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On the Enigmatic Hook of the Metaradiophryids (Alveolata, Ciliophora)

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Abstract. The astomatous metaradiophryids are ciliates which live endosymbiotically in earthworms (Annelida, Lumbricidae). Their prominent hook apparatus is demonstrated in detail in light micrographs of living organisms as well as in scanning electron micrographs of Parducz-fixed cells. Since it was first observed, this structure has been interpreted as a 'holdfast' organelle preventing ciliates from being expelled prematurely from the intestine of the worm along with its excrements. No active movement of the hook has been reported in earlier papers or in our recent studies. Nevertheless, a detailed description of different parts of the hook apparatus exists in older literature, including a hypothesis on how these elements interact with each other to function as a holdfast device – without any experimental evidence. The suspected mode of function of this structure is questioned and critically discussed.

Key words: Astomatous ciliates, *Metaradiophrya* spec., hook structure, holdfast organelle.

INTRODUCTION

Earthworms (Annelida, Lumbricidae) are known to harbour endosymbiotic astomatous ciliates in their intestines. For *Eisenia foetida*, which is abundant in dung and compost, the genera *Anoplophrya*, *Maupasella* and *Metaradiophrya* have been described (Dixon 1975, Günther 2013). The metaradiophryids are insofar of special interest as they exhibit a conspicuous hook (Fig. 1) which has, due to its obtruding anatomy, been interpreted as being a holdfast device since the beginning of its investigation (Stein 1854, Heidenreich 1935, Beers 1938, Williams 1942); this interpretation became readily established in early text books (e.g., Doflein and Reichenow 1953).

This study demonstrates in detail the microanatomy of the hook apparatus via the application of DIC light and conventional scanning electron microscopy. The suspected mode of function of this structure as a holdfast organelle is questioned and discussed in the light of our recent observation of living and fixed ciliates.

MATERIALS AND METHODS

Eisenia foetida was collected from a dung heap of an agricultural farm in Düsseldorf, Germany. To obtain ciliates, the worms were narcotized for 30 sec in 60% ethanol, transferred to a Petri dish filled with Locke-Ringer's solution (Hayat 2000), and then cross cut

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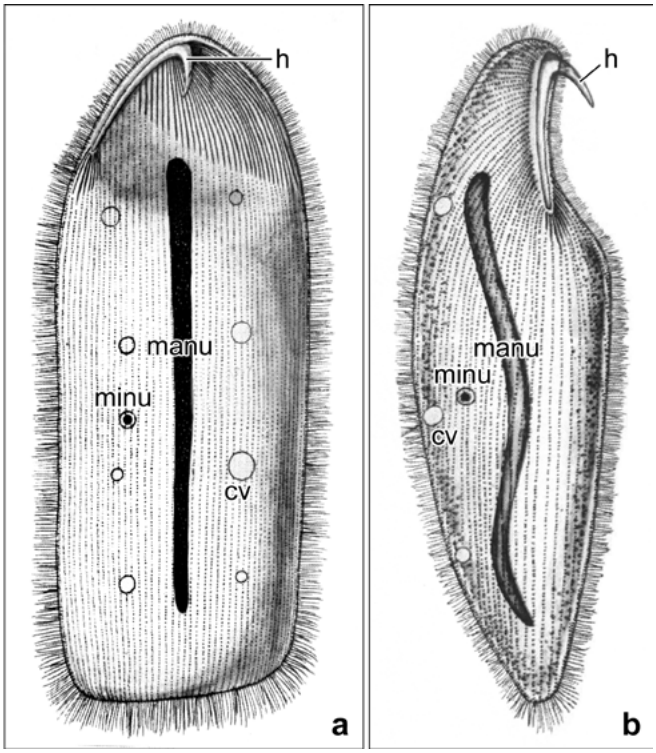


Fig. 1. a – ventral view of a medium-sized *Metaradiophrya asymmetrica* (135 µm × 55 µm) fixed in Schaudinn's fluid and stained in Mallory's anilin blue-orange G-acid fuchsin; **b** – right lateral view showing the normal shape of the ciliate; cv – contractile vacuole, h – hook, manu – macronucleus, minu – micronucleus (after Beers 1938).

in pieces of a few centimetres in length. The ciliates were obtained by squeezing out the intestinal contents of these pieces. The ciliates were isolated using a fine pipette and collected in a small glass dish.

Light microscopic examinations of living ciliates were made using a LEICA DMLB microscope equipped with differential interference contrast optics. Micrographs were taken with a CANON 5D Mk2 camera using a METZ 45 CT3 electronic flash for illumination.

