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Morphology, ultrastructure and functional anatomy of the branchial organ of *Terebellides stroemii* (Polychaeta: Trichobranchidae) and remarks on the systematic position of the genus *Terebellides*

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Abstract: The morphology, functional anatomy, ultrastructure and respiratory surface areas of the lamelliform branchiae of *Terebellides stroemii* were investigated. A comparison with a previous analysis on the branchiae of alvinellids gave evidence that a comparable type of lamellar branchiae exists in *Alvinella* and *Terebellides*. The observation of live *Terebellides stroemii* gave information on the directions of the blood circulation in the vessels of the two larger branchiae as well as on the water currents at the branchial surface. The specific gill surface area in *T. stroemii* is considerably smaller than that of the hydrothermal vent *Alvinella*. Due to the absence of a shallow blood network in the branchial lamellae, the diffusion distance between blood and external milieu is longer in *T. stroemii* than in *Alvinella* which possess such a vascular network. The similarities of the gills between *Terebellides* and *Alvinella* most probably represent an homoplasy through convergence. The segmental position of the branchiae, parapodia, gular membranes, nephridial and genital pores are summarized on a schematic comparative representation of the anterior segments of *Trichobranchus glacialis, Terebellides stroemii, Paralvinella grasslei* and *Alvinella pompejana*. The position of the genus *Terebellides* in the family Trichobranchidae is discussed.

Résumé : *Morphologie, ultrastructure et anatomie fonctionnelle de l'organe branchial de* Terebellides stroemii *(Polychaeta : Trichobranchidae) et remarques sur la position systématique du genre* Terebellides. La morphologie, l'ultrastructure et la surface respiratoire des branchies lamelliformes de *Terebellides stroemii* ont été étudiées. La comparaison avec une analyse précédente des branchies des alvinellidés montre qu'un type de branchie lamellaire comparable existe chez *Alvinella* et *Terebellides*. L'observation de spécimens vivants de *Terebellides stroemii* a montré le sens de la circulation du sang dans les vaisseaux branchiaux ainsi que le sens des courants d'eau à la surface des lamelles. La surface respiratoire spécifique de *T. stroemii* est nettement inférieure à celle d'*Alvinella*. De plus, en raison de l'absence de réseau sanguin superficiel dans les lamelles branchiales, la distance de diffusion entre le sang et le milieu extérieur est plus grande chez *T. stroemii* que chez *Alvinella* où ce réseau vasculaire est présent. Les similitudes branchiales entre *Terebellides* et *Alvinella* représentent probablement une homoplasie par convergence. L'emplacement des branchies, des parapodes, du septum antérieur, des pores néphridiens et génitaux est illustré sur un schéma comparatif des segments antérieurs de *Trichobranchus glacialis, Terebellides stroemii, Paralvinella grasslei* et *Alvinella pompejana*. La position du genre *Terebellides* dans la famille des Trichobranchidae est discutée.

Keywords: *Terebellides* • Branchial organ • Structure • Function • Systematics

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Introduction

The position and general morphology of the branchiae are morphological characters often used in phylogenetic analyses of polychaetes. The branchial organ of *Terebellides stroemii* Sars, 1835 located anteriorly, is dorsal and comprises a stalk bearing two pairs of lamelliform branchiae: anteriorly one pair, well developed, also called superior branchial lobes (lobes 1 and 2, according to Hutchings & Peart, 2000; Garraffoni & Lana, 2004) (Fig. 2A, B), covering a second pair of reduced branchiae, or inferior branchial lobes (lobes 3 and 4), posterior to the first ones (Fig. 2A). Due to its particular morphology, the branchial organ of *T. stroemii* is often considered difficult to interpret (Rousset et al., 2003). Following a morphological and ultrastructural study of the branchiae of two alvinellid species (Jouin & Gaill, 1990), we investigated the morphology, functional anatomy and fine structure of the branchiae of *T. stroemii* for a better understanding of this organ and with the goal of a comparative study with the stalked and lamellar branchiae of *Alvinella pompejana* Desbruyères & Laubier, 1980. A previous paper on the anatomy of anterior body segments and branchial vascular system in alvinellids (Jouin-Toulmond et al., 1996) has often been misinterpreted (Rouse & Pleijel, 2001; Rousset et al., 2003) and since morphological data on the anterior body segments and related organs are often used for phylogenetic studies of the Terebellida, we present these data in a synthetic diagram of *Terebellides stroemii*, *Paralvinella grasslei* Desbruyères & Laubier, 1982, *Alvinella pompejana*, together with data on *Trichobranchus glacialis* Malmgren, 1866, this last, for comparison with *Terebellides stroemii.*

Material and methods

Six specimens of *Terebellides stroemii* were collected (March 1989) in Banyuls-sur-mer at about 85 m depth (in a sediment characterized by the mollusc *Venus ovata*). Two other specimens came from the area of Roscoff-Baie de Morlaix (May 1990): one occurring in sediments of intertidal sea grass at northern beach of Térénez, one from subtidal coarse sediment of Baie de Morlaix. A third specimen, fixed with 10% formalin came from sublittoral sediments of the Rade de Brest. Some specimens are deposited in the Muséum National d'Histoire Naturelle in Paris: one complete specimen from Térénez (MNHN PNT01); another complete specimen from Rade de Brest (MNHN PNT02); two incomplete specimens (no branchiae) from Banyuls-sur–mer (MNHN PNT03 and PNT04).

The specimens were examined alive, when possible, then relaxed before fixation. One specimen from Banyuls-sur-mer was relaxed with a solution of $MgCl_2$ isotonic to seawater, then fixed with 10% neutral formalin in seawater for morphological study, drawings and calculation of the respiratory surface area. This specimen was measured and weighed after removal of the surface moisture, then the branchial organ was excised and one dorsal branchial lobe was separated. A drawing of profile of this lobe, with an excised part of lamellae, was made with a microscope fitted with a camera lucida and its surface measured using a planimeter. The excised lamellae (23) were isolated and also drawn at the microscope and their surfaces measured. The total respiratory surface area of the lobe was calculated by planimetry on the drawings as explained in Jouin & Gaill (1990) and the total surface area of the two symmetrical lobes was then evaluated. Another specimen from Banyuls-sur-mer was relaxed then fixed with 10% neutral formalin, then with Bouin fixative for histological sections. Three other specimens were relaxed, then the branchial organs were fixed for SEM and TEM studies with a cacodylate buffer (0.2M, pH 7.4) solution of 2% glutaraldehyde, adjusted with NaCl to about 1300 mosM, then post-fixed in buffered 1% OsO_4 and embedded in Epon. Similarly, one specimen from Roscoff was relaxed then fixed with the same fixatives and embedded in Epon for TEM studies. The sections were examined with a Philips 201 electron microscope. Preparations for SEM, were dried by the critical point method, coated with gold palladium and examined with a Jeol 840A scanning electron microscope.

