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Morphological reinvestigation and phylogenetic relationship of *Acanthobdella peledina* (Annelida, Clitellata)

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Summary. In recently collected specimens of *Acanthobdella peledina* the nervous system, the genital organs and the coelomic organisation were reinvestigated after complete serial sections. These anatomical results are schematically represented. In addition, the integument, the chaetae and the peripheral muscle layer were investigated by electron microscopy. In general, the results confirm Livanow's classic monograph (1906), with the exception of a few details. The body apparently possesses neither a prostomium nor an achaetous buccal region (peristomium). The number of 29 true segments is concluded from the number of segmental ganglia. The five anteriormost segments, each with four pairs of hook-shaped chaetae arranged around the mouth opening, are considered to be functionally equivalent to an anterior sucker. The ultrastructure of the integument and the chaetae generally conforms to the typical annelidan pattern. The muscle cells are of the typical hirudinean type. The outer male genital pore is positioned in segment 10; the female organs open in segment 11 directly behind the septum between segments 10 and 11. The main emphasis is laid on the evaluation of the position of the taxon within the Clitellata, including a discussion of the Branchiobdellida, and the cladograms presented show the Acanthobdellida to be the sister group of the Euhirudinea. Characters shared by the Branchiobdellida and Hirudinea (including *A. peledina*) are considered to be convergently evolved.

A. Introduction

The continuous phylogenetic interest in the monotypic annelid taxon Acanthobdellida is due to its mosaic of "oligochaetous" and hirudinean characters, giving the appearance of a transition stage between extremely derived "Oligochaeta" and very primitive "true" leeches. Our basic knowledge of *Acanthobdella peledina* Grube, 1851, almost exclusively goes back to Livanow's comprehensive and most detailed monograph of 1906. Additional information has been scarce and mostly refers to faunistic or biological details (Andersson 1965, 1988). The anatomy of this rare ectoparasite on salmonid fish has not been confirmed by recent morphological studies, except for an ultrastructural investigation of spermatozoa by Franzén (1991).

Livanow (1906, 1931) initially described *A. peledina* as an ancient but typical hirudinean. The phylogenetic discussion became controversial when Michaelsen (1919) classified the species within the "Oligochaeta" because of its apparent oligochaete-like male reproductive system and the possession of chaetae. He regarded the hirudinean-like characters (e.g. posterior sucker, annulated body surface and possession of an oblique muscle layer) as functional adaptations to an ectoparasitic life, convergently evolved in *Acanthobdella* and Hirudinea. The majority of systematists never followed him (for references see Scriban and Autrum 1928–34; Sawyer 1986a, b, c) so the textbook position of *Acanthobdella peledina* has remained within the Hirudinea. In the course of time, however, some of Livanow's (1906) observations have been questioned or differently interpreted, e.g. the number of segments and position of the genital openings (Livanow 1931; Brinkhurst and Gelder 1989), making a reinvestigation of *Acanthobdella* highly desirable. The availability of living specimens has permitted us to make detailed histological and ultrastructural studies, verifying most of Livanow's (1906) excellent descriptions. The musculature, the parenchyme, the coelom, the central nervous system and the genital organs were investigated by light microscopy; in addition, electron microscopic observations were made on the chaetae, epidermis and muscle cells. Only those structures are described which are especially important for phylogenetic considerations, which are different from Livanow's (1906) observations or which were not definitely recognized by him. The intention of the present contribution is to document some morphological details, to illustrate the general

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anatomy and to clarify the taxonomic position of the taxon within the Clitellata.

This problem, however, is inevitably connected with the taxonomic position of another ectosymbiont clitellate taxon, the Branchiobdellida. First considered to belong to the Hirudinea because of their constant number of segments (although only 15), absence of chaetae and the occurrence of a posterior sucker-like disc and of a dorsal and a ventral jaw (Odier 1823), they were later classified as "Oligochaeta" adapted to an ectocommensal life-style (Vejdowsky 1884; Michaelsen 1919). Holt (1953, 1965, 1989) considered the Branchiobdellida to be a taxon equivalent to the "Oligochaeta" and Hirudinea. Observations on spermatozoal ultrastructure support the hypothesis of a separate taxon Branchiobdellida besides "Oligochaeta" and Hirudinea (Ferraguti and Gelder 1991). Brinkhurst and Gelder (1989) retained this terminology pending a resolution of the relationships among the Clitellata, whereas Sawyer (1986a) classified them as a subclass of the Hirudinea.

B. Materials and methods

Several mature, living individuals of *Acanthobdella peledina* Grube, 1851, and a number of injured ones, which had been used in physiological experiments immediately prior to fixation, were kindly forwarded to the Osnabrück laboratory by Professor Dr. Irene Zerbst-Boroffka, Berlin. The animals were collected in the Pajeb Maskejaure Lake in Northern Sweden.

For light microscopical histology, individuals were narcotized with mineral water (CO_2), stretched to normal body length, pinned with small insect needles on the bottom of a petri dish and fixed with Bouin's fluid (see Westheide and Purschke 1988). After dehydration in ethanol and transfer into intermedia (methylbenzoate, butanol), specimens were embedded in Paraplast. Complete series of one cross-sectioned and one sagittally sectioned specimen were made with a Reichert-Jung Autocut rotary microtome (5 µm) and stained with Heidenhain's stain (=Azan, see Westheide and Purschke 1988). Three-dimensional reconstructions were done directly from the section series with a computer-aided programme (set up by Dr. Reinhard Windoffer, Osnabrück): contours of structure outlines under consideration were traced on a digitizer tablet via a camera lucida.

For electron microscopy, specimens were also narcotized and after initial fixation were dissected into smaller pieces. Specimens were fixed in a buffered (0.05 M sodium cacodylate) solution of 2.5% glutaraldehyde for 2 h at 21° C. After rinsing in buffer (7 changes, 1.5 h), specimens were incubated in 0.8% potassium ferricyanide in buffer for 0.5 h at room temperature and then postfixed in 0.5% OsO_4 plus 0.8% $K_3[Fe(CN)_6]$ in cacodylate buffer at 4° C (modified from McDonald (1984)). After rinsing in buffer specimens were dehydrated in a graded ethanol series. For scanning electron microscopy, they were then critical-point dried, mounted, and sputter-coated with gold. Examination took place in a Cambridge Stereoscan 250S. For transmission electron microscopy, tissue blocks were embedded in an Epon-Araldite mixture. Ultrathin sections were cut with a diamond knife (Reichert Ultracut E microtome), stained with aqueous solutions of uranyl acetate and lead citrate (LKB Ultrostainer) and examined in a Zeiss EM 109 electron microscope.

C. Results

Specimens of *Acanthobdella peledina* were 30–40 mm long and 2–3.5 mm wide. The cylindrical body is externally subdivided into annuli which do not correspond to the internal segmentation. In the mid-body each segment comprises four annuli.

I. Chaetae

The anterior end is ventrally flattened and bears five transverse rows of chaetae indicating the five anteriormost segments. Each row consists of four pairs of simple hook-shaped chaetae (Fig. 1 A). The first row is situated on the first visible annulus and there exists no achaetous annulus anterior to it. The chaetae of the first segment are arranged around the mouth opening and are about 250 µm long. In the following segments they measure 400 µm on average. At their base their diameter is 20–25 µm. The chaetae are anchored in deep follicles which cover about three-quarters of their length. Approximately in the middle of each chaeta there is a nodule-like thickening. Strong muscles attach to the chaetal root and near the nodule-like region. The chaetae consist of a bundle of long tubes which have the same diameter from the centre to the periphery (0.45 µm, 4–5 tubes per μm^2; Fig. 1 B, C). The tubes merge and form a more or less uniform inner structure without distinguishable cortical and medullar regions. The chaetae are surrounded by a thin, electron-dense coat. The individual tubes have a hexagonal outline and their narrow lumina (=chaetal channels; 75 nm in diameter) are completely filled with electron-dense material. The chaetal follicle is formed by a few lateral epidermal cells including the basal chaetoblast.

II. Epidermis

The epidermis is a monolayered epithelium, 15–20 µm thick, composed of supporting cells, sensory cells and gland cells, some of which are deeply sunken into the underlying tissues. There is a typical annelid-like cuticle consisting of a basal cuticle made up of a fine fibrillar matrix and 6–8 layers of non-striated collagen fibres (150 nm in diameter) and a 99-nm-thick epicuticle. It is traversed by microvilli and covered by a regular layer of membrane-bound epicuticular projections which form a dense border of elongated 0.5-µm-long rods.

