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Organisation of the praesoma in *Acanthocephalus anguillae* (Acanthocephala, Palaeacanthocephala) with special reference to the muscular system

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Abstract The organisation of the praesoma in the parasite *Acanthocephalus anguillae* was studied on the light and electron microscopic level, with emphasis on the morphology of the musculature. The study was compiled to add new data to the ground pattern of the Acanthocephala for analysis of the phylogenetic relationships within the Gnathifera. In *A. anguillae* the praesomal epidermis and lemnisci form a coherent syncytium, separated from the epidermis of the trunk. Hooks are seen to be derivatives of the subepidermal basal lamina and are covered by the praesomal epidermis. The praesomal circular body wall musculature forms a network of anastomosing muscle fibres that lines the proboscis; a praesomal longitudinal body wall musculature does not exist. The truncal circular and longitudinal body wall musculature rise up to the praesomal proboscis. The unpaired proboscis retractor, consisting of longitudinal circomyax fibres, forms an outer and an inner concentric tube; the latter extends through the entire praesoma and penetrates the receptacle wall. The sack-like receptacle is surrounded by a receptacle constrictor. The nervous system of the praesoma consists of a prominent cerebral ganglion, three nerves which extend anteriorly, ramify and end within the praesomal musculature, and two strong lateral posterior nerves. *A. anguillae* lacks an apical organ, lateral organs and a support cell. Many of the features present in the praesoma of *A. anguillae* can be assumed as ground-pattern characteristics of the Acanthocephala.

A. Introduction

Adult Acanthocephala are endoparasites of Gnathostomata with a worldwide distribution and common occurrence (Meyer 1933; Petrochenko 1956, 1958). Despite their

seriousness as the causes of diseases and epidemics (Sanford 1978) many aspects of their morphology and biology are not well known and their phylogenetic position still is a matter of discussion. Based on ultrastructural data, Ahlrichs (1995, 1997) considers the taxon *Seison* Grube, 1856 as the sister group of the Acanthocephala within a monophylum Syndermata. Molecular data support a position of the Acanthocephala within the Syndermata, but as a subtaxon of the Eurotatoria or Bdelloidea (Littlewood et al. 1998; Zrzavý et al. 1998; García-Varela et al. 2000). With respect to these attempts in analysing the sister group of the Acanthocephala it is surprising that there are only few detailed data on the ground pattern of this taxon. This study deals with the praesoma of *Acanthocephalus anguillae* (Müller, 1780) in order to substantiate the ground pattern of the Acanthocephala.

B. Materials and methods

Specimens of *A. anguillae* were extracted (in May 1998) from naturally infected barbels [*Barbus barbus* (Linné, 1758)] from the river Weser at Gimte near Göttingen (Germany), stunned by cooling at 4°C and brought to the laboratory in physiological saline solution.

For TEM two specimens were prefixed for 1 h at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), subsequently postfixed with 1% buffered osmium tetroxide and dehydrated in a graded acetone series. After embedding in Araldite (intermedium: propylene oxide) specimens were cut with diamond knives in serial ultrathin cross-sections (ca 70 nm) on a Reichert Ultracut microtome. Sections were stained with uranyl acetate and lead citrate in a LKB Ultrastainer and were examined with a Zeiss EM 900 electron microscope.

For light microscopy seven females and four males were fixed for 3 days at room temperature with Bouin, dehydrated in a graded ethanol series, butanol and methyl benzoate, and embedded in Paraplast (Roth). All specimens were cut with steel knives on a Reichert Jung microtome in serial semithin longitudinal or cross-sections (ca 5 µm) and dyed with aniline blue, orange G and nuclear red (Romeis 1989). Documentation was effected under an Axio-phot (Zeiss) light microscope. In the results, length data (arithmetic mean ± SD) and number of nuclei refer to these specimens.

Living specimens were immobilised by slight pressure under cover slides and examined under an Axioskop (Zeiss) light microscope.

