

N. Hehn · U. Ehlers · H. Herlyn

Ultrastructure of the acanthella of *Paratenuisentis ambiguus* (Acanthocephala)

Received: 7 October 2000 / Accepted: 18 January 2001 / Published online: 4 April 2001
© Springer-Verlag 2001

Abstract The fine structure of the early acanthella of *Paratenuisentis ambiguus* (Eoacanthocephala) was investigated. This developmental stage is characterised by losing its ability to move and by differentiation of adult structures. The frontal syncytium, present in the first developmental stage (the acanthor), is lost, while the epidermis and central syncytium persist. The epidermis of the acanthella contains a number of giant nuclei that are arranged into several small groups. The central syncytium is subdivided into different masses, containing nuclei that will give rise to the organs of the adult. The ‘uncinogenous bands’ extend into the anterior body of the acanthella. Formation of the hooks takes place within these strands. In all investigated stages no extracellular materials were observed. Posterior of the uncinogenous bands lies the brain anlage and the primordia of the reproductive system. Neither a sense organ nor a nervous system were found.

Introduction

The Acanthocephala were first named by Pallas (1760), who placed them within the Cestoda. At the end of the century, however, their unique organisation was recognised and they were considered as a new taxon. Many studies on adult Acanthocephala have followed. Their ontogenesis has been of hardly any interest; their life cycles, but not the structural organisation of the larvae, have been the main focus of attention. The first morphological studies of the larvae were made by Kaiser (1893). But this and all following studies have shown that light microscopic evidence leaves a lot of un-

answered questions. Specifically, the question of the origin of different structures, such as the hooks of the proboscis, has remained unanswered until now.

The development of Acanthocephala begins in the egg while it is still in the body cavity of the female. When it leaves the female, the acanthor has finished its development. The egg with the acanthor is discharged with the faeces of the definite host and is swallowed by the first host, an arthropod. After ingestion, the acanthor penetrates the intestinal wall of the arthropod host and enters into the body cavity, where further development takes place. Van Cleave (1935) defined different developmental stages and named the stage in the egg ‘acanthor’ and the following stage in the arthropod host ‘acanthella’; this latter was defined as the stage between acanthor and adult. Some authors refer to a ‘cysthacanth’, if an encapsulated form results at the end of development.

The acanthor was described following an ultrastructural examination by Albrecht et al. (1997, see also Taraschewski 2000). A shelled acanthor is enveloped by five eggshells, and consists of three syncytial layers: frontal, central and epidermal. The frontal syncytium probably has a glandular function and helps to break through the intestinal wall of the first host. The central syncytium forms the acanthor musculature and the epidermal layer surrounds both other syncytia. Hooks of the acanthor are epidermal differentiations.

After the penetration of the first host, the acanthor loses its musculature and hooks after 2–5 days and becomes immobile. All organs are formed new, except for the epidermis, which persists in the same mode as it was formed in the acanthor. The central syncytium is the starting point for organogenesis.

Materials and methods

Specimen collection

Adult females of *Paratenuisentis ambiguus* were collected from *Anguilla anguilla*, which were caught in the Weser near Gimte

N. Hehn (✉) · U. Ehlers · H. Herlyn
Institut für Zoologie und Anthropologie,
Universität Göttingen, Berlinerstrasse 28,
37073 Göttingen, Germany
E-mail: nhehn@gwdg.de
Tel.: +49-55-1395525

(Germany). The eggs were isolated from the body cavity under a stereo microscope and stored in 0.9% NaCl.

A few specimens of *Gammarus tigrinus* were isolated from an existing breeding colony and kept at a temperature of $15 \pm 2^\circ\text{C}$. After being left without food for 3 days the amphipods were fed with the acanthocephalan eggs and, after another day, normal feeding was resumed. About 5 weeks (41 days) p.i. the amphipods were dissected and acanthellae prepared for electron microscopy.

Electron microscope preparations

Three acanthellae were fixed in 2.5% glutaraldehyde in 0.1 mol sodium cacodylate buffer/l (pH 7.2) for 2 h at 4°C , dehydrated in a series of ethanol and embedded in Araldite for standard electron microscopy. Ultrathin sections were viewed with a Zeiss EM 900 electron microscope.

Results

The acanthella showed an unsegmented oval to elongated form. A dorso-ventral axis was not seen. The measurements of the examined specimens were 300–400 μm in length and 50–80 μm in width. Although all acanthellae were 41 days old, two larvae showed distinct signs of further differentiation than the third one.

