	Okinawa	Kojima et al. 2003
Arcovestia ivanovi	HV	Lau, Manus	Southward & Galkin 1997
Escarpia laminata	CS	GoM	Jones 1985
Escarpia southwardae	CS	GoG	Andersen et al. 2004
Escarpia spicata	SV, CS, WF	GoC, SCB	Jones 1985
Escarpia sp. *E1*	CS	Nankai	Kojima et al. 2002
Lamellibrachia barhami	SV, CS	GoC, JFR	Webb 1969
Lamellibrachia columna	SV	Lau	Southward 1991
Lamellibrachia juni	SV	Kermadec	Miura & Kojima 2006
Lamellibrachia luymesi	CS	GoM, Amazon	van der Land & Nørrevang 1975
Lamellibrachia satsuma	SV, CS	Kago, Nankai, Nikko	Miura et al. 1997
Lamellibrachia victori	CS	Uruguay	Mañé-Garzón & Montero 1985
Lamellibrachia sp. *L1*	SV	Okinawa	Kojima et al. 2001
Lamellibrachia sp. *L2*	CS	Nankai	Kojima et al. 2001
Lamellibrachia sp. *L4*	SV	Manus	Kojima et al. 2001
Oasisia alvinae	HV	EPR 21°N–18°S	Jones 1985
Oasisia fujikurai	HV	Kermadec	Miura & Kojima 2006
Paraescarpia echinospica	CS	PNG, Java	Southward et al. 2002
Ridgeia piscesae	HV	Explorer, JFR, GoC?	Jones 1985
Riftia pachyptila	HV	EPR 21°N–31°S, Galap, GoC	Jones 1981
Seepiophila jonesi	CS	GoM	Gardiner et al. 2001
Siphonobrachia lauensis	SV	Lau	Southward 1991
Tevnia jerichonana	HV	EPR 13°N–21°S	Jones 1985

[a] Kojima et al. (2001, 2002, 2003) mentioned unnamed species of *Lamellibrachia* (L1 to L7), *Escarpia* (E1, E2) and *Alaysia* (A1 to A4) in the western Pacific HV and CS sites and grouped them in several phylotypes based on CO1 molecular phylogeny.

[b] Distribution: Amazon = Amazon passive margin; EPR = East Pacific Rise; Explorer = Explorer Ridge; GSC = Galapagos Spreading Center; GoC = Gulf of California; GoG = Gulf of Guinea; GoM = Gulf of Mexico; JFR = Juan de Fuca Ridge; Kago = Kagoshima Bay; Kermadec = Kermadec back arc basin; Lau = Lau back arc basin; Manus = Manus back arc basin; Nankai = Nankai Trough; Nikko = Nikko Seamount; SCB = Santa Catalina Basin; Uruguay = Uruguay passive margin.

[c] Habitat: CS = cold seeps; HV = hydrothermal vents; SV = sedimented vents; WF = whale falls.

Species diversity is maximum in *Lamellibrachia* (nine spp.), followed with *Alaysia* (five spp.) and *Escarpia* (four spp.), whereas vent-endemic species are mostly represented by monospecific genera. However, recent molecular data point to several morphologically indistinguishable *Oasisia* species (Hurtado et al. 2002) and signal the need for a reexamination of monospecificity in vent species. This low species diversity within vent genera has been interpreted as evidence of a recent radiation of vent-endemic species from sediment-dwelling ancestors (e.g., Schulze & Halanych 2003).

Two alternative phylogenies are presented in Figure 2, summarising present ideas on the evolution within Vestimentifera. Broadly, all studies point to three major groups within Vestimentifera: the lamellibrachids, escarpids, and vent-endemic species as a crown group. Several molecular studies supported *Lamellibrachia* as the most basal extant vestimentiferan using 28S ribosomal DNA (rDNA; Williams et al. 1993), 18S rDNA (Halanych et al. 2001), or the CO1 gene (Black et al. 1997).

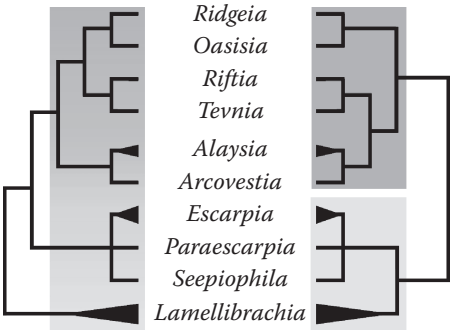

Figure 2 Alternative hypotheses for Vestimentifera phylogeny at the genus level. On the left side there is a continuum of species from seeps (light-grey shade) to vents (dark-grey shade) in contrast to the situation depicted on the right, showing two sister groups, one with seep species, the other with vent species. Clarification of the phylogenetic position of *Alaysia* and *Arcovestia* species would help resolve current uncertainty.

Using the same marker, CO1, Kojima et al. (2003) placed escarpids at the phylogenetic base but with no significant support. Cladistic approaches based on morphological characters are also in favour of a basal lamellibrachid clade (Schulze 2003) or, alternatively, a basal clade formed by lamellibrachids and escarpids (Rouse 2001). The existence of a vent-endemic species clade as the crown group of Vestimentifera has been repeatedly postulated from some molecular (Black et al. 1997) or morphological (Schulze 2003) studies, although this interpretation was weakly supported by other analyses (Williams et al. 1993, Halanych et al. 2001). The position of a clade uniting *Alaysia*-like and *Arcovestia* vestimentiferans (Kojima et al. 2003) is more problematic; it is seldom mentioned in the literature since these two taxa are generally not included (Halanych 2005). However, when they are included, they form a weakly supported clade with other vent-endemic species (Kojima et al. 2003), although morphological studies separated them, with *Arcovestia* closer to vent species and *Alaysia* closer to escarpids (Schulze 2003). The question remains regarding whether there is a clear-cut distinction between seep (and sedimented vents) and vent species (Figure 2, right), the latter including *Alaysia* and *Arcovestia* species, or if there is a continuous gradation from seep to vent species (Figure 2, left). To further resolve within-Vestimentifera phylogeny, other molecular markers or a combination of them is needed to increase the resolution of future analysis, which should include *Alaysia*-like species. Other arguments may come from a more comprehensive approach, namely, phylogeography, uniting the phylogenetic signal of molecular and morphological markers with our understanding of the biogeography and geological history of the oceans.

Where do vestimentiferan tubeworms live?

Vestimentiferans inhabit mainly hydrothermal vents and cold-seep environments in the deep sea, with the exception of *Lamellibrachia satsuma* living as shallow as 80 m in Kagoshima Bay (Hashimoto et al. 1993, Miura et al. 1997). Most commonly, species are restricted to one or the other habitat type. Some *Lamellibrachia* species, however, thrive in both environments (Jones 1985, Black et al. 1997, Southward & Galkin 1997, Tunnicliffe et al. 1998). *Escarpia spicata* exhibits the broadest habitat range, living at vents and seeps and on whale falls (Black et al. 1997).

Figure 3 shows the geographic and habitat distribution of known species on vent and seep sites around the world, based on the species and references list in Table 1. Cold-seep Vestimentifera have been found in all oceans to date except in the Indian Ocean (although *Paraescarpia echinospica* has been reported at a seep site of the Java Trench; Southward et al. 2002). In contrast, vent vestimentiferan tubeworms are only known from Pacific hydrothermal vents, with the crown

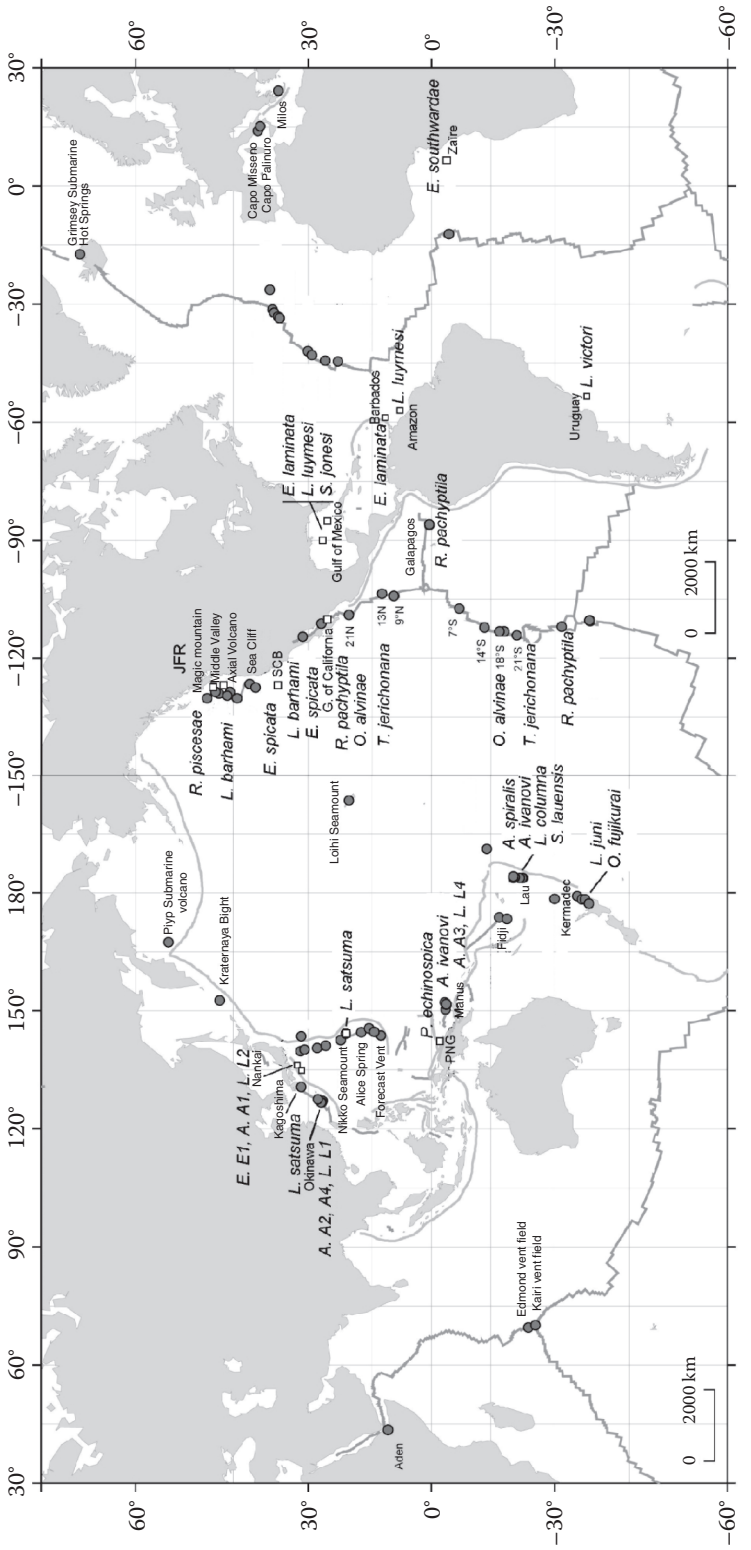

Figure 3 (See also Colour Figure 3 in the insert following page 212.) Geographic localisation of hydrothermal vent and cold seep sites explored to date (2008) with indication of identified Vestimentifera species. Refer to Table 1 for complete species names and references. (Map modified from Desbruyères et al. 2006.)

group species limited to the eastern Pacific. An interesting parallel exists between the western and eastern Atlantic: *Escarpia southwardae* found in the Gulf of Guinea is close to *E. laminata* (western Atlantic) and to *E. spicata* (eastern Pacific), suggesting an eastward route from the Pacific to the Atlantic (Van Dover et al. 2002, Schulze 2003, Andersen et al. 2004). This may be tentatively explained by larval dispersion and patterns of deep oceanic currents, which at present flow mainly from the Atlantic to the Pacific due to physical and geographical constraints (Van Dover et al. 2002). However, large areas are still poorly explored; the subduction zones of the eastern Pacific, the South Atlantic and Indian ridges and the circum-Antarctic ridges deserve more exploration. If vestimentiferan tubeworms are found in these areas, these data should yield important information regarding phylogeography of extant vent species. So far, mapping biogeographical data onto phylogeny (Schulze 2003) leads to ambiguous conclusions, except for the recent radiation of vent species on the East Pacific Rise (EPR).

A mention should be made about whale falls: only one vestimentiferan occurrence (*E. spicata*) has been documented off southern California (Feldman et al. 1998) despite considerable exploration and experimentation efforts in recent years (reviewed in Smith & Baco 2003 and Fujiwara et al. 2007). In addition, two occurrences of an unknown species of *Lamellibrachia* have been reported on rotting organic cargo from shipwrecks (Dando et al. 1992, Hughes & Crawford 2006).

Understanding the evolution and biogeographic patterns of Vestimentifera should also take fossil data into account. These have been reviewed (Campbell 2006) following reports about the apparent discrepancy between age estimates based on fossils and molecular phylogenies (Little & Vrijenhoek 2003). Molecular data indicated divergence estimates no older than mid-Mesozoic (about 100 million yr), whereas fossil data gave a Silurian or Devonian origin (over 400 million yr). The fossil remains consist mainly of preserved tubes that have been calcified (Little et al. 1997, Peckmann et al. 2005), and the comparison with chitinous tubes from living species is therefore difficult (Little & Vrijenhoek 2003, Campbell 2006).

From the various data reported here, it appears that Vestimentifera have recently radiated from seep species distributed worldwide to vent species restricted to the Pacific. The differences between these two habitats (illustrated in Figure 4) may prove to be an ecological constraint sufficient to drive the recent speciation events observed so far (Halanych et al. 2001, Schulze & Halanych 2003); the next section of the review details the ecological setting in which vestimentiferan tubeworms flourish.

Habitat and ecology

Contrasting habitat characteristics and environmental conditions distinguish the vent and seep habitats in which vestimentiferan tubeworms thrive. While vents are relatively short-lived, subject to major disturbances and waxing and waning of fluid flow, seeps are relatively long-lived and stable. Nevertheless, in both sulphide (i.e., $\sum H_2S$ total concentration of labile species of sulphide, and especially H_2S, the most toxic form for aerobic organisms; see Le Bris et al. 2003) is present and utilised by the endosymbionts of tubeworms. At vents, it is produced geothermally and emerges from cracks and crevices in the earth's crust (see Van Dover 2000). At seeps, the majority of sulphide is produced biologically via sulphate reduction utilising methane of other hydrocarbons as electron donors (Arvidson et al. 2004, Joye et al. 2004).

Most of our ecological knowledge in vestimentiferans is based on a few species, *Riftia pachyptila* and *Ridgeia piscesae* from vents and *Lamellibrachia luymesi* and *Seepiophila jonesi* from seeps. It has become apparent that some species from vents and seeps have evolved different life strategies in accordance with their different habitat types and characteristics, but some species can bridge the gap between these habitats and thrive under very broad environmental conditions. On the one hand, *Riftia pachyptila* grows extremely fast, but only in areas with relatively vigorous diffuse vent flow, and is relatively short-lived (Fisher et al. 1988, Hessler et al. 1988, Shank et al. 1998). In

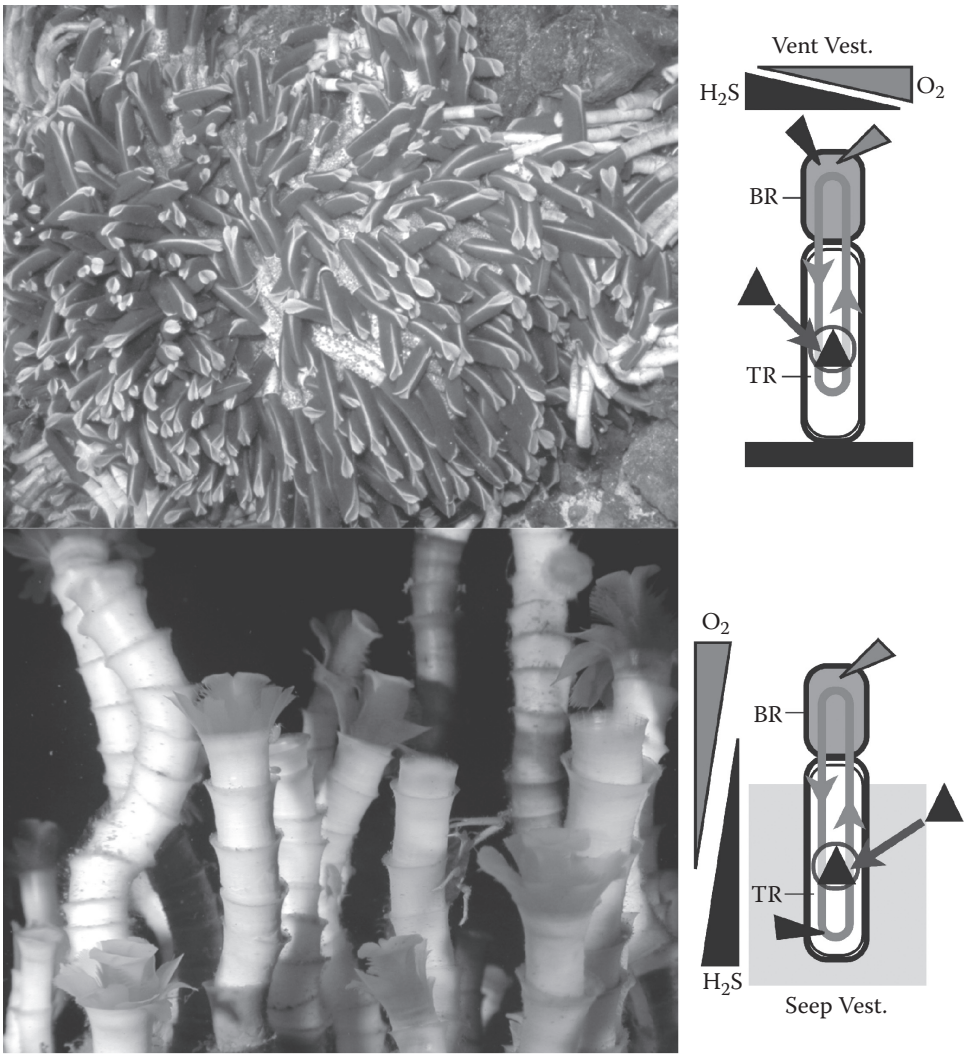

Figure 4 (See also Colour Figure 4 in the insert.) The two contrasted ecological settings of Vestimentifera are illustrated by the most-studied species: a bush of *Riftia pachyptila* from the EPR at 12°50′N (© Ifremer-Hope 1999) and one of *Lamellibrachia luymesi* from the Gulf of Mexico (© C.R. Fisher). Diagrams on the right show (top) how vent species such as *Riftia pachyptila*, fixed to the hard rock substratum, get both oxygen and sulphide through their branchial plume from the mixed fluid and deliver them through circulation to the internally located but environmentally acquired bacteria (black triangle) and (bottom) in seep species such as *Lamellibrachia luymesi,* sulphide is acquired from the sediment through the tapering, buried tube and trunk 'roots'. BR = branchial plume; TR = trophosome.

contrast, the seep tubeworms grow extremely slowly in areas with low seep flow and are extremely long-lived (Fisher et al. 1997, Julian et al. 1999, Bergquist et al. 2000, Cordes et al. 2005a, Cordes et al. 2007a). *Ridgeia piscesae*, however, can grow very rapidly when exposed to higher vent flow but is also capable of living in areas with lower vent flow, where it then grows much more slowly (Urcuyo et al. 2007).

