

Ultrastructure and overall organization of ligament sac, uterine bell, uterus and vagina in *Paratenuisentis ambiguus* (Acanthocephala, Eoacanthocephala) – the character evolution within the Acanthocephala

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Abstract

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As an adaptation to their endoparasitic lifestyle, Acanthocephala (Palaeacanthocephala, Eoacanthocephala, Polyacanthocephala, Archiacanthocephala) have evolved a highly specialized reproductive system. Most of our present knowledge of the efferent duct system of the female is based on palaeacanthocephalan and archiacanthocephalan representatives. In order to provide a basis for further elucidating the phylogenetic relationships within the Acanthocephala, we herein describe ultrastructure and overall organization of the ligament sac and efferent duct system in females of *Paratenuisentis ambiguus* (Eoacanthocephala, Neoechinorhynchida). Only one ligament sac was found. The uterine bell consists of two contractile binucleate syncytia (bell wall syncytium, lateral pocket syncytium), two pairs of contractile cells (lappet cells, uterine bell retractors) and three pairs of noncontractile cells (median cells). The contractile uterus bears four nuclei. The vagina is composed of a syncytial epithelium (four nuclei) and two binucleate sphincters. A comparison of the present findings with literature data leads to the following conclusions: except for the uterine bell retractors, the uterine bell components found in *P. ambiguus* can be assumed to be autapomorphies for the Acanthocephala. The sheathing syncytium and median dorsal cell belong to the basal pattern (*sensu* ground pattern) of a palaeacanthocephalan subclade termed the *Echinorhynchus*-group in the present study. The median oviduct syncytium and paired uterine bell retractors can be assumed to be basal pattern characteristics of the Archiacanthocephala and Neoechinorhynchida, respectively. The study includes a tabular survey of terminological synonyms used in the literature.

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Introduction

Acanthocephala (Palaeacanthocephala, Eoacanthocephala, Polyacanthocephala, Archiacanthocephala), as typical parasites, are distinguished from their free-living relatives by the presence of several reductions. Similar to tapeworms, for example, they lack an intestinal tract, with nutrients taken up

via a specialized epidermis. On the other hand, the parasitic lifestyle has also led to the evolution of highly elaborate features. This especially applies to the female reproductive system, where enormous numbers of eggs are produced to infect new hosts. Consequently, the reproductive system of female acanthocephalans differs considerably from that of their probable closest free-living and epizoic relatives, the

Eurotatoria and *Season*, respectively (Ahlrichs 1997, 1998; Herlyn *et al.* 2003). While the ovaria persist in the latter two taxa, ontogenesis reveals a degradation of the ovaria into the so-called ovarian balls in acanthocephalans (e.g. Ax 2003). Upon first insemination the ovarian balls release thousands of shelled eggs that, together with the ovarian balls, float freely within the body cavity. Before the eggs are voided into the intestinal lumen of the host they pass a tripartite efferent duct system consisting of a so-called uterine bell followed by a uterus and a vagina. The uterine bell probably functions as an egg-sorting apparatus that withholds most of the immature eggs (see Whitfield 1970).

Most of the previous studies of the overall organization of the efferent duct system of females were carried out on representatives of the Archiacanthocephala and Palaeacanthocephala (e.g. Kaiser 1893; Meyer 1933; Whitfield 1968; Asaolu 1980). On the other hand, relatively little is known regarding the organization of the efferent duct system in female eoacanthocephalans and polyacanthocephalans. Consequently, our ideas of how the female efferent duct system evolved within the Acanthocephala are still vague. Detailed analysis of morphology and overall organization of the reproductive system, however, promises further insights into the phylogenetic relationships of the acanthocephalan subtaxa. To provide a basis for such elucidation of the acanthocephalan phylogeny, we herein describe the ultrastructure and overall organization of ligament sac, uterine bell, uterus and vagina in the eoacanthocephalan *Paratenuisentis ambiguus* (Van Cleave, 1921); determine the overall organization of the efferent duct system of females, that we suggest is the basal pattern (*sensu* ground pattern, see Ax 1996) for acanthocephalans; and describe the morphological changes that we hypothesize to have occurred in the course of the evolution of the Acanthocephala. To facilitate comparison with existing information, the discussion includes a table of synonyms for terminology used in the literature (see Table 1).

Materials and Methods

Adult females of *P. ambiguus* (Eoacanthocephala, Neoechinorhynchida) were extracted (in 1997) from naturally infected European eels [*Anguilla anguilla* (Linneus, 1758)] from the river Weser near Göttingen, Germany, stunned at 4 °C in minimal essential medium (MEM, Life Technologies LTD) or physiological saline solution and brought to the laboratory.

For transmission electron microscopy (TEM), adult females were fixed in ruthenium red-tinted 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.2) at 4 °C for 1 h. Subsequently, the material was rinsed in a 0.1 M sodium cacodylate buffer, postfixed in 1% osmium tetroxide (same buffer) for 1 h at 4 °C, and dehydrated in a graded acetone series. After embedding in Araldite (propylene oxide as intermedium), a series of silver interfering transverse sections (about 70 nm) were cut with diamond knives on a Reichert Ultracut microtome. For documentation, selected

sections were stained automatically with uranyl acetate and lead citrate in an LKB Ultrastainer. Examination was carried out using a Zeiss EM 900 at 50 kV.