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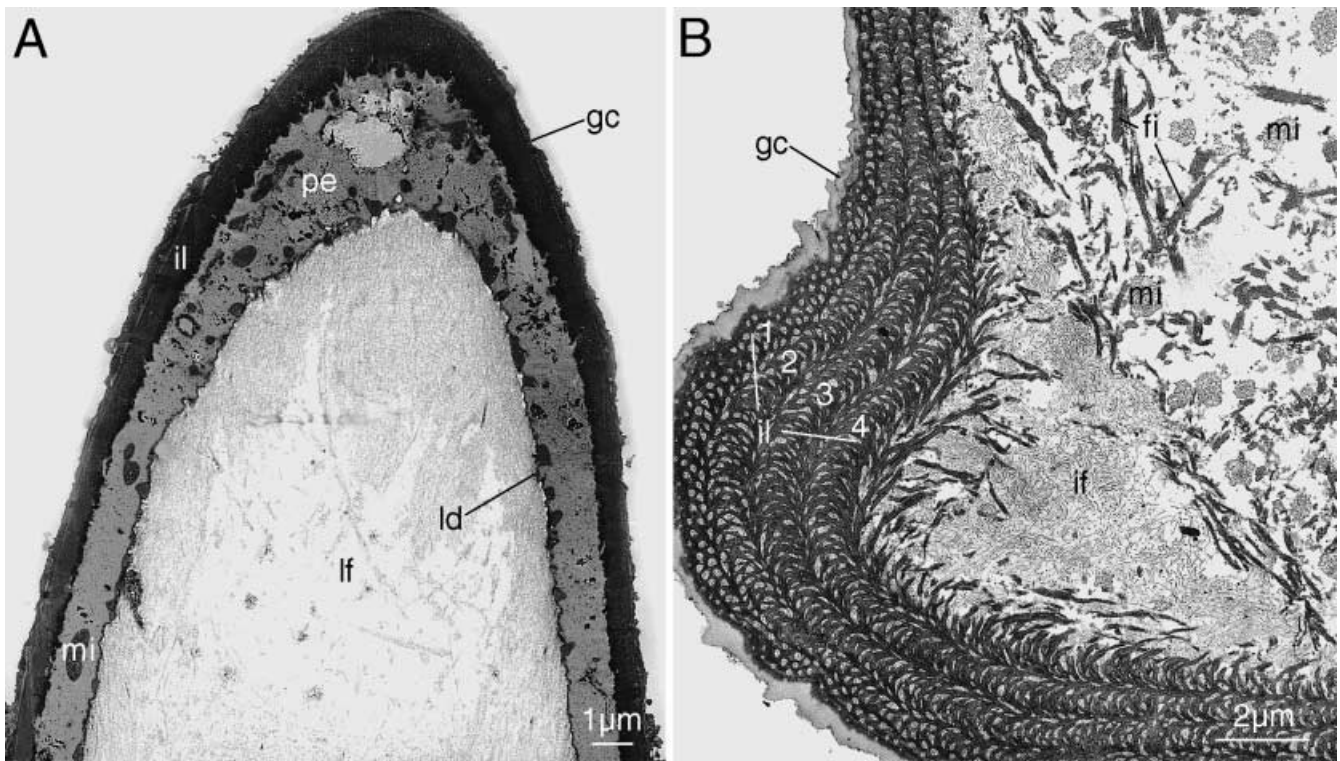


Fig. 1A, B *A. anguillae*; TEM micrographs. **A** Longitudinal section of a hook. **B** Oblique section through the periphery of the praesomal epidermis. *fi* Bundles of filaments, *gc* glycocalyx, *if* intermediate filaments, *il* internal layer, *ld* lamina densa, *lf* lamina fibroreticularis, *mi* mitochondrion, *pe* praesomal epidermis, 1–4 sublayers of the internal layer

C. Results

I. General praesomal features

In slightly contracted females of *A. anguillae* the praesomata, excluding the lemnisci, measured 1.7 ± 0.2 mm ($n=7$) in length (Fig. 3). In slightly contracted males the praesomata were 1.5 ± 0.2 mm ($n=4$) long, again excluding the lemnisci.