Results

We could not examine specimens of *Terebellides stroemii* Sars, 1835 from the type locality (Norway), but these have been re-examined and their description completed by several authors (Williams, 1984; Holthe 1986a; Hutchings, 2000; Hutchings & Peart, 2000). Our specimens, from Banyuls-sur-Mer (Mediterranean Sea) and Roscoff - Baie de Morlaix (English Channel), exhibit most of the specific characters of *T. stroemii*. Even if some morphological characters are different from those of the type species, we preferred to keep this species name and to only list the divergent characters (*) in Table 1. The morphology and size of the acicular chaetae from the first thoracic neuropod (chaetiger 6, segment VIII) are good specific characters. These acicular chaetae, larger and different in shape from the long shafted avicular uncini of all the following thoracic neuropodia, characterize the genus *Terebellides.* Both type of thoracic neurochaetae from our specimens are illustrated on Figure 1.

Most of the present data come from specimens collected in Banyuls-sur-Mer. We did not observe differences in the

Table 1. *Terebellides stroemii.* Main morphological characters observed on some specimens from the areas of Banyuls-sur-Mer and Roscoff (Baie de Morlaix). Body size and number of segments: Roscoff 2 specimens measured, Banyuls 4 specimens measured.

Tableau 1. *Terebellides stroemii.* Principaux caractères morphologiques observés sur les spécimens provenant des régions de Banyuls-sur-Mer et de Roscoff (Baie de Morlaix). Dimensions du corps et nombre de segments: Roscoff, 2 spécimens mesurés ; Banyuls, 4 spécimens mesurés.

Locality	L (mm)	N	N th	N ab	Relative length th/ab	Notochaetae chaetiger 1 (III)	N of acicular chaetae chaetiger 6 (VIII) & Length	Angle & tip of acicular chaetae	N avicular neurochaetae & Length	N branchiae (= lobes)	Marginal papillae on branchiae (*)	Terminal filament on branchiae (*)
Roscoff	35	50	18	32	1/1	small	7 & 1 mm	Sharp (110°) & pointed	14 ant. 20 post. & 0.43 mm	4 partly fused	+ (lobes 1-2)	+ (lobes 1-4)
Banyuls	36	53	18	34	Th > Ab	small	6 & 1.06 mm	Sharp (130°) & pointed	14 ant. 23 post. & 0.51mm	4 partly fused	+ (lobes 1-2)	+ (lobes 1-4)

L= total body-length; N = number of segments, chaetae or branchiae; th = thoracic; ab = abdominal; + = present.

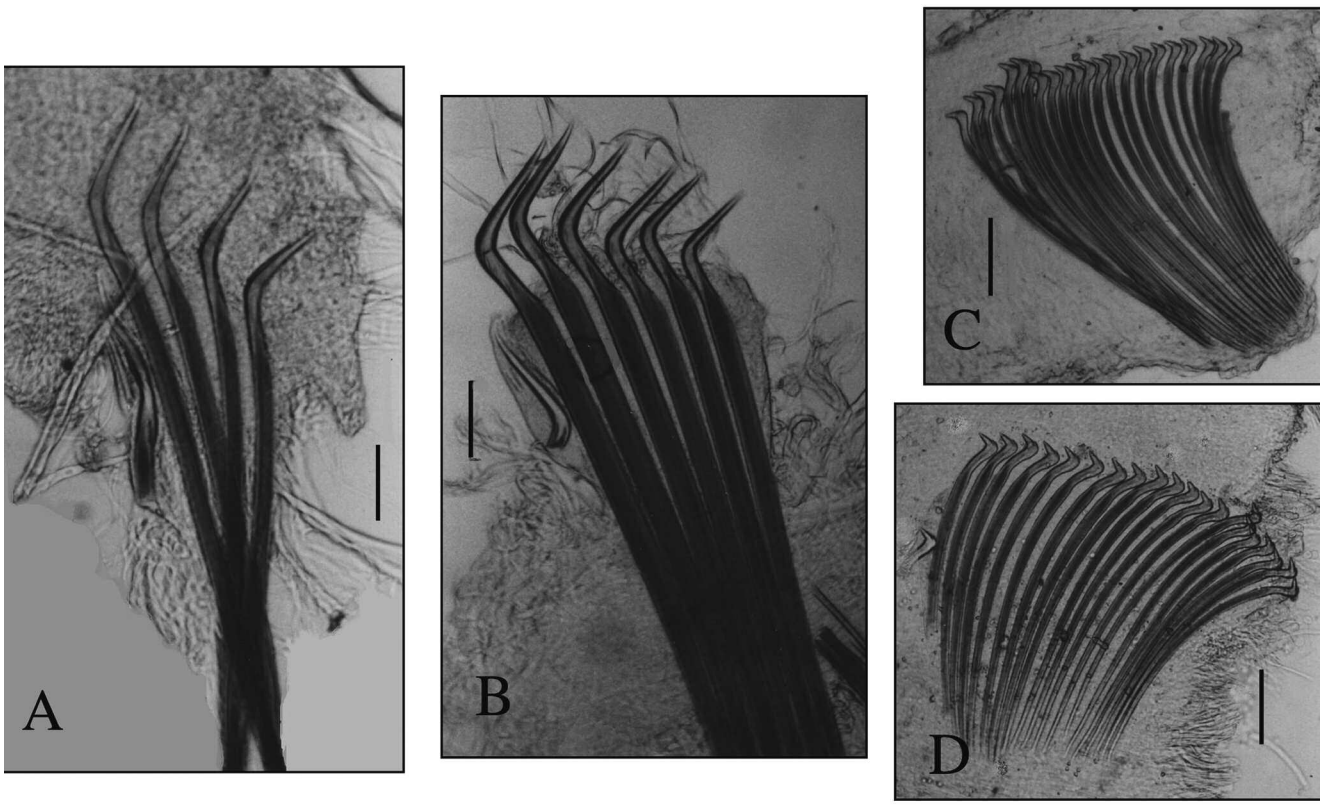


Figure 1. *Terebellides stroemii.* Photomicrographs of the acicular and avicular thoracic neurochaetae. **A, B.** Geniculate acicular chaetae of chaetiger 6. **C, D.** Long shafted avicular chaetae (chaetiger 17) (these are morphologically identical from chaetiger 7 to 18). A and C specimen from Banyuls-sur-mer; B and D specimen from Térénez (Baie de Morlaix). A-D: scale bar = 100 µm

Figure 1. *Terebellides stroemii.* Photomicrographies des neurochètes thoraciques aciculaire et aviculaire. **A, B.** Soies aciculaires coudées du sétigère 6. **C, D.** Soies aviculaires à longue hampe du sétigère 17 (morphologiquement identiques dans les sétigères 7 à 18). A et C spécimen de Banyuls-sur-mer; B et D spécimen de Térénez (Baie de Morlaix). A-D : échelle = 100 µm.

morphology and ultrastructure of the branchiae between the specimens from Banyuls-sur-Mer and those from Roscoff, except for the presence of a glandular area on the ventral surface of the two smaller branchial lobes on some specimens of Banyuls and Rade de Brest, a character never mentioned in the description of *T. stroemii*, nor in any other *Terebellides* species.