III. Musculature

The musculature of the body wall consists of a single layer of circular muscle fibres, two layers of diagonal fibres arranged nearly orthogonally and a varying number of longitudinal fibres. The muscle fibres are embedded in the extracellular matrix which is continuous with the parenchymal connective tissue. The fibres are clearly obliquely striated, between 1.5 and 2 mm long and of various diameters (10–200 µm) depending on the type of cell. In cross-sections most muscle fibres have an irregular outline (Fig. 2). They are circomyarian (see Lanzavecchia et al. 1988), consisting of a cortex contain-

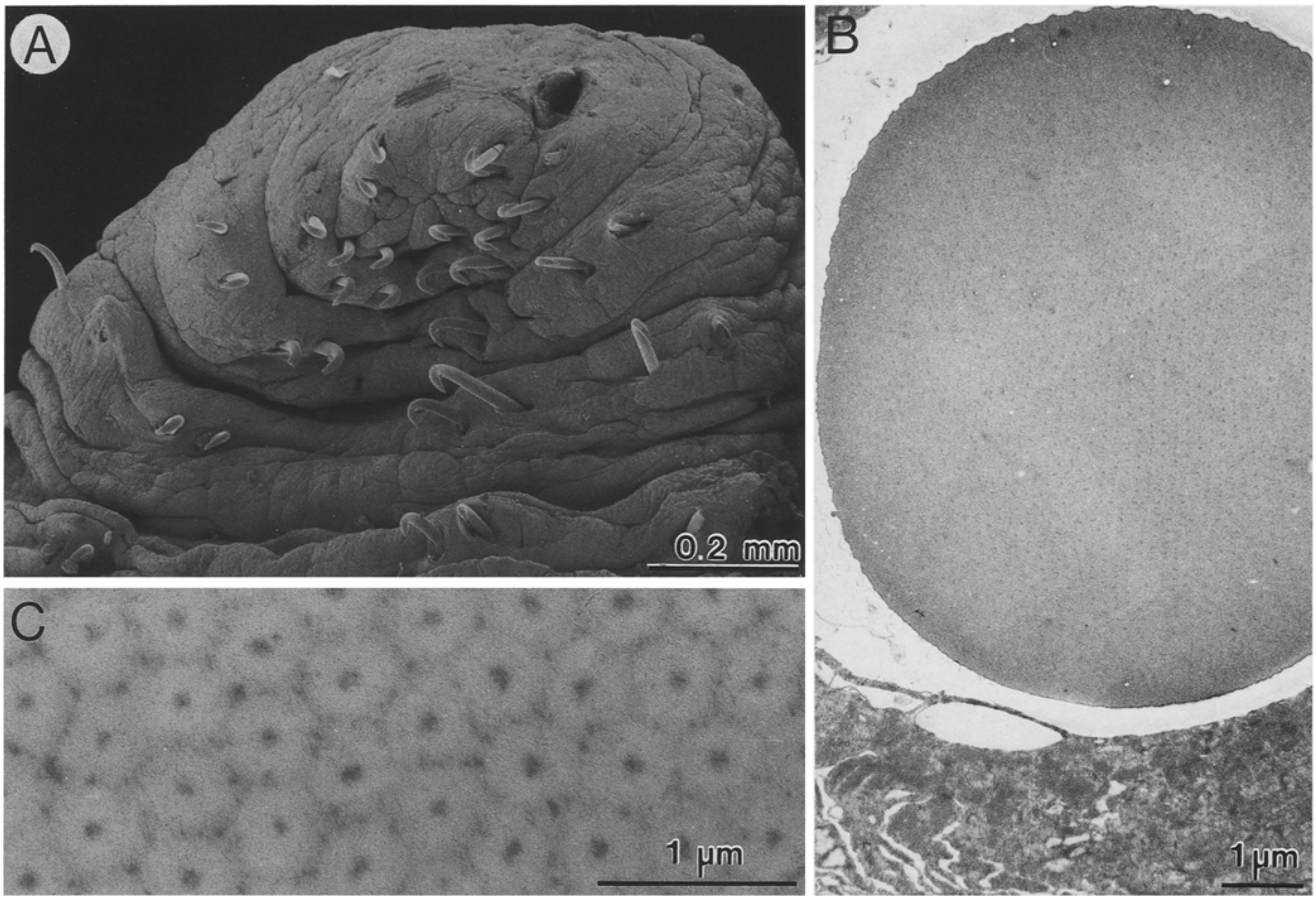

Fig. 1A–C. Chaetation. **A** SEM micrograph of the head region, ventral side. **B** TEM micrograph of cross-section near opening of follicle. **C** Higher magnification to show tubes typical of annelid chaetae

ing the contractile filaments and a sarcoplasmic core. Generally, the cortex is 2–3 µm thick. In the cortex thick (50 nm diameter) and thin (5–7 nm diameter) myofilaments, sarcoplasmic reticulum and Z-rods are arranged as is typical for obliquely striated muscles and a T-system is lacking. Each fibre possesses one fairly large nucleus which measures up to $15 \times 20 \times 80$ µm. Normally it is situated within the sarcoplasmic core in the middle of the fibres. However, in the circular muscle cells and very few longitudinal fibres the nucleus lies in an expansion of the sarcoplasmic core and, as a result, the contractile cortex is partly open describing a U-shaped arc in cross-sections through this part of the cell. In the oblique muscle cells, the perikarya are situated in a cytoplasmic protrusion outside the contractile cortex. In several longitudinal muscle cells, the cylinder of contractile material may be open at various sites where small peripheral sarcoplasmic zones devoid of myofilaments occur (Fig. 2).

IV. Coelom

The coelom of *A. peledina* is well developed and consists of segmentally arranged coelomic cavities lined by a peritoneum (Fig. 3A, B). The volume of the coelomic cavities is comparatively small because of the voluminous musculature, the connective tissue and the large gland cells. The coelomic cavities form neither channels nor branches. In the head region (the five anteriormost segments) there is a single confluent cavity around the pharynx with lateral septal vestiges only. In the following segments, the septa reach the digestive tract but are incomplete dorsally and ventrally to the intestine. Here apertures allow the coelothel-bound blood vessels and the nerve cord to pass into the following segment (Fig. 3B). Mesenteries are more or less reduced and vestigial; they have only been observed between the dorsal blood vessel and the gut. In the clitellar region (segments 9–11) each cavity is subdivided into a dorsal and a ventral part below the gut. In the segments of the posterior sucker, coelomic cavities are lacking.

V. Nervous system

The central nervous system of *A. peledina* has been reconstructed primarily in order to evaluate the number of ganglia and, thus, to determine the exact number of segments; as a result of which it was found that our observations generally confirm Livanow's (1906) results. The central nervous system consists of the brain (=su-