The praesomal epidermis and the two lateral lemnisci form a syncytium that is separated from the epidermis of the trunk by an infolding of the membrane (Figs. 2C, 3). At the anterior end of the praesoma the epidermis measures about 15 μ m in height. Posteriorly, the height of the epidermis increases up to about 40 μ m at the border to the epidermis of the trunk (Fig. 2A–C). Peripherally, the praesomal epidermis is covered by a thin glycocalyx (Fig. 1A, B). The cytoplasm of the praesomal epidermis contains bundles of filaments that measure about 200 nm in maximal diameter. In the peripheral epidermis these bundles form a so-called internal layer (see Ahlrichs 1997), termed striped layer in *Acanthocephala*, that is subdivided into four sublayers (Fig. 1B). Beneath the internal layer intermediate filaments are abundant (Fig. 1B). Mitochondria are the most frequent organelles within the epidermal cytoplasm (Fig. 1A, B), and fragments of dis-

solved nuclei are dispersed across the entire syncytium (Fig. 2A). Finally, channels of the lacunar system pervade the praesomal epidermis and the lemnisci (Fig. 2A, B).

The hooks and the basal lamina consist of extracellular matrix (Fig. 2A). Both can be subdivided into a strong electron-grey *lamina fibroreticularis* and a thin electron-dark *lamina densa* (Fig. 1A). A *lamina lucida* distal to the *lamina densa* is only partially visible, especially at the base of the hooks. The hooks are totally covered by a thin layer of epidermis (Fig. 1A).

The cerebral ganglion is situated within the inner tube of the proboscis retractor at the posterior end of the receptacle (Fig. 3B, C). In one female it measures about 0.14 mm in length. In the cerebral ganglia of all specimens, the perikarya are peripherally located whereas the inside of the cerebral ganglia is filled by the neuropile (Fig. 2D). Anteriorly, three nerves consisting of 5 or 6 neuronal fibres each stretch from the cerebral ganglion in an antierad direction. After iterative ramifications they end within the praesomal musculature. Posteriorly, two strong paired lateral posterior nerves with about 15 neuronal fibres each extend from the cerebral ganglion, penetrate the receptacle, cross the truncal body cavity and end within the body wall musculature (Fig. 2E). There are neither apical nor lateral organs. A support cell is also absent.

II. Musculature

The body wall musculature of the praesoma comprises three muscle syncytia: the praesomal circular musculature that is restricted to the proboscis, the circular mus-