For light microscopy, additional adult females of *P. ambiguus* were fixed for 3 days at room temperature with Bouin's solution, dehydrated in a graded series of ethanol and embedded in Paraplast (Roth) with butanol and methylbenzoate as intermedia. Series of semi-thin longitudinal and transverse sections (about 5 µm) were cut with steel knives on a Reichert Jung microtome, and stained with anilin blue, orange G and nuclear red (Romeis 1989). Live specimens were immobilized under cover-slips by slight pressure. Both semi-thin sections and live specimens were examined using Axiophot and Axioscop (Zeiss) light microscopes.

Additional observations using a light microscope were carried out on a series of semi-thin sections of *Aporhynchus aculeatus* Meyer (1931) (Archiacanthocephala, Aporhynchida) provided by the Museum of Natural History, Humboldt-Universität zu Berlin, Germany (Generalkatalog der Entozoen, ZMB 6018). With respect to the interpretation, however, it should be stated that some of the sections were of low quality.

Results

Ligament sac

Adult females of *P. ambiguus* have only one (ventral) ligament sac (Fig. 2C). It is lined by a thin layer of cytoplasm surrounded by extracellular matrix (Fig. 3C). The cytoplasm partly disintegrates into a mesh of anastomoses, whereby the 'meshes' are filled by extracellular matrix (Fig. 3B). The ligament sac is connected to the dorsal body cavity by an anterior opening. As a remnant of the primary body cavity (see Discussion), the dorsal body cavity lacks its own lining and thus borders directly onto the surrounding tissues (Figs 2C and 3C). Posteriorly, the ligament sac joins the efferent duct system, comprised of the uterine bell, uterus and vagina (Fig. 2A,B). The ligament strands that project into the uterine bell in some other acanthocephalan species (see Fig. 4C) are absent in *P. ambiguus*.

Uterine bell

The uterine bell of *P. ambiguus* consists of two contractile binucleate syncytia (bell wall syncytium, lateral pocket syncytium), two pairs of contractile cells (lappet cells, uterine bell retractors) and three pairs of cytoplasmic cells (median cells) (Fig. 1). Bell wall syncytium, lateral pocket syncytium and lappet cells form a funnel that encloses the longitudinally arranged median cells (Fig. 2A). Excluding the bell wall syncytium and uterine bell retractors, the uterine bell of *P. ambiguus* measures about 245 µm in length.

In vivo observations revealed peristaltic contractions of the uterine bell that start from the bell wall syncytium and

Table 1 Presence/absence and synonyms of the bell ground components in representatives of the Eoacanthocephala, Archiacanthocephala and Palaeacanthocephala

	Eoacanthocephala		Archiacanthocephala					
	<i>N. rutili</i> (Saeftigen 1884)	<i>N. rutili</i> (Meyer 1933)	<i>P. ambiguus</i> (present study)	<i>M. hirudinaceus</i> , <i>M. moniliformis</i> (Kaiser 1893)	<i>O. microcephala</i> (Kilian 1932)	<i>G. echinodiscus</i> , <i>O. thumbi</i> (von Haffner 1942a, b)	<i>M. moniliformis</i> (Asaolu 1980)	<i>O. atrata</i> (Dunagan and Rashed 1988)
m1	hinteres Achsenzellenpaar	2 Ventralzellen	median cell pair 1	2 dorsale Ligamentzellen	2 dorsale Wulstkörper, 1 n each	2 dorsale Wulstzellen	2 dorsal median cells	2 dorsal ligament attachment cells
m2	vorderes Achsenzellenpaar	(laterale) vordere Dorsalzellen	median cell pair 2	2 ventrale Ligamentzellen	2 ventrale Wulstkörper, 1 n each	2 ventrale Wulstzellen	2 anterior ventral median cells	2 ventral ligament attachment cells
m3	dorsales Zellenpaar des Glockenschlundes	2 hintere Dorsalzellen	median cell pair 3	2 ventrale Lippenzellen	2 Lippenzellen	2 Lippenzellen	2 posterior ventral median cells	2 median wall cells
lc	seitliche Zellen des Glockenschlundes	2 Lateralzellen	2 lappet cells	2 laterale Oviduktzellen	2 Uterusgangzellen	2 Oviduktzellen	2 lappet cells	2 lateral oviduct cells
br	laterale Längsmuskeln, 1 n each	2 Retraktor-muskeln, 1 n each	2 uterine bell retractors, 1 n each					
mo				mediane Oviduktzelle, 3–4 n	Uterusgangsyncytium, 4 n	Syncytium des Urogenitalkanals, 4 n	present (see <i>ov</i> in Figs 5, 9 and 10)	urogenital canal
<hr/>								
	Palaeacanthocephala							
	<i>A. lucii</i> , <i>P. laevis</i> (Baltzer 1880)	<i>A. lucii</i> , <i>P. laevis</i> (Saeftigen 1884)	<i>A. anguillae</i> , <i>A. ranae</i> , <i>C. strumosum</i> , <i>F. anatis</i> (Knüpfner 1888)	<i>A. lucii</i> , <i>A. ranae</i> , <i>A. trichocephalus</i> , <i>B. balaenae</i> , <i>C. strumosum</i> (Kaiser 1893)	<i>A. lucii</i> , <i>E. clavula</i> (Meyer 1933)	<i>Polymorphus minutus</i> (Whitfield 1968)		
m1	2 Zellen im Ligamentalstrang	vorderes Achsenzellenpaar	(...) Zellenpaar, Ligamentzellen	2 dorsale Ligamentzellen	2 innere Dorsalzellen	2 dorsal Ligament attachment cells		
m2	2 Zellen	hinteres Achsenzellenpaar	(...) Zellenpaar	2 ventrale Ligamentzellen	2 innere Ventralzellen	2 ventral Ligament attachment cells		
m3	2 Zellen	dorsales Zellenpaar des Glockenschlundes	(...) Zellenpaar	2 ventrale Lippenzellen	2 vordere Ventralzellen	2 median wall cells		
lc	2 Seitenzellen	seitliche Zellen des Glockenschlundes	2 Zellen	2 laterale Oviduktzellen	2 Lateralzellen	2 uterine duct cells		
ss	umgebene Lage, Maschenwerk, 2 n	2 unpaare Zellen (<i>A. lucii</i>), present, 2 n (<i>P. laevis</i>)	Muskelgewebe, 2 n, Zelle (<i>A. anguillae</i>)	syncytiale Hüllmasse, mediane Oviduktzelle, 3–4 n	2 Dorsalzellen	sheathing syncytium, 2 n		
md	unpaare/eingerollte Zelle	unpaare Zelle	unpaare Zelle	dorsale (...) Zelle	Dorsomedianzelle	median dorsal cell		