Often, hydrothermal vents and seeps are visually easily recognised through their conspicuous populations of megafauna. Among these are Vestimentifera (Figure 4), which build bush-like

aggregations and act as foundation species by creating this physical structure that provides living space for other species (Corliss et al. 1979, Paull et al. 1984, Kennicutt et al. 1985, Tunnicliffe 1991, Sarrazin & Juniper 1999, Tsurumi & Tunnicliffe 2003).

Hydrothermal vent tubeworms

Deep-sea hydrothermal vents are found at midocean ridges and back-arc basins. Catastrophic volcanic eruptions, tectonic disturbances and hydrothermal vent fluid circulation form a transient, relatively short-lived environment (see Van Dover 2000). At the fast-spreading EPR, large-scale disturbances that kill existing communities can occur on a decadal scale (Haymon et al. 1993, Shank et al. 1998, Tolstoy et al. 2006). Small-scale disturbances due to fluid flow alternation and changes in fluid composition occur over shorter time intervals (Fustec et al. 1987, Jollivet 1993, Shank et al. 1998). Vent fluid composition, flow rates and mixing with ambient seawater are variable in dramatic and unpredictable ways (see Childress & Fisher 1992, Fornari et al. 1998) and create unstable physicochemical conditions regarding temperature, sulphide, oxygen and pH gradients. Striking spatial patterns of typical macrofauna assemblages along a gradient of hydrothermal fluid flux can nevertheless be distinguished.

At the EPR and the Galapagos Spreading Center (GSC), the vestimentiferans *Riftia pachyptila*, *Tevnia jerichonana* and *Oasisia alvinae* live in conditions of vigorous diffuse flow (Hessler & Smithey 1983, Hessler et al. 1985, Haymon et al. 1991, Sarrazin et al. 1997, Shank et al. 1998). The fluids are generally enriched in sulphide, methane, hydrogen, carbon dioxide, silicate and in some cases ferrous iron. However, their temperature and chemical composition are highly variable in space over scales of centimetres and in time over scales of seconds (see Le Bris et al. 2006a). Most often, temperature was used as a proxy of the hydrothermal vent fluid contribution and related chemical parameters in diffuse flow habitats (Johnson et al. 1988a,b, Shank et al. 1998, Mullineaux et al. 2000, Urcuyo et al. 2003, Hunt et al. 2004). In addition, various techniques for *in situ* chemical measurements have considerably increased understanding of the conditions under which tubeworms live (Johnson et al. 1986, 1988a,b, Childress et al. 1993, Sarradin et al. 1998, Luther et al. 2001, 2008, Le Bris et al. 2003, 2006a,b).

In aggregations of *Riftia pachyptila*, temperature ranges from ambient deep-sea temperatures of about 2°C to a maximum of about 30°C, pH can be as low as 4.4, and sulphide can be as high as 330 µ*M* (Luther et al. 2001, Le Bris et al. 2003, 2006a,b). Within a site, the overall sulphide–temperature relationship varies only slightly. There is high temperature and chemical variability on small spatial and temporal scales. For example, at organism level, the branchial plume experiences higher pH and lower temperature conditions than the tube (Le Bris et al. 2003). Further, aggregations from different sites also showed considerable differences in correlations between temperature and sulphide or pH, not necessarily related to the age of a site (Le Bris et al. 2006a).

The density of *R. pachyptila* per surface area of basalt on which they grow has been reported as about 2000 ind. (individuals) m^{-2} (Shank et al. 1998) and between about 550 and 3500 ind. m^{-2} (calculated from data in Govenar et al. 2005). Individuals reached tube lengths of 3 m with individual biomasses up to 650 g wet weight (Grassle 1986, Fisher et al. 1988). The surface area of tubes of *R. pachyptila* was at least a magnitude higher than the surface area of the basalt (Govenar et al. 2005). The biomass correlated positively with the tube surface area. In most aggregations, some individuals of *Tevnia jerichonana* or *Oasisia alvinae* were found (Govenar et al. 2005); mostly, they grow on the basalt underneath large *Riftia pachyptila* bushes and are difficult to sample (M.B. personal observations).

Colonisation of *R. pachyptila* was found to be rapid and growth fast, and this species may be rapidly replaced by successional species (Hessler et al. 1988, Lutz et al. 1994, Shank et al. 1998).

The first time series study monitoring tubeworm aggregations was reported by Hessler et al. (1988) at Rose Garden, GSC, where fast tubeworm colonisation was followed by mussel overgrowth. Long-term photographic and video-recording observations along a 1.37-km long transect at the 9°50′N EPR region for a period of 5 yr after the 1991 eruption revealed that the most common pattern of sequential colonisation was from *Tevnia jerichonana* (11 mo posteruption), to *Riftia pachyptila* (32 mo posteruption), to the mussel *Bathymodiolus thermophilus,* which appears last and has sometimes been known to replace *Riftia pachyptila*. However, development of *R. pachyptila* aggregations without prior colonisation by *Tevnia jerichonana* (at least in the size visible in photographs and videos) was also observed (Shank et al. 1998).

Ridgeia piscesae lives at varying diffuse flow habitats in the Northeast Pacific. This species exhibits a greater tolerance to varying physicochemical conditions than any other known Vestimentifera. It inhabits sulphide edifices (smokers or chimeys) with a more dynamic, relatively high-temperature diffusive flow of hydrothermal fluid and basalt with relatively stable, low-temperature diffuse flow (Southward et al. 1995, Sarrazin et al. 1997, 1999, Urcuyo et al. 1998). Several growth forms or morphotypes can be distinguished in this species (Black 1991, Southward et al. 1995, Tunnicliffe et al. 1997, Tsurumi & Tunnicliffe 2003). Their occurrence is correlated with specific physicochemical characteristics (Sarrazin et al. 1997, Urcuyo et al. 1998). Environmental conditions at sulphide edifices are known to change on the scale of months (Sarrazin et al. 1997), while diffuse flow from basalt is much more stable in time. Also *R. piscesae* bushes exhibit a range of architectural types based on the degree of branching complexity (Tsurumi & Tunnicliffe 2003).

Using accepted terminology, the long-skinny morphotype of *R. piscesae* was exclusively found at low-temperature basalt. It is reported to grow over a metre in length (Urcuyo et al. 1998). The surface of tubes increases 200-fold compared with the surface of the basalt on which the aggregation grows (Urcuyo et al. 2003). There was a spatial gradient of temperature from the base of the tube to the plume, ranging from consistently elevated temperatures compared with ambient temperature (+0.07–22°C anomaly) to only slightly elevated temperatures (Urcuyo et al. 2003, 2007). Long-term temperature recording showed that over 90% of time temperature at the plume level was less than 1°C above ambient (Urcuyo et al. 2003). Plume-level sulphide concentration was less than 0.1 μ*M*, while measurements at the base were about 100 μ*M* (Urcuyo et al. 2003). Sulphide and temperature were correlated within each site, and this relationship was different between sites (Urcuyo et al. 2007). At one site, no sulphide was detected at plume level, while at the other site low levels of sulphide were measured in 60% of the measurements (Urcuyo et al. 2007).

Other morphotypes of *R. piscesae*, including the short-fat morphotype, occur on active sulphide structures exposed to high temperatures lower than 45°C (Martineu et al. 1997, Sarrazin et al. 1997, 1999).

A model of community succession describing six stages of succession was proposed by Sarrazin et al. (1997), later extended and supported by additional studies (Sarrazin & Juniper 1999, Sarrazin et al. 1999, Govenar et al. 2002). Following stages I and II lacking tubeworms, colonisation of *R. piscesae* can be found occasionally during stage III with low abundances (212 ind. m^{-2}), while at stage IV dense populations of small tubeworms are present. By growth of these tubeworms, the community turns into stage V LF (low flow). Alternatively, a stage V HF (high flow) community can directly develop from stage I or II with higher temperatures. In later successional stages IV,V-HF, and V-LF, tubeworm abundances range from 10,310 to 66,903 ind. m^{-2} (Sarrazin & Juniper 1999, Govenar et al. 2002). Surface areas provided by tubeworms were calculated for several collections and the data showed that in assemblages at stage IV the small tubeworms increased the surface area 1.5 times, in V-LF with large tubeworms by about 22 times and in V-HF, also with large tubeworms, by about 27 times (Sarrazin & Juniper 1999). Stage VI is characterised by senescent tubeworms. Mean temperatures were quite similar in all stages except the stage VI with senescent tubeworms (Sarrazin et al. 2002).

Seep tubeworms

At active and passive continental margins, cold seeps with seepage of higher hydrocarbons and gas are found throughout the world's oceans. Mostly, the expelled fluids are similar in temperature to the surrounding deep-sea water, but differ chemically. They lack oxygen and may contain methane or sulphide or high salt concentrations, but they lack the high levels of heavy metal concentrations typical of hydrothermal vents (see Sibuet & Olu 1998, Sibuet & Olu-Le Roy 2002, Levin 2005). Such seeps can persist for centuries; for example, in the Gulf of Mexico (GoM) estimates for stable seepage of individual sites are in excess of 10,000 yr (Roberts & Aharon 1994).

The vestimentiferan aggregations composed of *Lamellibrachia luymesi* and *Seepiophila jonesi* were studied in detail at the upper Louisiana slope, GoM (Bergquist et al. 2003, Cordes et al. 2005c). Most of these tubeworm bushes were dominated by *Lamellibrachia luymesi,* with an average of 74.3% (Bergquist et al. 2003) and 72.4% (Cordes et al. 2005b) of the species present in the bushes, but in some of the aggregations both species were almost equally distributed or highly dominated by *L. luymesi*. Abundance was from about 200 to over 9500 ind. m^{-2} surface area of sediment (Bergquist et al. 2003). The tube surface area increased the sediment surface from which the tubeworms grew between 2.6- to 26-fold (Bergquist et al. 2003). Such aggregations can extend 2 m above the sediment surface (MacDonald et al. 1989, 1990). Population sizes of individual aggregations varied between 150 and 1500 individuals, but aggregations covering tens to hundreds of square metres are common (see Cordes et al. 2005b). With their posterior extensions, termed *roots*, the animals penetrate deeply into the sediment. Such large animals were found to experience nearly ambient deep-sea conditions at the plume level resulting from little mixing of seep fluids with overlaying bottom water (MacDonald et al. 1989, Scott & Fisher 1995, Julian et al. 1999, Freytag et al. 2001). Sulphide concentrations rarely exceed 0.1 μ*M* at the plume level and usually are well below 0.1 μ*M*; sulphide concentrations are higher at the roots (Bergquist et al. 2003, Cordes et al. 2005b), which were found to acquire sulphide from the porewater of the sediments (Julian et al. 1999, Freytag et al. 2001). In general, a decrease in sulphide concentration with the age of the aggregations was found (Cordes et al. 2005b).

A model of community succession was proposed by Bergquist et al. (2003). Concerning tubeworms, active recruitment occurs when sulphide concentrations are high and authigenic carbonate precipitation allows larval settlement onto hard substrata. This recruitment phase lasts as long as sulphide is present and may last for several decades. When growth of aggregations depletes sulphide in bottom waters, animals use their roots to take up sulphide from the porewaters of the sediment. This phase may last for several centuries (Bergquist et al. 2003). In a diagenetic model, Cordes et al. (2005a) estimated that hypothetical release of sulphate from the roots fuelling sulphate reduction in the sediment could augment exogenous sulphide production and support moderate-sized aggregations for hundreds of years.

Role of tubeworms as foundation species

Despite the low number of tubeworm-associated community studies, which are restricted to a few foundation species, and difficulties in direct comparisons of data (differences in sampling methods, extraction of fauna, standardisation of abundance, classification and distinction between macro- and meiofauna size classes), it appears that the abundance of associated macrofauna is lower but the species richness is higher at seeps compared with vents. Further, as Tsurumi & Tunnicliffe (2003) pointed out and Gollner et al. (2007) later showed, the meiofauna size class considerably contributes to the total number of species colonising tubeworm aggregations. However, meiofaunal species occur in low abundances, in contrast to the highly abundant macrofauna.

Quantitative or semiquantiative studies of the associated communities are limited to a few species: *Ridgeia piscesae* (Sarrazin et al. 1999, Govenar et al. 2002, Tsurumi & Tunnicliffe 2003), *Riftia*

pachyptila (co-occurring with the smaller species *Tevnia jerichonana* and *Oasisia alvinae*) (Govenar et al. 2004, 2005, Gollner et al. 2007) and the mixed aggregations of *Lamellibrachia luymesi* and *Seepiophila jonesi* (Bergquist et al. 2003, Cordes et al. 2005b). The so-called Bushmaster junior/senior or Chimneymaster are hydraulically actuated collection devices of different sizes that are placed over the tubeworm aggregations and closed at the surface of the substratum to collect whole communities. In contrast, there is a higher risk of potential loss of parts of the community by sampling with grabs (Sarrazin et al. 1999, Tsurumi & Tunnicliffe 2003).

Separation of fauna from sediment and distinction between different faunal size classes also varied considerably between studies and limits a direct comparison of community structure. Sarrazin & Juniper (1999) distinguished, without sieving the samples, between the class of macroscopic mega- and macrofauna and submacroscopic macro- and meiofauna. Other researchers used nets of different sizes to separate the macrofauna from the meiofauna, while animals contained on a net with a mesh aperture of either 250 µm (Govenar et al. 2002), 1 mm (Bergquist et al. 2003, Govenar et al. 2005, Gollner et al. 2007, Tsurumi & Tunnicliffe 2003) or 2 mm (Cordes et al. 2005b) were considered to be macrofauna. So far, the entire community of mega-, macro-, and meiofauna at species level has been studied only in *Riftia pachyptila* aggregations (Govenar et al. 2005, Gollner et al. 2007).

The two different measures of abundance further limit the possibilities of direct comparisons. Either the abundance of associated fauna was calculated per tube surface area of the foundation species (Sarrazin et al. 1999, Tsurumi & Tunnicliffe 2003, Cordes et al. 2005c, Govenar et al. 2005) or abundances of foundation species and associated animals were standardised to the surface area of the substratum (basalt, sulphide chimneys or sediment) (Govenar et al. 2002, Bergquist et al. 2003, Gollner et al. 2007).

Macrofauna associated with *R. pachyptila* aggregations were studied at the hydrothermal vent 9°50′N EPR region (Govenar et al. 2004, 2005). A total of 46 associated macrofauna species were collected from eight aggregations (Govenar et al. 2005) and a single aggregation (Govenar et al. 2004). An aggregation of *R. pachyptila* that grew within a year after clearing the area artificially revealed the co-occurrence of *Tevnia jerichonana* (Govenar et al. 2004); however, other samples taken from the same location and another location revealed the presence of *T. jerichonana* and *Oasisia alvinae*. The abundance of associated macrofauna was between 1723 and 8216 ind. m^{-2} tube surface area (Govenar et al. 2005). The tubes increased the surface available for associated animals between about 7- and 144-fold. Species richness ranged from 19 to 35 species per aggregation and was positively correlated with the tube surface area. Despite differences in age and physicochemical characteristics of the two sites studied, the structure and composition of associated macrofauna communities were remarkably similar, and one of the univariate measures of diversity, the Shannon-Wiener index, was low and between H′ log e 1.23 to 2.14.