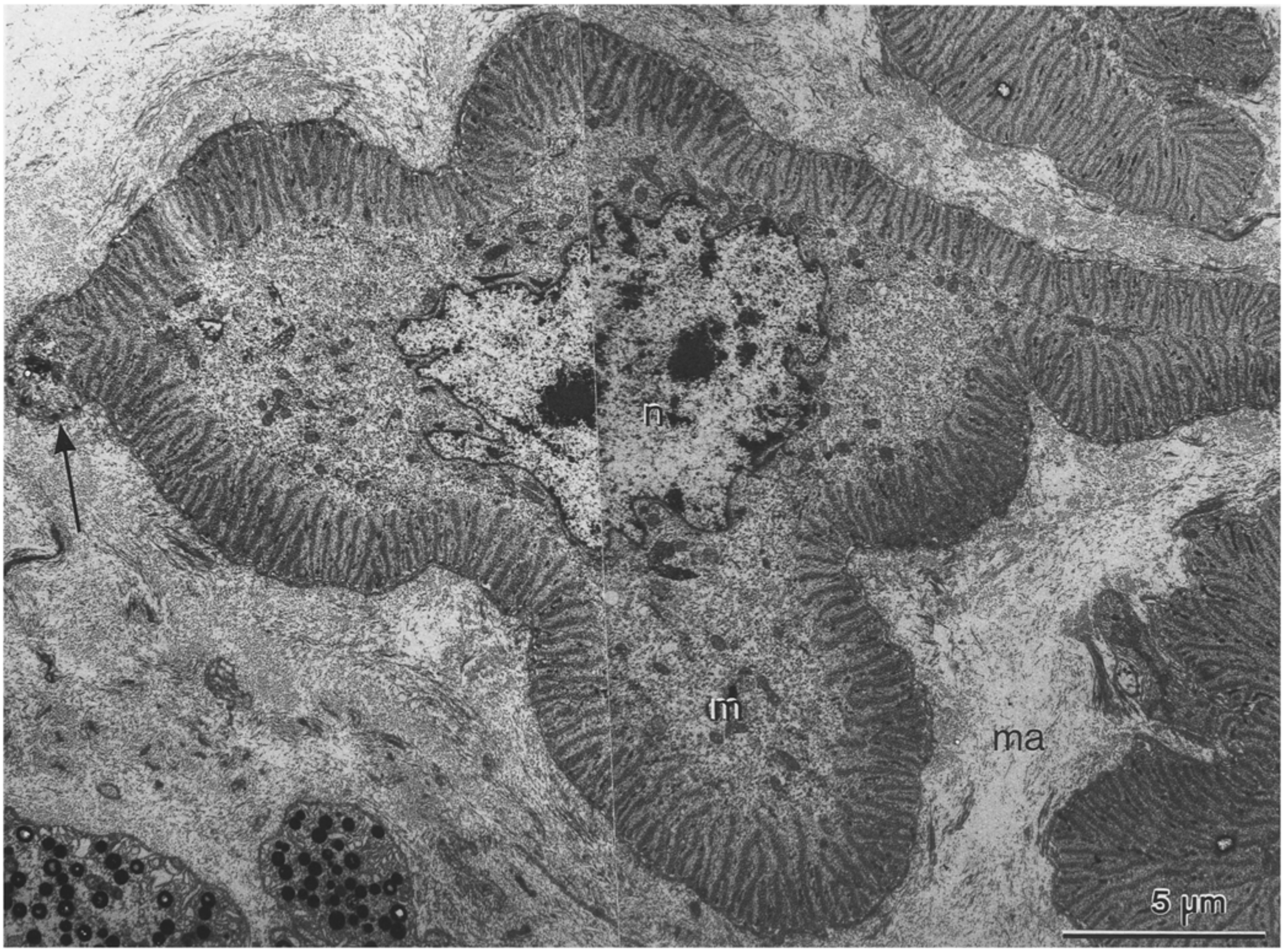

Fig. 2. Cross-section of longitudinal muscle cell embedded in extracellular matrix (*ma*). Contractile cortex and sarcoplasmic core with large nucleus (*n*), mitochondria (*m*) and numerous glycogen granules. In some fibres the mantle of contractile material shows a small area without myofilaments (*arrow*)

praoesophageal ganglionic mass), the suboesophageal ganglionic mass, the chain of free ganglia and the ganglionic mass of the posterior sucker (anal ganglionic mass) (Fig. 4A–C). The ventral chain comprises 21 free ganglia, the anteriormost of which lies in the posterior chaetae-bearing segment. As is typical of "true" leeches, each ganglion is made up of six discrete packets of neurons: unpaired anterior- and posterior-medioventral packets and paired anterolateral and posterolateral packets. Each ganglion is connected to the periphery by means of three pairs of lateral nerve roots. The anterior one innervates the first annulus, the median the second annulus and the third root divides and innervates the third and fourth annulus of a given segment. The presence of discrete packets of neurons and their constant number make it possible to estimate the number of segments constituting the coalesced ganglia of the anterior and posterior end. The suboesophageal ganglionic mass consists of 24 packets corresponding to four coalesced ganglia. Its posterior ganglion has two pairs of lateral nerves, the others have only one pair each. The anterior ganglion of this mass clearly innervates the first visible annulus with the first row of chaetae. The following three ganglia innervate the next three segments, each of which possesses a chaetae-bearing anterior annulus. The supraoesophageal ganglionic mass consists of 12 packets and hence represents the two anteriormost ganglia. Only two pairs of nerve roots emanate from this part of the central nervous system and they innervate the pharynx and the eyes in the third annulus, that is, there are no segmental regions innervated by these two anteriormost coalesced ganglia.

The posterior ganglionic mass lies anterior to the sucker and consists of four fused ganglia and segments. Apart from its posteriormost ganglion, which contains only four ganglionic packets, each of these ganglia is made up of six packets. Short connectives are still present, distinctly separating the ganglia. Each ganglion sends one pair of lateral nerves into the posterior sucker. In conclusion, the body of *A. peledina* consists of 29 segments but contains 31 pairs of ganglia.

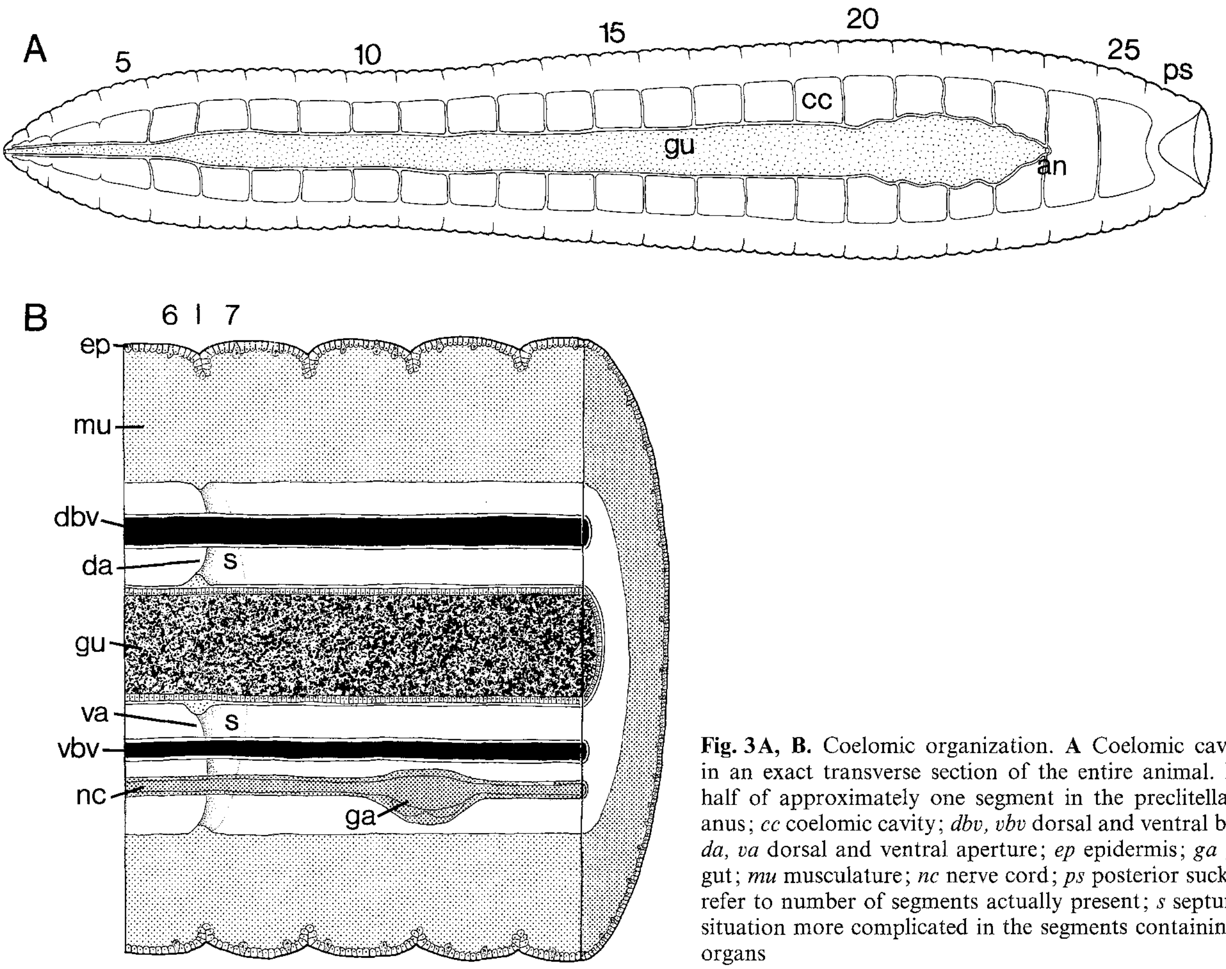

Fig. 3A, B. Coelomic organization. **A** Coelomic cavities as seen in an exact transverse section of the entire animal. **B** One body half of approximately one segment in the preclitellar region. *an* anus; *cc* coelomic cavity; *dbv, vbv* dorsal and ventral blood vessels; *da, va* dorsal and ventral aperture; *ep* epidermis; *ga* ganglion; *gu* gut; *mu* musculature; *nc* nerve cord; *ps* posterior sucker; *numerals* refer to number of segments actually present; *s* septum. Coelomic situation more complicated in the segments containing the genital organs