General anatomy of the branchial organ of T. stroemii

In the stalked branchial organ of *T. stroemii*, each branchia is made up of lamellae inserted on a musculo-epidermal stem surrounding a coelomic cavity that contains the blood vessels (Fig. 2A). Proximally the four stems run next to each other, forming a single cylindrical stalk surrounded by a musculo-epidermal layer (Figs 2A & 3A). The stalk (ca. 2. mm in length and 0.8 mm in width) connects the four branchiae to the dorsal part of the first chaetiger (segment III) that is fused dorsally to the second body segment (segment II, achaetous) and to the second chaetiger (segment IV). A detailed examination of the posterior part of the stalk shows that the two smaller stems of the lobes 3-4, are clearly posterior to the larger stems of lobes 1-2, and are inserted at the anterior limit of segment IV. The two pairs of branchiae are then connected dorsally to three successive segments II to IV by a common stalk. This is in agreement with a possible interpretation of the cross section of the stalk (Fig. 3A) that shows two pairs of branchial stems, with their coelomic cavities containing blood vessels, and corresponding to segments III and IV, plus a fifth one, located medially, possibly corresponding to segment II. This coelomic cavity (II) is reduced and does not contain any blood vessel since there is no corresponding branchiae on segment II.

The well developed branchiae (superior lobes 1 and 2), each about 4.5 mm in length and 1.3 mm in thickness at the widest, extend anteriorly as rounded shapes, are thinner posteriorly and terminate with a short filamentous tip (Fig. 2A). Dorsally, they have a convex shape and each comprises a single row of about 65 lamellae inserted on the dorsal part of a stem containing the blood vessels. The lamellae become smaller posteriorly. These two branchiae

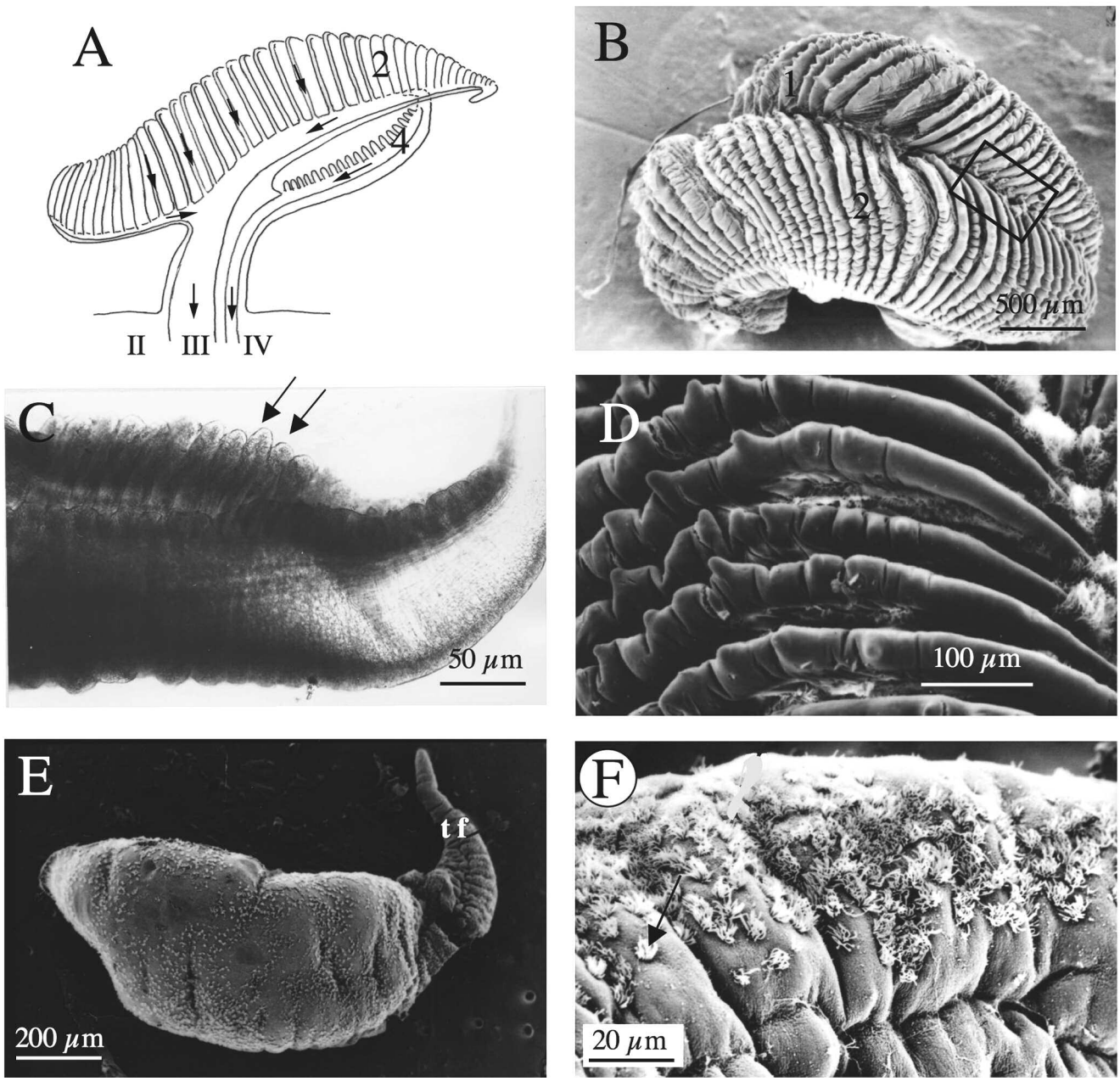


Figure 2. *Terebellides stroemii.* **A.** Schematic profile view of the left side of the branchial organ (branchiae 2 and 4) and the stalk inserted on the dorsal part of segments II-IV. Arrows show the direction of blood in the lamellar and branchial efferent vessels. **B.** Scanning electron micrograph (SEM): dorsal view of the larger branchiae (lobes 1 and 2) united along the branchial axis. Box indicates level of Fig. 3E. **C.** Light microscope view of apical part of the inferior lobe 4 with short branchial lamellae (arrows) and posterior terminal filament on the right. **D.** Dorsal view of lobe 2 (SEM) showing the marginal papillae of the lamellae, and the ciliated branchial axis on the right. **E.** SEM ventral view of an inferior lobe with terminal filament (tf), and glandular epithelium. **F.** Detail of E showing short tufts of cilia (arrow) on the glandular epithelium.

