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Glandular cell products in adult cestode: A new tale of tapeworm interaction with fish innate immune response

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ABSTRACT

The caryophyllidean tapeworm *Caryophyllaeus brachycollis* (Janiszewska, 1953) is indigenous to the Lake Blidinje in the west-central part of Bosnia-Herzegovina where it infects chub *Squalius tenellus* (Heckel, 1843). Of 22 chubs examined, 45% were infected with *C. brachycollis* and a total of 912 specimens of this worm were counted. Histopathological and ultrastructural investigations were conducted on interface region between chub intestine and cestode scolex. Different sizes of lipid droplets in cestode tegument, in interface region and in chub enterocytes were observed. *C. brachycollis* lacks any specialized attachment organs and with an expanded, flattened scolex goes deep in mucosal folds and firmly attaches to them. In the epithelium of fish intestine, near the site of worm attachment, a high number of mucous cells and several rodlet cells were noticed. Indeed, within the intestinal tunica propria-submucosa, beneath the site of scolex attachment, numerous neutrophils and mast cells were encountered. Transmission electron microscopy of the apical part of the scolex of *C. brachycollis* showed the occurrence of a multicellular, syncytial glandular complex, the scolex produced membrane-bound secretory granules and their fibrillar contents discharged by merocrine and apocrine secretion onto the host-parasite interface. Our results are among the first to provide evidence on the sophisticated relationship between fish intestine and amorphous-undefinable substance produced by scolex glandular complex.

1. Introduction

Fish in farms and in the wild can be infected with pathogens/parasites and the digestive tract is their main portal to the host body, the innate components of the immune system are the first barrier and protects host from the action of pathogens (Secombes and Chappell, 1996). The fish skin, gills, olfactory organ and gut are the most extended mucosal surfaces and the gut is covered by a mucus layer considered to be one of the most important components of fish mucosal immunity (Salinas, 2015; Wu et al., 2016; da Silva et al., 2017; Cabillon and Lazado, 2019; Bosi et al., 2022; Sayyaf Dezfuli et al., 2023). Among the most frequent reactions of fish to enteric helminths is hyperplasia of mucous cells which leads to increased mucus secretion (Scharsack et al., 2013; Souza et al., 2019; Bosi et al., 2022; Sayyaf Dezfuli et al., 2023, 2024).

The tapeworm, *Caryophyllaeus brachycollis* (Janiszewska, 1953) was the only helminth species encountered recently in the intestine of chub *Squalius tenellus* (Heckel, 1843) in Lake Blidinje in south of Bosnia-Herzegovina (Sayyaf Dezfuli et al., 2024). The Cestoda are one of the major groups of parasitic flatworms and as adults, the tapeworms primarily inhabit the alimentary canal of their definitive hosts with about 500 species occurring in freshwater and marine teleosts (Scholz and Kuchta, 2017). The tapeworms lack an alimentary canal and their bodies are covered with tegument and surface microtriches, which help the parasite in attachment to the host intestine and in obtaining the nutrients (Smyth and McManus, 1989; Chervy, 2009). The morphology of the scolex and its attachment structures are responsible for the extent of damage to the host intestine (Mackiewicz et al., 1972; Scholz et al., 2021). *C. brachycollis*, as other caryophyllidean cestodes, has an active and mobile scolex and by stretching and contracting movements the

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parasite changes the shape of the scolex and makes intimate contact with intestinal folds (Mackiewicz et al., 1972).

Tapeworm infection can provoke several alterations to the host immune response (Sharpe et al., 2018) and often induces an inflammation resulting in variable numbers and types of leucocytes subsequently being observed in the epithelium and lamina propria of host tissue (Karanis and Taraschewski, 1993; Williams et al., 2011; Scholz et al., 2021; Sayyaf Dezfuli et al., 2021, 2024). Helminths establish long-term infections by modulating the host immune response for survival, releasing excretory-secretory products (Scharsack et al., 2013; Hansen et al., 2019; Scott, 2023). The tegument of tapeworms performs multiple functions as protection, suppuration, secretion, excretion, and osmoregulation (Smyth and McManus, 1989).

Cestodes expel extracellular vesicles, which modulate the host response (Ancarola et al., 2017; Mazanec et al., 2021) and have effects on fish intestinal microbiota (Fu et al., 2019, 2022), and on proteolytic enzyme activity of fish gut (Frolova and Izvekova, 2022). The mode of mechanical attachment by suckers, hooks, tentacles and microtriches are well known in Cestoda (Caira and Littlewood, 2001). Beside attachment by above the structures, material secreted at the parasite-intestine interface occur in adult tapeworms (Hayunga, 1979b; Williams et al., 2011). The scolex glands, so-called frontal glands, are reported widely among cestodes of different orders (see Whittington and Cribb, 2001). The frontal glands are well-developed in tapeworms that have poor or totally lack mechanical attachment apparatus like monozoic gyrocotylids and caryophyllideans (see Hayunga, 1979b; Poddubnaya et al., 2008; Williams et al., 2011). The real function of the scolex glands is still uncertain (Whittington and Cribb, 2001).