In six of these eight aggregations, the meiofauna community was studied in detail (Gollner et al. 2007). Abundances were highly variable (<1 to 976 ind. 10-cm^{-2} surface area of basalt) and in the majority of samples were well below the average abundance values of meiofauna in deep-sea samples. Considering that the tube surface area increased the overall surface considerably, meiofauna can be considered comparatively rare in the habitat. Abundance was positively correlated with tube surface area and with the volume of sediment found within the bushes of tubeworms. A total of 33 species were found, increasing the overall number of macro- and meiofauna species in *Riftia pachyptila* aggregations to 79, with a ratio between macro- and meiofauna species of about 1.4:1. While the macrofauna communities were very similar at both sites, the meiofauna communities differed considerably, with H′ significantly higher at the older Riftia Field site (1.75 to 2.00) than at the younger Tica site (0.44 to 1.35). The average Bray-Curtis dissimilarity was almost 70%.

The associated fauna of *Ridgeia piscesae* aggregations from various locations of the Juan de Fuca Ridge was studied. Following the criteria of the community succession model of Sarrazin et al. (1997), detailed data are available for assemblages III and IV (one collection each Sarrazin &

Juniper 1999, Govenar et al. 2002), V-HF (one collection Sarrazin & Juniper 1999, three collections Govenar et al. 2002) and V-LF (one collection Sarrazin & Juniper 1999, one collection Bergquist et al. 2007). In addition, Tsurumi & Tunnicliffe (2003) based their analyses on 51 collections from a wide range of geographic sites and substratum types but did not use this classification. The abundance of associated macrofauna calculated per tubeworm surface area ranged from 200 to 50,000 ind. m^{-2} (Tsurumi & Tunnicliffe 2003), from 30,000 to about 250,000 ind. m^{-2} (Sarrazin & Juniper 1999), and from 12,000 to over 100,000 ind. m^{-2} (Govenar et al. 2002). Overall, the number of associated species in each aggregation had a large range (4–28), with a total species richness of 37 for the 51 samples studied (Tsurumi & Tunnicliffe 2003). The Shannon–Wiener index of species diversity H′ reported in earlier studies falls within the range 1.34–2.19 (Sarrazin & Juniper 1999) and 0.90–1.31 (Govenar et al. 2002) and corroborates a general trend of lower abundance and lower diversity on relatively short-lived sulphide edifices than on more stable basalt (Tsurumi & Tunnicliffe 2003, Bergquist et al. 2007).

At cold-seep sites on the upper Louisiana slope of the GoM, the macrofauna associated with tubeworm aggregations of mixed *Lamellibrachia luymesi* and *Seepiophila jonesi* was studied (Bergquist et al. 2003, Cordes et al. 2005b). The tube surface area increased the sediment surface from which the tubeworms grew 2.6- to 26-fold (Bergquist et al. 2003). After studying seven aggregations, the total number of associated species found was 65 (Bergquist et al. 2003). Later, by studying 13 additional aggregations, this number increased to at least 90 species (Cordes et al. 2005b). A single aggregation contained 22–44 (Bergquist et al. 2003) and 14–47 species (Cordes et al. 2005b). Species richness was correlated with habitat size (measured as tube surface area) (Cordes et al. 2005b). The Shannon–Wiener diversity index H′ ranged from 1.39 to 2.86 (Bergquist et al. 2003) and 1.37 to 3.10 (Cordes et al. 2005b). Applying a population growth model, Cordes et al. (2005b) estimated the age of the 13 studied aggregations to be between 8 and 157 yr. Faunal abundances decreased with increasing age of aggregations, species considered to be seep endemics dominated communities of young aggregations, while generalist species also known from the surrounding deep sea dominated older aggregations. Diversity was not linearly correlated with aggregation age; the lowest diversity was found in the youngest and oldest aggregations. These studies on the associated community together with laboratory experiments and modelling approaches suggest that *Lamellibrachia luymesi* not only provides habitat for an associated fauna but also can alter the biogeochemistry of the seep sites and reduce sulphide levels in the water around their tubes (Julian et al. 1999, Freytag et al. 2001, Bergquist et al. 2003, Cordes et al. 2003, 2005a,c).

Nutritional links between tubeworms and associated fauna

Lethal predation by killing and consuming whole large tubeworms has not been documented so far. However, considerable numbers of gametes and larvae released into the water column might be directly consumed in the pelagic environment (Bergquist et al. 2003); settled larvae and small juveniles might fall prey to mobile grazers (Micheli et al. 2002).

Partial predation by ‘nipping’ plume parts was observed directly by many scientists in the vent species *Riftia pachyptila* and *Ridgeia piscesae* (see Micheli et al. 2002, M.B. personal observations). In contrast, there is no evidence for predation in seep species (Bergquist et al. 2003, Cordes et al. 2007a). Feeding preference experiments showed that the brachyuran crab *Bythograea thermydron* and the galatheid crab *Munidopsis subsquamosa* were attracted by *Riftia pachyptila* as food and not as a biogenic structure (Micheli et al. 2002). Galatheid crabs and polynoid polychaetes (and possibly also the spider crab *Macroregonia macrochira)* forage on plumes of *Ridgeia piscesae* (Tunnicliffe et al. 1990, Juniper et al. 1992). Stable isotope ratios of crabs were consistent with a diet including *Riftia pachyptila* (Fisher et al. 1994). Similar studies identified *Ridgeia piscesae* as part of the diet of several polynoid polychaetes (Bergquist et al. 2007), whereas no predator was found to feed directly on *Lamellibrachia luymesi* and *Seepiophila jonesi* (MacAvoy et al. 2005).

Several parasitic siphonostomatoid copepods with typical mouth structures suitable for cutting round holes into host tissue were found associated with, and were thought to feed on, tubeworms (see Ivanenko & Defaye 2006). For example, several *Ceuthocetes* species were identified from aggregations of *Riftia pachyptila* (Gollner et al. 2006); *Dirivultus dentaneus* and *D. spinigulatus* were found associated with *Lamellibrachia barhami* and *Paraescarpia echinospica*, respectively (Humes & Dojiri 1980, Humes 1988, Southward et al. 2002). Other species that may feed on tubeworms are the phyllodocid polychaetes *Galapagomystides aristata* from vents and *Protomystides* sp. from seeps found associated with *Riftia pachyptila* and *Escarpia laminata*, respectively. These phyllodocids contained blood in their digestive systems, which was thought to stem from their tubeworm hosts (Jenkins et al. 2002, Cordes et al. 2007b).

Life cycle and symbiont transmission

In Vestimentifera, all evidence points to a biphasic life cycle with a pelagic larva and a benthic adult such as is common in many marine invertebrates. However, this pelago-benthic cycle is complicated by the uptake of the specific symbiont and the subsequent transformation from an aposymbiotic larva to a symbiotic entity comprised of a host with a specific endosymbiotic phylotype belonging to the subdivisison of *Gammaproteobacteria* (but also see 'Symbionts', p. 233). To take these overlapping but not identical life-cycle phases and the key events of settlement and symbiont transmission into account, tubeworms are considered in this review to have an aposymbiotic phase (fertilised egg within mother, embryonic and pelagic larval development, settlement of larva and metamorphosis) and a symbiotic phase (symbiont transmission in metamorphosing larva, juvenile and adult phase ending with the death of the individual) (Figure 5).

Further, at least four phases of nourishment can be distinguished: (1) lecithotrophy in the pelagic environment (Marsh et al. 2001), (2) microphagy after settlement (Southward 1988, Jones & Gardiner 1989, Nussbaumer et al. 2006) and maybe even in the late pelagic phase, (3) both microphagy and symbiotic nutrition and (4) only symbiotic nutrition via translocation of metabolites and direct digestion of symbionts (Bosch & Grassé 1984a, Felbeck & Jarchow 1998a, Bright et al. 2000).

Aposymbiotic phase

Spermatozoa and oocytes

Vestimentiferans are gonochoric with paired gonads (see Gardiner & Jones 1993, Southward 1999, Southward et al. 2005). The sex ratio was estimated to be 1:1 in several species (Young et al. 1996, Thiebaut et al. 2002). No evidence for periodicity in reproductive output was reported (see Tyler & Young 1999). All attempts to detect symbionts in eggs and ovaries of *Riftia pachyptila* and *Ridgeia piscesae* using *in situ* hybridisation techniques have failed (Cary et al. 1993, M.B. unpublished data). Also, testes of *Riftia pachyptila* did not contain symbionts (M.B. unpublished data).

According to Franzén (1956), the spermatozoa of all vestimentiferan species investigated so far are classified as 'modified sperm type'; following the more recent classification of Rouse & Jamieson (1987), such spermatozoa are defined as entaqua-sperm (van der Land & Nørrevang 1977, Gardiner & Jones 1985, 1993, Jones & Gardiner 1985, Cary et al. 1989, Southward 1993, Marotta et al. 2005). For this sperm type, release into the surrounding water is suggested, and from there they reach the female in some way (see Rouse 2005).

Mature oocytes are quite small: *Riftia pachyptila* eggs in the gonad have a diameter of 78 µm (Jones 1981) but are 105 µm after spontaneous release onboard various research vessels at ambient pressure (Cary et al. 1989), *Ridgeia piscesae* eggs are 90–100 µm (Southward et al. 1995), *Seepiophila jonesi* (as *Escarpia* sp.) are 115 µm, *Lamellibrachia luymesi* (as *Lamellibrachia* sp.) are 105 µm (Young et al. 1996) and *L. satsuma* fertilised eggs are 100 µm (Miyake et al. 2006).

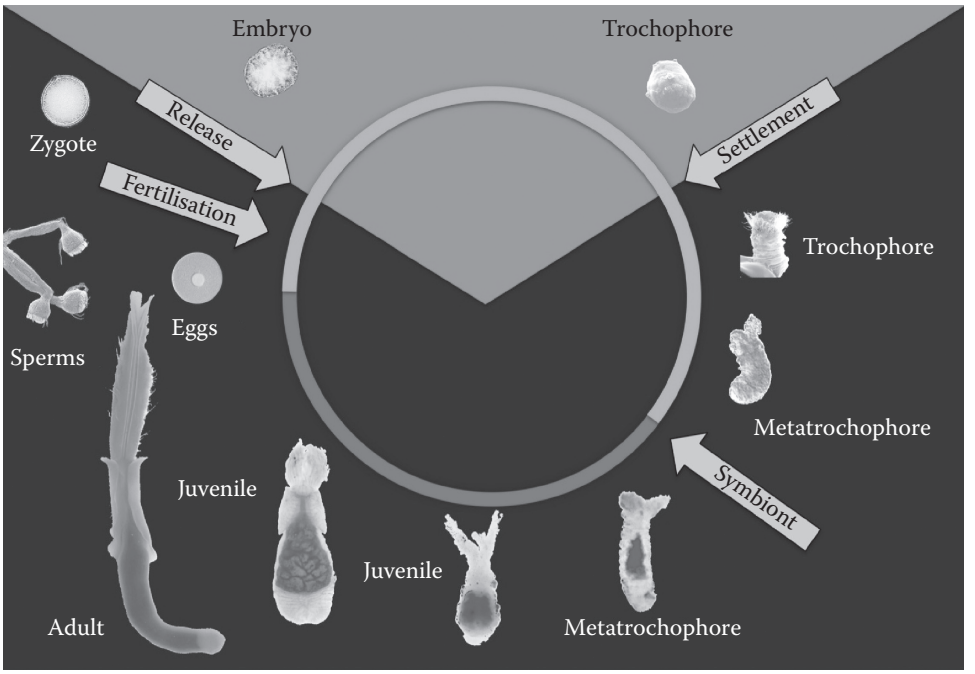

Figure 5 (See also Colour Figure 5 in the insert.) Schematic life cycle of Vestimentifera. Adults with separate sexes produce sperm and eggs. Sperm bundles are released and taken up by females, in which internal fertilisation takes place, and aposymbiotic zygotes are released into the water column and disperse. Embryonic and larval development into a trochophore takes place in the pelagic environment. On settlement and further larval development into a metatrochophore, metamorphosis and uptake of symbionts from the environment are initiated. The symbiotic metatrochophore with a trophosome develops into a small juvenile in which the trophosome is present as a one-lobule stage. On growth, the trophosome expands into a multilobule stage, and animals become mature. Green = aposymbiotic life stages; red = symbiotic stages (photographs of sperm bundles from Marotta et al. 2005; zygote, embryo, and pelagic trochophore from Marsh et al. 2001; sessile trochophore from Gardiner & Jones 1994).

Fertilisation and spawning

Internal fertilisation was first suggested by Gardiner & Jones (1985) and later by other authors (Malakhov et al. 1996a, Hilario et al. 2005). All studies were based on the finding of sperm in the female genital tract of *Riftia pachyptila* (Gardiner & Jones 1985), *Ridgeia piscesae* (Malakhov et al. 1996a), *Lamellibrachia satsuma* (Miyake et al. 2006) and *Riftia pachyptila*, *Ridgeia piscesae*, *Tevnia jerichonana*, *Lamellibrachia luymesi* and *Seepiophila jonesi* (Hilario et al. 2005). In *Ridgeia piscesae*, Southward & Coates (1989) had speculated that fertilisation should be either internal or just external to the female gonopores.

The findings of some sperm and eggs outside the body of vestimentiferans (Southward & Coates 1989, Southward 1999, MacDonald et al. 2002) as well as occasional *in situ* observations of expulsion of some products into the water column (Van Dover 1994, Hilario et al. 2005, M.B. personal observations) are in accordance with an internal fertilisation mode. As pointed out by Hilario et al. (2005), such exudates could be spermatozoa, sperm bundles, unfertilised eggs, zygotes, developing embryos or even larvae as they never have been collected directly.

The mode of release, however, apparently differs between tubeworm species. ‘Spawning’ events in the form of forceful expulsions have been observed in *Riftia pachyptila* (Van Dover 1994, M.B. personal observations). As described by Van Dover (1994) and captured by coincidence in a video

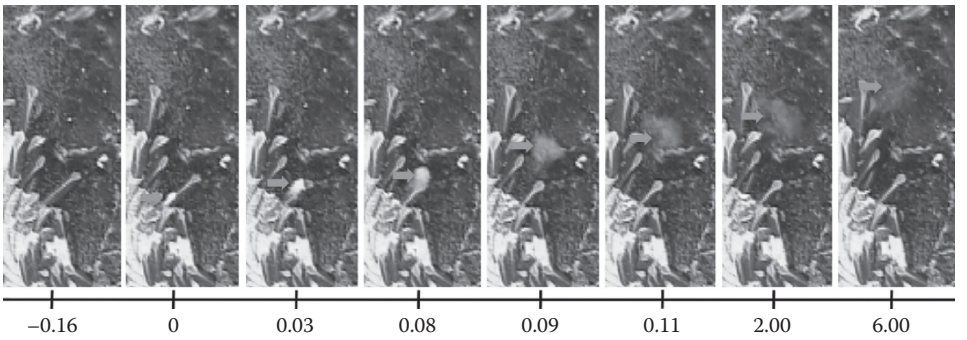

Figure 6 (See also Colour Figure 6 in the insert.) Video sequence of the expulsion of a white cloud from one individual of *Riftia pachyptila* at the East Pacific Rise 9°50′N region, location Tica, in December 2003, Alvin dive 3948. Red arrows mark the location of the released product. Time is given in seconds as ± from the time of release (0).

sequence (Figure 6), small, white clouds of 10- to 15-cm diameter were released forcefully. When originating from females, the presumed eggs were slightly negatively buoyant and sank between tubeworms, while such clouds of sperm released from males were neutrally buoyant and dispersed within 10 s in the water column. In *Ridgeia piscesae* (Southward & Coates 1989, MacDonald et al. 2002) and *Tevnia jerichonana* (Southward 1999), however, the transfer of spermatozoa appeared to be as clumps. These thread-like white objects obviously do not dissolve immediately after release and sink between other tubeworms so that they can be collected. They were identified as sperm masses (Southward & Coates 1989) found on plumes, the dorsal side of the obturaculum and vestimentum and sometimes even on the female gonopores or in the ovisacs. In addition, released eggs were found on plumes (MacDonald et al. 2002).

Embryonic development

Based on an *in situ* experiment, embryogenesis is suggested not to begin until inseminated oocytes are released into the water column (Hilario et al. 2005). Using artificial insemination techniques, larvae of *Lamellibrachia luymesi, Seepiophila jonesi* (Young et al. 1996), *Lamellibrachia satsuma* (Miura et al. 1997, Miyake et al. 2006) and *Riftia pachyptila* (Marsh et al. 2001) were reared and studied with light microscopy (LM) and scanning electron microscopy (SEM) techniques. In addition, some information based on transmission electron microscopy (TEM) sections is available for *Lamellibrachia satsuma* (Miyake et al. 2006). *Lamellibrachia luymesi* and *Seepiophila jonesi* were found to develop similarly at 1, 50 and 100 bar, 9°C. *Riftia pachyptila* was reared at 250 bar, 2°C. The shallow-water species *Lamellibrachia satsuma* was maintained at ambient pressure, 16°C.

In all species, the cleavage pattern was spiral. The development into ciliated larvae took only 3 days in *L. luymesi* and *Seepiophila jonesi* (Young et al. 1996), 5 days in *Lamellibrachia satsuma* (Miyake et al. 2006) and 34 days in *Riftia pachyptila* (Marsh et al. 2001).