Figure 2. *Terebellides stroemii*. **A.** Dessin schématique d'une vue latérale gauche des branchies (lobes 2 et 4) et du pédoncule inséré sur la partie dorsale des segments II-IV. Les flèches indiquent le trajet du sang dans les vaisseaux efférents des lamelles et des branchies. **B.** Vue dorsale en Microscopie à balayage (MEB) de la paire de branchies dorsales, bien developpées (lobes 1 et 2) accolées le long de l'axe de l'organe branchial. Le cadre indique le niveau de la Fig. 3E. **C.** Photomicrographie de la partie apicale du lobe inférieur 4 montrant dorsalement de courtes lamelles branchiales (flèches) et le filament postérieur à droite. **D.** Vue dorsale du lobe 2 (MEB) montrant les papilles marginales des lamelles branchiales et l'axe branchial cilié sur la droite. **E.** Vue ventrale (MEB) d'un lobe branchial inférieur montrant le filament terminal (tf), et l'épithélium glandulaire ventral. **F.** Détail (MEB) des petites touffes de cils (flèche) de l'épithélium glandulaire.

lie close together side by side and their stems are united basally for about half their length, so that the branchial organ appears as a single structure, convex in dorsal view, with bilateral symmetry and a longitudinal axis (Fig. 2B). The two reduced branchiae (inferior lobes 3 and 4), each about 2.3 mm in length and 0.8 mm in thickness, are located posterior and below the larger ones (Fig. 2A). They come out from the stalk at the level where the two superior branchial lobes separate from each other and they have a similar organization, each having a stem, bearing dorsally small ciliated lamellae (Fig. 2A, C), which terminate posteriorly with a filamentous tip. Although shorter than the dorsal branchiae, they extend nearly as far because they separate further from the common stalk. By contrast to the larger branchiae, the ventral epidermis of their stems comprises, in some specimens from Banyuls-sur-mer and Rade de Brest, white thickenings corresponding to glandular areas with short tufts of cilia (Fig. 2E, F).

The two superior branchial lobes as well as the two reduced lobes are each irrigated by one afferent and one efferent blood vessels, located in the coelomic cavity of their respective stems (Fig. 3A). The two reduced branchiae (lobes 3 and 4) have not been studied in detail, and only the larger, well-developed branchiae, probably the more efficient respiratory organs, are described in the present paper.

The branchial lamellae of T. stroemii

The curved lamellae, (about 800 µm by 600 µm for a lamella from the median part of the branchiae) are longer on their outer side than on the inner one, and terminate in an apical part located towards the longitudinal axis of the respiratory organ (Figs 2B & 3B, E). The flattened lamellae are thin (about 40 µm in thickness) and have a border of short papillae on their outer side (Figs 2D & 3B). Each lamella is heavily cilated on both faces, the ciliation being arranged, on a median lamella, in 9 dense longitudinal rows (i.e. from the base to the apex of the lamella) (Fig. 3C-D). There are tufts of cilia at the "apex" of the lamellae, so that the longitudinal axis between the lobes 1- 2 forms a densely ciliated groove (Fig. 3E).

Blood circulation in the lamellar vessels and water circulation at the lamellar surface

Each lamella possesses two marginal vessels, an "internal" vessel i.e. along the axial side and an "external" one, that are continuous with each other apically and connected basally to two longitudinal vessels (one afferent and one efferent) running in the branchial stems, then in the stalk. In addition, there is on both faces of each lamella, a network of parallel blood spaces (connecting vessels) forming anastomoses and running below the epidermis, perpendicular to the external rows of cilia (Fig. 3B, C).

Examination on live animals revealed that the blood contained amaebocytes that allowed us to determine the direction of the blood flow. On each lamella, the " internal" vessel is an afferent vessel, and the "external" is an efferent one (Fig. 3B). Similarly, in the stem of each branchiae, a longitudinal afferent vessel runs along the internal edge (i.e. near the longitudinal axis) and an efferent vessel runs along the external edge (Fig. 2A). These vessels extend in the branchial stalk and join the main longitudinal blood vessels of the body. On dissections of the anterior body part we could detect, on a dorsal view, only one pair of branchial afferent vessels originating from the anterior end of the contractile branchial heart; these two vessels each bifurcate further, giving two other afferent vessels, so that four afferent vessels run in the stalk (Fig. 3A). The connections of the efferent vessels to the ventral vessel could not be observed on dissected specimens.

On live specimens, the water currents at the surface of the branchiae, created by the cilia of the lamellae (Fig. 3C-D), form counter-currents with respect to the blood, i.e. from the external side to the axial side, so that along the ciliated branchial axis (Fig. 3E) there is often an accumulation of deposited particles.

Ultrastructure of the branchial lamellae

Each lamella comprises two epidermal layers separated by a central myoepithelial layer. There is a deep blood sinus located between the basal laminae of the epidermis and that of the central layer (Fig. 4A-C). The thickness of the epidermis and cuticle are about 17 µm and 1.7 µm respectively in the centre of a lamella, while the epidermis is thinner, about 2 µm, at the level of the marginal vessels (Fig. 4D). The epidermis comprises supporting cells and ciliated cells that form dense rows of cilia (Fig. 3D), visible on each side of a lamella (Fig. 4A).

The central layer of the lamellae has a varying thickness (1 to 5.5 µm) and comprises myoepithelial cells lining a central coelomic space that may contain circulating amaebocytes (Fig. 4B). The muscular part of the myoepithelial cells may form "pillars" uniting both sides of the central layer (Fig. 4C).

The blood sinus becomes wider in some places to form a network of connecting vessels running perpendicular to the external rows of cilia (Fig. 4B, C). These vessels connect the marginal afferent vessel to the marginal efferent one. They are located at the base of the epidermis and contrary to what was observed in alvinellid species they do not extend far into the epidermis. The diffusion distance between blood of connective vessels and external milieu is about 7 to 10 µm; it is thinner, up to 1.5 µm, at the level of the marginal vessels (Fig. 4D).