Concerning the cestode-fish intestine interface region, numerous light microscopical descriptions exist, whereas, very few ultrastructural studies have been done (e.g., Karanis and Taraschewski, 1993; Morley and Hoole, 1995; Williams et al., 2011; Dezfuli et al., 2011, 2012). In this investigation, we documented the presence of numerous lipid droplets within the tegument of the apical end of the scolex of *C. brachycollis*, in the interface region between tapeworm scolex and chub intestine and rarely also inside the fish enterocytes. The role of lipids in cestodes metabolism was mentioned in (Dandwate, 2020). A histochemical study on fish intestine harbouring a cestode compared the localization of carbohydrate, protein, lipid and glycogen in host cells and in an uninfected counterpart (Dama and Pathan, 2019). Our results are among the first to provide data on the sophisticated relationship between *S. tenellus* intestine and *C. brachycollis*. The objectives of this investigation were histological and ultrastructural observations of the tapeworm tegument and fish intestine in search of evidence on occurrence of secretory material from scolex frontal glands and its accumulation in parasite-intestine interface region.

2. Material and methods

On July 30, 2023, a sub-population of 22 individuals of chubs *Squalius tenellus* were examined from Lake Blidinje in Blidinje Natural Park in Southern Bosnia-Herzegovina Federation (43°36′25″N 17°29′48″E). Fish sampling was performed in a semi-quantitative way using gill nets of two mesh sizes (24 and 40 mm). In the field, chubs were anesthetized using MS222 (125 mg L−1, tricaine methanesulfonate, Sandoz, Basel, Switzerland) and weighed (235,6 ± 26,4 g, mean ± S.E.), measured (total length 25,47 ± 1,01 cm, mean ± S.E.) and sexed (10 males, 12 females), then the spinal cords were severed and fish were dissected ventrally. The body cavity and visceral organs were examined by visual inspection in search of helminths, then, the digestive tract was removed, opened longitudinally, and the position and number of parasites were recorded.

Pieces of infected intestines measuring up to 15 × 15 mm in size, were excised and fixed in 10% neutral buffered formalin for 24 h. In the laboratory, the samples were paraffin wax-embedded using a Shandon Citadel 2000 tissue processor according to routine methods. Multiple 5 μm sections were taken from each tissue block, stained with Haematoxylin and Eosin and Alcian Blue, Giemsa, Grimelius and examined and photographed using a Nikon Microscope ECLIPSE 80i. Pieces of intestine of uninfected fish for comparative purpose were processed as mentioned above. Multiple histological sections were taken from each tissue block, observed and photographed using an optical microscope (Nikon Eclipse 80i; Nikon, Tokyo, Japan). For comparison of number of mucous cells and their type in infected/uninfected intestine, slides stained with alcian blue 8GX pH2.5 (AB) followed by periodic acid–Schiff (PAS) (Bosi et al., 2015, 2017a) were examined. The different stained mucous cells (blue = AB-positive, magenta = PAS-positive, violet = AB/PAS-positive) were counted on images obtained with a Nikon Microscope ECLIPSE 80i (Nikon) with objective 40x. Five areas from 2 slides for 5 uninfected and 5 infected chubs were used for mucous cells quantification. The statistical comparison between number of mucous cells per 100,000 μm^2 of epithelial area in infected/uninfected chub for each stained group (AB, PAS, and AB/PAS) was performed by the software GraphPad Prism v.5.0. Data sets were compared by the non-parametric Mann-Whitney test and the level of significance was set at $p < 0.01$.

For scanning electron microscopy (SEM), ten specimens of *C. brachycollis* fixed alive in 10% neutral buffered formalin were dehydrated in a graded ethanol series with a final change to absolute acetone. After dehydration, specimens were critical-point-dried desiccated using a HCP-2 critical point dryer (Hitachi, Tokyo, Japan). Later, dehydrated big specimens were cut into pieces and mounted on stubs, sputter-coated using an JFC 1600 Auto Fine Coater (JEOL Ltd., Tokyo, Japan) with gold-palladium (15–20 μm in thickness), and examined using a JEOL-JSM-6510 L V microscope (JEOL Ltd., Tokyo, Japan) at 15 kV.

For transmission electron microscopy (TEM), ten *C. brachycollis* specimens preserved in formalin were further fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 5 h at 5 °C, rinsed twice for 20 min in the same buffer, and post-fixed in 1% osmium tetroxide for 1 h. Fixed specimens were dehydrated in a graded ethanol series with a final change to absolute acetone and embedded in a mixture of Araldite and Epon using the instructions provided by the Araldite/Embed-812 E M Embedding Kit (EMS) (Sigma Aldrich, Buenos Aires, Argentina). Ultrathin sections (50–90 nm in thickness) were cut on a Leica MZ6 ultramicrotome (Leica Microsystems, Wetzlar, Germany) mounted on formvar coated copper and stained in 4% aqueous solution of uranyl acetate and 4% aqueous solution of lead citrate before being examined with a JEOL JEM 1011microscope (JEOL Ltd., Tokyo, Japan) at 80 KV.