Epidermis

The epidermis was a thick layer of 5–25 μm and showed many vesicles in the older specimen. In the younger larva the vesicles were confined to an area of 1.3 μm at the outer layer of the epidermis, which, in all species, bears many invaginations, opening via pores onto the body surface. The inner surface of the body wall showed many condensations.

Within the epidermis 14–19 giant nuclei and an undefined number of smaller nuclei were present. The giant nuclei form several groups during development. Three

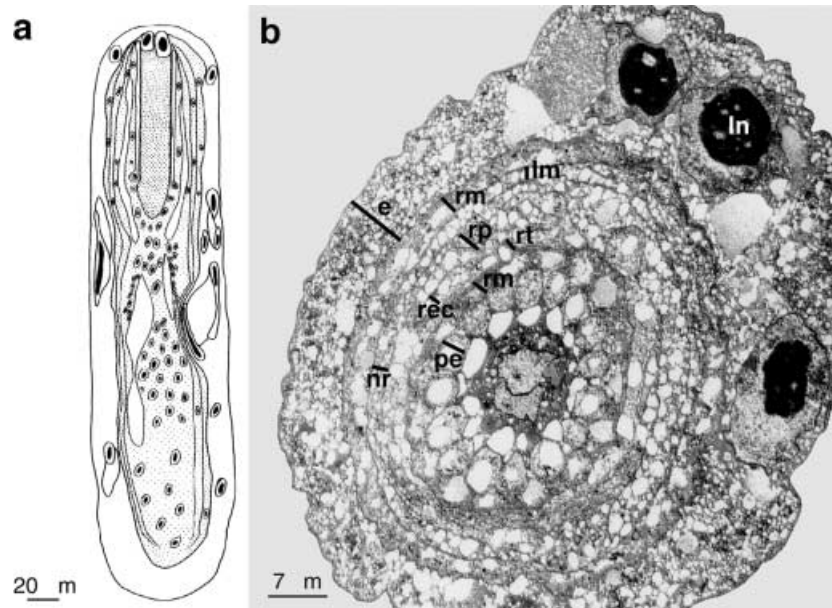
nuclei are found in the apical tip, six more in two groups of three nuclei further behind in the anterior half of the body. The rest of the nuclei were irregularly distributed over the posterior half of the body. Large cavities were found next to the most posterior-lying giant nuclei (Figs. 1a, 2b, c).

Central syncytium

The central syncytium of the youngest larva was hardly differentiated. It showed the beginnings of dividing into small masses and forming uncinogenous bands.

The central syncytium of the older larvae is subdivided into groups of nuclei and their surrounding cytoplasm. The groups are separated from each other by membranes. The cytoplasm that surrounds the three apical nuclei forms a cone of about 140 μm in length. This cone is electron-dense and in some areas shows diluting tendencies (Fig. 1b). It contains no cell organelles and is separated by a membrane from the more distally lying structures. The cone is surrounded by 16–18 circularly arranged uncinogenous bands, which are separated from each other by membranes. These strands expand from eight cytoplasmic bulbs, which contain the nuclei (Figs. 1a, 2a). Small cavities

Fig. 1 **a** Schematic drawing of the acanthella, showing the layers and the cytoplasmic strands in the frontal part and the lacunar system developing near the giant nuclei of the epidermis. **b** The cross-section through the anterior end shows the thick epidermis (*e*) and the layers separated from the central syncytium. The cytoplasmic strands (*cs*), surrounding the centre, represent the future praesomal epidermis (*pe*), as well as the region where the hooks are segregated. The strands are surrounded by the layers in which central syncytium is subdivided. (*an* apical nuclei, *cm* circular muscles, *lm* longitudinal muscles, *nr* neck retractor, *pp* proboscis protruser, *r* retractor, *rec* receptuculum)