Aposymbiotic, pelagic trochophore

The larvae of all these artificially reared species had a trochus in the anterior body region, interpreted as a prototroch. A second ciliary band, located posteriorly to the prototroch, was described in *Lamellibrachia luymesi*, *Seepiophila jonesi* (Young et al. 1996) and *Riftia pachyptila* (Marsh et al. 2001) but was lacking in *Lamellibrachia satsuma* (Miura et al. 1997, Young 2002). Following the progress of development in detail, 15-day-old larvae of *Lamellibrachia* sp. exhibited a wide prototroch (Young 2002). After 21 days, two ciliary bands had developed (C.M. Young personal communication). Whether these bands represented the prototroch and the metatroch or a wide prototroch that later separated into two is not yet clear. No apical plate, mouth opening or anus was detected in

any species, although Young (2002) suggested that in the 21-day-old larva of *Lamellibrachia* sp. a mouth might be present. A telotroch was also described in both *Lamellibrachia* species (Miura et al. 1997, Young 2002, Miyake et al. 2006). According to Rouse (1999), these larvae (with a prototroch, protonephridia and an apical plate, which is lost in some) are definitely of the trochophore type.

Dispersal and settlement

No larvae such as those described have ever been found in the water column despite intensive searches (Mullineaux et al. 2005). However, Harmer et al. (2008) detected a tubeworm genetic sequence in a pelagic water sample taken in the vicinity of vents at the EPR 9°50′N region. *In situ* hybridisation of 18S rRNA techniques to identify larvae have been developed (Pradillon et al. 2007), but appropriate material has yet to be made available for testing.

Artificially reared *Riftia pachyptila* larvae were estimated to disperse more than 100 km along the axial summit collapse trough over a period of 38 days, passively for about the first 3 wk and then actively using their cilia for the next 2 wk (Marsh et al. 2001). This calculation was based on the assumption that these larvae are exclusively lecithotrophic during their entire pelagic phase, based on measurements of protein and lipid content, respiration rates and a model of the flow regime along the ridge axis of the 9°50′N EPR region. Modelling the flow regime in two different regions of the EPR using current records showed that the dispersal potential of *R. pachyptila* can be quite different even when the average daily current speed is similar. Maximal dispersal distance at 13°N was 241 km but only 103 km at 9°50′N (Mullineaux et al. 2002).

Artificially reared *Lamellibrachia satsuma* larvae could be maintained in the laboratory for 45 days (Miyake et al. 2006). They swam in Petri dishes for about a month when no suitable substratum was offered. They attached rapidly to any substratum offered 10 days after fertilisation; however, there was no further development. With a current speed model, Miyake et al. (2006) estimated that larvae can disperse about 2000 km in the Kuroshio Subgyre area.

Knowledge is scarce on habitat selection, spatial and temporal recruitment and settlement processes during which vestimentiferan larvae move to the substratum, explore, attach and begin their benthic life (Qian 1999). Regardless of inhabiting soft sediments (seeps or vents) or hard substrata (basalt, sulphide chimneys at vents or whale carcasses), vestimentiferans appear to need a hard substratum on which to settle. Vestimentiferan tubeworms also recruit to artificial substrata as scientists have often noted using many experimental devices made of plastic, glass or ropes deployed at locations with appropriate environmental conditions. At sedimented seeps, larvae are suggested to settle on carbonate rocks, the by-products of hydrocarbon degradation (Fisher et al. 1997). Only *Paraescarpia echinospica* was also collected from mud without obvious hard substratum being present (Southward et al. 2002). At vents, small specimens are found on basalt but also very often on tubes of larger specimens. This shows that recruitment to those places obviously is possible, but not that larvae are capable of selectively choosing this location. Discontinuous and synchronised recruitment within a vent site was reported for *Riftia pachyptila* based on size–frequency histograms (Thiebaut et al. 2002). However, a different population structure study using size–frequency histograms came to the conclusion that recruitment occurred throughout the year (Govenar et al. 2004).

The co-occurring vent species *Tevnia jerichonana*, *Riftia pachyptila* and *Oasisia alvinae* were the subject of several studies. Deploying artificial substrata at sites with different environmental conditions at the 9°50′N EPR vent region during a period of 3 yr, two of five substrata were colonised by small tubeworms located in ‘warm’ vent areas (Mullineaux et al. 1998). Subsequently, basalt blocks were deployed in this region in three different tubeworm aggregations. Identifying the settled tubeworms by molecular methods, it was found that all three species colonised the experimental blocks regardless of the dominance of tubeworm species in the initial assemblage and independent of temperature measured on the blocks. *Riftia pachyptila* and *Oasisia alvinae* were never found on blocks without *Tevnia jerichonana*, but *T. jerichonana* colonised blocks also without the

other two species, even when these blocks were deployed in *Riftia*-dominated clumps. This pattern strongly supported the facilitation/competition hypothesis suggesting that *Tevnia jerichonana* facilitates colonisation of *Riftia pachyptila* and *Oasisia alvinae* (Mullineaux et al. 2000). An earlier long-term photo- and videographic documentation of community succession in this area showed that at the macroscopic level, most often *Tevnia jerichonana* clumps preceded the establishment of *Riftia pachyptila*; however, *R. pachyptila* clumps without pre-existing *Tevnia jerichonana* were also documented (Shank et al. 1998). Also, the findings of many small individuals of *Riftia pachyptila* settled on tubes of larger conspecific but not on *Tevnia jerichonana* tubes are in contrast to the hypothesis (Thiebaut et al. 2002).

In the following years, another experiment was conducted to test the facilitation/competition hypothesis at a later successional community stage (Hunt et al. 2004). When using tubes of *T. jerichonana* or *Riftia pachyptila* or artificial plastic tubes glued to blocks and deployed for several months, the species pattern of colonisation differed from previous studies. Colonists of *R. pachyptila* and *Oasisia alvinae* were found on blocks without *Tevnia jerichonana*. Further, no difference in the number of colonists between blocks with natural and artificial tubes was found, although it is important to note that tubes of live *Riftia pachyptila* touched all blocks due to the specific deployment in *R. pachyptila* aggregations. The authors suggested that *Tevnia jerichonana* might be important for colonisation of new vents and young developing communities, while *Riftia pachyptila* might act as settlement cue in later successional community stages. Predator exclusion experiments using the same blocks, caged to exclude predators, uncaged or controls with cages having one side open, revealed that tubeworms colonised not only cages in the vestimentiferan zone, as expected, but also the lower-flow bivalve zone. Colonisation in the vestimentiferan zone was statistically similar regardless of treatment after 5 and 8 mo (Micheli et al. 2002).

Aposymbiotic, sessile trochophore

The smallest sessile larval stage collected from washing of large *Ridgeia piscesae* aggregations was only 58 µm long. Based on the prototroch and two types of larval chaetae (ciliary and hooked uncini), it was identified as a trochophore. No metatroch, neurotroch, telotroch or apical organ was detected. Also, neither mouth opening nor anus was apparent when examining this specimen using SEM techniques (Jones & Gardiner 1989).

Aposymbiotic, sessile metatrochophore

The next developmental stage can be classified as a segmented larva (metatrochophore) according to Heimler (1988). This stage is characterised by an elongated hyposphere, larval segments and adult organs such as head appendages being formed. The smallest of these metatrochophore larvae were aposymbiotic. Two specimens 150 and 270 µm long (Southward 1988) and two specimens 200 and 250 µm long (Nussbaumer et al. 2006) were studied in detail (Figures 7A,B, 8A–C). Although named 'juvenile' by Southward (1988) and Jones and Gardiner (1989), such specimens of *Ridgeia piscesae* (Southward 1988, Jones & Gardiner 1989), *Riftia pachyptila* (Jones & Gardiner 1989) and unidentified vestimentiferan tubeworms from the 9°50′N EPR region (co-occurring *R. pachyptila*, *Tevnia jerichonana* or *Oasisia alvinae*) (Nussbaumer et al. 2006) exhibited a prototroch. They had a transient digestive system with mouth and anus, cilia at the position of a neurotroch, two palps also termed tentacles (Southward 1988, Nussbaumer et al. 2006) or branchial filaments (Jones & Gardiner 1989) and larval chaetae termed setae by Jones & Gardiner (1989). No trophosome was present. Feeding on bacteria (Southward 1988) or bacteria and diatoms (Nussbaumer et al. 2006) was evident from remnants found in endodermal midgut cells.

Adopting the terminology of Rouse & Fauchald (1997), the body is divided into (1) a presegmental region (termed cephalic region in Southward 1988) with a prostomium containing the brain and a peristomium, the area surrounding the mouth also containing a corresponding unpaired, minute peristomial coelomic cavity and the prototroch, and (2) a segmental region with either two or three

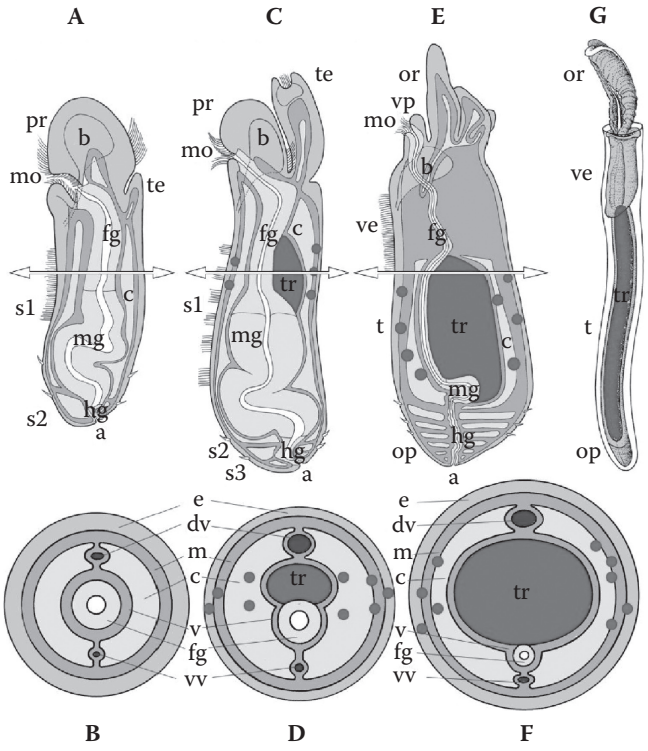

Figure 7 (See also Colour Figure 7 in the insert.) Schematic drawing of life-history stages: (A) aposymbiotic metatrochophore with (B) corresponding cross section; (C) symbiotic metatrochophore with symbionts invading and (D) corresponding cross section; (E) symbiotic juvenile with trophosome in one-lobule stage and (F) corresponding cross section; (G) adult with trophosome in multilobule stage. Pink, symbiont-housing trophosome or symbionts (tr); blue, digestive system, mouth opening (mo), ventral process (vp), foregut (fg), midgut (mg), hindgut (hg) and anus (a); purple, blood vascular system with dorsal blood vessel (dv) and ventral blood vessel (vv). Body regions of larvae: pr = prototroch; s1–3 = chaetigers 1 to 3; and te = palps. Body regions of juvenile and adult: or = obturacular region; ve = vestimentum; t = trunk; op = opisthosome. Tissues: e = epidermis; m = muscles; c = coelom; v = visceral mesoderm. (From Nussbaumer et al. 2006.)

segments and, correspondingly, paired coelomic cavities and larval chaetae. Only the first two segments were chaetigers having four sets of larval chaetae each. The postsegmental region, the pygidium, including the growth zone at the posterior end of the body, was minute and quite indistinct.

While Nussbaumer et al. (2006) clearly described the palps developing from the first segment, confirming Rouse's (2001) interpretation based on the fact that palps are located behind the prototroch, Southward (1988) speculated that they either originate from the presegmental region (first segment in Southward 1988) or the first segment (second segment in Southward 1988). Both Southward (1988) and Nussbaumer et al. (2006), however, clearly saw the elongated region, which on symbiont transmission will develop into the trunk containing the trophosome, as a single, highly elongated segment.

Symbiont transmission

Symbionts

In investigations to date, the symbionts of vestimentiferan tubeworms have been detected by molecular methods free living in the benthic and pelagic environments and found associated with the

developing tube of small individuals (but not large ones), in the skin of the metatrochophore larvae and juveniles and in the trophosome of metatrochophore larvae, juveniles and adults.

The majority of molecular studies of the identity of the symbionts revealed that each host species houses a single, specific endosymbiont (but see next paragraph). Based on 16S rRNA sequences, two phylotypes of *Gammaproteobacteria* with 4.3% sequence divergence are known (Feldman et al. 1997, Di Meo et al. 2000, Nelson & Fisher 2000, McMullin et al. 2003, Thornhill et al. 2008, Vrijenhoek 2010). No information is yet published for the symbionts of the host genera *Arcovestia*, *Alaysia* and *Paraescarpia*. Phylotype 1 has a larger geographic distribution (Atlantic, Pacific, GoM), diversity of host species (species of *Lamellibrachia, Escarpia, Seepiophila*, *Arcovestia* and *Alaysia*; S. Johnson unpublished observations) and habitat type (seeps, vents, one example whale fall) than phylotype 2. Three groups of symbionts can be distinguished in phylotype 1. Group 1 contains the symbionts of *Lamellibrachia barhami* from the Oregon Slope and Middle Valley, *Escarpia laminata* from Florida Escarpment and *Lamellibrachia columna* from Lau Basin. The symbionts of group 2 are present in *Lamellibrachia* sp. and *Escarpia laminata* from Alaminos Canyon, *Lamellibrachia barhami* from Vancouver Margin, Monterey Bay, and *Escarpia spicata* from a whale fall in Santa Catalina, California. Group 3 symbionts are found in *Seepiophila jonesi*, *Lamellibrachia* cf. *luymesi* and a new escarpid species from the Louisiana slope. Phylotype 2 has been found so far only in the East Pacific vent species *Ridgeia piscesae* and the co-occurring *Riftia pachyptila*, *Tevnia jerichonana* and *Oasisia alvinae*. The genome of this symbiont, Candidatus *Endoriftia persephone* from *Riftia pachyptila*, has been published (Robidart et al. 2008).

Two studies claimed that several symbiotic phylotypes were extracted from tubeworm trophosomes. Two sequences of *Epsilonproteobacteria* were obtained from *Lamellibrachia* sp. from a cold seep in the Sagami Bay and visualised in the trophosome by *in situ* hybridisation (Naganuma et al. 1997a,b). Later, a picture was published (Figure 3 in Naganuma et al. 2005) in which the different types of these presumed bacteria were, in the authors' opinion, sperm of the testes. Using terminal-restriction fragment length polymorphism (t-RFLP), *Ridgeia piscesae* collected from Juan de Fuca Ridge, Axial Caldera, Explorer Ridge and Magic Mountain were found to contain either only the previously known γ-proteobacterial endosymbiont or up to four additional operational transcribed units belonging to other γ-proteobacterial clusters, *Alphaproteobacteria*, and Cytophaga-Flavobacterium-Bacteroides. However, no fluorescent *in situ* hybridisation (FISH) was applied in this study, and in our opinion a conclusive proof for multiple symbionts remains to be established.

Recently, the symbiont of the vent tubeworms was detected free living in the environment at the EPR 9°50′N sites. Natural collections of basalt, artificially deployed rocks and glass slides from various locations within and next to tubeworms, but also about 100 m away from the axial summit collapse trough on bare basalt, and water samples taken about 1 m away from tubeworm aggregations with a pelagic pump revealed the presence of the free-living stage (Harmer et al. 2008). Various growth types, such as rods and chains of rods, were stained with a symbiont-specific probe applying *in situ* hybridisation (Harmer et al. 2008, Nussbaumer personal communication) (Figure 8A).

A diverse bacterial community of *Epsilon-*, *Alpha-*, *Delta-*, and *Gammaproteobacteria* inhabits the tube of adult *Riftia pachyptila*. The specific symbiont was not part of this community (Lopez-Garcia et al. 2002, Nussbaumer et al. 2006). The developing tube of small specimens, however, also harbours the symbiont (Nussbaumer et al. 2006) (Figure 8B). This finding suggests that prior to symbiont uptake, the free-living symbionts aggregate in the developing tube and remain there only for a certain time.