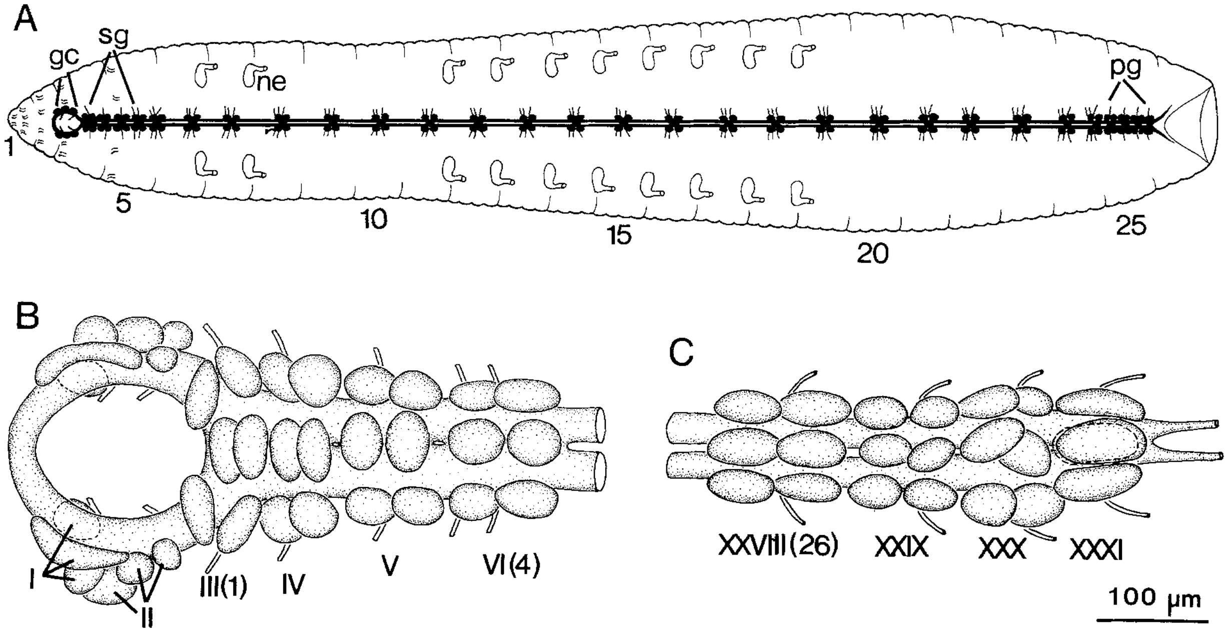

Fig. 4. **A** Nervous system and position of functioning nephridia (*ne*). **B** Supra- and suboesophageal ganglionic mass, each of the coalesced ganglia is made up of six packets; ventral view. **C** Posterior ganglionic mass, ventral view. *gc* ganglia connected with circumoesophageal connectives; *sg* four ganglia constituting the suboesophageal ganglionic mass; *pg* ganglionic mass (ganglia of segments 26–29) of posterior sucker, *arabic numerals* denote the segments actually present, *roman numerals* follow Sawyer's (1986a) nomenclature, *hatched lines* refer to dorsal ganglionic packets. Same scale in **B** and **C**

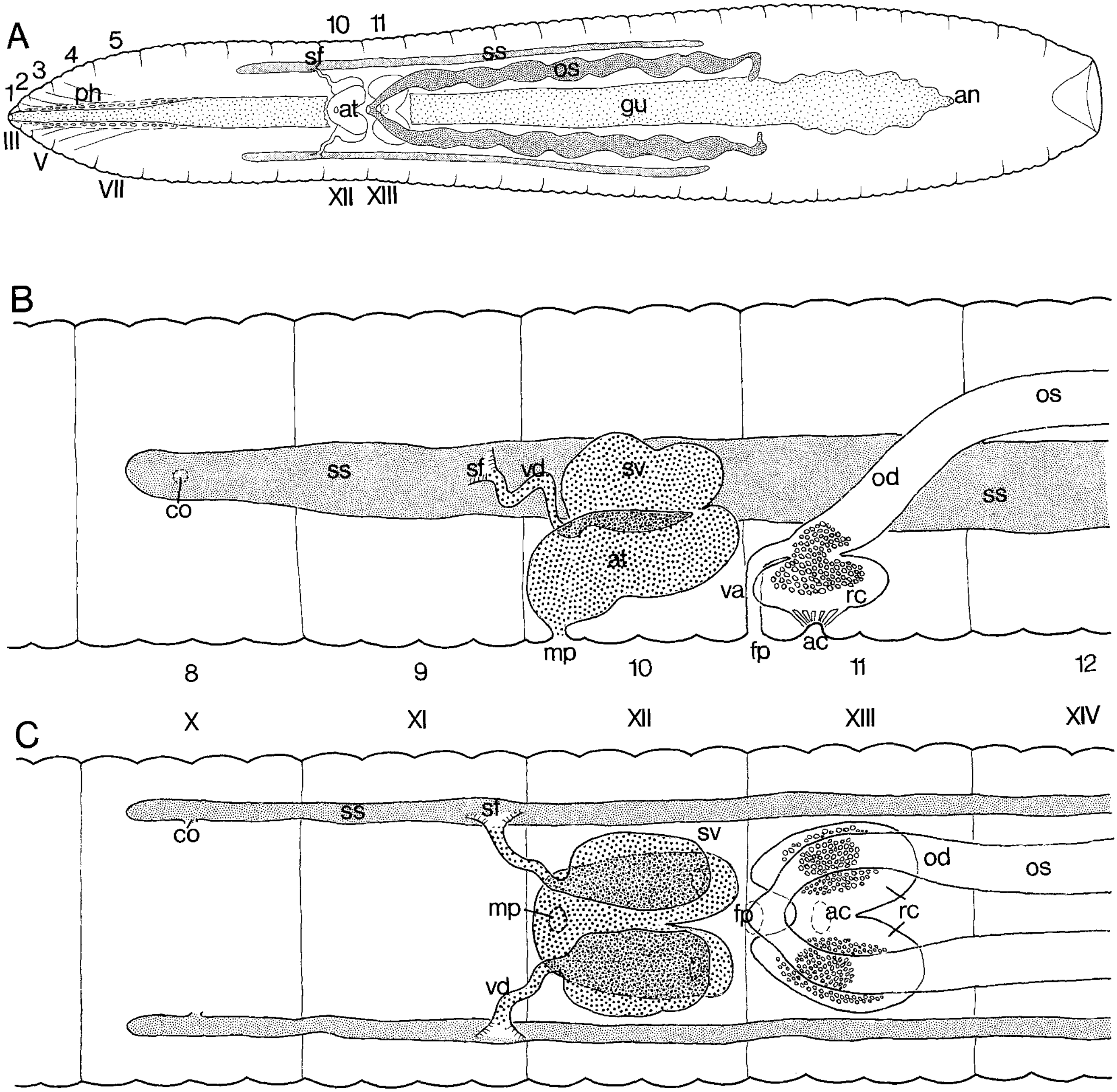

Fig. 5A–C. Genital organization. **A** Position of male and female organs and genital openings. View from dorsal side. *Roman numbering* considers prostomium and peristomium to be reduced; *arabic numerals* denote the segments actually present. **B** Position of genital organs in segments 8–12; lateral view. **C** Dorsal view. *ac* area copulatrix; *an* anus; *at* atrium; *co* connection between sperm sac and coelom; *fp* female pore; *gu* gut; *mp* male pore; *od* oviduct; *os* ovisac; *ph* pharynx; *rc* receptacular cavity; *sf* seminal funnel; *ss* sperm sac; *sv* seminal vesicle; *va* vagina; *vd* vas deferens