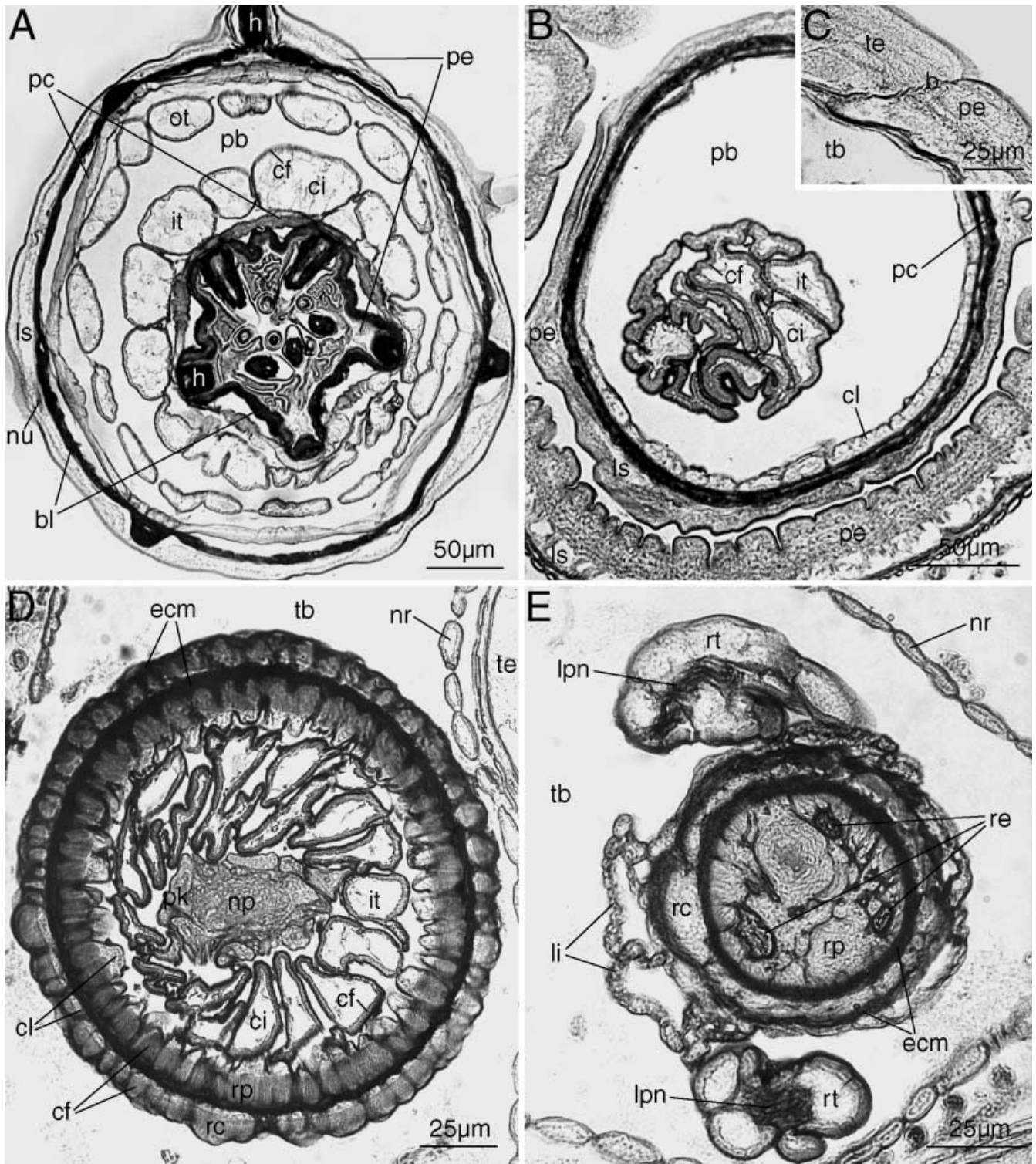


Fig. 2A–E *Acanthocephalus anguillae*; light micrographs of cross-sections through the praesoma. **A** Partly retracted proboscis. **B** Retracted neck. **C** Border between the praesomal epidermis and that one of the trunk. **D** Receptacle within the body cavity of the trunk. **E** Posterior end of the receptacle. *b* Border between the praesomal epidermis and that one of the trunk, *bl* basal lamina, *cf* layer of contractile filaments, *ci* cytoplasmic inside, *cl* cytoplasmic lining, *ecm* extracellular matrix, *h* hook, *it* inner tube of the

proboscis retractor, *lpn* lateral posterior nerve, *li* ligament sacks, *ls* channels of the lacunar system, *np* neuropile, *nr* neck retractor, *nu* nucleus, *ot* outer tube of the proboscis retractor, *pb* praesomal body cavity, *pc* praesomal circular musculature, *pe* praesomal epidermis, *pk* perikarya, *rc* receptacle constrictor, *re* process of the retractor, *rp* receptacle, *rt* retinacle muscle, *tb* body cavity of the trunk, *te* epidermis of the trunk