The terms 'cell(s)' and 'Zelle(n)' implicate the presence of one nucleus (if not otherwise stated). 'Zellenpaar' = 'cell pair', br, uterine bell retractors; lc, lappet cells; m1–3, median cell pair 1–3; md, median dorsal cell; mo, median oviduct syncytium; n, number of nuclei; ss, sheathing syncytium.

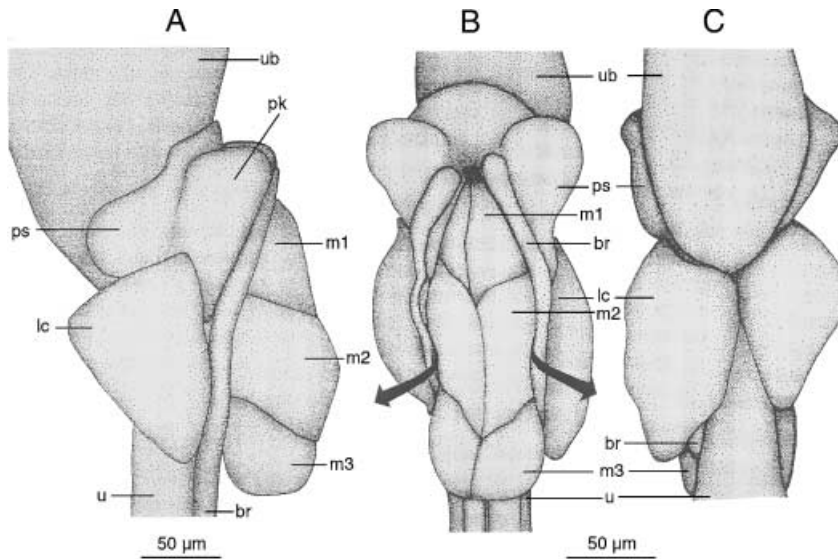


Fig. 1—**A–C.** *Paratenuisentis ambiguus*, drawings of the uterine bell. —**A.** Lateral view. —**B.** Dorsal view, arrows indicate openings. —**C.** Ventral view. Abbreviations: br, uterine bell retractor; lc, lappet cell; m1–3, median cell pair 1–3; pk, perikaryon; ps, lateral pocket syncytium; u, uterus, ub, bell wall syncytium.

proceed posteriorly via the lateral pocket syncytium towards the lappet cells. Beyond this functional co-action bell wall syncytium, lateral pocket syncytium and lappet cells share common morphology in that they consist of two types of morphologically consistent subunits. The first type is interspersed with contractile filaments that extend between z-discs (termed ‘contractile layer’ in the following). The second type of layer is mainly cytoplasmic and only contains isolated strands of contractile filaments (termed ‘cytoplasmic layer’ or ‘cytoplasmic core’ in the following). The cytoplasmic subunit bears organelles such as nuclei, endoplasmic reticulum and mitochondria, the last of which accumulate along the isolated contractile filaments (Fig. 3A).

Bell wall syncytium The bell wall syncytium represents the anteriormost component of the uterine bell (Figs 1 and 2A). Its fan-shaped anterior rim joins the lining of the ligament sac. Consequently, the lumen enclosed by the bell wall syncytium represents the posterior continuation of the ligament sac lumen. In the bell wall syncytium, one distal contractile layer surrounds one proximal cytoplasmic layer. However, contractions produce partly the appearance of having more than two layers (Fig. 2D).