For a horizontally transmitted bacterium, symbiotic life is facultative. Such bacteria exhibit a population in the environment as well as a population associated with the host. The free-living population serves as an inoculum for the symbiosis. Symbiont release mechanisms from the host into the environment are quite common in symbiosis with horizontal transmission; however, in vestimentiferan tubeworms they have not been found yet. The free-living counterpart of the symbionts

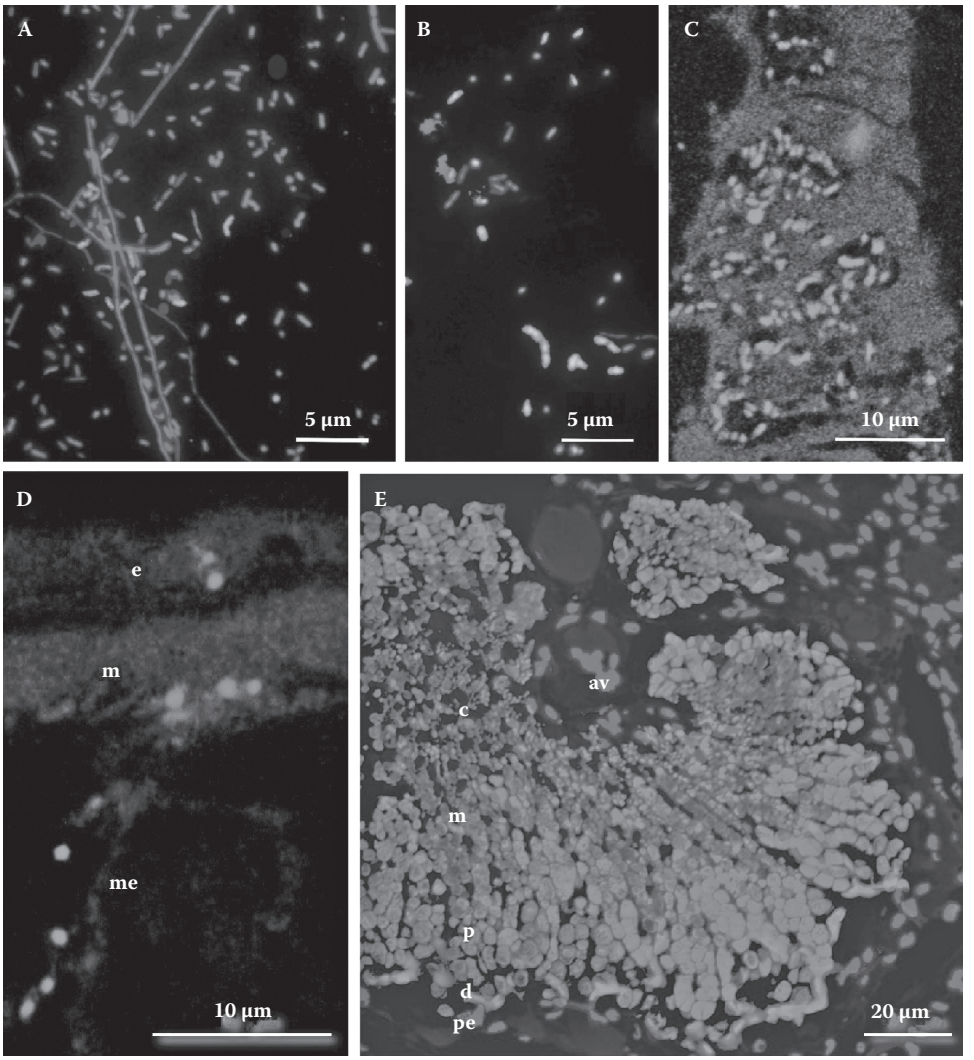

Figure 8 (See also Colour Figure 8 in the insert.) Fluorescent *in situ* hybridisation with symbiont-specific (pink) and eubacterial (blue) probes. (A) Free-living bacterial community containing symbionts (pink) colonising on glass slide during 1-yr deployment (courtesy of A.D. Nussbaumer). (B) Free-living bacterial community containing symbionts (pink) on and in developing tube of metatrochophore (courtesy of A.D. Nussbaumer). (C) Symbionts (pink) in developing trophosome in metatrochophore. (D) Symbionts (pink) in epidermis (e), muscles (m), and undifferentiated mesoblastem (me). (E) Juvenile one-lobule stage with symbionts (pink) and host nuclei (blue): av = axial blood vessel; distinct zonation of central (c), median (m), peripheral (p) and few degrading bacteriocytes (d), peritoneum (pe) (C, D and E from Nussbaumer et al. 2006).

is part of the free-living microbial community, and selection pressure relies on successful competition under present environmental conditions. At the same time, such a versatile bacterium exhibits the necessary repertoire for associating with their hosts. The metagenome of Candidatus *Endoriftia persephone* of *Riftia pachyptila* displays remarkable motility (Hughes et al. 1998) and chemoreception, suggesting that the free-living counterparts of these endosymbionts can use chemotaxis to reach their respective hosts (Robidart et al. 2008). The horizontally acquired microbes do not experience a significant selective pressure for genome reduction, as opposed to strictly vertically transmitted

symbionts, which do not exist in a free-living state. The genome of Candidatus *Endoriftia persephone* is 3.3 Mb, and its guanosine plus cytosine (GC) content is 60% (Robidart et al. 2008).

Transmission

Evidence for horizontal transmission came from series of sessile developmental stages and was also supported by the finding of the free-living symbiont in the environment (Harmer et al. 2008). The locations of entry for bacteria and the tissue developing into the trophosome were hypothesised to be either the mouth and the endodermal midgut (Southward 1988, Jones & Gardiner 1989) or the skin and the visceral mesoderm (Nussbaumer et al. 2006). Consequently, the evolution of symbiosis is pictured quite differently. One interpretation is that a feeding larva ingests bacteria and encloses them in food vacuoles in the midgut. One bacterial species, however, evades digestion and starts proliferating in the endodermal cells, which as a consequence transform into the trophosome (Southward 1988, Jones & Gardiner 1989). Another explanation is that one bacterial species manages to invade the skin of the larva in a way similar to a pathogen infection process and migrates through several tissues (epidermis, musculature, undifferentiated mesoblastem) until it reaches the visceral mesoderm between dorsal blood vessel and gut, which initiates proliferating into the trophosome (Nussbaumer et al. 2006). While the former hypothesis is based solely on microscopy, identifying the occurrence and location of bacteria, the latter hypothesis also relies on FISH using symbiont-specific and general γ-proteobacterial and eubacterial probes.

Another line of evidence for horizontal transmission is the lack of phylogenetic congruence and cospeciation between symbiont and host, which would reflect cotransmission over evolutionary timescales. Furthermore, the time depth of diversification of host and symbiont phylogenies is often dissimilar. Vestimentiferans reveal no evidence of cospeciation when comparing symbiont 16S rRNA gene- and host COI-based trees (Feldman et al. 1997, Nelson & Fisher 2000, McMullin et al. 2003). The sequence divergence of the two symbiotic phylotypes suggests a separation over 200 million years ago (mya), whereas host radiation was possibly as recent as 60 mya (Vrijenhoek 2010).

Symbiotic phase

Symbiotic metatrochophore

The overall morphology of the only specimen studied (unidentified vestimentiferan from the 9°50′N EPR region) by reconstruction from serial TEM/LM sections revealed how the development from the aposymbiotic to the symbiotic metatrochophore proceeded. The symbiotic metatrochophore developed the first two palps, the peristomal coelomic cavity was closed, and a set of midventrally located patches of cilia in the first chaetiger developed (Nussbaumer et al. 2006) (Figures 7C,D, 8D, 9C,D). Also, the trophosome started to develop and was composed only of peritoneal cells and bacteriocytes containing rod-shaped symbionts. The digestive system was transient, composed of a slit-like mouth opening, a fore-, mid-, and hindgut and an anus. Apparently, the gut was still functioning, as evidenced by degrading bacteria and diatoms. A few symbionts, identified by FISH using a set of symbiont-specific, γ-proteobacterial and eubacterial probes, were found in the epidermis, the muscles and the undifferentiated mesoblastem in the trunk region containing part of the foregut (Figure 9D). In contrast to the symbionts in the prospective trophosome being contained in vacuoles, in the other tissues the symbionts are either intracellular but free in the cytoplasm or intercellular between various tissues (Nussbaumer et al. 2006). These findings suggest that the symbionts first aggregate in the developing tube, invade the host larva specifically in the trunk region and migrate through several tissues by entering and exiting host cells until they reach the prospective trophosome area where they establish and proliferate. Interpartner recognition processes, known to play a pivotal role in horizontally transmitted symbionts, have not been studied in this symbiosis yet. Further, symbionts invading and travelling within a host are usually subjected to a vigorous host

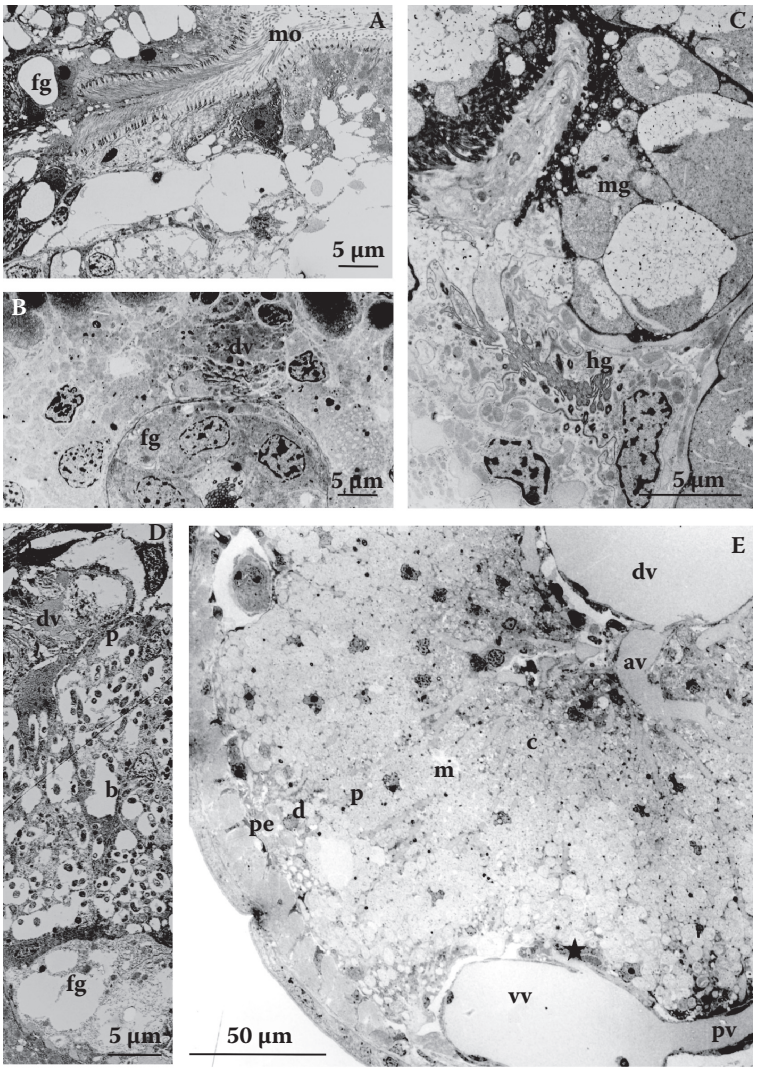

Figure 9 Transmission electron micrographs of aposymbiotic metatrochophore. (A) Mouth opening (mo) and foregut (fg). (B) Foregut (fg) and dorsal blood vessel (dv). (C) Midgut (mg) and hindgut (hg). (D) Symbiotic metatrochophore with dorsal blood vessel (dv), foregut (fg), and developing trophosome: bacteriocytes (b) containing rods and peritoneum (p). (E) Symbiotic juvenile with trophosome in one-lobule stage exhibiting axial blood vessel (av), distinct zonation of central (c), median (m), peripheral (p), and few degrading bacteriocytes (d), peritoneum (pe), and peripheral blood vessels (pv); note dorsal blood vessel extends to axial blood vessel and ventral blood vessel (vv) extends to peripheral blood vessels; star indicates foregut. (From Nussbaumer et al. 2006.)

immune response. Interestingly, Candidatus *E. persephone* possesses a wide repertoire of defence-associated genes (Robidart et al. 2008).

Metamorphosis

The onset of metamorphosis, the process during which larvae go through morphological and physiological changes to complete transition from the pelagic larva to the benthic juvenile, is known to commence before, concurrently or immediately after settlement (Qian 1999). The termination of

metamorphosis is marked by the loss of the prototroch (Heimler 1988) and is accomplished in vent vestimentiferans (*Ridgeia piscesae* and unidentified specimens from the 9°50′N EPR) during the symbiotic phase. The exact onset of metamorphosis is not known as there is not enough information on the morphology of the planktonic and benthic trochophore available. Thus, the development of a functioning digestive system, for example, could either happen on settlement or already be present during the planktonic phase. The latter scenario would imply a switch from lecithotrophic to planktotrophic lifestyle and thus potentially a prolongation of the possible time period to reside and disperse in the water column. Further, delayed settlement has been suggested by Jones & Gardiner (1989) as the examination of small sessile specimens with SEM revealed quite a plasticity in occurrence of mouth, anus and other features related to the size (and possibly age?).

Symbiotic early juveniles

Previously unpublished observations by one of us (M.B.) have shown that, at the early juvenile stage of unidentified specimens from the 9°50′N EPR), a small, functioning trophosome with all cell cycle stages of bacteriocytes and associated aposymbiotic tissues is present (one-lobule stage) concurrently with a mouth opening on the tip of the so-called ventral process and a transient, still-functioning digestive system. The palps increase in number during development, and the vestimentum develops (Figures 7E,F, 8E, 9E).

This so-called one-lobule stage of the trophosome in such small juveniles resembles a single lobule of the typical multilobed organ of larger juveniles and adults. The entire one-lobule organ takes up most of the inner trunk region. The single central axial blood vessel is a direct extension of the dorsal blood vessel with a myoepithelium composed of myocytes, so-called non-bacteriocytes (epithelial cells) and central bacteriocytes. Most of the bacteriocytes are apolar and show a distinct zonation of central bacteriocytes containing rods, median ones with small cocci and peripheral ones with large cocci and a zone of degradation, similar to the multilobule stage, described by Bright & Sorgo (2003) (Figure 10A). The bacteriocytes are enclosed by a peritoneum. Numerous small blood vessels extend from the ventral blood vessel to surround and connect with the peritoneum,

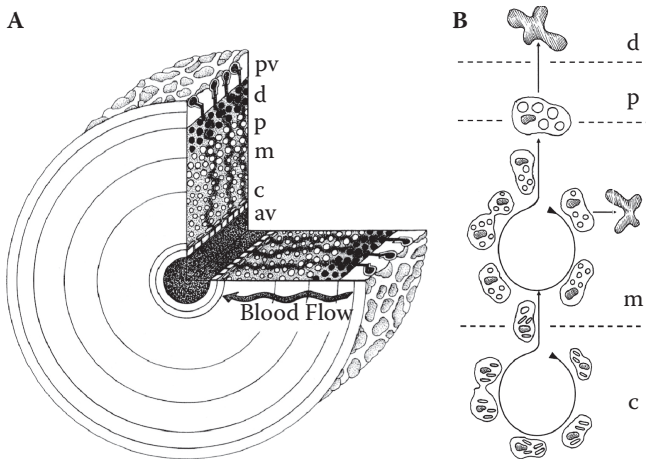

Figure 10 (A) Schematic drawing of one lobule of trophosome in larger juveniles and adult (from Bright & Sorgo 2003 with permission from John Wiley & Sons). (B) Schematic drawing of bacteriocyte cell cycle with terminal differentiation (from Pflugfelder et al. 2009 with permission from Springer Science): av = axial blood vessel, distinct zonation of central (c), median (m), peripheral (p), degrading bacteriocytes (d), peritoneum basal to peripheral blood vessels (pv); note blood flow is from periphery to the central region.

thus allowing vascular blood (VB) to circulate between these peritoneal cells but also into the interspaces between the bacteriocytes.

Termination of transmission was evidenced by massive apoptosis in the epidermis, somatic musculature and undifferentiated mesoblastem in the trunk region. Further symbiont uptake was evidently stopped by this apoptosis since in larger juveniles symbionts were never found in these tissues. It remains to be studied in detail if and how the host regulates this process.

Symbiotic late juveniles and adults

In large juveniles and adults, the digestive system ceases to function. Remnants of the gut, however, can still be found in juveniles of *Riftia pachyptila* with body lengths of at least a few millimetres (M.B., unpublished data). The trophosome was studied in detail in several species: *Lamellibrachia luymesi* (van der Land & Nørrevang 1975, 1977), *Riftia pachyptila* (Cavanaugh et al. 1981, Bosch & Grassé 1984a,b, Hand 1987, Gardiner & Jones 1993, Bright & Sorgo 2003), *Ridgeia piscesae* (Jones 1985, De Burgh 1986, De Burgh et al. 1989, Malakhov et al. 1996b) and several *Lamellibrachia* species (Kim & Ohta 2000).

The trophosome is multilobed in larger juveniles and adults instead of being composed only of a single lobule as in small juveniles. However, the cellular organisation is identical. Several ultrastructural findings led to the hypothesis of a bacteriocyte cell cycle with terminal differentiation, initially formulated by Bosch & Grassé (1984a,b) and later supported by Gardiner & Jones (1993) and modified by Bright & Sorgo (2003). Finally, using immunohistochemistry to identify the location and abundance of proliferating and mitotically active host cells, as well as cells undergoing apoptosis in *Riftia pachyptila* and *Lamellibrachia luymesi*, Pflugfelder et al. (2009) could verify the existence of such a cell cycle (Figure 10B). Bacteriocytes undergoing DNA synthesis and mitosis are restricted to central and median bacteriocytes but are completely lacking in the periphery; apoptosis is mainly in the periphery. Thus, the authors proposed that tissue-specific unipotent bacteriocyte stem cells containing rod-shaped symbionts undergo a cell cycle with division in the central lobule region. Semidifferentiated daughter cells containing small coccoid symbionts then migrate into the median region, where they either undergo another round of cell division or initiate programmed cell death. Migration of these differentiated cells containing large coccoid symbionts to the periphery is followed by the fully differentiated cells entering apoptosis, including digestion of the symbiont. As the diameter of each lobule is constant (resulting from cell proliferation in the centre being in balance with cell death in the periphery), net growth of the organ is accomplished by lobules growing in length or by ramification of lobules, both from proliferating cells in the centre and median regions (Pflugfelder et al. 2009; see next section, 'Growth and lifespan').