VI. Genital organs

The genital organs lie in the parenchymal connective tissue or in the coelom. The male organs consist of paired sperm sacs and vasa deferentia which open into the unpaired atrium (Figs. 5A–C, 6). The male opening is situated ventrally on the first annulus of segment 10, behind the septum between segments 9 and 10. The sperm sacs are elongated and extend from segments 8 to 18. They are filled with spermatozoa and morulae of spermatocytes as well as spermatids at various stages of development. In the fully mature specimens investigated, a clearly demarcated testis was not detectable. In a small area of segment 8 epithelia of sperm sacs and coelomic cavities come close together but an open connection between them could not be observed. Distinctly ciliated sperm funnels project into the sperm sacs in the posterior part of segment 9. From here the efferent ducts meander posteriorly into the following segment, where each forms a seminal vesicle filled with masses of spermatozoa. The seminal vesicles lead via a sphincter into the male atrium. Its epithelium consists of support-

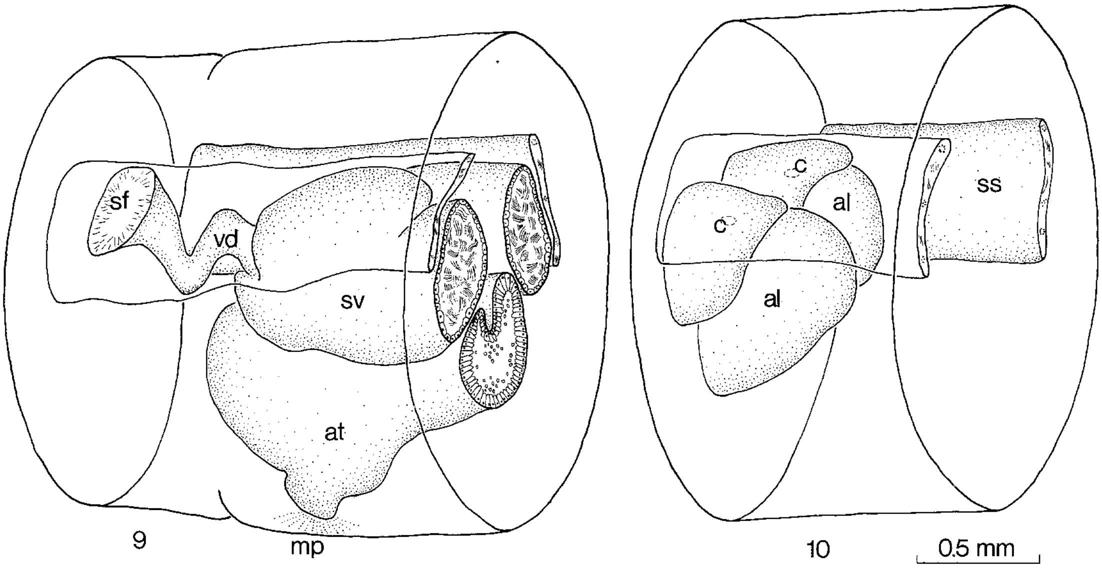

Fig. 6. Male genital organs. Three-dimensional reconstruction within segments 9 to 10. *al* posterior atrial lobes; *at* atrium; *c* connection between *sv* and *al; mp* male pore; *sf* sperm funnels; *ss* sperm sac; *sv* seminal vesicle; *vd* as deferens

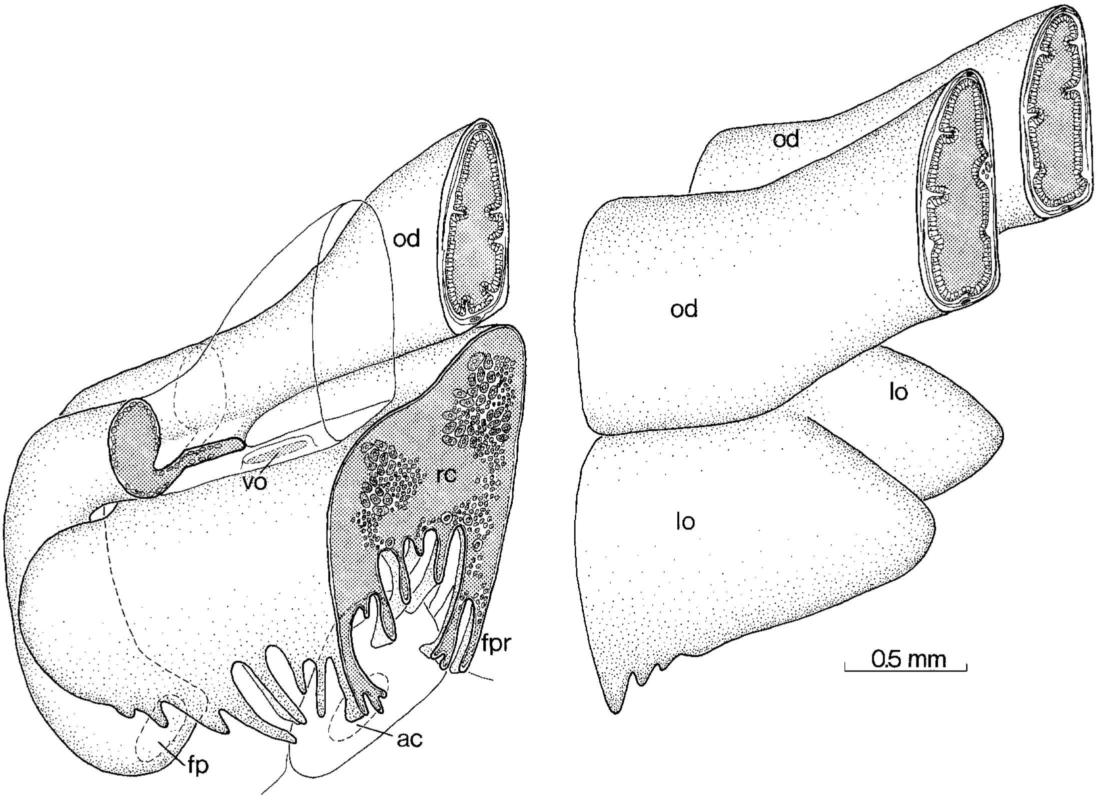

Fig. 7. Female genital organs. Three-dimensional reconstruction within segment 11. Left oviduct transparent to demonstrate that prior to the fusion of the two oviducts (*od*) they each possess a ventral opening (*vo*) which leads into a large common chamber. *ac* area copulatrix; *fp* female pore; *fpr* finger-like projections; *lo* lobules of receptacular cavity; *rc* probable receptacular cavity

ing cells and two types of gland cells. It is surrounded by a thin layer of circular muscle cells. An ejaculatory part is not distinguishable within the male duct.

The female organs are made up of paired ovisacs, paired oviducts and an unpaired vagina (Figs. 5A–C, 7). A so-called receptacular cavity situated in segment 11 is closely associated with the genital organs proper. The female opening lies in the first annulus of segment 11 just posterior to the intersegmental furrow between segments 10 and 11. The elongated ovisacs lie within the coelom from segments 12 to 19. Constrictions mark the areas where the sacs pass through the septa. Developing oocytes were found only in the posterior part (segments 15–19). The oviduct is characterized by its thick