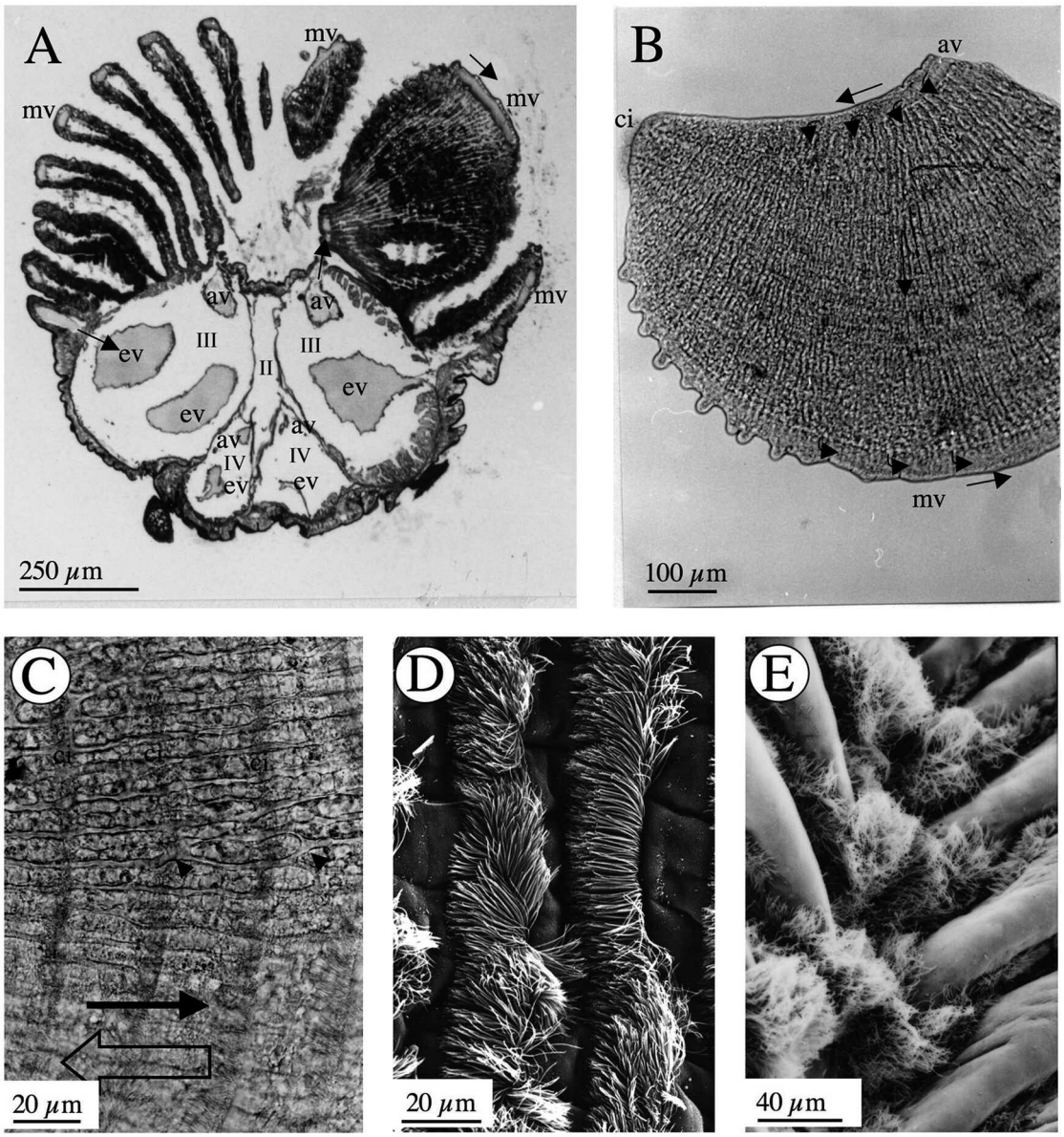
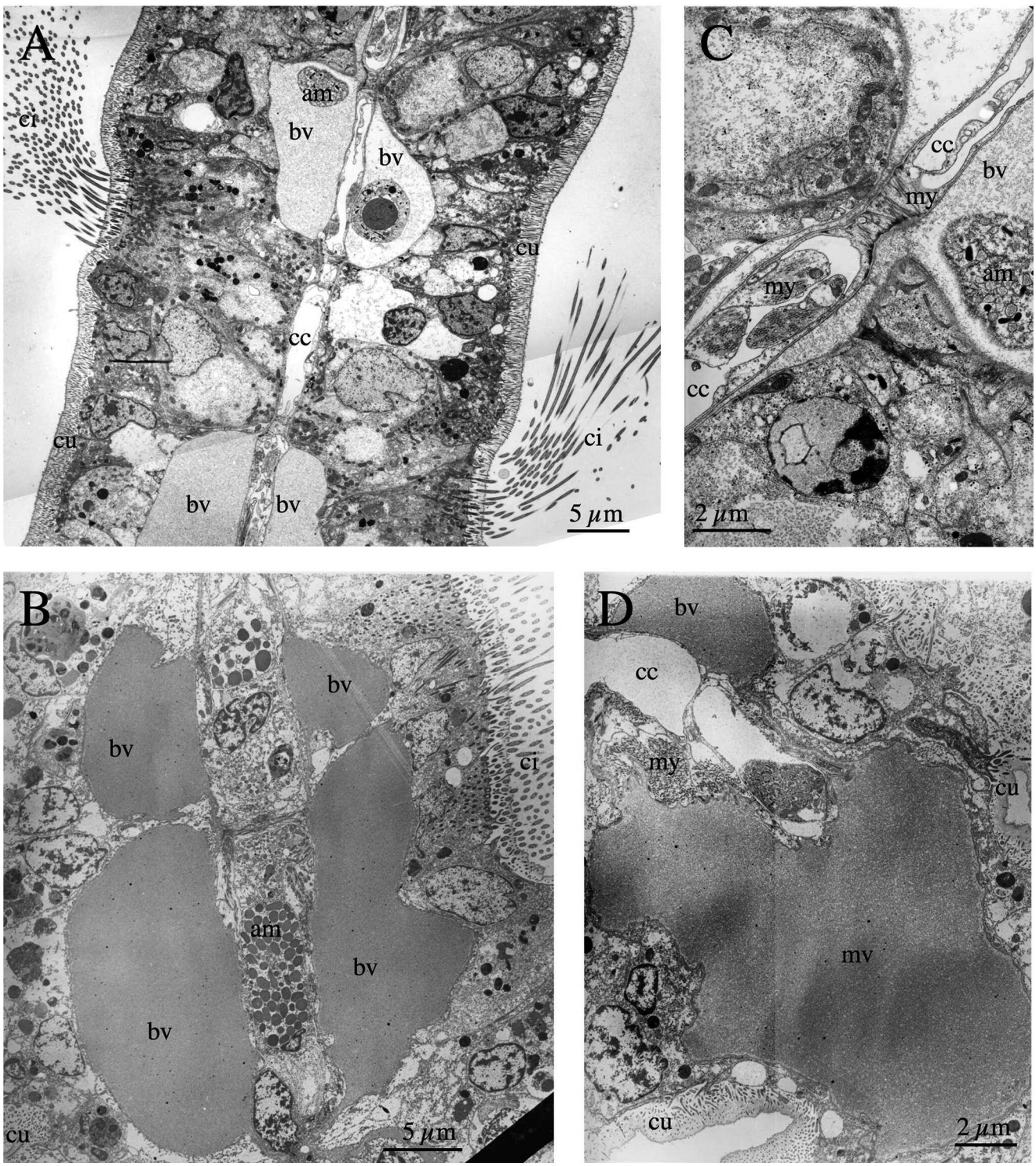


Figure 3. *Terebellides stroemii*. **A.** Histological cross section of the dorsal lobes 1 and 2, each with a large stem and coelomic cavity (connected with segment III) containing one afferent (av) and one efferent vessel (ev). The smaller stems of lobes 3 and 4 have small coelomic cavities (IV) containing also afferent and efferent vessels. Arrows indicate the direction of blood flow. Note the small residual and median coelomic cavity (II). **B.** Light microscope view of a lamella from lobe 1. Arrows indicate the direction of blood flow from the marginal afferent vessel (av) to the marginal efferent vessel (mv). The apical tuft of cilia (ci) indicates the dorsal axis. **C.** Enlargement, perpendicular to B, showing the transverse connecting blood vessels forming anastomoses (arrowheads) and the longitudinal external rows of cilia, much more visible on D. In C black arrows indicate the direction of blood flow, open arrow the direction of water circulation. **D.** SEM view of two rows of cilia on a lamella. **E.** SEM view of the ciliated dorsal branchial axis (enlargement of the box on Fig. 1B).