Growth and lifespan

Various morphological characters were used to measure tubeworm size. The tube length has proved to be a valid character in several species (e.g., *Lamellibrachia luymesi, Seepiophila jonesi*, *Tevnia jerichonana*, *Ridgeia piscesae*) since the worms consistently occupy the entire tube (Fisher et al. 1997, Shank et al. 1998, Bergquist et al. 2000, 2002, Govenar et al. 2002, Cordes et al. 2005b, 2007a).

In *Riftia pachyptila* tube length has been shown not to be a reliable measure of animal size. This species is capable of moving within its tube and usually occupies only a fraction of its tube (Gaill et al. 1997, Ravaux et al. 1998, 2000). Fisher et al. (1988), for example, found a tube 2 m long with an animal only 80 cm in length. Within the tube, sometimes chitinous septa are built (Gaill et al. 1997, Ravaux et al. 2000). Moreover, *R. pachyptila* is capable of contracting its trunk region considerably and thus decreases its overall length, so that especially in fixed material, the total length is an expression of the state in which the animal died (M.B. personal observation). Further, fixation in formalin or ethanol also leads to overall shrinkage of the tissue. Fisher et al. (1988) found that the only reliable size measure in *R. pachyptila* was the relation between the volume of the CF and the total wet weight. Thiebaut et al. (2002), in contrast, found that the length of the plume and

that of the vestimentum as well as the width of the plume, vestimentum and opisthosome are unaffected by formalin fixation, although it affects total length. The width of the vestimentum was then used by these authors as a biometric index representative of the individual size. Moreover, they found that the tube length and vestimentum width were correlated. Govenar et al. (2004) found a strong negative correlation between the length an animal occupies in its tube and the tube length in one aggregation of *Riftia pachyptila* but pointed out that this relationship was unlikely to hold true for animals from other microhabitats.

Despite these limitations, growth rates have been estimated from the length of the tube, even in *R. pachyptila*. In time series studies taking *in situ* photographs, Lutz et al. (1994) estimated the increase in tube length at 85 cm yr^{-1}, thus making *R. pachyptila* the fastest-growing invertebrate. Monitoring tubeworm aggregations at the 9°50′N EPR region for several years posteruption, tubes of *R. pachyptila* exceeding 2 m in length were found 3.5 yr after their appearance. In contrast, the smaller *Tevnia jerichonana* was found to grow about 30 cm yr^{-1} (Shank et al. 1998). Another study to estimate growth was carried out using vestimentum width of three populations collected at the same vent site (Riftia Field, 9°50′N EPR) over a period of 22 days. Although changes in environmental conditions, known to influence the growth rate, cannot be neglected as pointed out by the authors, they proposed that the differences in size–frequency histograms revealing several cohorts are due to growth and aging of the population since the time period considered in this study was short and the samples came from the same vent field. They estimated a mean increase in vestimentum width of 0.193 mm d^{-1}. To compare these growth rates with the one using time series photographs (Lutz et al. 1994), Thiebaut et al. (2002) used the relation between vestimentum width and tube length, which was found to be correlated, and estimated a tube growth rate of as much as 160 cm yr^{-1}.

Direct measurement of growth using cable ties as a banding device for individual tubeworms as well as staining the tubes of entire tubeworm aggregations have been extremely useful. The increase of growth was monitored with cable ties and video analyses in two cold-seep species over a period of 4 yr. The majority of *Lamellibrachia luymesi* grew very little in at least one of these 4 yr, on average only 0.77 cm yr^{-1}, while *Seepiophila jonesi* did not grow at all (Fisher et al. 1997). *In situ* staining and measurements of tube-length increase over time confirmed earlier studies. Large individuals with tube lengths over 2 m of *Lamellibrachia luymesi* are estimated to be older than 200 yr (Bergquist et al. 2000, 2002). Growth in this species was best described by a model of declining growth rates with individual size (Cordes et al. 2005a). *Seepiophila jonesi* also grows extremely slowly and reaches ages comparable to *Lamellibrachia luymesi*, as established with a growth model including a size-specific probability of growth and an average growth rate that does not vary with individual size (Cordes et al. 2007a). In accordance with the wide range of environmental conditions under which *Ridgeia piscesae* can thrive and develop different growth forms, it is not surprising that growth rates can also vary from as much as 95 cm yr^{-1} (Tunnicliffe et al. 1997) to as little as 3 mm yr^{-1} in the long-skinny morphotype (Urcuyo et al. 2003, 2007).

A different approach was used by estimating cell proliferation and cell death in the epidermis applying immunohistochemical techniques as a measure of animal growth in *Riftia pachyptila* and *Lamellibrachia luymesi* (Pflugfelder et al. 2009). Interestingly, proliferation activities in both species are higher than in any other invertebrate studied so far and are comparable only to wound-healing processes or tumour growth. While in *Riftia pachyptila* cell death is downregulated, leading to fast growth, in *Lamellibrachia luymesi* it is upregulated, leading to a fast turnover of tissues and rejuvenation, which might be important for this extremely long-lived species.

Physiology and host–symbiont relationships

The originality of the Vestimentifera as far as functional physiology of the symbiosis is concerned relies on the following paradoxical situation:

- Symbionts are acquired from the environment, implying they are able to live freely in the vent or seep environments and suggesting their association with the vestimentiferan is facultative, although they are obviously growing well within their host.
- But for the hosts, it is an obligatory symbiosis, at least at the juvenile and adult stages (see 'Symbiotic phase', pp. 235–239), with bacteria remotely located within an internal organ, thus prompting sophisticated adaptations of the host systems to provide the bacteria with the inorganic, and potentially toxic, molecules they need.
- Or, as stated by Fisher et al. (1989): "The host tube-worms reap the benefits of an autotrophic life style, while providing their symbionts with an environment which free-living sulfide-oxidising bacteria can only regard with envy".

To our knowledge, nobody has been able to cultivate these bacteria so far, strongly limiting physiological studies and suggesting a profound transformation on the onset of symbiosis. Yet, the trophosome of vestimentiferan tubeworms is actually an extremely efficient organ to cultivate these bacteria, with tremendous yields reaching 10^9 to 10^{11} bacterial cells per gram of tissue (Powell & Somero 1986). In this section, what is known about the metabolic requirements and performance of the bacterial symbionts, including recent insights from genomic studies, is first reviewed. The host adaptations to fulfil these requirements and take advantage of the symbiosis are then examined, and responses to additional environmental constraints within the hydrothermal vents and cold-seep ecosystems are considered. Over the last decade, and starting from the state-of-the-art situation depicted in the review by Childress and Fisher (1992), the processes by which metabolites (carbon, sulphide, oxygen, nitrogen) and waste products (protons, sulphate) cycle among environment, host and symbionts and the corresponding enzymes involved in both host and symbiont metabolism have been progressively, yet incompletely, unravelled. Figures 11 and 12 summarise these findings for vent (*Riftia pachyptila*) and seep (*Lamellibrachia luymesi*) model tubeworms and should be referred to throughout this section.

Metabolic requirements and performance of the symbionts

The initial description of symbionts of *Riftia pachyptila* inferred their chemoautotrophic potential from microscopy (Cavanaugh et al. 1981), biochemistry (Felbeck 1981) and stable isotope ratios (Rau 1981). The symbionts looked like thiotrophic free-living gram-negative bacteria with sulphur inclusions (Cavanaugh et al. 1981), the trophosome tissue demonstrated highly significant adenosine triphosphate (ATP) sulphurylase and RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) activities (Felbeck 1981), with peculiar stable isotope signatures for ^{13}C (Rau 1981), *R. pachyptila* tissues being less depleted in ^{13}C than other thiotrophic symbioses. Mussels and clams have $\partial^{13}C$ values of about –30 per mil (‰), in the range that is expected for carbon derived from chemoautotrophic bacteria, but the $\partial^{13}C$ value of *R. pachyptila* was much higher (~–15‰).

Live symbiont metabolism

Since the symbionts could not, and still cannot, be cultivated, subsequent studies of these bacteria have been limited to those performed on freshly isolated or preserved material. Isolation and purification of symbiotic bacteria from live tubeworms is possible through dissection of trophosomal tissue and size fractionation on a Percoll gradient (Distel & Felbeck 1988). The bacterial suspension obtained is relatively pure, and bacteria stay metabolically active for a few hours, depending on the pressure, temperature and composition of the incubation medium (Distel & Felbeck 1988, Wilmot & Vetter 1990, Scott et al. 1994). These experiments showed that the symbionts of *R. pachyptila* were very specific in their needs, metabolising only sulphide, and not thiosulphate as other

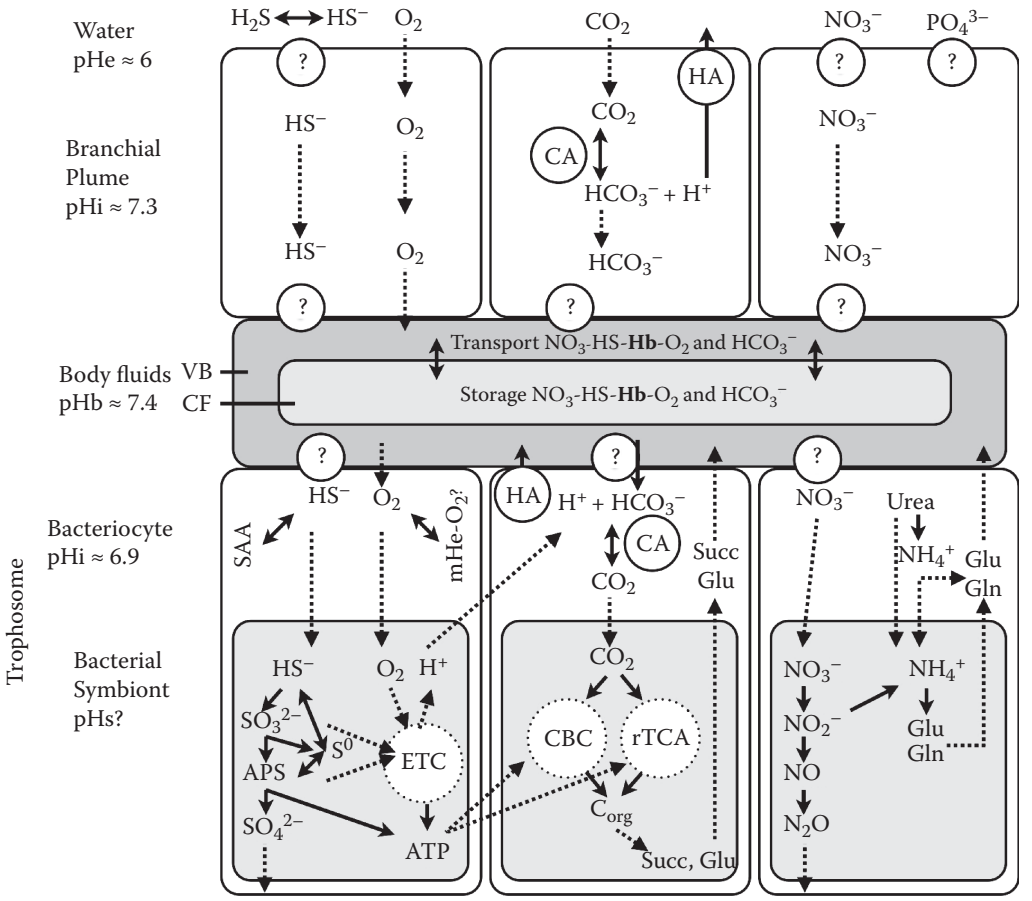

Figure 11 Schematic physiological model for the symbiosis between the vent tubeworm *Riftia pachyptila* and its thiotrophic bacteria regarding the principal metabolites: oxygen, sulphide, carbon dioxide and nitrate. For clarity, the various metabolic pathways (O_2 and H_2S, CO_2, NO_3^-) are shown on separate cells but co-occur in plume epithelial or trophosome bacteriocyte cells. See text for details. APS = adenosine phosphosulphate; CA = carbonic anhydrase; CBC = Calvin-Benson cycle; CF = coelomic fluid; ETC = electron transport chain; HA = proton-ATPase; mHe = myohemerythrin; rTCA = reverse tricarboxylic acid cycle; SAA = sulphur-amino acids; VB = vascular blood.

free-living or symbiotic thioautotrophic bacteria (Wilmot & Vetter 1990), and fixing carbon only from molecular carbon dioxide and not bicarbonate (Scott et al. 1998, 1999).

By measuring oxygen consumption rates of isolated symbiont suspension from *R. pachyptila,* it was possible to demonstrate that they oxidise sulphide but not thiosulphate or sulphite (Wilmot & Vetter 1990). Heterotrophy was also tested using malate, succinate, pyruvate or fumarate as substrata, all of which were not oxidised. The bacteria are able to oxidise even small amounts of sulphide (5 μ*M*) and have maximal respiration rates at concentrations greater than 1 m*M* sulphide, showing no inhibition in oxygen consumption up to sulphide concentrations of 2 m*M*. The products of sulphide oxidation are mostly insoluble elemental sulphur and, to a lesser extent, soluble sulphate and polysulphide (Wilmot & Vetter 1990). Polysulphide increase is only transient, and the final soluble product of sulphide oxidation is sulphate. Similar results were obtained by measuring carbon fixation in a trophosome incubation experiment (Fisher et al. 1989); carbon fixation occurred

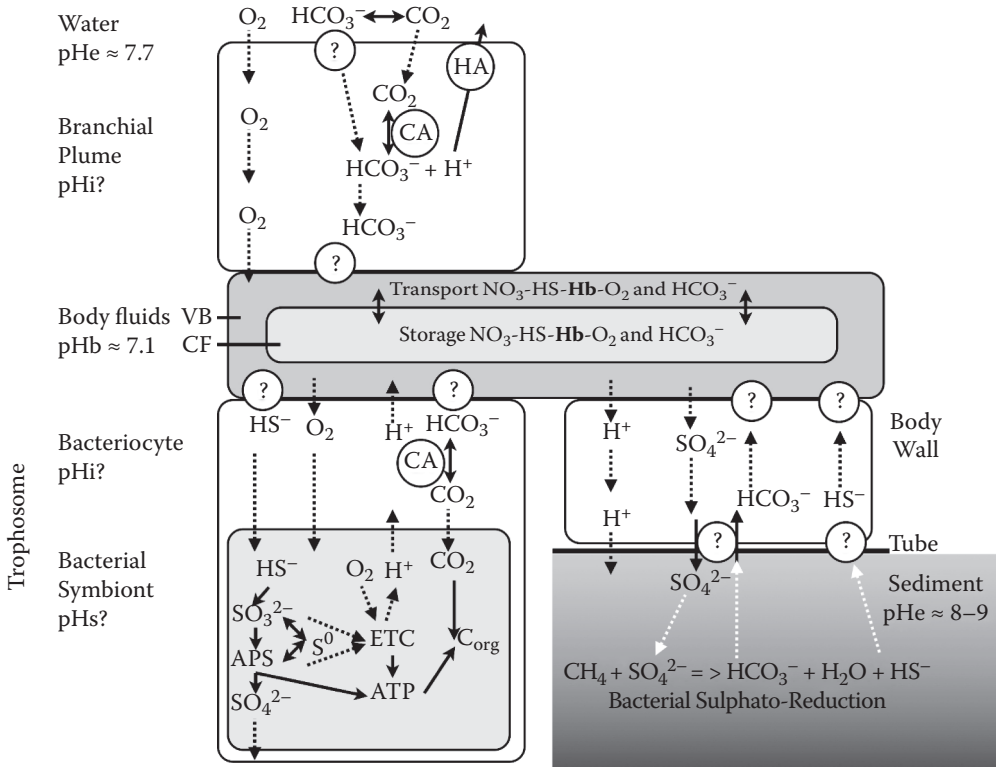

Figure 12 Schematic physiological model for the symbiosis between the seep tubeworm *Lamellibrachia luymesi* and its thiotrophic bacteria regarding the principal metabolites: oxygen, sulphide, carbon dioxide and nitrate. See text for details. Abbreviations as in Figure 11. (Adapted from Dattagupta et al. 2006.)

only with sulphide, but it was obviously inhibited by increasing oxygen concentrations, suggesting that sulphide oxidation and carbon fixation are not tightly coupled.

Carbon fixation by isolated symbionts was also measured by ^{14}C-inorganic carbon incubation and subsequent analysis of ^{14}C incorporation rates and apparent half-saturation constant ($K_{½}$) (Scott et al. 1998, 1999). By setting various pH and total inorganic carbon levels in the suspension medium, it is possible to discriminate which form of carbon is preferred: carbon dioxide or bicarbonate. Reliance on CO_2 with relatively low affinity is characteristic of cells adapted to growth with abundant total inorganic carbon. Alternatively, higher affinity and reliance on both CO_2 and HCO_3^- are typical of organisms adapted to growth with low total inorganic carbon. The three vestimentiferan symbionts tested (from *R. pachyptila*, *Ridgeia piscesae* and *Seepiophila jonesi*) all incorporated carbon from CO_2 and not HCO_3^-. This is related to the absence of carboxysome in these symbiotic bacteria, in contrast to the free-living, sulphide-oxidising *Thiomicrospira crunogena,* which is able to use both CO_2 and HCO_3^- (Scott et al. 1998). However, symbionts from *Ridgeia piscesae* had a higher affinity for CO_2 than those from *Riftia pachyptila* ($K_{½}$ of 7.6 µ*M* vs. 49 µ*M*; Scott et al. 1999). Since both symbionts belong to the same phylotype, this difference has been attributed to substratum availability, in relation to the higher inorganic carbon content in the blood of *R. pachyptila* (often above 30 m*M*; Childress et al. 1993, Toulmond et al. 1994) compared with *Ridgeia piscesae* (<10 m*M*; Scott et al. 1998).

