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RESEARCH NOTE

ULTRASTRUCTURE OF MICROTRICHES ON THE SCOLEX OF *CYATHOCEPHALUS TRUNCATUS* (CESTODA: SPATHEBOTHRIIDEA)

Céline Levron[1], **Tomáš Scholz**[1] and **Bahram S. Dezfuli**[2]

[1]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic;

[2]Department of Biology and Evolution, University of Ferrara, St. Borsari 46, 44100 Ferrara, Italy

Abstract. The scolex surface of the mature spathebothriidean *Cyathocephalus truncatus* (Pallas, 1781), a parasite of the brown trout *Salmo trutta fario* L., was studied using scanning and transmission electron microscopy. A particular attention was paid to microtriches, unique structure on the surface of the Cestoda. The scolex of *C. truncatus* is covered with two types of filiform microtriches (filitriches): aciculate (≈ 3 µm long) and capillate (≈ 10 µm long). Capillate microtriches, which have never been reported in any other spathebothriideans, are described for the first time using transmission electron microscopy. The tegument covered with filiform microtriches only (no spiniform microtriches are present) is typical of cestode groups supposed to be the most basal, e.g., Gyrocotylidea, Spathebothriidea, and Caryophyllidea.

The tegument of tapeworms (Cestoda) is covered with a specialised structure named microtriches, which represent an autapomorphy of cestodes. Studies on microtriches suggest that they may be of great systematic and phylogenetic value (Richmond and Caira 1991). Microtriches have multiple functions such as amplification of the surface area for absorption and digestion, excretion, movement and external protection (Jones 1998, Palm 2004).

The morphology of microtriches, mainly their shape, has been found to vary among species, life-cycle stages and body regions, particularly on the scolex (MacKinnon and Burt 1983, Žďárská and Nebesářová 1999). Two main types of microtriches have been described in cestodes: filiform microtriches (or filitriches) and spiniform microtriches (spinitriches), each including several subtypes (Faliex et al. 2000, Palm 2004). In the basal groups of the Cestoda, including Spathebothriidea, filiform microtriches have been observed (Charles and Orr 1968, Burt and Sanderman 1974, Hayunga and Mackiewicz 1975, Richards and Arme 1981, Kuperman 1988, Davydov et al. 1997, Poddubnaya et al. 2006). However, few exceptions with spiniform microtriches have been mentioned in Caryophyllidea (Poddubnaya and Izvekova 2005).

The Spathebothriidea is a small group of cestodes with only five genera (Gibson 1994) and may represent the earliest eucestode order (Olson and Caira 1999). They are uncommon and most aspects of their biology remain largely unknown (Marques et al. 2007). They are polyzoic but lack external segmentation (Gibson 1994). The tegument of spathebothriideans has been studied using transmission electron microscopy in *Cyathocephalus truncatus* (Pallas, 1781), *Didymobothrium rudolphii* (Monticelli, 1890) and *Diplocotyle olrikii* Krabbe, 1874 (Burt and Sanderman 1974, Kuperman 1988, Protasova and Roytman 1995, Davydov et al. 1997, Marques et al. 2007), but much information on the ultrastructure of members of this phylogenetically pivotal group of tapeworms is still missing. Therefore, the main objective of this study is to provide information on the ultrastructure of microtriches on the scolex of the spathebothriidean cestode *Cyathocephalus truncatus*, using scanning and transmission electron microscopy.

Address for correspondence: *C. Levron*, *Institute of Parasitology*, *Biology Centre of the Academy of Sciences of the Czech Republic*, *Branišovská 31*, *370 05 České Budějovice*, *Czech Republic*. *Phone*: *+420 387 775 410*; *Fax*: *+420 385 310 388*; *E-mail*: *levron@paru.cas.cz*