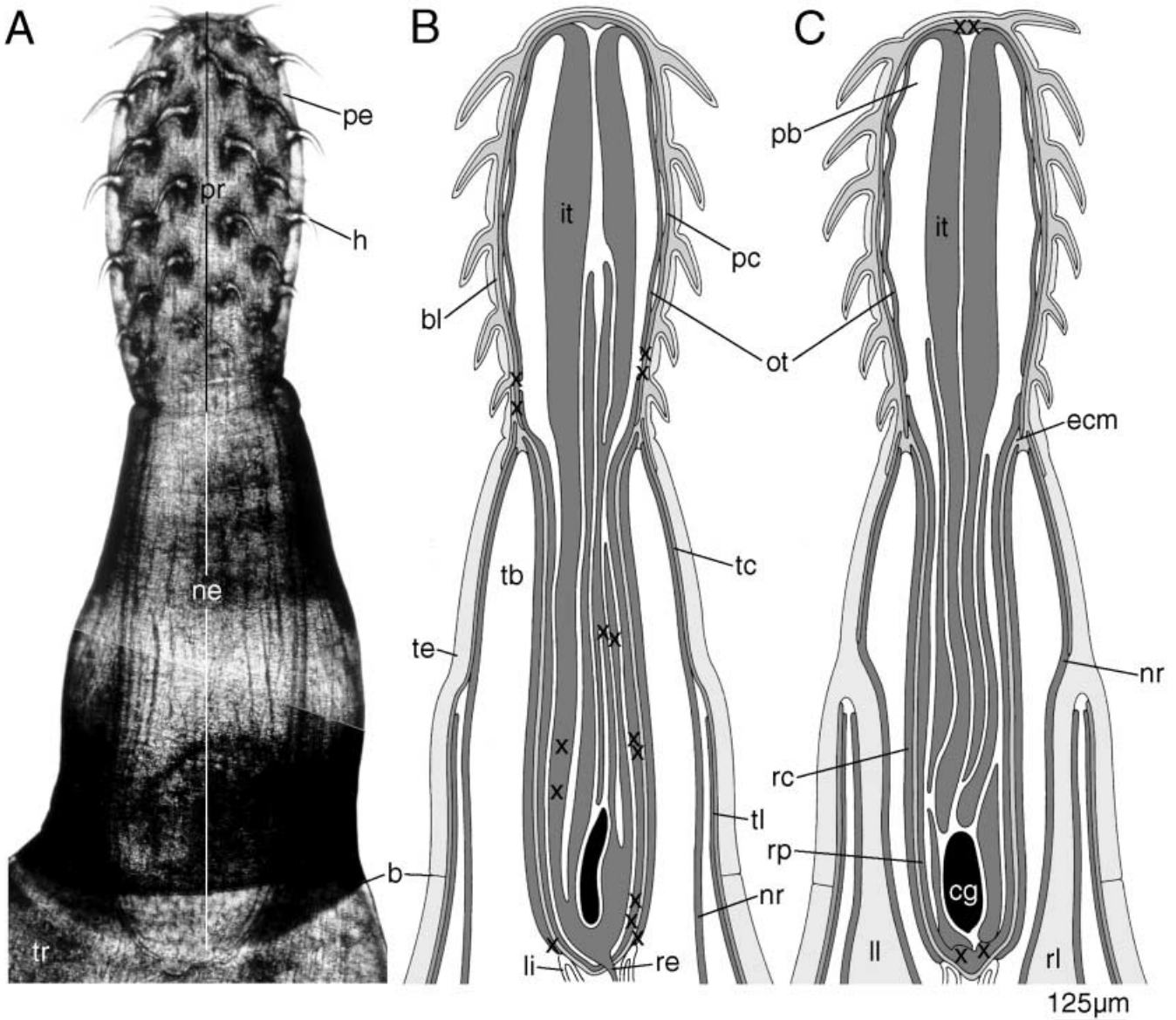


Fig. 3A–C *A. anguillae*; praesoma and anterior trunk of a slightly contracted female. **A** Light micrograph of a living specimen. **B, C** Drawings of a longitudinal (**B**) and a horizontal (**C**) section on the basis of cross-sections (5 µm); every *x* corresponds to a nucleus. *b* Border between the praesomal epidermis and that one of the trunk, *bl* basal lamina, *cg* cerebral ganglion, *ecm* extracellular matrix, *h* hook, *it* inner tube of the proboscis retractor, *li* ligament sacks, *ll* left lemniscus, *ne* neck, *nr* neck retractor, *ot* outer tube of the proboscis retractor, *pb* praesomal body cavity, *pc* praesomal circular musculature, *pe* praesomal epidermis, *pr* proboscis, *re* process of the proboscis retractor, *rc* receptacle constrictor, *rl* right lemniscus, *rp* receptacle, *tb* body cavity of the trunk, *tc* circular musculature of the trunk, *te* epidermis of the trunk, *tl* longitudinal musculature of the trunk, *tr* trunk

culature of the trunk that lines the trunk and the entire praesomal neck, and the longitudinal musculature of the trunk that lines the trunk and the neck up to the lemnisci. A praesomal longitudinal body wall musculature is absent. At the base of the proboscis, the praesomal circular musculature and that one of the trunk overlap for a distance of almost 10 µm (Fig. 3B, C). Both circular muscle

syncytia as well as the longitudinal musculature of the trunk consist of networks of anastomosing muscle fibres. Most of the muscle fibres are circomyary (Fig. 2A, B).

In nine specimens (all seven females and two male ones), the praesomal circular musculature contained six nuclei whereas in two specimens, which were males, only four nuclei were present. In all specimens two of the nuclei were located in a cytoplasmic swelling at the proboscis apex (Fig. 3B, C).

The unpaired proboscis retractor consists of anastomosing longitudinal circomyary muscle fibres that form two concentric tubes within the proboscis (Fig. 2A). The peripheral layer of contractile filaments of the muscle fibres is thin compared to the cytoplasmic inside (Fig. 2A, B, D). Both tubes of the proboscis retractor fuse at the proboscis apex (Fig. 3B, C). Posteriorly, the outer tube extends up to the anterior end of the receptacle whereas the inner tube stretches throughout the entire praesoma (Figs. 2A, B, D, 3B, C), pene-