Lateral pocket syncytium Posteriorly, the bell wall syncytium borders on the lateral pocket syncytium (Figs 1 and 2A). The most prominent feature of this syncytium is its two ventrally connected pocket-like bulges. Each of these uninucleate ‘pockets’ encloses a small cavity that communicates with the remainder of the bell lumen. The lateral pocket syncytium consists of a cytoplasmic core that is surrounded by a peripheral contractile layer. Medianly, the contractile layer is strongest around the nuclei, unlike laterally, where it is of similar strength (Fig. 2E).

Lappet cells Posterior to the lateral pocket syncytium, a pair of lappet cells constitutes the uterine bell (Figs 1 and 2A). Apart from an anterior ventral and a posterior dorsal interconnection, the lappet cells are detached from each other. In the lappet cells, the circumferential contractile layer around the cytoplasmic core is of nearly constant strength over its entire extension (Fig. 2A,F).

Median cells The lumen between the lateral pockets and the lappet cells is vertically bipartitioned by two pairs of median cells (Fig. 2A,F). Together with a third pair they form a longitudinal bar (Fig. 1A,B). The relatively electron-dense cytoplasm of the median cells is interspersed with vacuoles (Fig. 3E). The median cells are interconnected by desmosomal cell contacts. Apart from these cell contacts, the intercellular space is rather inflated and forms protrusions that penetrate the median cells (Figs 2F and 3E). Among granula and droplets of different size, spermatids are visible in the intercellular space (Fig. 3F).

Bell ground The two anterior-most median cell pairs and the lappet cells enclose two lumina that are commonly referred to as oviducts (Figs 2A and 3D). The oviducts are connected to the primary body cavity via narrow dorsal clefts between the second median cell pair and the lappet cells (Fig. 1B). More posteriorly, the oviducts fuse into a single duct that meets the uterus (Fig. 2A).

Uterine bell retractors These stretched cells extend from the lateral pocket syncytium to the gonopore (Figs 1A,B and 2A,B,E,G–J). Each uterine bell retractor is composed of a peripheral contractile layer that is strengthened posteriorly and a central cytoplasmic core that bears the nucleus (Fig. 2G).