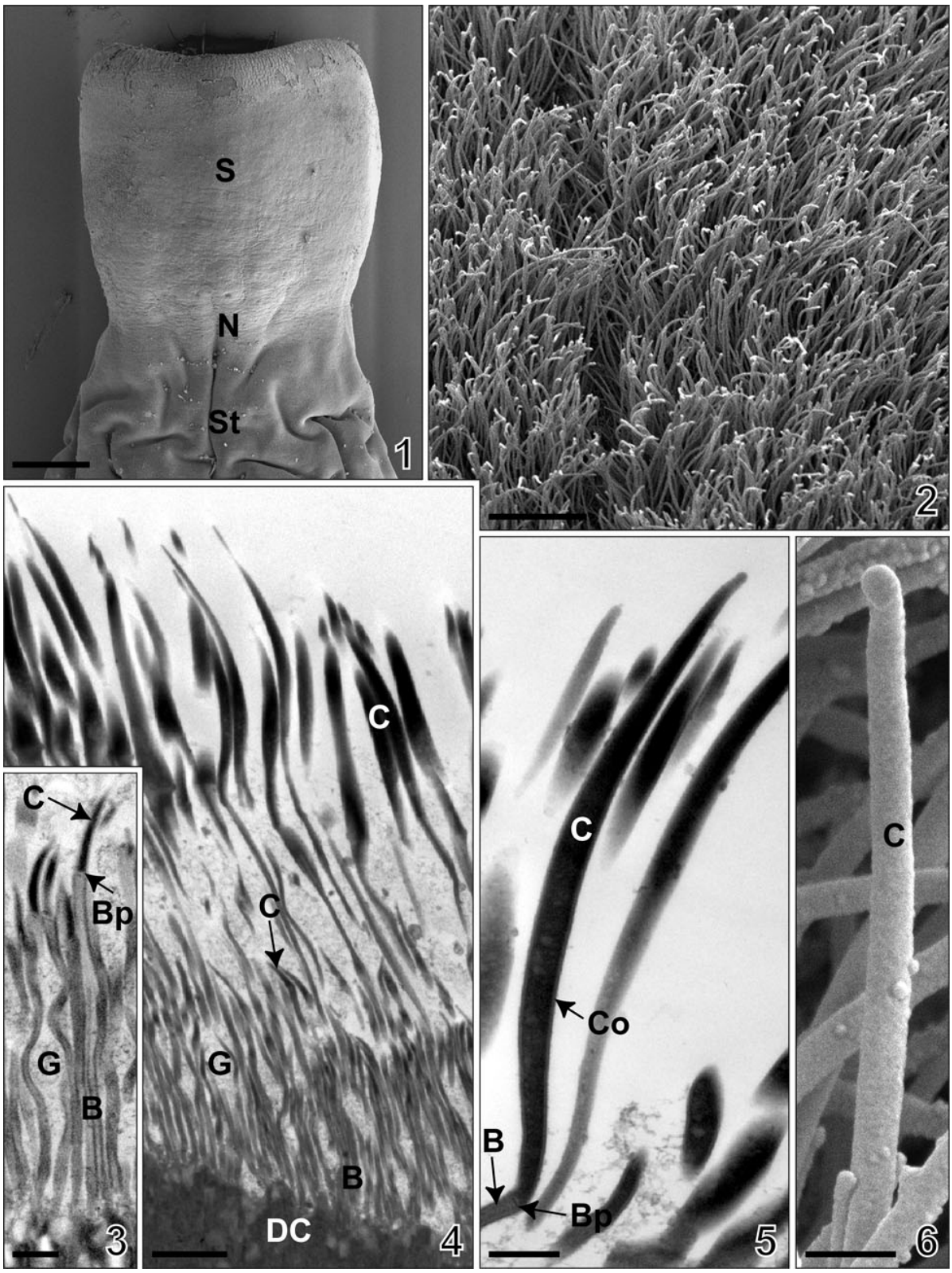
Adult specimens of *C. truncatus* were obtained from the intestine of brown trout, *Salmo trutta fario* L., collected from the Brenta River, northern Italy in February 2007. Three scolices were fixed with 2.5% glutaraldehyde in cacodylate buffer during one day, washed overnight in 0.1 M sodium cacodylate buffer at pH 7.4, postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1 h, dehydrated in ethanol and propylene oxide and embedded in Araldite and Epon. Ultrathin sections (60–90 nm in thickness) were cut on a Leica Ultracut UCT ultramicrotome, placed on copper grids and stained with uranyl acetate and lead citrate. The grids were examined using a JEOL 1010 transmission electron microscope operating at 80 kV.

For SEM investigations, five scolices of *C. truncatus* were fixed with hot formaldehyde solution (4%). Fixed worms were dehydrated in a graded ethanol series with final change in absolute ethanol. The cestodes were critical point-dried with liquid CO_2, sputter-coated with gold-palladium and examined using a JEOL JSM 6700F (accelerating voltage 3 kV).

Measurements were made from micrographs and include the range followed by the standard deviation and the number of measurements (n) in parentheses. The terminology of individual parts of microtriches follows that proposed at the Sixth International Workshop on Cestode Systematics and Phylogeny (Smolenice, Slovakia, 15–20 June 2008), i.e. "base" corresponding to the basal part, "basal plate" representing the junctional region, and "cap" corresponding to the distal part (see also Žďárská and Nebesářová 1999, Levron et al. 2008).

The scolex of *C. truncatus* is 600 ± 55 µm (n = 5) long and 710 ± 70 µm wide and it is funnel-shaped with an apical attachment organ on its distal part (Fig. 1). Observations of the whole surface of the scolex (from the apical part to the neck) did not show any variability in the morphology of microtriches and revealed the presence of two types of filiform microtriches: aciculate and capillate (Figs. 2–4). The basal part of microtriches is covered with glycocalyx (Figs. 3, 4).

