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On the presence of the sessilid peritrich *Apiosoma* in the oro-pharynx of *Lissotriton vulgaris* (Urodela, Vertebrata)

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Abstract

We report on a population of smooth newts (*Lissotriton vulgaris*) from the vicinity of Jena (Germany), in which we detected the presence of sessile peritrich ciliates of the genus *Apiosoma*. The zooids of the ciliates were found in the oropharynx of the urodelans during their aquatic annual phase. We performed histological and SEM investigations to document and identify the protists. The histological studies indicated that the ciliate zooids were firmly attached to the oral epithelium of the newts and were abundant throughout the oral cavity. The SEM investigation demonstrated that the ciliates densely covered the dorsal epithelium of the newt's tongue. We discuss the potential implications of the presence of numerous relatively large ciliate zooids on the movement of the water volumes during the aquatic prey capture and prey transport in the newts.

Key Words

Amphibia, ecology, histology, limnology, microscopy, symbiont

Introduction

Species of sessilid peritrich ciliates (Lynn 2008; Foissner et al. 2009; Jiang et al. 2025) are known to live as epibionts on a variety of aquatic invertebrates (Green 1965; Smith 1986; Dias et al. 2007; Dias et al. 2009; Cabral et al. 2010; Álvarez-Campos et al. 2014; Corbi et al. 2016; Cabral et al. 2018; Chatterjee et al. 2018; Lu et al. 2020; Malfatti et al. 2022), as well as on vertebrates (Yuryshynets et al. 2019). Some of the peritrichs may live as commensals or as ectoparasites (Tanahashi et al. 2017), but may also lead to fatal end for the host (Gouda 2006).

Among the peritrichs, trichodinids are well known to inhabit the gills and the skin of aquatic amphibians and some were even detected as endoparasites (de Jager et al. 2019; Zhou et al. 2021). Sessil peritrichs are known as fish parasites affecting the body surface and the gills of their hosts (Reichenbach-Klinke and Elkan 1965; Viljoen and Van As 1985; Akpinar and Didiner 2020; Latif et al. 2023). Wenrich (1924) reported a variety of sessile protozoan species from the order Peritrichia to inhabit the skin and the gills of anuran larvae. According to that author, some of the species from the genera *Vorticella*, *Opercularia*, *Rhabdostyla*, and *Epistylis* were found only on the surface of the host tadpoles. Banina (1982) found seven species of peritrichs on the skin of agile frog tadpoles (*Rana dalmatina*). The author also reported that one of the ciliate species (*Apiosoma tintinnabulum*) was found on the skin of the smooth newt.

In the present study we report on numerous sessile ciliates that were detected in the oral cavity of the smooth newt *Lissotriton vulgaris* from an isolated newt population near the city of Jena, Germany. We discuss the possible impact of the ciliate zooids on the function of the feeding apparatus in the newts during their aquatic annual phase.

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Material and methods

Two female and three male adult smooth newts (*Lissotriton vulgaris*) were collected in their aquatic stage in June 2017 in temporary ponds of the periphery of Jena (Germany) with collection permission AV02_AG01-17 granted by the nature conservation authority of the city of Jena. The preliminary goal of the study was to gain information on the function of the feeding system of the species. The animals were brought to the lab of the Zoological Institute of the University of Jena, where they were anesthetized and killed in 0.5% aqueous MS222 solution, cut in two pieces approximately 10 mm posterior to the shoulder girdle and immersed into fixation solution as described below. All treatments were in strict accordance with national and international laws.

Histology

For histology, samples of one male and one female *L. vulgaris* were immersed in Bouin's solution (Böck 1989) for two months, changing the solution once a week. After decalcification, the samples were dehydrated in a graded ethanol-isopropanol series and embedded in paraffin. Next, 7 µm semi serial-sections were made on a Reichert-Jung 2030 (Reichert-Jung, Bensheim, Germany) and a MH 360 (Zeiss, Germany) rotatory microtome. The sections were mounted on glass slides and, after removing the paraffin, stained with periodic acid Schiff (PAS) and a combination of hematoxylin-eosin (HE) according to standard protocols after Böck (1989) and Kiernan (2003). The sections were analysed by using a digital camera mounted to an Axiolab microscope (Carl Zeiss Jena, Germany).