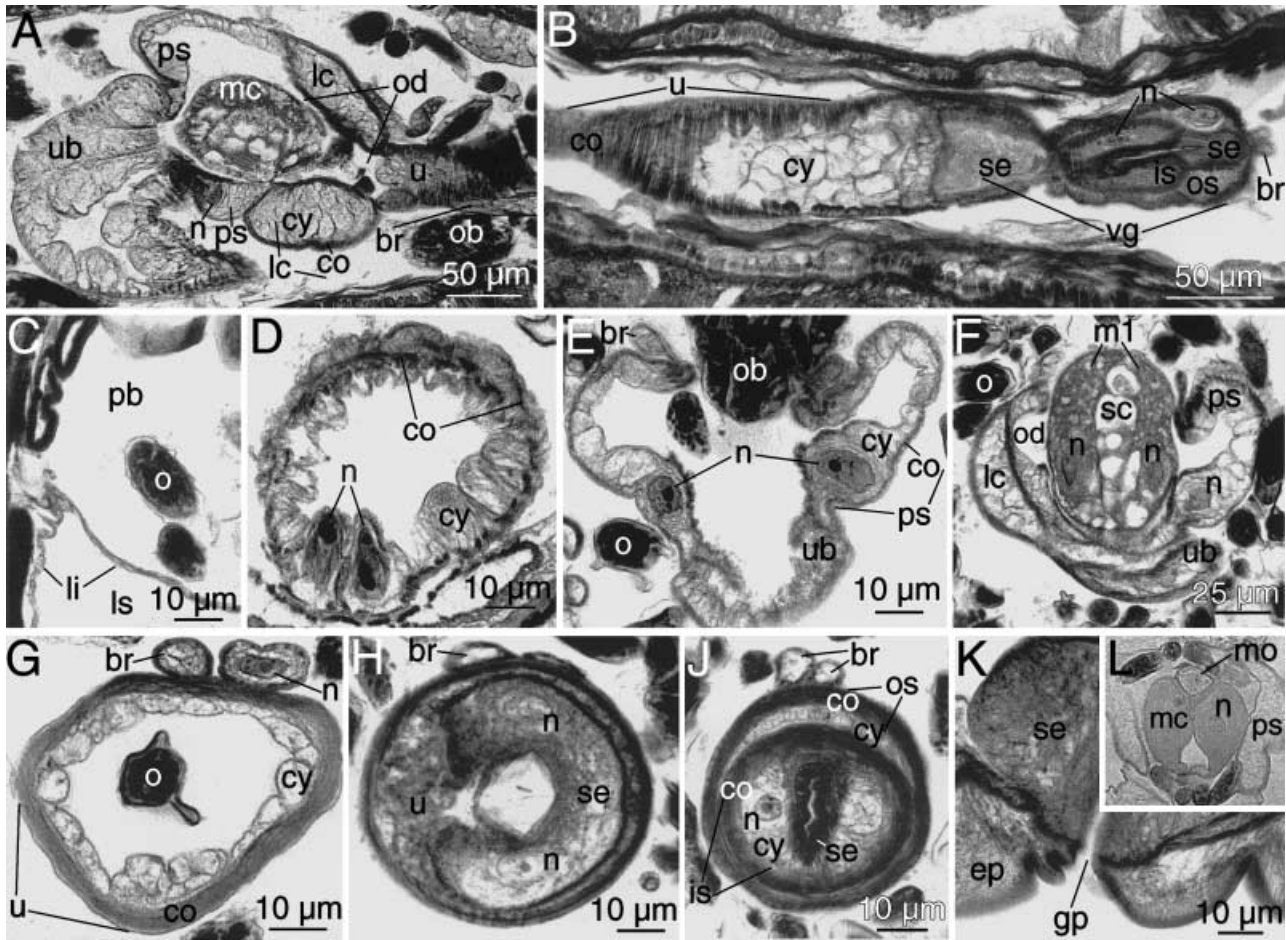


Fig. 2—A–K. *Paratenuisentis ambiguus*, light micrographs of horizontal (A,B) and transverse sections (C–K, ventral side below). —A. Overview of the uterine bell. —B. Overview of uterus and vagina. —C. Dorsal primary and ventral secondary body cavity (= ligament sac). —D. Bell wall syncytium. —E. Lateral pocket syncytium. —F. Bell ground. —G. Uterus and uterine bell retractors. —H. Transition-zone of uterus and vagina. —J. Vagina. —K. Gonopore. —L. *Aporhynchus aculeatus*, light micrograph showing the presumed median oviduct syncytium.

Abbreviations: br, uterine bell retractor; co, contractile layer; cy, cytoplasmic layer/core; ep, epidermis; gp, gonopore; is, inner sphincter; lc, lappet cell; li, ligament; ls, ligament sac; m1, median cell pair 1; mc, median cell; mo, presumed median oviduct syncytium; n, nucleus; o, egg; ob, ovarian ball; od, oviduct; os, outer sphincter; pb, primary body cavity; ps, lateral pocket syncytium; sc, intercellular space; se, syncytial vagina-epithelium; u, uterus; ub, bell wall syncytium; vg, vagina.

Uterus and vagina

A tubiform muscle syncytium (two nuclei) about 260 μm long and extending from the lappet cells to the vagina (Figs 1 and 2A,B), constitutes the uterus. It consists of a peripheral layer of contractile filaments and a stronger proximal layer with only isolated strands of contractile filaments (Fig. 2B,G).

The vagina measures about 160 μm in length. It is composed of three concentric syncytia (Fig. 2B): the innermost epithelium (four nuclei) appears dumbbell-shaped in longitudinal sections. Its anterior end projects into the uterus lumen while the posterior end forms the ventral-subterminal gonopore (Fig. 2H). This noncontractile syncytium is surrounded by two binucleate sphincters that convergently

consist of a strong distal layer of contractile filaments and a mainly cytoplasmic proximal layer (Fig. 2J). While the outer sphincter extends from the uterus to the gonopore, the inner sphincter is restricted to the middle part of the vagina (Fig. 2B).

Discussion

The most evident characteristic of the efferent duct system in female eoacanthocephalans is a 180° rotation along the longitudinal axis compared to that of the other acanthocephalan clades. This inverted orientation, however, does not hamper the comparison between the overall organization of the uterine bell in the different clades (see Fig. 4). Thereby, we