trates the receptacle with one to three processes, depending on the specimen (Figs. 2E, 3B), and – now called receptacle retractor – inserts at the body wall of the trunk. Within the praesoma the proboscis retractor constantly includes two pairs of nuclei which are located within the central cytoplasm of distinct muscle fibres (see Fig. 3B).

The receptacle inserts at the base of the proboscis at the body wall. Its posterior end extends into the body cavity of the trunk. Apart from one female with eight nuclei the receptacle contains six nuclei that are located within swellings of the cytoplasm (Fig. 3B, C). A strong layer of extracellular matrix separates the receptacle from the binucleate receptacle constrictor (Fig. 3B, C). The receptacle constrictor and the receptacle principally show a corresponding organisation; both consist of a strong layer of circular contractile filaments and a thin central layer of cytoplasm (Fig. 2D).

The syncytial neck retractor again consists of longitudinal circomyary muscle fibres that are arranged like a meshwork of anastomosing tubes. The neck retractor inserts posteriorly to the receptacle constrictor and runs through the body cavity of the trunk to its posterior insertion at the body wall (Figs. 2D, E, 3B, C).

The lateral posterior nerves are surrounded by two retinacle muscles with a single nucleus between the receptacle and the receptacle constrictor each (Fig. 2E).

D. Discussion

I. General praesomal features

The syncytial epidermis with bundles of filaments and an internal layer as well as the hooked proboscis of *A. anguillae* belongs to the ground pattern of the Acanthocephala. Internal or striped layer, bundles of filaments and sperm ultrastructure support the hypothesis that *Seison* is the sister taxon of the Acanthocephala. Based on this hypothesis the syncytial epidermis with an internal layer and bundles of filaments is a plesiomorphic characteristic of the Acanthocephala, while a hooked proboscis is an autapomorphy of this taxon (Ahlrichs 1995, 1997).