Scanning electron microscopy (SEM)

For SEM, three *L. vulgaris* (one female and two males) were used. After anesthesia and euthanasia as described above, the floor of the mouth was removed and fixed in modified Karnovsky solution (2.5% glutaraldehyde, 2% formaldehyde in 0.1 M cacodylate buffer with 2% sucrose) for 18 hours (Karnovsky 1965). After fixation, samples were rinsed and postfixed in 1% osmium tetroxide for 2 hours, rinsed in distilled water and immersed for 30 minutes in 25% HCl at 40 °C in a Falcon tube, agitating the tube slightly to wash the mucus from the surface. The samples were then rinsed in distilled water and dehydrated in a graded ethanol series, immersed in 100% acetone and critically point dried (Emitech K850 critical point dryer). Next, samples were coated with gold in a sputtercoater (Emitech K500) and analysed in a XL 30 ESEM scanning electron microscope (Philips, Eindhoven, Netherlands).

The identification of the ciliates was performed after Li et al. (2008, 2016).

Results

Scanning electron microscopy and histological investigations revealed numerous ciliates attached to the oral epithelium of the newts (Figs 1, 2). The greatest number of ciliates (about 15 per 50 µm^2) were detected on the dorsal face of the tongue (Fig. 2). Some of the ciliates were in a contracted state and others in a more elongated state. Generally, the ciliates were funnel-like shaped with a narrow stalk attached to the substrate epithelium, and the broad peristomal disc with numerous cilia (Figs 1, 2). Scanning electron microscopy revealed the pattern of the horizontal (relative to the body axis) annular striations (Fig. 2b). Histology showed granular structures in the ciliates body, which stained positive for both alcian blue and PAS (Fig. 1). Based on the morphological features of the ciliate individuals (especially based on SEM) we were able to identify them as belonging to the Phylum Ciliophora, Class Oligohymenophora Subclass Peritrichia, Order Peritrichida, Suborder Sessilina, Genus *Apiosoma*.

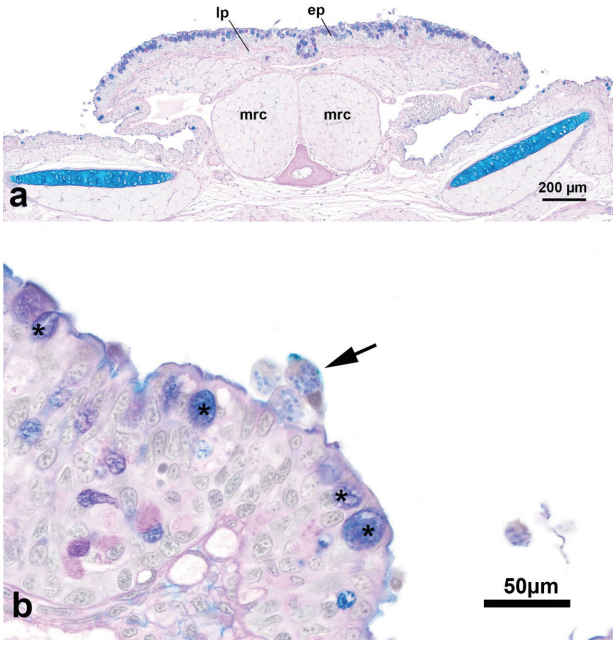

Figure 1. Light micrographs (combined PAS/AB staining) of the tongue of *Lissotriton vulgaris*, covered with sessilid peritrich ciliates: **a.** Cross-section of the tongue, ep indicate epithelium, lp indicate lamina propria, mrc indicate musculus rectus cervicis; **b.** Detailed view of a ciliate attached to the tongue epithelium of a smooth newt, asterisks indicate goblet cells, arrow indicates ciliates.