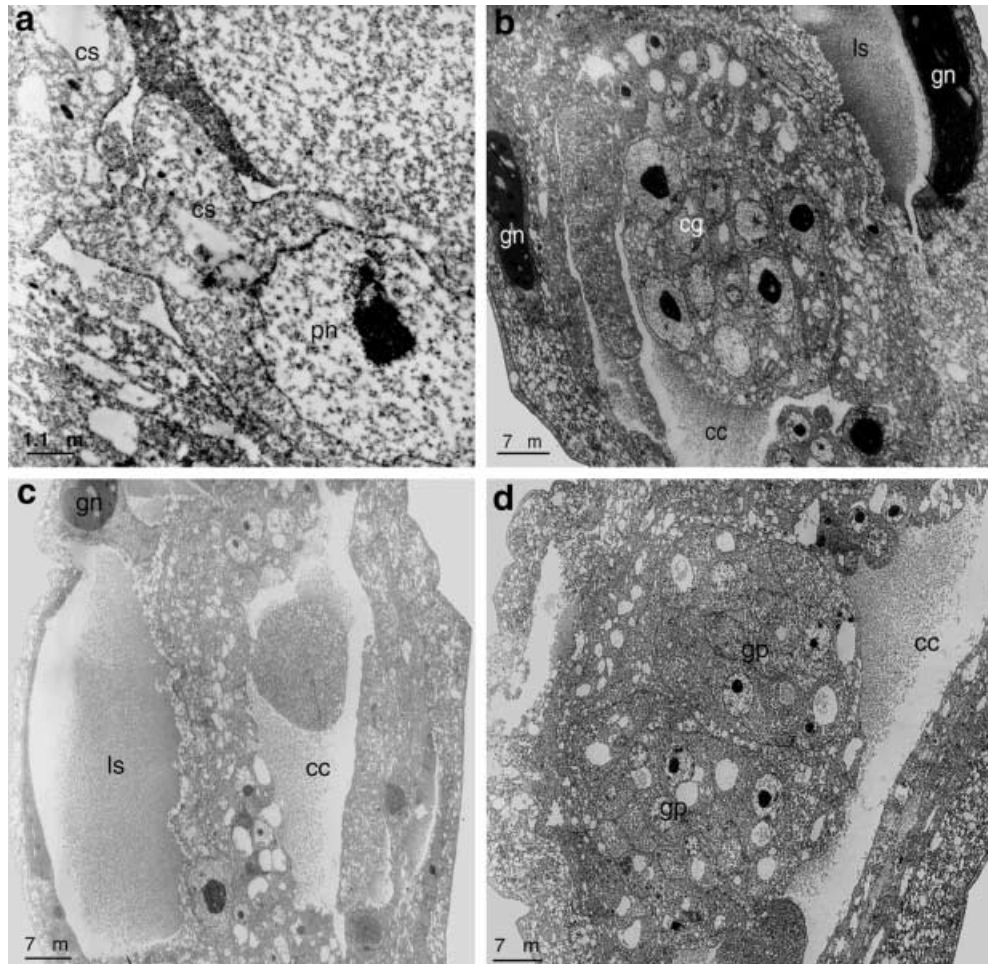


Fig. 2 **a** Cross-section through the end of the cytoplasmic strands (*cs*) showing the proboscis nuclei (*pn*) lie within cytoplasmic bulbs, from which the cytoplasmic strands extend. **b** Posterior of the proboscis nuclei, a nuclear mass of the central syncytium forms the primordia of the cerebral ganglion (*cg*). Next to it begins a cavity that represents the forming of the pseudocoel (*cc*). In the epidermis the giant nuclei (*gn*) and the lacunar system (*ls*) are to be seen. **c** The body cavities (*cc*) and the lacunar system (*ls*) expand widthwise and forge the central syncytium into a small strand between them. **d** Cross-section through the posterior end, displaying two major groups of central nuclear masses, probably forming the genital primordia (*gp*)

arranged like a string of beads are to be seen within the strands.

Distal of the strands, the central syncytium is divided into layers (Fig. 2). It starts with the separation into four main layers, which can be subdivided so that nine layers arise. These layers show no differences in structure, containing vesicles and nuclei in different amounts and sizes. Only two of the layers persist throughout the animal up to the posterior end. Caudal of the cytoplasmic strands are to be seen different groups of nuclei that are separated from each other by membranes. Two central groups in the posterior end are particularly obvious (Fig. 2d). In the larvae examined, no further differentiated brain anlage or nerves were observed.

Central cavities

Besides the cavities in the epidermis, a few more cavities are present in the central syncytium. The youngest of the three examined specimens showed a big cavity in the middle part of the body, surrounded by nuclei containing cytoplasm. The older larvae, however, had smaller cavities that were separated from each other by parts of the central syncytium.

The biggest cavity in one of the older larvae began at 170 μm between the two innermost layers. In this area the central syncytium is shaped like a small band between the central cavity and the lacunar system (Fig. 2c). Anterior and posterior of this big cavity are smaller ones.