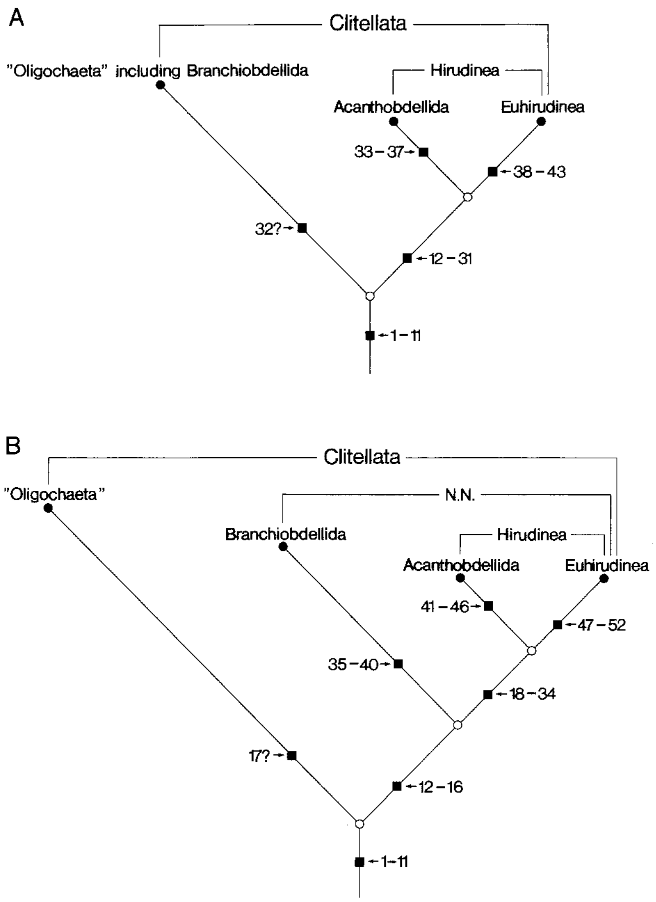

Fig. 8 A, B. Tentative dendrograms to clarify the phylogenetic relationships of Branchiobdellida, Acanthobdellida and Euhirudinea. *Black squares* = apomorphic characters. **A** 1–11. Autapomorphies Clitellata: (1) eversible pharynx roof, (2) hermaphroditism, (3) gonads in specific segments, (4) clitellum and production of cocoons, (5) plesiopore male ducts, (6) spermathecae, (7) specific development, (8) loss of trochophora, (9) spermatozoa with specific ultrastructural features (see, e.g. Ferraguti 1984 for details), (10) reduction of prostomium and loss of prostomial appendages, (11) brain shifted backwards. 12–31. Autapomorphies Hirudinea: (12) fixed number of 32 segments plus pro- and peristomium (I–XXXIV following Sawyer's (1986) nomenclature), (13) chaetae in anterior segments III–VII, (14) posterior sucker consisting of the seven posteriormost segments, (15) perikarya of ventral ganglia grouped into six discrete packets, (16) two anteriormost ganglia coalesced to form brain, (17) ganglia of segments III–VI coalesced to form suboesophageal ganglionic mass, (18) ventral nerve cord comprising 21 free ganglia with the first free ganglion in segment VII, (19) posterior ganglionic mass formed by ganglia XXVIII and following, (20) loss of pygidium, (21) typical structure of muscle cells, (22) extensive development of oblique muscle layer between circular and longitudinal layer, (23) parenchymal connective tissue, (24) reduction of mesenteries – reduction of coelom, (25) specific nephridia (separation of nephrostome and nephridial duct, nephridial duct without cilia), (26) fused male pores, (27) fused female pores, (28) loss of spermathecae and internal fertilization, (29) area copulatrix, (30) spermatophores, (31) specific mode of locomotion. 32. Autapomorphies "Oligochaeta" including Branchiobdellida: ? 33–37. Autapomorphies Acanthobdellida: (33) body consisting of only 29 visible segments, (34) prostomium and so-called peristomium not visible as separate annuli (either lacking except ganglia or coalesced with segment III), (35) posterior sucker built up of only four segments (XXVIII–XXXI), (36) chaetae with specific sucker-like function, (37) loss of ciliated funnel in nephridia. 38–43. Autapomorphies Euhirudinea: (38) loss of chaetae, (39) anterior sucker (I–IV), (40) coelom restricted to system of channels, (41) sperm sacs specifically arranged, (42) male opening in XI, (43) female opening in XII. **B** 1–11. Autapomorphies Clitellata: see **A** 1–11. 12–16. Autapomorphies N.N.: (12) fixed number of segments, (13) chaetae restricted to anterior segments, (14) posterior sucker, (15) typical structure of muscle cells, (16) fused male pores. 17. Autapomorphies "Oligochaeta": ? 18–34. Autapomorphies Hirudinea: (18) fixed number of 32 segments (I–XXXIV), (19) posterior sucker consisting of seven posteriormost segments, (20) perikarya of ventral ganglia grouped into six discrete packets, (21) two anteriormost ganglia coalesced to form brain, (22) ganglia III–VI coalesced to form suboesophageal ganglionic mass, (23) ventral nerve cord comprising 21 free ganglia with first free ganglion in VII, (24) posterior ganglionic mass formed by ganglia XXVIII–XXXIV, (25) loss of pygidium, (26) extensive development of oblique muscle layer, (27) parenchymal connective tissue, (28) reduction of mesenteries and reduction of coelom, (29) specific nephridia (separation of nephrostome and nephridial duct, nephridial duct without cilia), (30) fused female pores, (31) loss of spermathecae and internal fertilization, (32) area copulatrix, (33) spermatophores, (34) specific mode of locomotion. 35–40. Autapomorphies Branchiobdellida: (35) body consisting of constant number of 15 segments, (36) loss of prostomium, (37) anterior adhesive organ ("anterior sucker"), (38) two jaws, (39) nephridioducts fused and opening dorsally, (40) sperm ducts specifically arranged. 41–46. Autapomorphies Acanthobdellida: see **A** 33–37. 47–52. Autapomorphies Euhirudinea: see **A** 38–43

glandular epithelium and prominent outer muscle envelope. Anteriorly each oviduct communicates with a cavity interpreted as a receptacle for foreign sperm (Fig. 7). This chamber embraces the vaginal complex and posteriorly it ends in two tapering lobules. Its ventral side shows a complex system of finger-like projections which penetrate the body wall muscle layers. A part of the underlying body wall forms a 0.5 mm wide recessed area (area copulatrix). It is arranged as a transverse cleft and separated by only one annulus from the female pore. The receptacular cavity is bordered only by a peritoneum. A loose mass of at least three different types of cells was found within its lumen. These cells partly protruded into the oviduct.

D. Discussion

I. Phylogenetic position of Acanthobdellida

The proposed phylogenetic position of the Acanthobdellida (Fig. 8 A, B) is based on all characters available, especially including those of our present investigations. As will be substantiated below, a series of highly probable synapomorphies show that the Acanthobdellida represent the sister group of the Euhirudinea, which comprise the "true" leeches – Rhynchobdellida, Pharyngobdellida and Gnathobdellida. In the case of the Branchiobdellida, however, lack of convincing synapomorphies between this group and any other clitellate taxon results in various cladograms of almost equal probability. In the first one (Fig. 8 A) the Branchiobdellida are considered to be evolved independently within the "Oligochaeta" whereas the Acanthobdellida and Euhirudinea form the monophylum Hirudinea. The second cladogram (Fig. 8 B) shows the Branchiobdellida to be more closely related to Acanthobdellida and Euhirudinea. A third grouping, which places the Branchiobdellida as the sister group of the "Oligochaeta", may have the lowest probability. The cladogram in Brusca and Brusca (1990) showing the Euhirudinea to be the sister group of the Branchiobdellida which in turn form the sister group of the Acanthobdellida, has now to be considered obsolete: characters supposed to be synapomorphies (anterior body sucker, loss of remaining body setae, evolution of "mesenchymal" tissue and reduction of the coelom) can more parsimoniously be explained as convergencies compared with the more than 20 synapomorphies which we found for Acanthobdellida and Euhirudinea (see below).

In both cladograms presented here, the "Oligochaeta" are characterized by quotation marks, indicating their paraphyletic status (Brinkhurst and Nemec 1987; Erséus 1987). For the time being we are not aware of any certain apomorphic feature unique to these non-hirudinean clitellates; they are characterized entirely by the retention of plesiomorphies and by the lack of hirudinean features.

Nevertheless, "Oligochaeta" is still retained because the "oligochaetous" sister group of the Hirudinea has not yet been recognized. The former preference for the Lumbriculidae as this sister group is for various reasons seriously questioned (Brinkhurst and Gelder 1989). Since this problem is not relevant to the present investigation it will not be discussed further here.