Symbiont metabolic capabilities

A number of discrete biochemical studies have improved understanding of vestimentiferan symbiont capabilities for CO_2 fixation (RuBisCO; Robinson et al. 1998, 2003), sulphide oxidation (ATP sulphurylase; Renosto et al. 1991, Laue & Nelson 1994, Beynon et al. 2001) or nitrogen metabolism (Lee & Childress 1994, Lee et al. 1999, Minic et al. 2001, 2002, Minic & Herve 2003).

Until recently (see 'Genomics of the symbionts', p. 244), and since the initial characterisation of RuBisCO activity in vestimentiferan symbionts (Felbeck 1981), it has been postulated that fixation of CO_2 occurred through the Calvin-Benson cycle. Further investigations showed that vent vestimentiferan symbionts (from *Riftia pachyptila* and *Tevnia jerichonana*) did not express the most common form I RuBisCO (*cbbL* gene) but instead the form II RuBisCO (*cbbM* gene) in contrast with bivalve symbionts (Robinson & Cavanaugh 1995). A form II RuBisCO was also expressed in *Lamellibrachia* sp. (probably *Lamellibrachia* sp. L1; Table 1) (Elsaied et al. 2002), but *in situ* hybridisation analyses were not convincing due to confusion between sperm and bacteria on trophosome sections (see 'Symbionts', p. 233). Form II RuBisCO, a dimer of large subunits, has a lower specificity for CO_2 and thus is more effective under CO_2-rich conditions (Tabita et al. 2007). The kinetic properties of form II RuBisCO from *Riftia pachyptila* symbionts demonstrate a typical low affinity (K_{CO2} = 240 µmol L^{-1}) and a low CO_2/O_2 specificity factor (Ω = 8.6) compared with form I (Robinson et al. 2003). The degree of isotopic discrimination by the enzyme was determined to be 19.5‰, lower than that for form I (Robinson et al. 2003). Together, these properties may help explain the particular $\partial^{13}C$ signature in vestimentiferans: the isotopic discrimination by form II RuBisCO combines with a large CO_2 gradient between the host and the symbiont that drives an enrichment in ^{13}C of the CO_2 pool available to the symbionts (Scott 2003).

Felbeck (1981) initially reported high activities of adenosine phosphosulphate (APS) reductase and ATP sulphurylase in trophosome extracts from *R. pachyptila*, indicating that symbiont production of ATP could occur through the APS pathway. However, this pathway may also function in the reverse direction, as in dissimilatory sulphate reduction and assimilatory biosynthetic pathways (Madigan et al. 2009). High activity and kinetic properties of ATP sulphurylase of *R. pachyptila* (Renosto et al. 1991), specifically a high K_{cat} (296 s^{-1}) for ATP synthesis that may be related to the specific nucleotide sequence of the *sopT* gene (Laue & Nelson 1994) and structure of the active site (Beynon et al. 2001), strongly support the idea that this pathway derives sufficient energy from sulphide oxidation to sustain the chemoautotrophic metabolism of the symbiont. Elemental sulphur (S^0) accumulates in symbiont-containing tissues of thiotrophs (Vetter & Fry 1998) and has been localised to membrane-bound vesicles in the symbionts (Pflugfelder et al. 2005), where it may serve as an internal store of partially oxidised sulphur. This store may be further oxidised (i.e., re-enter the APS pathway) if oxidants are present (mainly O_2 and eventually NO_3^-; Hentschel & Felbeck 1993) but may also generate sulphide in case of anoxia (Arndt et al. 2001). The complete oxidation of sulphide to sulphate produces electrons shuttled through an electron transport chain (ETC) system, and the resulting proton gradient generates ATP through oxidative phosphorylation.

Nitrogen metabolism has also been the subject of several studies (Lee & Childress 1994, Lee et al. 1999, Minic et al. 2001, 2002, Minic & Herve 2003). It appears that the main source of nitrogen for the symbiont may be nitrate (NO_3^-) that can be reduced to nitrite (NO_2^-) with the enzyme nitrate reductase (Lee et al. 1999), eventually converted to ammonia (NH_3) and ultimately be incorporated into amino acids due to the activities of glutamine synthetase and glutamate dehydrogenase (Lee et al. 1999). As well as amino acids, nucleotide synthesis needs nitrogen. Interestingly, the symbiont possesses the enzymatic equipment for the biosynthesis of pyrimidine nucleotides through the *de novo* pathway but lacks the enzymes of the salvage and catabolic pathways (Minic et al. 2001, 2002).

Numerous other nutrients (e.g., phosphorus, vitamins, metals) are obviously necessary for the complete metabolism of the symbionts, but the assimilation and conversion pathways involved have not been studied so far.

Genomics of the symbionts

A long-awaited major breakthrough is the publication of the metagenome of the symbiont of *R. pachyptila* (Robidart et al. 2008) and incidentally its denomination as Candidatus *Endoriftia persephone*. The main findings reported by Robidart et al. relate to the metabolic possibilities that could be expressed in the free-living form of *E. persephone* (e.g., heterotrophic capacity, flagellum synthesis) and, in addition to the confirmation of the different pathways described, the existence of an alternative carbon fixation pathway, the reductive tricarboxylic acid (reverse TCA or rTCA) cycle. Energy requirements to fix CO_2 differ among these two pathways: the rTCA cycle requires only one molecule of ATP for one of CO_2, whereas the Calvin-Benson cycle requires three molecules of ATP (reviewed by Nakagawa & Takai 2008). Moreover, as mentioned by Markert et al. (2007), the stable carbon isotopic fractionation between inorganic CO_2 and biomass by the rTCA cycle is less than that by the Calvin-Benson cycle, providing an attractive alternative explanation for the anomalously high carbon isotope values found in Vestimentifera. It should be noted that oxygen-sensitive enzymes are involved in the rTCA cycle (Nakagawa & Takai 2008), prompting a reexamination of the experiments conducted on isolated symbionts regarding the oxygenation characteristics of the suspension medium.

Genome knowledge allows for expression (transcriptome and proteome) studies with a reliable identification of major proteins produced under various conditions. In the first study of this kind, Markert et al. (2007) confirmed the quantitative importance of the sulphide oxidation pathway and the reality of the rTCA cycle for carbon fixation. The three major enzymes DsrA (dissimilatory sulphite reductase), AprA/AprB (APS reductase) and SopT (ATP-sulphurylase), catalysing the oxidation of H_2S to SO_4^{2-} and yielding ATP, constitute more than 12% of the total cytosolic symbiont proteome (Markert et al. 2007). Several genes encoding sulphur transferases are also present (Robidart et al. 2008) and would allow direct use of thiosulphate by the symbionts, in contrast to findings from earlier physiological studies (Wilmot & Vetter 1990). The use of elemental sulphur as an electron sink when oxygen is absent, postulated by Arndt et al. (2001), may be possible via a sulphur reductase (Robidart et al. 2008). The four enzymes allowing the rTCA cycle are abundant and show a high activity level, providing strong evidence that symbionts of *R. pachyptila* effectively use the rTCA cycle for autotrophic carbon fixation as well as the Calvin-Benson cycle (Markert et al. 2007). Future physiological studies are now needed to determine the relative importance of each carbon fixation pathway as well as numerous other metabolic pathways revealed by genomic sequence annotation (Robidart et al. 2008).

Host adaptations to fulfil symbiont metabolic needs

From this information, the requirements of symbionts for chemoautotrophy may be summarised as follows: they need carbon, in the form of molecular carbon dioxide, sulphide and oxygen for energy, and nitrogen, mainly in the form of nitrate and ammonium. The acquisition from the environment and subsequent transport of these molecules by the host worm to the internal location of the symbionts has been puzzling researchers ever since the chemotrophic nature of the symbiosis was postulated. Evidence for host adaptations to perform these functions has mainly been brought by whole-organism experiments carried out in pressurised aquaria, discrete biochemical analyses and, more recently, broad-range gene and protein expression studies.

Autotrophic balance of intact animals

The first whole-organism experiments that gave evidence for autotrophy of the *R. pachyptila* symbiosis, by analysing gas fluxes in and out of flow-through pressurised aquaria, were those of Fisher et al. (1988) and Childress et al. (1991). These experiments established that, given appropriate (i.e., environmentally realistic) amounts of oxygen and sulphide, the *R. pachyptila* symbiosis could eventually consume carbon dioxide instead of producing it as in other animals. Without sulphide, O_2 consumption was around 2.7 μmol g^{-1} h^{-1} with a corresponding ΣCO_2 production of 2.2 μmol g^{-1} h^{-1}, whereas consumption of 2.5 μmol g^{-1} h^{-1} sulphide (and 5.5 μmol g^{-1} h^{-1} O_2) neutralised net ΣCO_2 fluxes, and maximum ΣCO_2 uptake reached 2.7 μmol g^{-1} h^{-1} for 5.5 μmol g^{-1} h^{-1} sulphide and 9 μmol g^{-1} h^{-1} O_2 consumptions (Childress et al. 1991).

These initial experiments have been repeated several times since this work was reported, and the last refinements, using semicontinuous mass spectrometry measurements in addition to gas chromatography calibration, were performed and published by Girguis & Childress (2006). Their results showed that substrata concentrations and environmental temperature can strongly govern oxygen and sulphide uptake rates of *R. pachyptila* as well as net carbon uptake: symbiont carbon fixation was observed to be highest after sufficient oxygen and sulphide had been acquired by *R. pachyptila* and when temperatures were relatively high (Girguis & Childress 2006). Under similar conditions to those used by Childress et al. (1991) (conditions termed "better" in Girguis & Childress 2006), they obtained somewhat higher yet comparable flux values considering temperature effects (8–13°C in Childress et al. 1991; cf. 15°C in Girguis & Childress 2006) with a maximum ΣCO_2 uptake of 3.7 μmol g^{-1} h^{-1} for 7.8 μmol g^{-1} h^{-1} sulphide uptake and 12.8 μmol g^{-1} h^{-1} O_2 uptake. Pushing the symbiosis in *R. pachyptila* to its limits ("best" conditions in Girguis & Childress 2006) with external concentrations of 256 μmol L^{-1} ΣH_2S, 197 μmol L^{-1} O_2 and 10.8 μmol L^{-1} ΣCO_2 at 15°C), they measured fluxes of 12.7 μmol g^{-1} h^{-1} ΣCO_2 for 11.9 μmol g^{-1} h^{-1} ΣH_2S and 25.1 μmol g^{-1} h^{-1} O_2. Optimal temperature for maximal CO_2 uptake was around 26°C, close to the maximal thermal tolerance of *R. pachyptila,* which is above 30°C (Girguis & Childress 2006); at the optimal temperature, O_2 and sulphide consumptions of 35 and 16 μmol g^{-1} h^{-1} yielded a ΣCO_2 uptake of nearly 16 μmol g^{-1} h^{-1}.

In cold-seep species, as stated, there is not enough sulphide in the water column around the plumes of vestimentiferan tubeworms to sustain a sulphide-driven autotrophic mode of life. However, sulphide present in the sediment has been proposed as the source of sulphide for adult *Lamellibrachia luymesi* taken up through the buried part of their tubes and body (Julian et al. 1999). In a modified setup taking this into account with a split-vessel, flow-through respirometry chamber (Freytag et al. 2001), it was possible to show net CO_2 (and O_2) uptake at the plume level when sulphide was passed over the roots. At 5°C, consumption rates were lower than in *Riftia pachyptila*; using sulphide concentrations around the roots in the range 200–400 μmol L^{-1}, Freytag et al. measured 1–3 μmol g^{-1} h^{-1} CO_2 uptake for 3–5 μmol g^{-1} h^{-1} sulphide and 7–10 μmol g^{-1} h^{-1} O_2 consumptions.

Acquisition of inorganic substrata from the environment

As shown, two organs of vestimentiferan hosts may serve as an entry point for the necessary metabolites to be delivered to the symbionts: the branchial plume and, in the case of cold-seep tubeworms, the posterior end of the trunk, or root. The mechanisms by which these substances cross the epithelium and enter the body fluids have been the subject of several studies using direct, physiological or indirect, biochemical approaches.

As for any exchange surface and for uncharged molecules such as O_2, H_2S or CO_2, the first uptake mechanism is diffusion, which is proportional to the surface area exposed to the external medium and inversely proportional to the diffusion distance (Fick's law). Although a

respiratory role for the branchial plume of Vestimentifera is obvious based on anatomical considerations (e.g., Gardiner & Jones 1993), actual surface area estimates of complicated surfaces such as respiratory organs are extremely tedious and, as a result, have seldom been performed. For Vestimentifera, one study dealt with *R. pachyptila* (Andersen et al. 2002) and another with the two morphotypes of *Ridgeia piscesae* (Andersen et al. 2006). *Riftia pachyptila* has a mean specific branchial surface area (SBSA) of 22 $cm^2\ g^{-1}$, second highest among all aquatic animals. Mean diffusion distance for the branchial filament wall was 15 µm, with a minimal diffusion distance of 2 µm in the pinnules that cover the free tip of the filaments (Andersen et al. 2002). In *Ridgeia piscesae*, two morphotypes occur: a short-fat, chimney-dwelling and a long-skinny, basalt-dwelling morphotype (see 'Hydrothermal vent tubeworms', p. 223 and Southward et al. 1995). Mean SBSA (24 $cm^2\ g^{-1}$) is similar in both morphotypes and comparable to the values obtained for *Riftia pachyptila* as well as the diffusion distances for the chimney-dwelling morphotype, whereas there is a 20% increase in diffusion distance for the basalt-dwelling morphotype (Andersen et al. 2006). To date, the branchial plume of seep species and the root part of the trunk in seep species or basalt-dwelling *Ridgeia piscesae* have not been subjected to detailed studies of their surface area or diffusion distances, preventing substantiated comparisons. The only estimate based on average geometric considerations yields a specific root surface area of 8 $cm^2\ g^{-1}$ in *Lamellibrachia luymesi* (Dattagupta et al. 2006), about a third of the SBSA in vent species.

Diffusion is the only uptake mechanism known for uncharged molecules such as oxygen (but see discussion of the role of haemoglobin [Hb] in facilitated diffusion and transport), but alternative pathways may exist for chemicals that also occur in ionic form. In water, this is the case for carbon dioxide ($CO_2 \Leftrightarrow HCO_3^-$; p$K \approx 6.2$ at 10°C), sulphide ($H_2S \Leftrightarrow HS^-$, p$K \approx 6.8$ at 10°C) and the various forms of nitrogen (NH_4^+, NO_2^-, NO_3^-), the dissociation of which mainly depends on the environmental pH and temperature around the exchange organ. Using whole-organism experiments as described and by varying the external conditions while measuring VB and CF substrata concentrations, it has been possible to trace the most plausible acquisition pathways for carbon dioxide and sulphide.

Carbon dioxide total concentration in the body fluids of *Riftia pachyptila* can reach extremely high values, up to 50 mmol L^{-1} (Childress et al. 1993), and CO_2 uptake is correlated to environmental CO_2 and not HCO_3^- concentrations (Goffredi et al. 1997b, Girguis & Childress 2006). CO_2 diffusion is facilitated in the vent environment, where pH values are lower and ΣCO_2 higher than in normal seawater due to mixing with acidic and CO_2-enriched hydrothermal vent fluid, thus providing a high CO_2 partial pressure Pco_2 gradient across the branchial epithelium. Diffusion alone can account for the high ΣCO_2 values measured in CF and VB because the Pco_2 gradient is maintained by rapid internal transformation of CO_2 into HCO_3^- through carbonic anhydrase (CA) activity and by the active elimination of protons through H^+-ATPase (adenosine triphosphatase) activity (Goffredi et al. 1997b, 1999, Goffredi & Childress 2001), thus maintaining a higher pH in the body fluids relative to the external pH. These findings have been substantiated by similar studies on *R. pachyptila* and other species (Girguis & Childress 1998, 2006, Girguis et al. 2002, Dattagupta et al. 2009) and by more detailed examination of ATPase and CA in branchial plume tissue. *Riftia pachyptila* has unusually high ATPase activity (646 $\mu molP_i\ g^{-1}\ h^{-1}$), with a large proportion of its ATPases (38%) being P- and V-H^+ATPases, the forms devoted to proton transport (Goffredi & Childress 2001). In *R. pachyptila*, branchial epithelium V-H^+ATPase is mostly colocalised with CA on the apical side, and both enzymes probably allow CO_2 entry while regulating intracellular pH (De Cian et al. 2003a). In comparison, seep species have much lower ATPase activities (48–57 $\mu molP_i\ g^{-1}\ h^{-1}$ including plume and root), suggesting that other routes may be active for proton elimination and carbon uptake (Dattagupta et al. 2009), such as proton channels and HCO_3^- transport across the body wall epithelium. The hypothesis is supported by the presence of vascular connections between the trophosome and the body wall in *Lamellibrachia luymesi* that are absent in *Riftia pachyptila* (van der Land & Nørrevang 1977, Gardiner & Jones 1993).