For scanning electron microscopy (SEM), the ciliates were fixed for 30 min at room temperature with Parducz's fixative (Parducz 1967). Fixed cells were washed 3 × 30 min in distilled water and dehydrated in a graded series of ethanol. Cells were dried in a BAL-TEC CPD 030 apparatus. After coating with gold in a BALZERS UNION SCD 040 sputter device, cells were examined with a FEI Quanta 200 ESEM.

RESULTS

1. Light microscopy

As revealed by light microscopical analyses of living organisms, most of the examined metaradiophryids were about 150 µm long and 100 µm wide, in extremis 200 µm long and 150 µm wide. The anterior end of the cell body is rounded (Fig. 2a–c) while the posterior end is either rounded (Fig. 2a) or truncated (Fig. 2b). Two rows of contractile vacuoles (4–8 vacuoles per row) are located laterally to the oblong macronucleus (Fig. 2a–c). Frequently division stages are seen (Fig. 2c) with a horizontal division furrow (Fig. 2c, d). Most specimens belonged to *Metaradiophrya lumbrici*; very few were identified as *M. falcifera* (not shown).

The most prominent structure of the ciliate is the hook apparatus at the anterior cell pole (Fig. 2e). In ventral views, the 13–15 µm-long hook is connected to a 3.5–4.0 µm-wide strip which extends over a length of 45 µm along the right margin of the cell. Towards the left margin, no strip (at least not comparable with that of the right margin) is seen, but rather a 30 µm-long and 2.5–2.7 µm-wide band-like, somewhat darker, fuzzy structure. In the antero-ventral region, below the hook, a cell excavation or depression is obvious.

We were able to observe neither any active movement of the hook apparatus nor any body contraction.

2. Scanning electron microscopy

In scanning electron micrographs of the anterior, depressed part of metaradiophryids, the hook area is characterized by a ciliature which differs from that of the other parts of the cell. A tuft of relatively short cilia is evident in the right area of the depression (Fig. 3a, b, e, asterisk). Their length varies between 1.5–10 µm in different cells. The somatic cilia close to this tuft are markedly longer and exhibit another beat pattern (Fig. 3e, arrowhead).

The hook is a very prominent structure, measuring 10–13 µm in length. It shows the same position and the same inclination from the cell surface in all examined specimens of *M. lumbrici* (Fig. 3b–d). There is no clear indication of movement. The hook is located between two strips which form an inverted V-structure (Fig. 3f). This is in contrast to what could be observed using light microscopy, where exclusively the right strip is visible as a very prominent structure. Consequently, this right strip is intracellularly underlined by a solid, high-contrast material, whereas the left strip is mainly visible from outside underlined by a faint, low-contrast material as indicated by the band-like structure in light micrographs. In the SEM, the right strip appears as a 20–45 µm long and 3 µm broad structure giving rise to about 24 skeletal rays. The left strip is a bit shorter (19–35 µm) and gives rise to only about 11 rays. Generally, there was a smooth transition between the strips