Figure 3. *Terebellides stroemii*. **A.** Coupe histologique transversale des branchies et des pédoncules branchiaux des lobes 1 et 2 chacun avec une large cavité coelomique (reliée au segment III) contenant un vaisseau afferent (av) et un vaisseau efferent (ev). Les pédoncules des lobes 3 et 4 ont de petites cavités coelomiques (IV) contenant aussi des vaisseaux afférents et efférents, Les flèches indiquent le sens de la circulation sanguine. Noter la petite cavité coelomique médiane résiduelle (II). **B.** Photomicroscopie d'une lamelle branchiale du lobe 1. Les flèches indiquent le sens de la circulation sanguine du vaisseau marginal afférent (av) vers le vaisseau marginal efférent (mv). La touffe ciliée apicale (ci) indique l'axe dorsal. **C.** Agrandissement de la Fig. B montrant les vaisseaux sanguins transversaux formant des anastomoses (têtes de flèche) et les rangées ciliaires longitudinales, mieux visibles en D. En C la flèche noire indique le sens de la circulation sanguine, la flèche claire le sens des courants d'eau de surface. **D.** Vue en MEB de deux rangées ciliaires sur une lamelle. **E.** Détail au MEB de la ciliature de l'axe branchial (agrandissement du cadre de la Fig. 1B).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Evaluation of the specific branchial surface area

The estimated branchial respiratory surface area of the two larger branchiae, for a *T. stroemii* specimen of 0.1537 g wet weight (specimen from Banyuls-sur-mer with total length: 35 mm and maximum width: 4 mm) was 0.93 cm^2. The specific branchial surface area is then 6 cm^2 per gram of body wet weight (a minimal value since the respiratory surface area of the two smaller branchiae has not been taken into account). This value is of the same order of magnitude as for the intertidal polychaete *Arenicola marina* which was 4 cm^2 per gram of body wet weight (Jouin & Toulmond, 1989) and considerably lower than the specific branchial surface areas of two species of hydrothermal vent alvinellids (see Discussion).

Discussion

The branchial lamellae of *Terebellides stroemii* exhibit morphological and functional features similar to those of *Alvinella* (Jouin & Gaill, 1990). Observations on live *T. stroemii* revealed the direction of the seawater flow at the surface of the two large ciliated branchiae, as well as the direction of the branchial blood circulation. The cilia of the lamellae help the water flow from the lateral parts of the two branchiae towards the dorsal axis of the branchial organ. Conversely, the circulation of the blood in the lamellae and in the whole branchiae is going in opposite direction, from the axial area to the lateral area, and thereby forms a counter-current. The water currents produce an accumulation of particles in the dorsal axis of the branchiae. It is therefore likely that particles can be collected by the large tentacular lobe (upper lip) located just in front of the branchiae in *T. stroemii*. In addition to its respiratory function, the branchial organ may then participate in the collection of particles for feeding or for elaboration of the tube. All this could not be previously observed on the lamellar branchiae of alvinellids because the collected animals on board the ship were not alive by the time they reached the surface. However collection of particles by the branchiae has previously been suggested by Desbruyères & Laubier (1986) for *Paralvinella pandorae*.

The organization of the circulatory system in the branchial lamellae of *T. stroemii* is similar, but less complex, than in *Alvinella*. *Terebellides stroemii* does not possess intraepidermal loops forming a shallower blood network in the lamellae such that observed in *Alvinella pompejana* (Jouin & Gaill, 1990). The minimal diffusion distance in the lamellae is therefore longer in *T. stroemii* than in alvinellids : 7 to 10 µm instead of 2-3 µm, respectively. This could have an adaptive value in alvinellids that are exposed to very different and challenging environmental conditions. This view is also supported by the higher specific branchial surface areas in alvinellids: 12 and up to 47 $cm^2\ g^{-1}$ wet weight in *Alvinella pompejana* and *Paralvinella grasslei* respectively (Jouin & Gaill, 1990) as compared to *Terebellides stroemii* with 6 $cm^2\ g^{-1}$.

A small coelomic cavity was observed in the central part of the branchial lamella of *T. stroemii*. It is very limited and occupied by cell bodies of the lining myoepithelium and some amoebocytes. Such a small coelomic cavity is also found in alvinellids but was first unnoticed due to its small size (Jouin & Gaill, 1990).

According to several authors, the genus *Terebellides* is characterized by having branchiae as a single mid-dorsal stalked structure on segments II to IV (Fauvel, 1927; Day, 1967; Fauchald, 1977) or on segment III, i.e. the first chaetigerous segment (Holthe, 1986a, b; Garraffoni & Lana, 2003) or on segments III-IV (Hutchings & Peart, 2000). Actually in *T. stroemii*, the stalk, inserted dorsally on

Figure 4. *Terebellides stroemii*. Transmission electron micrographs (TEM) of cross sections through branchial lamellae. **A.** Median part of a lamella. The connecting blood vessels (bv), containing amaebocytes (am), are located at the base of the epidermis, along a muscular central layer of myoepithelial cells lining a coelomic cavity (cc). Note the rows of epidermal ciliated cells (ci) and the cuticle (cu). **B.** The central coelomic cavity is sometimes invaded by amaebocytes (am) containing numerous electron-dense granules. bv: connecting blood vessels. **C.** Basal part of epidermis and thin central coelomic cavity (cc) with myoepithelial cells (my). The connecting blood vessels (bv) are bordered by the basal laminae of epidermis and central coelomic layer. **D.** The epidermis is thinner at the level of the marginal vessel (mv). (bv) connecting blood vessel; (cc) coelomic cavity; (cu) cuticle; (my) myoepithelial cells.