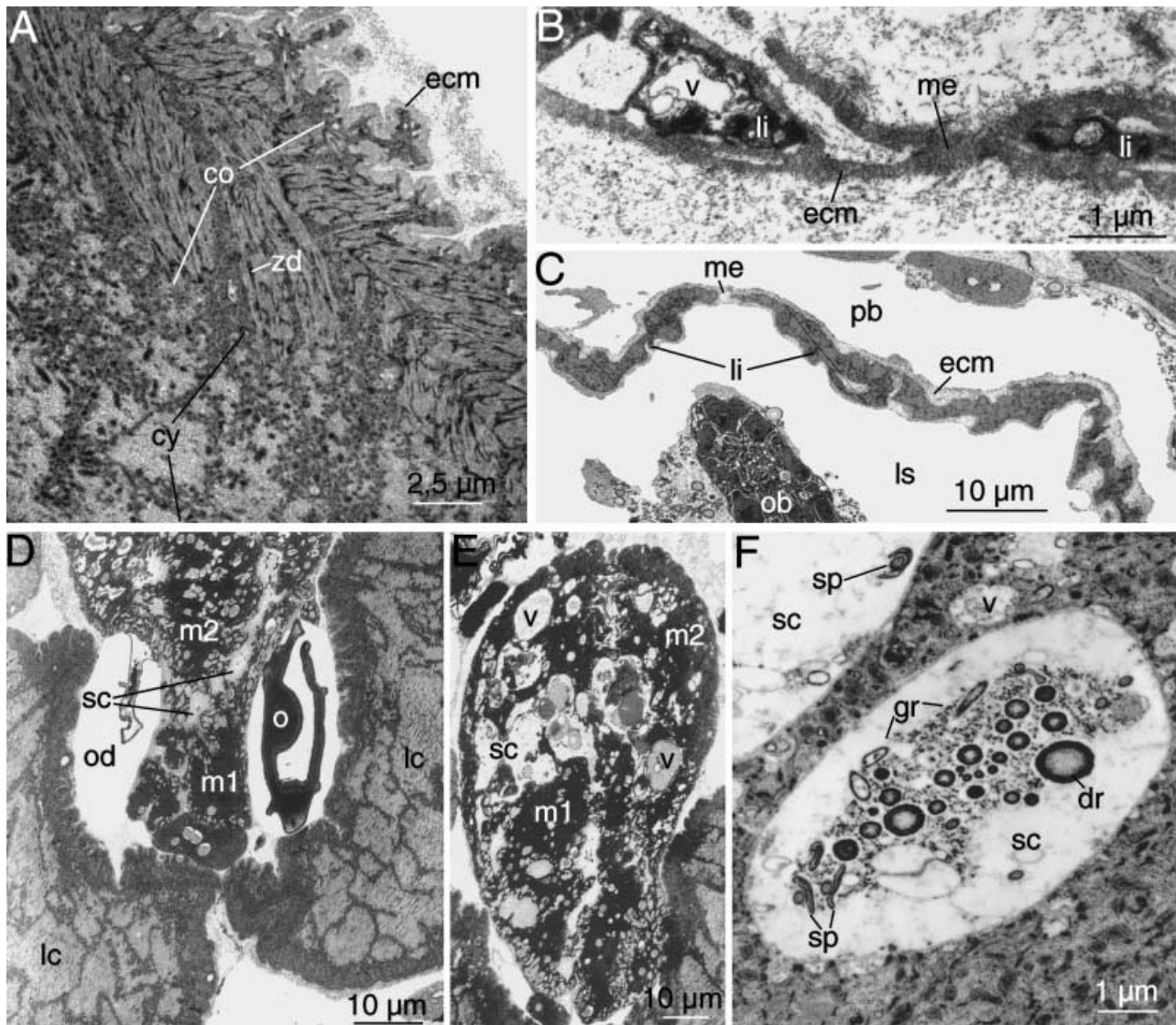


Fig. 3—**A–F.** *Paratenuisentis ambiguus*, electron micrographs of transverse sections. —**A.** Bipartite organization of a lappet cell. —**B,C.** Detail and overview showing the mesh-like organization of the ligament. —**D.** Oviducts. —**E.** Median cells. —**F.** Protrusion of the intercellular space. Abbreviations: co, contractile layer; cy,

cytoplasmic layer; dr, droplet; ecm, extracellular matrix; gr, granules; lc, lappet cell; li, ligament; ls, ligament sac; m1, 2, median cell pair 1 and 2; me, mesh-like interruption of the ligament; o, egg; ob, ovarian ball; od, oviduct; pb, primary body cavity; sc, intercellular space; sp, spermatid; v, vacuole; zd, z-disc.

consider herein as ‘ventral’ the body side to which the cerebral ganglion and the two main longitudinal nerves of the trunk (lateral posterior nerves) are shifted in adults of *P. ambiguus* as well as in many other acanthocephalans (for *P. ambiguus*, see e.g. Herlyn *et al.* 2001, Fig. 1G), a character state inherited from the basal pattern of the Gastroneuralia (see Ahlrichs 1995) or Protostomia (see Nielsen 2001).

Ligament sac

In the Acanthocephala, those body cavities of trunk and praesoma that lack their own lining ontogenetically originate

from splittings of primordial syncytia, that give rise to the adult organs (for example, see Meyer 1928; for *P. ambiguus* see also Hehn *et al.* 2001). As a result of this schizocoelic formation, they are often referred to as ‘pseudocoel’ (see Schmidt 1985). However, regardless of the mode of formation, these body cavities still lack their own lining, and, inasmuch, represent remnants of the blastocoel. Therefore, we prefer in continuation with Meyer (1928; p. 215) the term ‘primary body cavity’ for body cavities of the Acanthocephala without an own lining. In contrast, we denote those body cavities as ‘secondary’ that are surrounded by an own lining or coelothelium (see Bartolomaeus 1994).