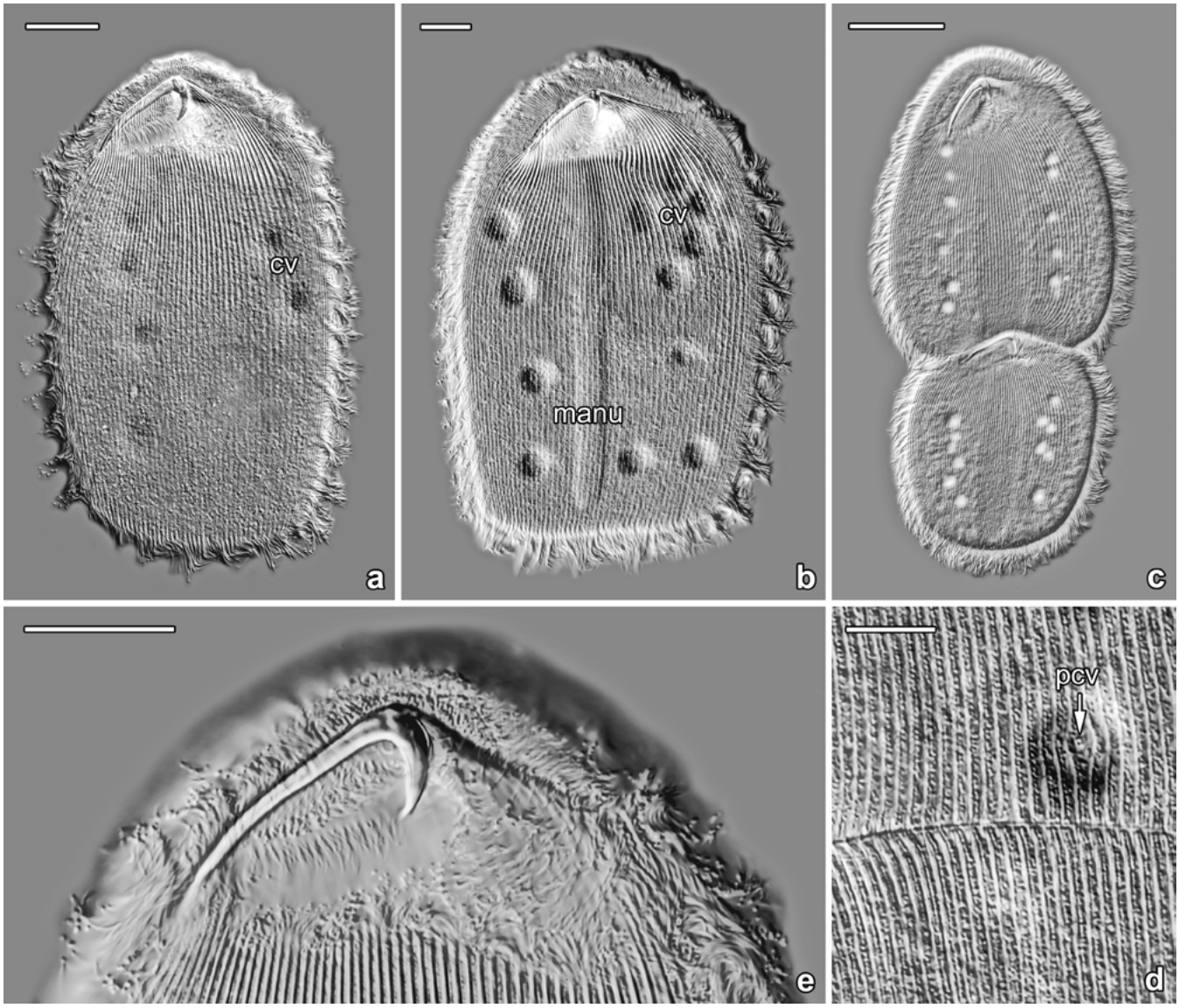
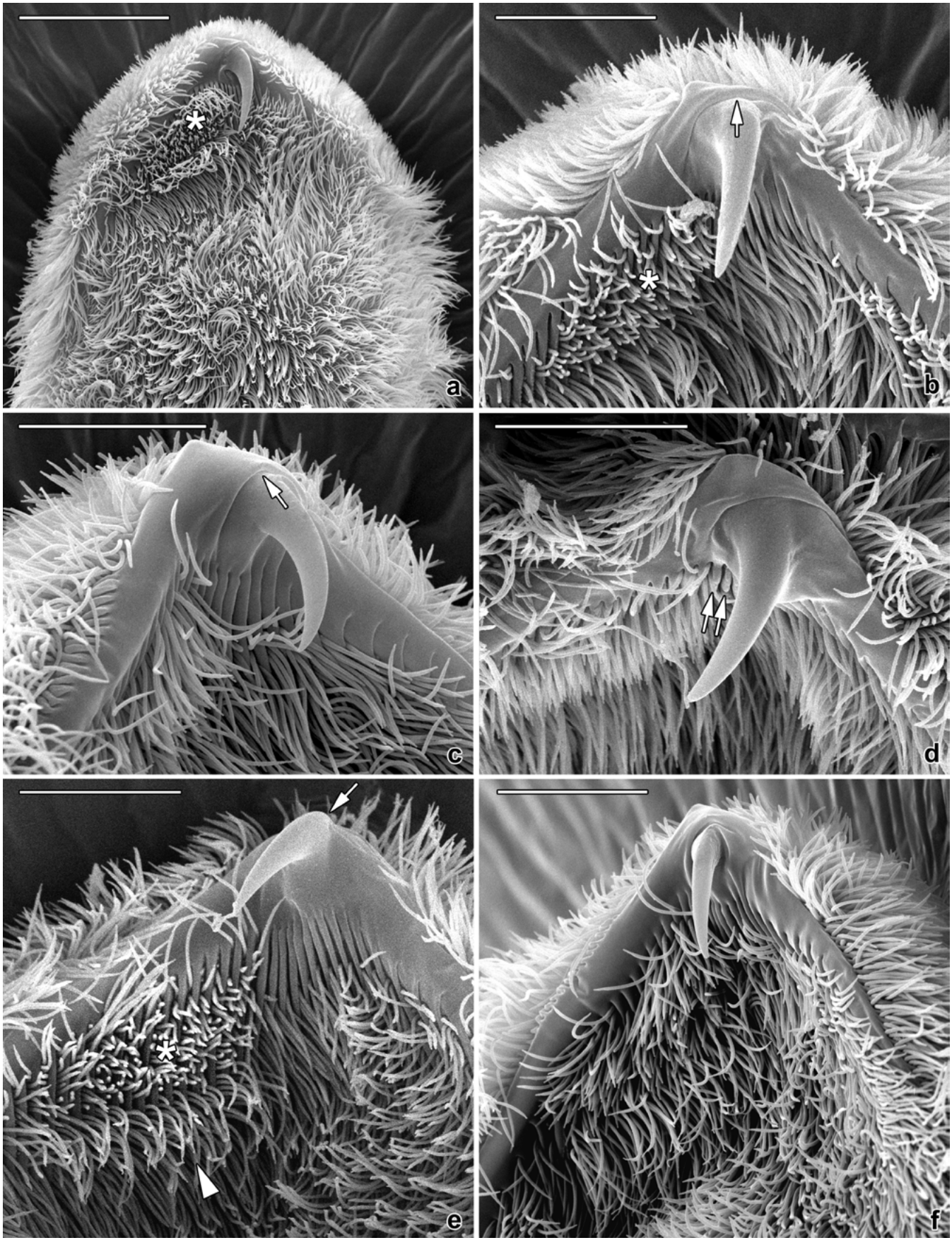