The aciculate filiform microtriches are hidden within capillate when observed by scanning electron microscopy (Fig. 2). However, using transmission electron microscopy, it is possible to distinguish them (Fig. 4). They are 2.8 ± 0.3 µm (n = 10) long and approximately 80 nm wide (base). The electron-lucent base is 2.3 ± 0.2 µm (n = 10) long. The basal plate



Figs. 1–6. *Cyathocephalus truncatus*, scanning (SEM) and transmission (TEM) electron micrographs of the scolex. **Fig. 1.** Anterior part of the body showing the scolex, neck and the proximal part of the strobila; SEM. **Fig. 2.** Capillate filiform microtriches; SEM. **Fig. 3.** Aciculate filiform microtriches; TEM. **Fig. 4.** Longitudinal section of the two types of filiform microtriches; TEM. **Fig. 5.** Details of the electron-dense cap of capillate filiform microtriches; TEM. **Fig. 6.** Distal extremity of capillate filiform microtriches; SEM. *Abbreviations*: B – base; Bp – basal plate; C – cap; Co – cortex; DC – distal cytoplasm; G – glycocalyx; N – neck; S – scolex; St – strobila. Scale bars: Fig. 1 = 200 μm; Fig. 2 = 5 μm; Figs. 3, 5, 6 = 0.3 μm; Fig. 4 = 1 μm.

separates the base from the electron-dense cap, which measures 0.5 ± 0.2 μm (n = 10) in length (Fig. 3).

Long intermingled capillate filiform microtriches are 9.7 ± 1.1 μm (n = 10) long and approximately 90 nm wide (base) (Figs. 2, 4). Due to their length, no complete longitudinal sections of microtriches were observed by TEM (Fig. 5). The base (≈ 6 μm long) is electron-lucent (Figs. 4, 5). The electron-dense cap, separated by a basal plate from the base, measures approximately 3.7 μm (Figs. 4, 5). An outer electron-lucent cortex surrounds an internal electron-dense medulla (Fig. 5). In TEM, the electron-dense cap is slightly thickened in the middle part ("club-shaped") (Figs. 4, 5). However, this thickening was not visible in SEM micrographs (Figs. 2, 6).

Results of the present study, i.e. observation of two types of filiform microtriches on the scolex of the spathebothriidean cestode *C. truncatus*, are in contradiction with the results of Kuperman (1988) and Protasova and Roytman (1995), who reported microvilli on the scolex of *C. truncatus*. However, Kuperman (1988) mentioned that the tegument of the specimens he studied had been damaged (see figs. 35 and 36 in Kuperman 1988) and thus it is not relevant to compare his results with the present observations. Nevertheless, it is reasonable to assume that the structures he called "microvilli" were in fact microtriches lacking electron-dense cap in ultrathin sections.

Filiform microtriches, present in all major cestode groups, are considered to be an ancestral type and they can vary considerably in size (Palm et al. 2000, Palm 2004). During the Sixth International Workshop on Cestode Systematics and Phylogeny (Smolenice, Slovakia, 15–20 June 2008), participants proposed to classify filiform microtriches to three main types depending on their length: papillate (short), aciculate and capillate (long) (L. Chervy – in preparation). The exact function of filiform microtriches remains unclear, but a nutritional function for this structure was suggested (MacKinnon and Burt 1983).

The capillate filiform microtriches on the scolex of *C. truncatus* are now described using transmission electron microscopy for the first time. They have not been reported in any other spathebothriideans. Much shorter microtriches (2–4 μm long) were observed on the scolex of the spathebothriideans *Diplocotyle olrikii* and *Didymobothrium rudolphii* (Burt and Sanderman 1974, Marques et al. 2007). Comparatively long or longer, slender microtriches (7–50 μm long) as those found in *C. truncatus* have been described from the tegument of plerocercoids of *Diphyllobothrium dendriticum*, *D. ditremum* and *D. vogeli* (Diphyllobothriidea) and also on the proximal end of the scolex peduncle of many larval trypanorhynchs (Andersen 1975, Palm 2004). Elongate or hair-like filiform microtriches have also been reported on the scolices of some adult trypanorhynchs (*Pseudolacistorhynchus noodti*, *Poecilancistrum caryophyllum*), diphyllideans (*Macrobothridium euterpes* and *M. syrtensis*) and tetraphyllideans (*Paraorygmatobothrium janineae*, *Paraorygmatobothrium kirstenae* and *Ruhnkecestus latipi*) (Palm et al. 2000, Neifar et al. 2001, Caira and Durkin 2006, Ruhnke et al. 2006). The length of capillate microtriches, which are fairly slender and may agitate the surrounding environment, increases the surface area, which suggests their nutritional function.

Morphological and size variation of microtriches is most common on the scolex of tapeworms (Jones 1998), but microtriches on the scolex of *C. truncatus* are relatively uniform. A similar uniformity of microtriches has been observed in other cestodes considered to be basal (Charles and Orr 1968, Burt and Sanderman 1974, Hayunga and Mackiewicz 1975, Richards and Arme 1981, Kuperman 1988, Davydov et al. 1997, Poddubnaya et al. 2006). These cestodes are also devoid of spiniform microtriches similarly as *C. truncatus*. Therefore, our results represent another evidence about the placement of spathebothriideans among the most basal groups of cestodes, as suggested by molecular data (Olson and Caira 1999).

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