In accordance with the present findings for *A. anguillae* the hooks of *Paratenuisentis ambiguus* (Van Cleave, 1921) (Eoacanthocephala) and *Moniliformis moniliformis* (Bremser, 1811) (Archiacanthocephala) consist of a distal *lamina densa* and a proximal *lamina fibroreticularis* (Taraschewski et al. 1989; Herlyn 2000). In all Acanthocephala species investigated so far the hooks border to the basal lamina (Taraschewski et al. 1989; Herlyn 2000; Taraschewski 2000). Thus, the ground pattern of the Acanthocephala can be substantiated to that effect that the hooks of the autapomorphic proboscis consist of a *lamina fibroreticularis* and a *lamina densa*, both derivatives of the basal lamina.

The present study confirms earlier observations that the cerebral ganglion of Acanthocephala species is sub-

divided into a central neuropile and peripherally located perikarya. Lateral posterior nerves as present in *A. anguillae* are also known from other Acanthocephala species (see, for example, Meyer 1933). Both structures, an unpaired cerebral ganglion and paired lateral posterior nerves, represent ground-pattern characteristics of the Acanthocephala (Ahlrichs 1995).

II. Musculature

The existence of a praesomal circular musculature combined with the absence of a praesomal longitudinal musculature, as described here for *A. anguillae*, is known for representatives of the Palaeacanthocephala, Eoacanthocephala and Archiacanthocephala (Kilian 1932; Meyer 1933; Herlyn 2000). Otherwise, Nickol and Holloway (1968) described a longitudinal body wall musculature for the proboscis of *Corynosoma hamanni* (Linstow, 1892) (Palaeacanthocephala), and Hammond (1967) mentioned for *Acanthocephalus ranae* (Schrank, 1788) (Palaeacanthocephala) that the muscular fibres beneath the basal lamina of the praesoma “appear to be longitudinally orientated”. However, the line drawings of *C. hamanni* (Nickol and Holloway 1968 Figs. 6, 9) show that the description refers to the outer tube of the retractor. Therefore, it can be assumed that the existence of a praesomal circular musculature combined with the absence of a praesomal longitudinal musculature belongs to the ground-pattern characteristics of the Acanthocephala, whereas the indication of Hammond (1967) of longitudinally orientated muscle fibres beneath the praesomal epidermis of *A. ranae* needs re-examination.

Regarding the number of proboscis retractors within the praesoma of a single specimen, differing data can be found in the literature. There are reports of two or more proboscis retractors in one specimen for *Macracanthorhynchus hirudinaceus* (Pallas, 1781) (Archiacanthocephala) and *Neoechinorhynchus emydis* (Leidy, 1851) (Eoacanthocephala) (Van Cleave and Bullock 1950; Dunagan and Miller 1974). On the other hand, there are descriptions of an unpaired proboscis retractor in *A. anguillae*, *P. ambiguus* and, again, *M. hirudinaceus* (Meyer 1933; Herlyn 2000). In accordance with the latter findings, Harada (1929) mentioned that the muscle fibres of the proboscis retractor of *Bolbosoma turbinella* (Diesing, 1851) (Palaeacanthocephala) are interconnected. The inconsistency can be solved as the reports on *M. hirudinaceus* may show. Dunagan and Miller (1974) termed the different fibres of the proboscis retractor with own names. This can be helpful, especially with respect to the function of the fibres. However, one should be aware that all the fibres are interconnected by anastomoses, thus, forming an unpaired proboscis retractor (see Meyer 1933). Probably, such an unpaired proboscis retractor belongs to the ground-pattern characteristics of the Acanthocephala.

An unpaired receptacle as described here in *A. anguillae* is known for many species including representatives

of the genus *Aporhynchus* (Archiacanthocephala), even though it is very small in this taxon (Meyer 1933). Therefore, a receptacle without doubt also belongs to the ground pattern of the Acanthocephala. Finally, because of its obligate presence within all Acanthocephala species (Meyer 1933) a neck retractor can be assumed for the ground pattern of the Acanthocephala. Outside the Acanthocephala such a receptacle and neck retractor are unknown. Thus, they probably represent autapomorphies of the Acanthocephala.

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