Fig. 2. *Metaradiophrya* from life under differential interference contrast illumination (DIC). **a, b** – ventral views of the ciliate; **c** – division stage; **d** – division furrow; **e** – anterior part of the ciliate showing the details of the hook region. cv – contractile vacuole, manu – macronucleus, pcv – porus of contractile vacuole. Scale bars: 20 µm (a, b, e), 50 µm (c), 10 µm (d).

and rays (Fig. 3b, c, e, f) but sometimes the rays exhibited a knee-like bending proximally (Fig. 3d, arrows). The apex of the hook frequently shows a conspicuous fold (Fig. 3b–d, arrow). Occasionally this fold is missing (Fig. 3e, arrow).

DISCUSSION

Despite the fact that astomatous ciliates from earthworms have been known for numerous decades (e.g. Stein 1854), this is to our knowledge the first report illustrating living metaradiophryids. We agree with the statement made by Beers in 1938: "Undoubtedly the skeletal elements are the structures of greatest interest in *Metaradiophrya*." From its first description this structure has been interpreted as a holdfast organelle preventing the ciliates from being expelled prematurely from the intestine of the worm along with its excrements. This explanation is very tempting due to the obtruding anatomy of the hook region. However, Beers reports later on in the same article: "I have been


a
b
c
d
e
f

quite unable to identify either in the living animals or in stained whole mounts and sections any myonemes for moving the hook. Neither have I been able to observe in living specimens any movement of the hook." We confirm by our observation Beers' last statement. Moreover, we tried – unsuccessfully – to find convincing ultrastructural indications for a holdfast action, e.g. holes or depressions in the wall of the intestine (unpublished data).

Nevertheless, in 1942 Williams elaborated a hypothesis of the movement and mode of function of the holdfast apparatus, interpreting the structures found in his fixed, stained and therefore dead, specimens. In addition to hook movement, he also proposed an attachment and a detachment manipulator, all of which articulate with each other. The movement would be caused by so-called contractile fibrils. This hypothesis has not been evidenced by any experimental data or by any live observation, and is thus pure speculation.

On the other hand, four of our electron microscopic findings might be of interest in the context of a holdfast activity: i. In the right area of the apical depression, a tuft of cilia different from the somatic ones is found; these are possibly involved in a sucker-like action and by this in anchoring the hook in the intestinal wall. ii. The fold on the apex of the hook might be an indication that some (possibly very rapid and therefore not visible to the naked eye) movements of the hook occur. iii. Also the presence of smooth and knee-like bended rays at the transition region to the strips might hint to acting forces. iiii. Varying lengths of cilia between the rays in the anterior depressed part might be due to lifting or lowering of the strip-and-ray apparatus. However, these are merely hypotheses which require experimental support.

Even if the hook is indeed a holdfast organelle to prevent the metaradiophryids from being swept out from the worm's intestine, the question arises as to how the ciliates of the other genera which live in earthworms and do not have this device manage to stay inside their host.

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Fig. 3. Scanning electron micrographs of *Metaradiophrya*. **a** – anterior part of the cell. An area of the hook region (asterisk) shows cilia of a different length and arrangement from the somatic ciliature. **b–d** – the anterior part of the hook is covered by a fold (arrow); **e** – occasionally the apical fold of the hook is missing (arrow). Somatic cilia in close vicinity to the tuft of cilia are longer (arrowhead); **f** – the hook is flanked by a right and a left strip, resulting in an inverted V-structure. Scale bars: 25 μm (a), 10 μm (b–f).