Discussion

According to the literature on the development of the inner organisation (DeGiusti 1949; Moore 1962; Harms 1967; Wanson and Nickol 1975), the examined acanthellae are apparently in a relatively early stage of development. Samuel and Bullock (1981) found that the development of the acanthella of *Paratenuisentis ambiguus* lasts 32 days at a temperature of 22–23°C,

while at a temperature of $13 \pm 2^\circ\text{C}$ the development of adult structures progressed much more slowly. The examined species had a delayed development, due to the low temperature at which the amphipod host was kept.

The epidermis

The epidermis of the acanthor seems to persist in the acanthella. In the stages examined it is not differentiated in different regions (striped or canal layer, fibrous felt layer, radial layer) as described for the adult (Crompton and Lee 1965; Hammond 1967; Cheng 1986; Taraschewski 2000).

The number of nuclei is not constant in *P. ambiguus*, but the distribution seems to be mostly consistent. The six giant nuclei in the first third of the body represent the nuclei of the lemniscs, which correlates with the results of Samuel and Bullock (1980).

The epidermal cavities that are attached to the more posterior-lying six to eight giant nuclei are the developing lacunar system that, in the adult, consists mainly of two large channels that lie near the epidermal nuclei (Lühe 1911; Herlyn 2000). The cavities come into contact during larval development.

Proboscis and hooks

The three nuclei found in the apical tip correspond with the apical nuclei identified in other species (Hopp 1953; Harms 1965; Samuel and Bullock 1980; Amin 1982), whose function is unknown. They serve as a limiting structure of the apical epidermis and are diluted at the end of development. The uncinogenous strands were also seen in light microscopic studies (DeGiusti 1949; Cable and Dill 1967; Samuel and Bullock 1980, for example). From their position and number of the layers it is probable that these strands represent the region where the hooks will be formed. Hynes and Nicholas (1957) described a balloon-like riffled structure that is subdivided into smaller parts, each of which emanates a hook. The lack of extracellular material prevents the exact region for the forming of the hook from being determined. The tip of the hook probably develops within the strand, whereas the root is a derivative of the basal lamina. This would support the results of Crompton and Lee (1965) and Taraschewski (2000), who found that the hooks consist of cytoplasmic and connective tissue parts. Alternatively, as the hooks in the adult are covered by the epidermis, there is the possibility that they develop within the strand.

The number of developing layers in the acanthella corresponds to the number of tissue layers in the adult; it can therefore be said that the uncinogenous bands are the region of hook development and represent the epidermis of the proboscis. This means that the proboscis, including the proboscis epidermis, does not only

develop inverted, but as a division of the central syncytium. The epidermis of the body and the epidermis of the proboscis therefore will fuse during the extrusion of the proboscis.

The musculature

Although no muscle fibres are found in this developing stage, it is obvious that the musculature of proboscis and body will arise from the nine layers around the cytoplasmic strands, because of their number and position. In the inverted state of the proboscis, the muscle layers are found multiplied. The innermost layer represents the circular muscles, distally follows the proboscis retractor, then the receptaculum, the proboscis protruder and the neck retractor. The outermost layers are the longitudinal and circular muscles of the body.

Hynes and Nicholas (1957) and Crompton and Nickol (1985) described the differentiation of the retractor from a central strand of proboscis tissue that remains after the protrusion of the proboscis. The body musculature is positioned directly under the epidermis, which matches the results of Hynes and Nicholas (1957) who found a cylindrical structure, with a central strand, said to form the ligament for *Polymorphus minutus*. The cylinder expands and comes to lie under the epidermis, where it forms the body musculature.

Development of the posteriorly lying organs

The nuclear strand within the central syncytium is divided into smaller groups of nuclei that will form the ligament, the reproductive organs and the genital primordium. Nothing was found of the ligament, but in one of the examined animals the probable genital primordium, the central syncytium, was observed to be divided into two major groups of nuclei. These are probably the primordia of the testes, as described by Harms (1965), Samuel and Bullock (1980) and Amin (1982). In another animal of the same age there was one compact mass of nuclei in the corresponding position, which could represent the primordia of the ovaries, as referred to by Hynes and Nicholas (1957), Cable and Dill (1967) and Samuel and Bullock (1980).

The cavities in the central syncytium seem to be the developing pseudocoel. The results of Meyer (1933), who states that the pseudocoel is not a real coelom because it originates in the break of the central syncytium and is not surrounded by membranes, could not be confirmed. The different sizes of the cavities in the larvae indicate the partitioning of the big cavity by forming tissue and moving cytoplasm.