II. Synapomorphies of "Oligochaeta" and Acanthobdellida?

The clitellate nature of the taxa considered in the present paper is beyond doubt. They share a series of features, the synapomorphic state of which is unanimously accepted (e.g. Brinkhurst and Jamieson 1971; Jamieson 1981; Sawyer 1986a) (autapomorphies 1–11, Fig. 8A, B). Michaelsen's (1919) opinion that *Acanthobdella* is an "oligochaete" evolved convergently to leeches has to be rejected and is without any substantial foundation. The characters considered for this classification are: the occurrence of chaetae, a segmental coelom, a blood vascular system and sperm sacs not divided into so-called testisacs, all of which have to be regarded as plesiomorphies taken from the stem species of clitellates. Since the ultrastructure of the chaetae in *Acanthobdella* does not differ from the pattern generally observed in annelids (Specht 1988), there is also no specific structure (synapomorphy) in the chaetae which is common to "Oligochaeta" and Acanthobdellida.

The segmental organization of the coelom and a blood vascular system belong to the ground pattern of annelids as well. Moreover, in *Acanthobdella* the coelom is modified, representing an initial state that fits very well into a morphological sequence of progressive reduction of the coelom. The same holds true for the structure of the sperm sacs which extend far posteriorly in the parenchymal connective tissue, parallel to and completely separated from the ovisacs – a pattern which has not been observed in "oligochaetes" so far.

III. The sister group relationship of Acanthobdellida and Euhirudinea

On the other hand, in our first phylogenetic hypothesis *Acanthobdella* and the "true leeches" share a set of not less than 20 synapomorphies (12–31, Fig. 8A). Characters not present in any other clitellate are: (1) the reduction of the coelom due to a great expansion of the muscle layers, starting with a reduction of the mesenteries and finally resulting in a system of canals, (2) nephridia without an open connection to the coelom, (3) extensive development of diagonal muscles between circular and longitudinal fibres, (4) a loss of spermathecae, (5) the occurrence of an area copulatrix, (6) the transfer of sperm by spermatophores, (7) ganglionic cells form six discrete packets in the ganglia (Livanow 1904; Gray and Guillery 1963; Vagnetti and Farnesi 1978; Payton 1981), (8) a nervous system consisting of brain (two coalesced ganglia), suboesophageal ganglionic mass (four coalesced ganglia), 21 free body ganglia and a posterior ganglionic mass formed by the ganglia of seven segments, (9) a parenchymal connective tissue with special cell types (Abeloos 1925; Babaskin 1931; Bradbury 1956), (10) fused female pores and (11) a specific mode of locomotion. (The rare occurrence of spermatophores and loss of spermathecae in a few oligochaetous clitellates must be considered to be convergencies.) Since these characters are restricted to Acanthobdellida and Euhirudinea, in both cladograms suggested they clearly establish the sister-group relationship of these taxa (Fig. 8A, autapomorphies 12–31; Fig. 8B, autapomorphies 18–34). The probability of this suggested relationship is quite high due to the large number of synapomorphies.

IV. Characters common between Hirudinea and Branchiobdellida

The characters: (1) a fixed numbers of segments, (2) a posterior sucker, (3) the typical structure of the muscle cells (Röhlich 1962; Farnesi and Vagnetti 1972; Lanzavecchia and De Eguileor 1976) and (4) fused male pores, have been observed in Branchiobdellida as well. In addition, loss of chaetae (at least in posterior segments) occurs in several "oligochaete" taxa besides Branchiobdellida, Acanthobdellida and Euhirudinea. What is the likelihood that these similarities between Branchiobdellida and Hirudinea are either convergencies related to the ectocommensal or ectoparasitic life-style (Fig. 8A) or synapomorphies (Fig. 8B)?

1. Posterior sucker

The occurrence of a posterior adhesive structure is certainly correlated with a fixed number of segments. In annelids, the number of segments generally varies and new segments are formed teloblastically anterior to the pygidium throughout life. Such a zone of embryonic and undifferentiated tissues would not allow a firm connection between the posterior sucker and the trunk, which is definitely required for attachment to actively moving hosts such as fishes or crayfishes. Since there are obviously only a few structural solutions for this particular problem, the probability of convergence is high (Westheide and Rieger 1987; Rieger and Tyler 1985). The probability of convergence for this feature is further supported by the different number of segments involved and by the fact that different segments form these structures [Branchiobdellida 1–2 (=XV–XVI according to Sawyer's (1986a) nomenclature), but 7 (=XXVIII–XXXIV) in Hirudinea and only 4 in Acanthobdellida (=XXVIII–XXXI)]. Moreover, the mode of operation and structure of the adhesive organs in Branchiobdellida may differ from that of the Hirudinae; the former has been described as a duo-gland adhesive structure by Gelder and Rowe (1988).

2. Number of segments

Concerning the fixed number of segments, the probability of convergence is also high since the number of segments actually present varies between Acanthobdellida (29), Euhirudinea (32 without pro- and peristomium), and Branchiobdellida (15). However, the different number of segments in Acanthobdellida and Euhirudinea can easily be derived from each other because *Acanthobdella* still possesses a nervous system which has a ganglionic arrangement and compartmentation identical to those of the "true" leeches apart from the lack of the three posteriormost pairs of ganglia.

3. Jaws

The occurrence of jaws in a few Hirudinea and the Branchiobdellida has also been discussed as a possible synapomorphy between these two taxa. Among leeches jaws are only present in Gnathobdellida, a highly derived taxon within the Euhirudinea, and to our knowledge there is no evidence for jaws in the stem species of the taxon. In contrast to the other Euhirudinea and to the Acanthobdellida, the Gnathobdellida are ectoparasites on vertebrates with a thick keratinized epidermis. Their jaws have a triradiate arrangement whereas in Branchiobdellida there are only two jaws, dorsally and ventrally positioned. Although they are similarly formed from thickenings of the stomodaeal cuticle, the structural differences are great enough that the occurrence of jaws must be considered as a convergence, dispelling the probability of any close affinities between these two groups (Jennings and Gelder 1979; Damas 1984).

4. Genital organs

The only remaining characters which might have a certain probability of synapomorphy for Acanthobdellida and Branchiobdellida are: (1) the fused male pores and (2) the position of the genital openings. As already pointed out by Brinkhurst and Gelder (1989), fusion of male ducts is different in Lumbriculidae and Branchiobdellida. In the former the ducts open separately and symmetrically into the atrium, in the latter they are fused to an unpaired duct before entering the atrium and they are positioned asymmetrically. The structure of the male atrium in Acanthobdellida is more similar to that in Lumbriculidae with fused male pores. Thus, the likelihood of convergence of fusion of male systems in Acanthobdellida and in Branchiobdellida seems to be higher than that of homology.

Specification of the positions of the genital openings remains difficult. Following Sawyer's (1986a) labelling, the first annulus in the Euhirudinea (considered to be the prostomium) is marked I and the female system opens in XII. With the same labelling system, in the Branchiobdellida the female pore is situated at the intersegmental furrow between XII and XIII (Sawyer 1986a, Brinkhurst and Gelder 1989). It is not unlikely that in the Acanthobdellida, the prostomium and the following so-called peristomium have been lost or fused with the following segment (III). The most convincing evidence for this assumption is twofold: the occurrence of chaetae in the first visible annulus and the identical structure and position of the nervous system in the anterior part of both Acanthobdellida and Euhirudinea. Thus, the first two annuli of the Acanthobdellida are not homologous either to the two achaetous portions of the anterior end of the "Oligochaeta" [usually labelled "0" (=prostomium) and "1" (=peristomium)] or to the first two annuli of the Euhirudinea (I and II). In this case Sawyer's (1986a) numerical designation must be changed: in *Acanthobdella* then the male pore lies in segment XII and the female in segment XIII. (This would also mean that the position of the genital openings in the Euhirudinea is different from that in *Acanthobdella:* male pore in XI, female in XII). It should be mentioned that the position of the genital openings is not constant in the Euhirudinea (see Sawyer 1986a).