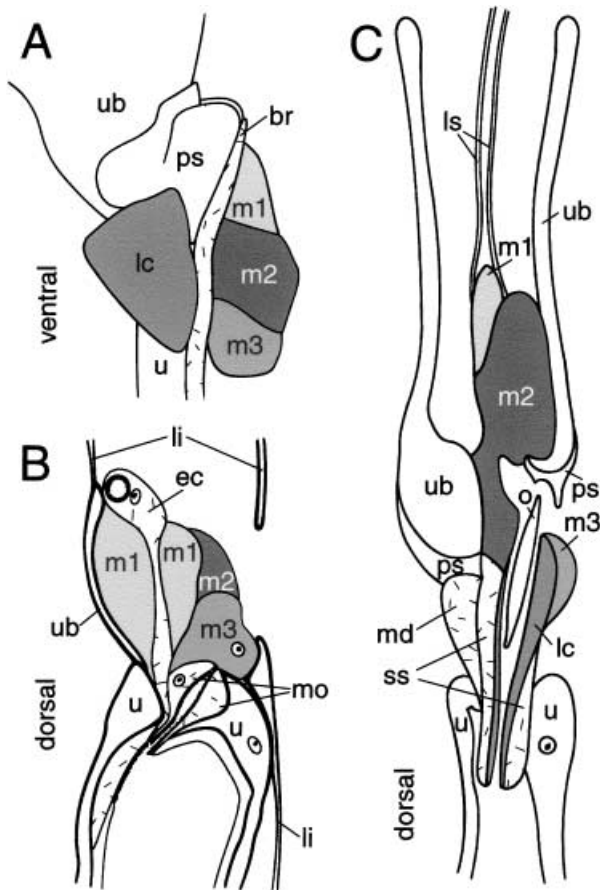


Fig. 4—Character states of the uterine bell; bell ground components assumed for the acanthocephalan basal pattern in shades of grey, derived characters speckled. —**A.** *Paratenuisentis ambiguus* (Eoacanthocephala, Neoechinorhynchida), lateral view. —**B.** *Gigantorhynchus echinodiscus* (Archiacanthocephala, Gigantorhynchida), sagittal section (modified after von Haffner 1942b). —**C.** *Acanthocephalus ranae* (Palaeacanthocephala, Echinorhynchida; *Echinorhynchus*-group), sagittal section (modified after Kaiser 1893), lateral pocket syncytium and lappet cells out of the plane of section. Abbreviations: br, uterine bell retractor; ec, excretory cell; lc, lappet cell; li, ligament; ls, ligament strand; m1–3, median cell pair 1–3; md, median dorsal cell; mo, median oviduct syncytium; o, egg; ps, lateral pocket syncytium; ss, sheathing syncytium; u, uterus; ub, bell wall syncytium.

According to these definitions, only the ventral body cavity of females of *P. ambiguus* represents a secondary body cavity or ligament cavity, as it is termed in acanthocephalans. In contrast, the dorsal body cavity borders directly onto the surrounding tissues (see Figs 2C and 3C), and, thus, can be regarded as a remnant of the primary body cavity. If one considers that the previous descriptions of two ligament sacs in eoacanthocephalans were mostly based on light microscope observations, often of whole mounts (see, e.g. Petrochenko 1956; Golvan 1959), it appears possible that the description

of a dorsal ligament sac in other eoacanthocephalan species actually refers to the primary body cavity. Consequently, additional ultrastructural data on the ligament sacs of other eoacanthocephalan species might shed light on the presently unresolved question of how the alternative character states one ligament sac versus two ligament sacs evolved within the Acanthocephala (see Monks 2001).

Uterine bell

The following analysis of the overall organization of the efferent duct system in female acanthocephalans is based on a total of 26 detailed descriptions comprising two eoacanthocephalan, 10 palaeacanthocephalan and six archiacanthocephalan species (see Table 1). Except for *Bolbosoma balanae* (Gmelin, 1790) and *Pomphorhynchus laevis* (Zoega, 1776), the palaeacanthocephalans considered belong to a subclade (termed the *Echinorhynchus*-group in the following) that is characterized by the lack of lateral sense organs at the base of the proboscis (Herlyn *et al.* 2001) and that appears monophyletic in molecular analyses (see García-Varela *et al.* 2002; Herlyn *et al.* 2003).

As a result of the relative position, number of nuclei and/or contractile (dis)ability, most of the uterine bell components found in *P. ambiguus* can be homologized with structures described in other species of Acanthocephala (see Fig. 4, Table 1). Thus, it can be assumed that the doubtlessly apomorphic uterine bell of the Acanthocephala (e.g. Ax 2003) originally consisted of two contractile binucleate syncytia (bell wall syncytium, lateral pocket syncytium), one pair of contractile cells (lappet cells) and three pairs of cytoplasmic cells (median cells) (see also Miller and Dunagan 1985).

As far as we are aware, paired uterine bell retractors are described exclusively for the representatives of the Eoacanthocephala, *Neoechinorhynchus rutili* (Müller, 1780) and *P. ambiguus* (both Neoechinorhynchida) (see Table 1, Fig. 4; see also Meyer 1931). Furthermore, they can be seen in females of *Neoechinorhynchus carinatus* Buckner & Buckner, 1993 (see Oetinger and Buckner 1993). Against the background of the widely accepted monophyly of the Eoacanthocephala (see García-Varela *et al.* 2000, 2002; Herlyn 2001; Monks 2001; Herlyn *et al.* 2003), the uterine bell retractors might belong to the basal pattern of the Eoacanthocephala. In any case, the character can be assumed as part of the basal pattern of the Neoechinorhynchida.

Sheathing syncytium and median dorsal cell are found solely in the representatives of the Palaeacanthocephala (see Table 1, Fig. 4). Descriptions of two separate cells instead of a sheathing syncytium in *Acanthocephalus lucii* (Müller, 1776) and *Echinorhynchus clavula* Dujardin, 1845 (see Saeftigen 1884; Meyer 1933) apparently refer to the perikarya of this mostly binucleate syncytium since other authors describe a binucleate sheathing syncytium in *Acanthocephalus anguillae* (Müller, 1780), *Acanthocephalus ranae* (Schrank,

1788), *Arhythmorhynchus trichocephalus* (Leuckart, 1876), *Bolbosoma balaenae* (Gmelin, 1790), *Corynosoma strumosum* (Rudolphi, 1802), *Filicollis anatis* (Schrank, 1788), *Polymorphus minutus* (Goeze, 1782), *P. laevis* and in other studies of *A. lucii* (see Baltzer 1880; Knüppfer 1888; Kaiser 1893; Whitfield 1968; see also Table 1). Moreover, a sheathing syncytium instead of two separate cells can also be found in the uterine bell of *Echinorhynchus truttiae* Schrank, 1788 (personal observations, unpublished).