High levels of CA activity (254 μmol CO_2 g^{-1} min^{-1}) have been reported for branchial plume tissue of *Riftia pachyptila* (Kochevar et al. 1993, Kochevar & Childress 1996, Goffredi et al. 1999). Similar activity levels occur in *Tevnia jerichonana* branchial plume tissue, but much lower activities have been measured in seep-species plume tissue (Kochevar & Childress 1996). A protein sequence for *Riftia pachyptila* plume CA clustered with cytosolic CA isoforms (De Cian et al. 2003c), and immunolocalisation showed it is mostly present at the apical side of the branchial epithelium (De Cian et al. 2003a). Confirmation of high transcription levels of CA messenger RNA have been reported in transcriptomic approaches in *R. pachyptila* (Sanchez et al. 2007b) and *Ridgeia piscesae* (Ruan et al. 2008), and two different CA genes are expressed in the branchial plume of *Riftia pachyptila*, with one form specifically expressed in this tissue, the other common with the CA gene expressed in the trophosome (Sanchez et al. 2007a). Further functional characterisation and physiological studies are needed to clarify the precise role of each of these isoforms, with some of these specialised for CO_2 conversion or better poised for acid–base regulation (Henry 1996).

Because sulphide is toxic to aerobic organisms (Grieshaber & Volkel 1998), thiotrophic symbioses constitute very unusual models by which sulphide becomes an essential nutriment. Initially, experiments with *R. pachyptila* exposed to various external sulphide concentrations and pH showed a correlation of body fluid total sulphide (ΣH_2S) with the ionic bisulphide ion HS^- and not with the diffusible H_2S form (Goffredi et al. 1997a). However, additional measurements and refinements in analytical procedures revealed that both H_2S and HS^- could be acquired (Girguis & Childress 2006) depending on the external pH and the corresponding main form of sulphide in the water (H_2S below pK_S, HS^- above pK_S). Within an environmentally realistic concentration range (10–400 μmol L^{-1}), ΣH_2S and O_2 uptake are highly correlated, which is consistent with the sulphide-oxidising metabolism of the symbionts (Girguis & Childress 2006).

In seep species, there is hardly any sulphide in the water surrounding the branchial plume high above the sediment. Sulphide is sometimes detectable at the base of tubes or at the surface of sediment and increases sharply with sediment depth, reaching 1.5 mmol L^{-1} at 20 cm (Julian et al. 1999). The thin (70-μm) tube of the root is permeable to H_2S (Julian et al. 1999), and sulphide uptake does occur under these conditions, allowing inorganic carbon uptake (Freytag et al. 2001). Based on these results and additional measurements of sulphate and proton concentrations, Dattagupta et al. (2006) proposed a model (see also Figure 12) in which sulphate excretion fuels microbial consortia in the sediment (Boetius et al. 2000); the resulting sulphide production may diffuse through the thin tube and enter the tubeworm through the body wall epithelium. This sulphate/sulphide cycle would be sufficient to support the long life of seep tubeworms according to growth models (Cordes et al. 2005a). In vent tubeworms, and even more so in seep tubeworms, further studies that examine membrane transport are needed to confirm and detail these pathways in plume and body wall epithelia, both at the apical (cell–environment interface) and basal (cell–body fluid interface) membranes (as illustrated by the numerous question marks in Figures 11 and 12).

Nitrogen uptake in *R. pachyptila* takes place in the form of nitrate, not ammonium or dinitrogen (Lee & Childress 1994, Hentschel et al. 1998, Girguis et al. 2000). With environmental nitrate concentrations around 40 μmol L^{-1} around vents (Lee & Childress 1994), nitrate uptake amounts to 3.5 μmol g^{-1} h^{-1} (Girguis et al. 2000). However, the mechanism of nitrate uptake, a highly unusual function in animals, is yet unknown. *Riftia pachyptila* can also take up free amino acids (Childress et al. 1984), but this is not believed to be important in the normal physiology of the worm. To our knowledge, there are no data on the uptake of nitrogen compounds in seep species, whereas both ammonium and nitrate might be present in the environment (Lee & Childress 1994).

Transport of inorganic metabolites in the body fluids

The body fluids of *R. pachyptila* accumulate high amounts of oxygen, sulphide, carbon dioxide and nitrate, well above the concentration of these molecules in the surrounding environment. The central role of Hb in the transport of oxygen and sulphide was suspected very early, which is not

surprising considering the large amount (4% blood and 26% CF, W/W; Childress et al. 1984) and bright red colour of these fluids in *R. pachyptila*. The role of body fluids in sulphide accumulation and transport (Arp & Childress 1981, 1983), and subsequently its reversible binding to Hb, independently of oxygen binding was established early (Arp et al. 1987). Vestimentifera have extracellular Hbs in both the VB and CF. VB contains two forms, HbV1 and HbV2, whereas CF contains one form, HbC1 (Zal et al. 1996b). HbV1 is similar to the high molecular weight (~3500 kDa) hexagonal bilayer haemoglobin (HBLHb), which is specific of annelids, while HbV2 and HbC1 are extracellular Hb of lower molecular weight (~400 kDa) (Zal et al. 1996b). Analysis of their subunit composition demonstrated that these two forms differ slightly, one globin chain being absent from HbC1 (Zal et al. 1996a). The Hb concentration is also higher in VB ([haem] ≈ 3 mmol L^{-1}) than in CF (0.5 mmol L^{-1}) (F.L. personal observations). The involvement of cysteins in the mechanism of sulphide binding, initially proposed (Arp et al. 1987), was substantiated by the finding and localisation of free cysteins on some of the globin chains constituting both VB and CF Hbs (Zal et al. 1996a, 1997) and convincing equilibration studies (Zal et al. 1998). An evolutionary scenario was proposed (Bailly et al. 2002, 2003) by which sulphide binding could have appeared in annelid Hbs to avoid its toxicity and evolved in reversible binding for sulphide transport in thiotrophic Siboglinidae. However, crystallographic data showed that these cystein residues are located beneath the surface of the molecule, questioning their efficient role in sulphide binding (Flores et al. 2005). Instead, these authors have proposed that zinc ions bound to HbC1 may chelate sulphide, but their experiments explained only 57% of sulphide binding with this Hb and only 18% with HbV1. Increasing the zinc chelator concentration in a subsequent analysis, these authors obtained a complete inhibition of sulphide binding in HbC1 (Flores & Hourdez 2006). At the same time, the structure of the 400-kDa Hb from the Frenulata *Oligobrachia mashikoi* was also obtained (Numoto et al. 2005), and mercury binding to free cystein residues showed that these sites were readily accessible to mercury and that sulphide binding could be stabilised by close-by phenylalanine residues. Additional work is clearly needed to fully understand the possibly multiple mechanisms of sulphide binding to Vestimentifera Hb and their precise functional characteristics under physiologically relevant conditions to get the whole picture of combined oxygen and sulphide transport, which is so important for the symbiosis to operate.

Carbon dioxide also accumulates in high amounts in body fluids of *Riftia pachyptila* (Childress et al. 1993), and most of it is not bound to Hb as carbamate but is present in the ionic form bicarbonate, the most preponderant form at body fluid pH values (7.1–7.4) (Toulmond et al. 1994). The two body fluids, VB and CF, have different behaviour in relation to CO_2; gel filtration analysis of the fluids revealed that the protein-free fractions retained 64% of the CO_2 in VB and 80% in CF, in relation with their respective Hb content (Toulmond et al. 1994). An alternative hypothesis for CO_2 transport as HCO_3^- was proposed early (Felbeck 1985), involving branchial carboxylation of pyruvate into C4 compounds (Felbeck & Turner 1995) malate and succinate, which are readily labelled in the effluent following perfusion experiments with dissected plumes and vestimentums under atmospheric pressure. Further studies with catheterised worms maintained under pressure have shown that CO_2 from the environment is apparently transported initially as inorganic carbon in the blood of *R. pachyptila* (Felbeck et al. 2004). Nevertheless, the carbon stored in the large quantities of organic acids found in the body fluids, and which may originate either from the branchial plume or the trophosome based on enzymatic capacities (Felbeck 1985), could function as a store to maintain carbon fixation in the trophosome during periods of low CO_2 availability (Felbeck et al. 2004).

Nitrate transport must also occur if nitrate is utilised as the main nitrogen source for bacterial biosynthetic metabolism (Lee et al. 1999) or occasional respiration (Hentschel & Felbeck 1993), and nitrate is found at elevated concentrations in the body fluids of *R. pachyptila*: up to 0.2 mmol L^{-1} in CF and in the range 1–4.5 mmol L^{-1} in VB (Hentschel et al. 1998, Pospesel et al. 1998). Hb, or a blood-borne protein in sufficient amount to accommodate the high nitrate concentrations measured, is a serious candidate to bind nitrate (Hahlbeck et al. 2005), but the mechanism is as yet unknown.

Utilisation of inorganic metabolites and bacteriocyte–symbiont relationships

In the trophosome, bacteriocytes must take up available molecules in the blood to provide them to the symbionts localised within the vacuoles. In addition, organic molecules produced by the symbionts must transit through the bacteriocytes and be delivered to the body fluids to benefit the host metabolism. The cellular physiology of bacteriocytes is thus central to the functioning of the symbiosis in Vestimentifera. Although bacteriocyte isolation has proved feasible in *R. pachyptila* (De Cian et al. 2003b), few studies have exploited this approach, and most of these have been done on trophosome tissue incubations. The rest of current knowledge on bacteriocyte–symbiont relationships is based on indirect evidence, mostly from biochemical and molecular studies.

Regarding oxygen and considering the microaerophilic nature of the bacteria (see, e.g., Fisher et al. 1989, Hentschel & Felbeck 1993, Girguis & Childress 2006), the free oxygen concentration should be maintained very low within the bacteriocytes, but no direct measurements are yet available. The high affinity of extracellular Hbs in VB and CF may help to limit the amount of free oxygen in the bacteriocytes. Interestingly, the expression level of myohemerythrin (mHe), a non-haem, di-iron oxygen-binding protein present in *R. pachyptila* (Bailly et al. 2008) and *Ridgeia piscesae* (Ruan et al. 2008), appears especially high in the trophosome (Sanchez et al. 2007b) and could play a role in oxygenation regulation in the vicinity of bacteria. Further studies on the functional properties of mHe relative to those of VB and CF Hbs are needed.

Intracellular storage of sulphide in the bacteriocytes could be mediated by sulphur-amino acids (SAAs). Early on, high thiotaurine levels were reported in symbiotic tissues of Vestimentifera (Alberic 1986, Pruski et al. 2000, Yin et al. 2000). Thiotaurine synthesis from hypotaurine appears to be a specific adaptation to the thiotrophic mode of life (Pruski et al. 2001), and its rate is related to sulphide exposure, either experimentally (*in vitro* tissue incubation; Pruski & Fiala-Medioni 2003) or naturally *in situ* (Brand et al. 2007). While the precise function (i.e., transport or storage of sulphide) of hypotaurine and thiotaurine has yet to be established, it seems to be a host attribute: free-living sulphide-oxidising bacteria (*Thiobacillus hydrothermalis*) do not metabolise thiotaurine unless trophosome extract is added to the medium (Pruski et al. 2001). However, recent evidence showed that hypotaurine and thiotaurine also accumulate in non-symbiotic annelids exposed to high-sulphide environments, redefining their role in sulphide detoxication (Ortega et al. 2008, Yancey et al. 2009). In the symbionts, sulphide may also be stored as elemental sulphur (S^0) granules, as stated, and be further oxidised to sulphate with oxygen or nitrate as final electron acceptors. However, in case of anoxia, it seems that elemental sulphur may be reduced back to H_2S that accumulates in the blood (Arndt et al. 2001). The extent of anaerobic sulphide production relates to the colour of the trophosome (Pflugfelder et al. 2005) and hence on the elemental sulphur content of symbionts (Arndt et al. 2001).

To provide molecular CO_2 to the symbionts, bacteriocytes have to draw from the large pool of HCO_3^- in the body fluids and convert it with the help of CA. Metabolically produced CO_2 may also participate in inorganic carbon supply to the bacteria. CA is highly active in trophosome extracts from *Riftia pachyptila* with a level similar to that observed in the plume (Kochevar et al. 1993, Kochevar & Childress 1996, Goffredi et al. 1999). In *Tevnia jerichonana*, CA activity in the trophosome is lower than that in the plume, but in seep species (*Lamellibrachia luymesi* cited as *Lamellibrachia* sp. and *Seepiophila jonesi* cited as Escarpid, undescribed), CA activity is higher in the trophosome compared with the plume (Kochevar & Childress 1996).

Immunolocalisation of cytosolic CA and *in situ* hybridisation with CA-cDNA probe in *Riftia pachyptila* trophosome lobules demonstrated that CA is expressed and found in bacteriocytes and peritoneal cells (De Cian et al. 2003a). A physiological investigation using various inhibitors on isolated bacteriocyte suspensions from the trophosome tissue of *R. pachyptila* revealed a complex interaction of CA with two important ion-transporting enzymes: the vacuolar-type V-H^+ATPase and the Na^+K^+-ATPase (De Cian et al. 2003b). These enzymes seem to be involved in the transepithelial

transport processes for electrolytes and CO_2, and the authors suggested that in the trophosome, the proton efflux might generate a local acidification of the outer layer of the membrane, facilitating the influx of CO_2. There is one cytosolic isoform of CA (RpCAtr) highly expressed in the trophosome (De Cian et al. 2003c, Sanchez et al. 2007a), in contrast with the branchial plume where this isoform is much less expressed than another, more specific branchial isoform (RpCAbr) (Sanchez et al. 2007a). In addition, immunoblots of membrane-bound protein fractions from the trophosome of *R. pachyptila* revealed proteins with CA activity, but the sequence of this putative membrane-bound CA is yet unknown (De Cian et al. 2003c). Similar characterisation and localisation studies on CA in seep species are lacking.

Based on purified symbiont incubations, the transfer of organic carbon from the symbionts to the host appears to be mainly in the form of succinate and glutamate (Felbeck & Jarchow 1998a,b). Succinate plays a pivotal role in the *R. pachyptila* symbiosis since it is also the main end product of anaerobic metabolism during the first 40 h of anoxic exposure (Arndt et al. 1998a,b). The phosphagen creatine phosphate, not analysed in these studies, should also play a role on the onset of anaerobiosis, as suggested by large amounts of creatinine (1128 µmol L^{-1}) and high level of creatine kinase (176 µmol.g^{-1}.min^{-1}) in the trophosome (De Cian et al. 2000). The trophosome also has high levels of glycogen, up to 100 µmol glycosyl units g^{-1} (Arndt et al. 1998b). Glycogen reserves are localised in both host cells and symbionts, which contribute equally to the total glycogen content of the trophosome (Sorgo et al. 2002). However, utilisation of glycogen only appeared after long-term (48-h) anoxic exposure (Arndt et al. 1998b).

Regarding nitrogen compounds, glutamate and glutamine may be the main molecules derived from the ammonia issued from nitrate reduction (Lee et al. 1999) and that could be excreted to the host. However, the complex sharing of enzymatic activities between host and symbionts for many N-compound pathways has neen noted (see 'Symbiont metabolic capabilities', p. 243 and Minic & Herve 2004). In addition, unusually high levels of uric acid (1–2 mmol L^{-1}) and urea (0.9 mmol L^{-1}) have been reported in the trophosome of *R. pachyptila* (De Cian et al. 2000). While the role of uric acid is still elusive, urea transporters have been found in the genome of the symbiont (Robidart et al. 2008), and urease activity is present in the trophosome (De Cian et al. 2000), allowing for a complex shuttle of urea and ammonia between the two partners.

Conclusion

For a group that was little known some 30 yr ago, and remains difficult to access due to its mostly deep-sea habitat, much understanding has been gained in all aspects of the biology of Vestimentifera. Of course, knowledge of their biodiversity, as well as that of many other deep-sea-dwelling organisms, is still far from complete. Exploration of unknown areas of the ocean bottom, especially in the Southern Hemisphere, is necessary to identify new species and supplement knowledge of biogeographic traits and the evolutionary history in this taxon. This effort is considered by the international community in programmes such as ChESS (Census of Marine Life program on Biogeography of Deep-Water Chemosynthetic Ecosystems) (Tyler et al. 2002).

To develop a global approach of symbiosis-related gene expression (transcriptome) and protein production (proteome) in vestimentiferan tubeworms, there is an urgent need for host genome sequencing. First attempts using suppression subtractive hybridisation (SSH) libraries in *R. pachyptila* (Sanchez et al. 2007b) or expressed sequence tag (EST) libraries in *Ridgeia piscesae* (Nyholm et al. 2008) have been confronted with too many unknown sequences due to the lack of phylogenetically close model organisms. The recent availability of some annelid genomes (e.g., *Capitella capitata*) should help in this respect. However, with the present development of new high-throughput techniques, genome or transcriptome sequencing of the host in a model vestimentiferan species should be accessible in the near future. Choosing model vestimentiferan tubeworms for this purpose should rely on ecological (vent and seep species), evolutionary (crown and basal groups) and

physiological (acquired knowledge) bases, and for this purpose there are two obvious species, *Riftia pachyptila* and *Lamellibrachia luymesi*.

The problem of the control of symbiont genome expression by the host, necessary for a stable and efficient mutualistic interaction, is one of general biological significance. Vestimentiferan representatives certainly stand out as model systems to study such problems as the infectious mode of symbiont transmission, the effective elimination of bacteria from cells outside trophosome tissue and the very efficient growth and metabolic control of bacteria. Controlling bacterial growth within a single cell type may indeed prove useful in designing novel ways of dealing with infectious bacteria, including those eliciting disease in humans (Hentschel et al. 2000).

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