Figure 4. *Terebellides stroemii*. Micrographies électroniques à transmission (TEM) de coupes transversales de lamelles branchiales. **A.** Partie médiane d'une lamelle. Les vaisseaux sanguins connectifs (bv) contenant des amébocytes circulants (am) sont situés à la base de l'épiderme le long de la couche musculaire centrale de cellules myoépithéliales bordant une cavité coelomique (cc). Noter les cellules épidermiques ciliées (ci) et la cuticule (cu). **B.** La cavité coelomique centrale (cc) est parfois envahie d'amébocytes (am) contenant de nombreux granules denses aux électrons. (bv): vaisseaux sanguins connectifs; **C.** Base de l'épiderme et couche musculaire centrale avec des cellules myoépithéliales (my) et une mince cavité coelomique (cc). Les vaisseaux sanguins connectifs (bv) sont bordés par la lame basale de l'épiderme et celle de la couche centrale. **D.** L'épiderme est plus mince au niveau du vaisseau marginal (mv). (bv) vaisseaux sanguins; (cu) cuticule; (my) cellules myoépithéliales.

segments II to IV, comprises a muscular epidermal wall surrounding the four parallel coelomic stems of the branchiae. The presence of a possible residual extension of the coelomic cavity of segment II in the branchial stalk (Fig. 3A) might indicate the loss of a pair of gills and that three pairs of gills was probably the plesiomorphic condition.

In alvinellids the branchiae are located on segments II to V (peristomium considered as segment I) or on segments III to VI in some *Paralvinella* species (Desbruyères & Laubier, 1986, 1989, 1991, 1993; Rouse & Pleijel, 2001; Rousset et al., 2003; Glasby et al., 2004) as in several Terebellomorpha (Holthe, 1986b). The branchial organ is much more developed in alvinellids than in *T. stroemii*, since there are four pairs of pinnate branchiae (two rows of lamellae on each branchia) in *Alvinella pompejana* and *A. caudata*, while there are only two pairs of simple lamellar branchiae (one row of lamellae on each branchia) in *T. stroemii*.

In conclusion, the position, morphology and structure of the lamellar branchiae and of the anterior circulatory system in *Terebellides stroemii* can be compared to that of *Alvinella*. As in other Terebellida (Fauvel, 1897; Picton, 1899) each branchia receives an afferent vessel coming from the contractile heart (branchial heart) containing a rod-like heart-body (Jouin-Toulmond et al., 1996) and the branchial efferent vessels return to the ventral vessel, although this last connection could not be observed at present on *T. stroemii*. Such a branchial circulatory system connected to a branchial heart containing a heart-body is common in the Terebellomorpha (a plesiomorphic character for the group). The lamelliform branchiae of *T. stroemii* represent a simple model, each having only one row of lamellae, whereas in *Alvinella* each pinnate branchiae has two rows of lamellae. Stalked and lamellate branchiae are rare in polychaetes; they are present for example in the ampharetid *Isolda* Müller, 1858 and in the pectinariid, *Pectinaria* Savigny, 1818. These types of branchiae have obviously evolved several times independently within the Terebellomorpha and the similarities of the branchiae between *Terebellides* and *Alvinella* most probably represent an homoplasy through convergence.

In a study on the anatomy of the anterior circulatory system of alvinellids, in which the location and arrangement of the branchial and segmental blood vessels indicate the anterior metamery of the body (Jouin-Toulmond et al., 1996, Figures 1-3), the chaetigers of *Alvinella* labelled 1, 4 and 5 (Figs 1 & 2) correspond to segments VI, IX and X, and those numbered 4 and 7 in *Paralvinella* (Fig. 3) correspond to segments VI and IX. All the branchial segments II to V are achaetous in *Alvinella*, while only segment II is achaetous in *Paralvinella,* the first notopodium being present on segment III (see present Fig. 5). Consequently and in accordance with Glasby et al. (2004), the characteristic notopodial acicular hooks of alvinellids ("straight spines" in Rousset et al., 2003) are present on segment IX in *Paralvinella* (Rouse & Pleijel, 2001) and on segments IX and X in *Alvinella* (Fig. 5). This is different from the interpretations of Desbruyères & Laubier (1991), and Rousset et al. (2003). New observations on the position of the septum and nephridiopores in *Alvinella* are reported in Fig. 5. The septum (= wall of the perioesophageal pouch, in Jouin-Toulmond et al., 1996) has a position, between chaetigers 1 and 2, different from that in our previous paper (between chaetigers 3 and 4). The positions of the nephridiopores (on segment V), anterior to the gonopores (on segment VI) are also indicated in *Alvinella* and *Paralvinella.*

Remarks on the systematic position of the genus Terebellides

The family Trichobranchidae Malmgren, 1866, including *Terebellides*, is generally considered monophyletic on the basis of the long-shafted uncini on the thoracic neuropodia and the absence of ventral shields (Garraffoni & Lana, 2004). According to Garrafoni et al . (2005), the family Trichobranchinae comprises at present four genera: *Trichobranchus* Malmgren, 1866 (synonym *Filibranchus* Malm, 1874 according to Hessle, 1917; Holthe, 1986a ; Garraffoni & Lana, 2002; another synonym is *Artacamella* Hartman, 1955, according to Garraffoni & Lana, 2004); *Octobranchus* Marion & Bobretzky, 1875 (synonym *Novobranchus* Berkeley & Berkeley, 1954 according to Kingston & Mackie, 1980); *Unobranchus* Hartman, 1965, and *Terebellides* (synonym *Ampharetides* Ehlers, 1913, according to Holthe, 1986a).

At least three autapomorphies separate *Terebellides* from all other trichobranchids (Fig. 5):

1. the stalked lamellar branchial organ (two associated pairs of branchiae) on segments II-IV; 2. the anterior thoracic notopodial region of five segments (a similar notopodial region has three segments in *Octobranchus* and *Unobranchus* and is absent in *Trichobranchus*) and the location of the first neuropodium on chaetiger 6 (= segment VIII) ; 3. the smooth geniculate acicular neurochaetae of these first neuropodium, different from the long shafted avicular uncini of all other thoracic neuropodia (Figs 1 & 5) and never present in other genera of the family.

These geniculate acicular neurochaetae are few on chaetiger 6 but larger than the following long shafted uncini equipped with a short main fang and secondary teeth (Table 1 and Fig. 1). All the species of *Terebellides*, - at present 34 valid species, according to Garraffoni et al. (2005) - exhibit these characters which are absent in all other Trichobranchidae.