Discussion

The impact of the sessile ciliates on aquatic vertebrates is not entirely understood (see Li et al. 2016). The external sessile ciliates which infest fish may impact the tissue on the skin and the gills and may cause excess mucus production, loss of condition, flashing and piping (see Pet-

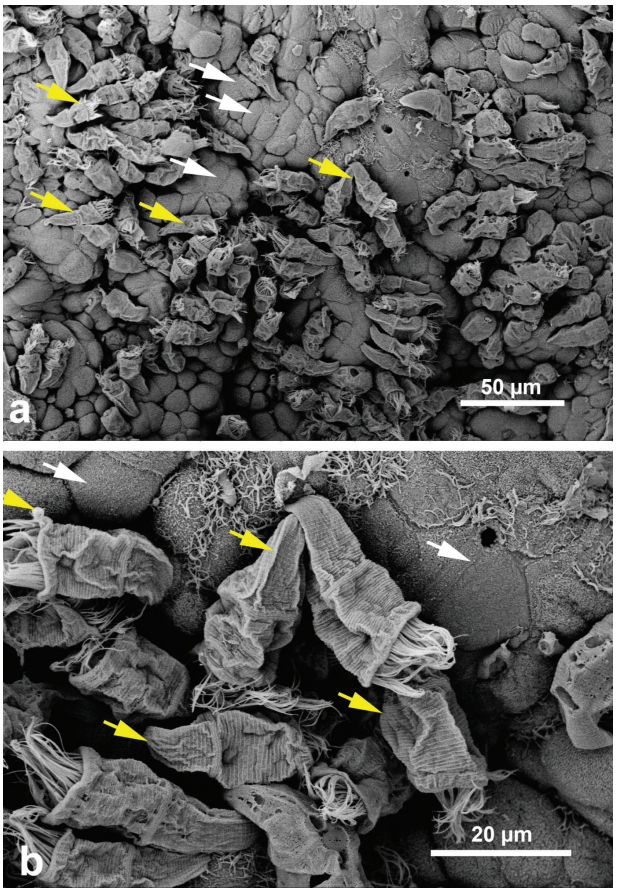

Figure 2. SEM micrograph of the dorsal lingual surface of *Lissotriton vulgaris*, covered with peritrich ciliates: **a.** Dorsal view of the tongue of the smooth newt; **b.** Detailed view of a dorsal section of the tongue of the smooth newt. White arrows indicate superficial epithelial cells; yellow arrows indicate ciliates.

ty et al. 2022). According to Akpinar and Didiner (2020), the presence of numerous parasitic ciliates might indicate a deteriorated water quality. As the smooth newts rely on water dependent mechanisms for food uptake and intraoral food transport during their aquatic annual phase (see Heiss et al. 2015), we hypothesize that the oropharyngeal infestation may impact the feeding process. Specifically, vertebrates which rely on efficient suction in underwater feeding lack prominent oropharyngeal morphological structures as they may negatively impact the intraoral water flow during the fast suction feeding process (see Lemell et al. 2002). Smooth newts are known to exhibit a remarkable form of phenotypic flexibility where they have a smooth lingual surface in their aquatic seasonal stage, but grow a carpet of densely packed longitudinal lingual papillae as they change to their terrestrial stage. These seasonal morphological changes of the lingual surface are assumed to benefit the terrestrial and aquatic feeding mechanism in the corresponding seasonal stage (Heiss et al. 2015, 2017). In the case of the infested newts caught in their aquatic stage, the ciliates were about the size of the dorsal lingual papillae the newts would develop in their terrestrial stage (see Heiss et al. 2017). The massive infestation with sessile ciliates on the actual smooth tongue surface might functionally counteract the sophisticated seasonal flexibility. Specifically, the smooth tongue surface might provide hydrodynamic benefits for suction feeding. If the smooth tongue surface is covered with numerous ciliates, they might negatively impact the water flows during suction feeding and thus prevent optimal hunting performance. The relatively large ciliates that cover densely the tongue epithelium (Fig. 2) may cause drag within the waterflow in bidirectional feeding situation (Miller and Larsen 1989) and may induce turbulent aquatic microflows within the lingual section of the mouth.

In conclusion, the presence of cilitates in oropharyngeal cavity of amphibians is a rarely described phenomenon. Here, we report on a heavy "infestation" of the oropharyngeal cavity of smooth newts with *Apiosoma* ciliates. We hypothesize that a oropharngeal cavity packed with ciliates might have a negative impact on the ecological performance of the individual newt. To our knowledge, this is the first report of sessile ciliates infesting the oropharyngeal cavity of newts; however, such infestations may be frequently overlooked, as the ciliates are not visible to the naked eye and more elaborate methods are required that are rarely available in the field.

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