Thus, both a sheathing syncytium and a median dorsal cell can be assumed as characteristics of the *Echinorhynchus*-group. Considering the possible paraphyly of the Palaeacanthocephala (Herlyn *et al.* 2001, 2003; but see also García-Varela *et al.* 2000, 2002; Monks 2001), both characters might represent autapomorphies of a larger clade comprising the *Echinorhynchus*-group as well as *P. laevis* and *B. balaenae*.

A peculiarity of the Archiacanthocephala is the presence of the median oviduct syncytium [named after the German term 'mediane Oviduktzelle' used by Kaiser (1893); see Table 1, Fig. 4]. It has been found in the oligacanthorhynchids *Oligacanthorhynchus atrata* (Meyer 1931), *O. microcephala* (Rudolphi, 1819), *O. thumbi* Haffner, 1939, *Macracanthorhynchus hirudinaceus* (Pallas, 1781), in the gigantorhynchid *Gigantorhynchus echinodiscus* (Diesing, 1851) and in the moniliformid *Moniliformis moniliformis* (Bremser, 1811) (see Table 1). As it appears to be present also in the aporhynchid *Aporhynchus aculeatus* (see Fig. 2L), the character median oviduct syncytium probably belongs to the basal pattern of the Archiacanthocephala. Whether or not this is the case, the close association of median oviduct syncytium and excretory organs in representatives of the Gigantorhynchida and Oligacanthorhynchida (see Fig. 4) suggests a monophyletic origin of both clades, an assumption that previously has been made on the basis of peculiarities of the presomal musculature (i.e. posteriad elongation of the receptacle, and the relative proposition of the proboscis retractor penetration sites; see Herlyn 2000) and that has received support also from a recent 18S rDNA analysis (García-Varela *et al.* 2002). With respect to those uterine bell components that have so far only been described for single species (i.e. 'anterior chamber cells' and 'ligament attachment syncytium' in *M. moniliformis*, see Asaolu 1980), however, it would be premature to draw any phylogenetic conclusions.

Uterus and vagina

Morphology and overall organization of uterus and vagina appear to vary little within the Acanthocephala. For example, in *P. ambiguus*, *O. atrata* and *M. moniliformis* the uterus consistently was found to be a tubelike muscular syncytium with two nuclei, and the vagina is always described as being composed of three concentric syncytia (binucleate outer and inner sphincter, epithelium mostly with four nuclei) (see Asaolu 1980; Dunagan and Rashed 1988; present study). Corresponding numbers of uterine and vaginal nuclei can

also be found in the descriptions of other acanthocephalan species, that are representatives of the *Echinorhynchus*-group (see Hamann 1891; Kilian 1932; Meyer 1933; Whitfield 1968). On the other hand, descriptions in which the epithelium represents the caudal continuation of the uterus in some archiacanthocephalans (see Kilian 1932; Meyer 1933) probably resulted from misinterpreting the protrusion of the vaginal epithelium into the uterus lumen (see Fig. 2B,H). Thus, the presence of a binucleate uterus as well as a vagina composed of an outer binucleate sphincter, an inner binucleate sphincter and an innermost epithelium with four nuclei can be assumed to be basal pattern characters of the Acanthocephala. The lack of a uterus and a vagina in the Eurotatoria and in *Seison*, and in the Gnathostomulida (see Ax 1996), suggest that both characters represent additional evolutionary novelties of the Acanthocephala.

Conclusion (see Fig. 4)

TEM observations reveal only one secondary body cavity, or ligament sac in females of *P. ambiguus*. This observation poses the question of whether there are actually two ligament sacs in other eoacanthocephalan species, as has been reported occasionally in the literature.

The uterine bell of *P. ambiguus* consists of a binucleate bell wall syncytium, a binucleate lateral pocket syncytium, two lappet cells, three pairs of median cells and a pair of uterine bell retractors that stretch along uterus and vagina. Except for the uterine bell retractors, that belong to the basal pattern of the Neoechinorhynchida, the listed components represent autapomorphies of the Acanthocephala. Within the Acanthocephala, the palaeacanthocephalan *Echinorhynchus*-group is characterized by an additional median dorsal cell and a sheathing syncytium. A median oviduct syncytium is probably part of the basal pattern of the Archiacanthocephala. The close association of the median oviduct syncytium and excretory organs in species of gigantorhynchids and oligacanthorhynchids corroborates a monophyletic origin of both taxa.

The uterus of acanthocephalans is a single binucleate syncytium whereas the vagina consists of three different syncytia (outer binucleate sphincter, inner binucleate sphincter, innermost epithelium with four nuclei). Both, the uterus and the vagina can be assumed to be apomorphic for the Acanthocephala.

Additional data on the overall organization of the uterine bell, especially of the genera *Koronacantha* Monks & Pérez-Ponce de León, 1996 and *Leptorhynchoides* Kostylev, 1924 (both genera belong to the second subclade of palaeacanthocephalans, which appears to be monophyletic in molecular analyses; see García-Varela *et al.* 2000) and members of the fourth clade of the Acanthocephala ranked as class (Polyacanthorhynchida), are required for further resolving the phylogenetic relationships within the Acanthocephala against the background of the present knowledge of the uterine bell evolution.

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