The distinction of *Terebellides*, indicated in Colgan et al. (2001) as well as in Glasby et al. (2004) and in Garraffoni

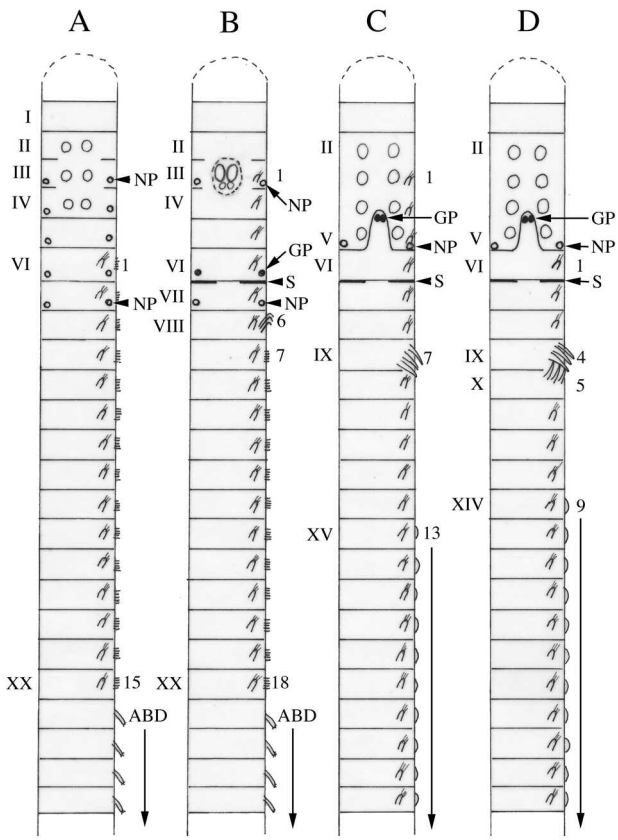

Figure 5. Schematic representation of the anterior metamery for two trichobranchids **A.** *Trichobranchus glacialis*, **B.** *Terebellides stroemii,* and two alvinellids **C.** *Paralvinella grasslei* and **D**. *Alvinella pompejana*. "Cephalic" part, anterior to segment I (= peristomium) not represented. Paired branchiae (open circles) are located dorsally. Segments are numbered on the left part in roman, and chaetigers, only drawn on the right hand side, in normal characters. The positions of the septum (gular membrane) (S) and nephridial pores (NP) correspond to new observations on alvinellids. The corresponding data on *Terebellides stroemii* are those of Duchêne (1982) and Holthe (1986a), those on *Trichobranchus glacialis*, are those of Holthe (1986a). Thoracic neuropods comprise long shafted avicular uncini in **A** and **B** except in segment VIII (chaetiger 6) of *T stroemii* that exhibit the typical bent acicular chaetae of the genus. ABD: abdominal region in *Trichobranchus* and *Terebellides* with neuropodia only (short avicular uncini on pinnules) to the end of body. Alvinellids in **C** and **D** have large notopodial hooks in segments IX or IX-X and neuropodia appear as low tori from segments XV or XIV to the end of body, together with notopodia. (GP) genital pores ; (NP) nephridial pores; (S) septum.

Figure 5. Représentation schématique de la métamérie de la région antérieure du corps de deux Trichobranchidae **A.** *Trichobranchus glacialis*, **B.** *Terebellides stroemii,* et deux Alvinellidae **C.** *Paralvinella grasslei* et **D.** *Alvinella pompejana*. Région « céphalique » antérieure au segment I (= péristomium) non représentée. Les branchies paires (cercles) sont dorsales. Les segments sont numérotés à gauche en chiffres romains, les sétigères, indiqués seulement à droite, sont en caractères normaux. La position du septum antérieur (S) et des pores néphridiens (NP) correspondent à de nouvelles observations sur les Alvinellidés. Les données correspondantes sur *Terebellides stroemii* sont celles de Duchêne (1982) et Holthe (1986a), celles sur *Trichobranchus* sont de Holthe (1986a). Les neuropodes thoraciques portent des uncini aviculaires à longue hampe chez les Trichobranchidae sauf dans le segment VIII (sétigère 6) de *T. stoemii* qui porte des soies aciculaires coudées, typiques du genre. ABD: région abdominale chez les Trichobranchidae avec seulement des neuropodes (uncini aviculaires courts à l'extrémité de pinnules) jusqu'au pygidium. Les Alvinellidae, en **C** and **D**, ont de grosses soies notopodiales en crochet (segments IX ou IX-X) et des neuropodes en tores uncinigères non saillants, accompagnés de notopodes tout le long du corps, à partir des segments XV ou XIV. (GP) pore genitaux ; (NP) pores néphridiens; (S) septum.

& Lana (2004) based on both molecular and morphological data, is generally unnoticed in papers dealing with the phylogenetic analysis of the Terebellida. The features of *Terebellides,* namely the presence of geniculate acicular neurochaetae as first thoracic neurochaetae on segment VIII are often erroneously given as a character (and even an apomorphy) of the family Trichobranchidae (McHugh, 1995; Fauchald & Rouse, 1997; Rouse & Pleijel, 2001). These acicular chaetae in *Terebellides* ("spine-like neurosetae" in McHugh, 1995; "curved or bent spines" in Fauchald & Rouse, 1997; "geniculate acicular hooks" in Hutchings & Peart, 2000), due to their position in the first neuropodium can be considered homologous to the long shafted avicular neurochaetae of other subsequent thoracic segments (Garraffoni & Lana, 2004). But they are simply considered as "long shafted uncini with an elongated main fang" by these authors (see Table 2 and 3), a view that diminishes the peculiarity of the character. Similarly in Rousset et al. (2003) these first neurochaetae are not clearly distinguished from the subsequent thoracic avicular uncini.

Together with the above-mentioned autapomorphies, the geniculate acicular neurochaetae, absent in all other Trichobranchidae, give evidence for the monophyly of *Terebellides*. We suggest that *Terebellides*, is clearly distinct from all other trichobranchids and should be placed in the family Trichobranchidae, apart from the subfamily Trichobranchinae sensu Fauvel 1927, now including *Trichobranchus, Octobranchus* and *Unobranchus*. The family Trichobranchidae must be maintained as distinct to the Terebellidae, as indicated by Hessle (1917), Fauchald (1977), Holthe (1986a), Hutchings (2000), Glasby et al. (2004) and against the opinion of Rouse & Pleijel (2001)

who consider the group as included in the family Terebellidae.

New investigations on other morphological and anatomical characters on these polychaetes, especially studies of the digestive, nephridial and reproductive sytems, together with molecular data will provide useful additional informations for new phylogenetic studies.

At present, Rousset et al. (2003) in their phylogenetic analysis of the Alvinellidae based on combined morphological and molecular data, although they eliminated the character branchiae (considered not well established in *Terebellides*), unexpectedly found a sister group relationship between Alvinellidae and *Terebellides*. On the other hand, Colgan et al. (2001) on the basis of molecular data, found *Terebellides* topologically very distinct to *Trichobranchus*. Our present morphological data on the location and structure of the branchiae of *T. stroemii*, on the position of the first notopodium, gular membrane, nephridial and genital pores in *Terebellides, Paralvinella* and *Alvinella*, as well as in *Trichobranchus,* summarized in a comparative diagram of the anterior metamery of the body (Fig. 5) do not contradict the results of these authors.

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