Acknowledgements This work was supported by the DFG (EH 175/2-1) and the Akademie der Wissenschaften und der Literatur Mainz.

References

- Albrecht H, Ehlers U, Taraschewski H (1997) Syncytial organisation of Acanthors of *Polymorphus minutus* (Palaeacanthocephala), *Neoechinorhynchus rutili* (Eoacanthocephala) and *Moniliformis moniliformis* (Archiacanthocephala) (Acanthocephala). *Parasitol Res* 83:326–338
- Amin OM (1982) Description of larval *Acanthocephalus parksidae* Amin 1975 (Acanthocephala, Echinorhynchidae) from its isopod intermediate host. *Proc Helminthol Soc Wash* 49:235–245
- Cable RM, Dill WT (1967) The morphology and life history of *Paulisentis fractus* VanCleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). *J Parasitol* 53:810–817
- Cheng TC (1986) Acanthocephala: the spiny-headed worms. In: Cheng TC (ed) *General parasitology*. Academic Press, Orlando, pp 445–463
- Crompton DWT, Lee DL (1965) The fine structure of the body wall of *Polymorphus minutus* (Goeze 1782) (Acanthocephala). *Parasitology* 55:357–364
- Crompton DWT, Nickol BB (eds) (1985) *Biology of the Acanthocephala*. Cambridge University Press, Cambridge
- DeGiusti DL (1949) The life cycle of *Leptorhynchoides thecatus* (Linton), an Acanthocephala of fish. *J Parasitol* 35:437–460
- Hammond RA (1967) The fine structure of the trunk and praesoma wall of *Acanthocephalus ranae* (Schrank 1788) Lühe 1911. *Parasitology* 57:475–486
- Harms CE (1965) The life cycle and larval development of *Octospinifer macilentis* (Acanthocephala: Neoechinorhynchidae). *J Parasitol* 51:286–293
- Herlyn H (2000) *Zur Ultrastruktur, Morphologie und Phylogenie der Acanthocephala* (dissertation). Logos Verlag, Berlin
- Hopp WB (1953) Studies on the morphology and life cycle of *Neoechinorhynchus emydis* (Leidy), an Acanthocephalan parasite of the Map Turtle *Gratemys geographica* (Le Sueur). *J Parasitol* 39:284–299
- Hynes HBN, Nicholas WL (1957) The development of *Polymorphus minutus* (Goeze 1782) (Acanthocephala) in the intermediate host. *Ann Trop Med Parasitol* 51:380–391
- Kaiser J (1893) Die Acanthocephalen und ihre Entwicklung. *Bibl Zool* 7:148 ff
- Kates KC (1943) Development of the swine thorn-headed worm, *Macracanthorhynchus hirudinaceus*, in its intermediate host. *Am J Vet Res* 4:173–181
- Lühe M (1911) Acanthocephalen. Register der Acanthocephalen und parasitischen Plattwürmer, geordnet nach ihren Wirten. In: Brauer A (ed) *Die Süßwasserfauna Deutschlands. Eine Exkursionsfauna*, vol 16. Fischer, Jena, pp 1–116
- Meyer A (1933) Acanthocephala. In: Bronn HG (ed) *Bronns Klassen und Ordnungen des Tierreiches*, vol 4(2/2). Akademische Verlagsgesellschaft, Leipzig
- Moore DV (1962) Morphology, life history and development of the Acanthocephalan *Mediorhynchus grandis* Van Cleave 1916. *J Parasitol* 78:76–85
- Pallas PS (1760) *Dissertatio medica inauguralis de infestis viventibus intra viventia* (thesis). Lugdunum Batavorum, Haak
- Samuel G, Bullock WL (1981) Life cycle of *Paratenuisentis ambiguus* (Van Cleave 1921) Bullock and Samuel 1975 (Acanthocephala: Tenuisentidae). *J Parasitol* 67:214–217
- Taraschewski H (2000) Host–parasite interaction in Acanthocephala: a morphological approach. *Adv Parasitol* 46:1–179
- Van Cleave HJ (1935) The larval stages of Acanthocephala. *J Parasitol* 21:435–436
- Wanson WW, Nickol BB (1975) Presomal morphology and development of *Postorhynchus formosus*, *Prothenorchis elegans*, and *Moniliformis dubius* (Acanthocephala). *J Parasitol* 145:75–83