V. Conclusions

In any case the Branchiobdellida, the Acanthobdellida and the Euhirudinea are monophyla, each characterized by several autapomorphies (33–37, 38–42 in Fig. 8A and 35–40, 41–46, 47–52 in Fig. 8B). In both of the suggested cladograms the Branchiobdellida share convergencies either with Acanthobdellida, or Euhirudinea and in our first hypothesis there are also convergently evolved characters between Branchiobdellida and Hirudinea (see above for estimation of phylogenetic importance). On the other hand, the Branchiobdellida possess characters not present in other taxa, which represent high level autapomorphies: (1) a body consisting of a constant number of 15 segments including the so-called peristomium, (2) sperm ducts specifically arranged, (3) nephridioducts partly fused (the anterior ones are fused or separate and the posterior ones are all separate) and opening dorsally and (4) possession of an anterior adhesive organ on the ventral surface of the ventral lip.-

Characters clearly establishing the monophylum Acanthobdellida are: (1) a body consisting of 29 actually present segments and (2) chaetae used for attachment and adhesion to the hosts and possibly for assistance in feeding. The other characters mentioned occur convergently in one of the other taxa. Characters similarly only present in Euhirudinea are: (1) an anterior sucker built up of four segments, (2) a coelom restricted to a system of channels and complete reduction of septa and (3) sperm sacs subdivided into numerous vesicles (testisacs).

We consider the Acanthobdellida to represent the sister group of the Euhirudinea (Rhynchobdellida, Pharyngobdellida and Gnathobdellida). A sister group relationship of the Branchiobdellida to these Hirudinea appears to be possible. However, it is highly probable that the characters common to Branchiobdellida and Hirudinea are convergencies. Therefore, these two taxa very likely represent ectosymbionts on different hosts evolved independently from different "oligochaete" ancestors.

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References

Abeloos M (1925) Recherches histologiques et histophysiologiques sur le parenchyme et les néphridies des Hirudinées Rhynchobdelles. Bull Biol Fr Belg 59:436–97

Andersson E (1965) Ecological notes on *Acanthobdella peledina* Grube found on grayling and brown trout. Rep Inst Freshwater Res Drottningholm 46:185–199

Andersson E (1988) The biology of the fish leech *Acanthobdella peledina* Grube. Zool Beitr 32:31–50

Babaskin AW (1931) Über das Bindegewebe der Hirudineen. Zool Jahrb Anat 53:1–102

Bradbury S (1956) A histochemical study of the adipose cell of the leech, *Glossiphonia complanata.* Q J Microsc Sci 97:499–517

Brinkhurst RO, Gelder SR (1989) Did the lumbriculids provide the ancestors of the branchiobdellidans, acanthobdellidans and leeches? Hydrobiologia 180:7–15

Brinkhurst RO, Jamieson BGM (1971) Aquatic oligochaeta of the world. Oliver and Boyd, Edinburgh, pp 1–860

Brinkhurst RO, Nemec AFL (1987) A comparison of phenetic and phylogenetic methods applied to the systematics of Oligochaeta. Hydrobiologia 155:65–74

Brusca RC, Brusca GJ (1990) Invertebrates. Sinauer Associates, Sunderland, pp 1–922

Damas D (1984) Approche histochemique, microanalytique et ultrastructurale de la calcification du collagène cuticulaire des dents de la sangsue medicinale. Bull Soc Zool Fr 109:269–278

Erséus C (1987) Phylogenetic analysis of the aquatic Oligochaeta under the principle of parsimony. Hydrobiologia 155:75–89

Farnesi RM, Vagnetti D (1972) Morphological investigations on the parietal musculature in *Branchiobdella pentodonta* Whitman. Boll Zool 39:343–350

Ferraguti M (1984) The comparative ultrastructure of sperm flagella central sheath in Clitellata reveals a new autapomorphy of the group. Zool Scr 13:201–207

Ferraguti M, Gelder SR (1991) The comparative ultrastructure of spermatozoa from five branchiobdellidans (Annelida: Clitellata). Can J Zool 69:1945–1956

Franzén A (1991) Spermiogenesis and sperm ultrastructure in *Acanthobdella peledina* (Hirudinea) with some phylogenetic considerations. Inv Repr Developm 19:245–256

Gelder SR, Rowe JP (1988) Light microscopical study on the adhesive and epidermal gland cell secretions of the branchiobdellid *Cambarincola fallax* (Annelida, Clitellata). Can J Zool 66:2057–2064

Gray EG, Guillery RW (1963) An electron microscopical study of the ventral nerve cord of the leech. Z Zellforsch 60:826–49

Holt PC (1953) Characters of systematic importance in the family Branchiobdellidae (Oligochaeta). Virginia J Sci 4:57–61

Holt PC (1965) The systematic position of the Branchiobdellidae (Annelida: Clitellata). Syst Zool 14:25–32

Holt PC (1989) Comments on the classification of the Clitellata. Hydrobiologia 180:1–5

Jamieson BGM (1981) The ultrastructure of the Oligochaeta. Academic Press, London, pp 1–462

Jennings JB, Gelder SR (1979) Gut structure, feeding and digestion in the branchiobdellid oligochaete *Cambarincola macrodonta* Ellis 1912, an ectosymbionte of the freshwater crayfish *Procambarus clarkii.* Biol Bull 156:300–314

Lanzavecchia G, De Eguileor M (1976) Studies on helical and paramyosinic muscles. V. Ultrastructural morphology and contraction speed of muscle fibers of *Erpobdella octoculata* and *Erpobdella testacea* (Annelida, Hirudinea). J Submicrosc Cytol 8:69–88

Lanzavecchia G, De Eguileor M, Valvassori R (1988) Muscles. In: Westheide W, Hermans CO (eds) The ultrastructure of Polychaeta. Microfauna Marina 4:71–88

Livanow N (1904) Untersuchungen zur Morphologie der Hirudineen, II. Das Nervensystem des vorderen Körperendes und seine Metamerie. Zool Jahrb Anat 20:153–226

Livanow N (1906) *Acanthobdella peledina* Grube, 1851. Zool Jahrb Anat 22:637–866

Livanow N (1931) Die Organisation der Hirudineen und die Beziehungen dieser Gruppe zu den Oligochaeten. Erg Fortschr Zool 7:378–484

McDonald K (1984) Osmium ferricyanide fixation improves microfilament preservation and membrane visualization in a variety of animal cell types. J Ultrastruct Res 86:107–108

Michaelsen W (1919) Über die Beziehungen der Hirudineen zu den Oligochaeten. Mitt Zool Mus Hamburg 36:131–153

Odier AM (1823) Memoire sur les Branchiobdelle, nouveau genre d'Annelides de la famille des Hirudinées. Mem Soc Hist Nat Paris 1:69–78

Payton BW (1981) Structure of the leech nervous system. In: Muller KJ, Nicholls JG, Stent GS (eds) The neurobiology of the leech. Cold Spring Harbor, pp 35–50

Rieger R, Tyler S (1985) Das Homologietheorem in der Ultrastrukturforschung. In: Ott JA, Wagner GP, Wuketits FM (eds) Evolution, Ordnung und Erkenntnis. Paul Parey, Berlin Hamburg, pp 21–36

Röhlich P (1962) The fine structure of the muscle fiber of the leech *Hirudo medicinalis.* J Ultrastruct Res 7:399–408

Sawyer RT (1986a) Leech biology and behaviour I. Anatomy, physiology and behaviour. Clarendon Press, Oxford, pp 1–418

Sawyer RT (1986b) Leech biology and behaviour II. Feeding biology, ecology and systematics. Clarendon Press, Oxford, pp 1–374

Sawyer RT (1986c) Leech biology and behaviour III. Bibliography. Clarendon Press, Oxford, pp 1–266

Scriban IA, Autrum H (1928–1934) 2. Ordnung der Clitellata: Hirudinea = Egel. In: Kükenthal W, Krumbach T (eds) Handbuch der Zoologie. 2.2. Walter de Gruyter, Berlin Leipzig, pp 119–352

Specht A (1988) Chaetae. In: Westheide W, Hermans CO (eds) The ultrastructure of Polychaeta. Microfauna Marina 4:45–59

Vagnetti D, Farnesi RM (1978) Morphological and ultrastructural study of the ventral nerve cord in *Branchiobdella pentodonta* Whitman (Annelida, Oligochaeta). J Comp Neurol 178:365–382

Vejdowsky F (1884) System und Morphologie der Oligochaeten. F. Rivnac, Prague, 1884, pp 1–212

Westheide W, Purschke G (1988) Organism processing. In: Higgins RP, Thiel H (eds) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington, pp 146–160

Westheide W, Rieger R (1987) Systematics of the amphiatlantic *Microphthalmus-listensis*-species-group (Polychaeta: Hesionidae): Facts and concepts for reconstruction of phylogeny and speciation. Z Zool Syst Evolutionsforsch 25:12–39