3. Results

A total of 912 specimens of the tapeworm *C. brachycollis* were counted in the intestine of ten infected chubs (4 females, 6 males). The prevalence was 45% and the intensity of infection ranged from 7 to 260 worms per host (92.4 ± 28.7, mean ± S.E.), with six fish harbouring over 60 cestodes. The parasites were observed in all the regions of the alimentary canal, frequently in large cluster (Fig. 1A and C) or singularly (Fig. 1B), and a density of 5 worms per cm^2 was common (Fig. 1A and D). In infected chubs, *in situ*, often an excessive brownish mucus/catarrh was noticed over or around the parasites which in histological sections appeared as a thick blanket of mucus that gave an intense positive signal when stained with alcian blue (Fig. 1C and see further). During dissection of the intestine, it was noticed that the vast majority of the worms were attached to the fish gut wall by their scolex (Fig. 1A). This aspect was confirmed in fixed pieces of the intestine (Fig. 1D and see below).

Observations of histological sections revealed that likely all attached specimens of *C. brachycollis* enter with their necks and scolexes in the deep folds and did not penetrate beyond the mucosal layer (Fig. 2A–C). In *C. brachycollis*, their club-shaped scolex lack specialized attachment organs thus the parasite adjusts the shape of the scolex to improve their hold to the folds (Fig. 2A–C). Nonetheless, the tight adherence of the scolex and neck to the intestinal epithelium was impressive (Fig. 2A–F).

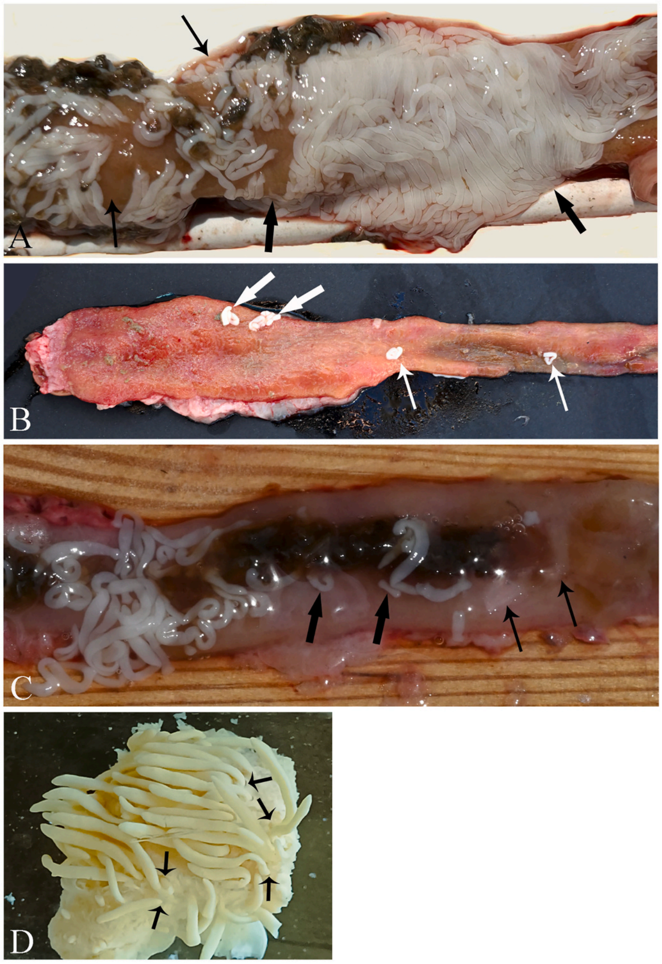

Fig. 1. Images after necropsy of chub *Squalius tenellus* infected with cestode *Caryophyllaeus brachycollis.* **(A).** High infection of the tapeworm in intestine of one of most infected host specimens, anterior intestine (thick arrows) suffered overcrowding of *C. brachycollis*, in middle intestine (arrows) parasites appeared as clusters. **(B).** One of less infected fish, *C. brachycollis* appeared in small clusters (thick arrows) or as single tapeworm (arrows), the largest part of the intestine is without parasite. **(C).** Opened intestine *in situ*, presence of excessive brownish mucus/catarrh over the worms (arrows) or encircled them (thick arrows) is visible. **(D).** One fixed piece of infected intestine of chub, the image revealed tapeworms occurred as clusters in a small area of the intestine, note very close attachment sites of the scolexes (arrows).

Often, the tapeworm uses the anterior fold of the scolex to grasp some villi and made intimate contact with adjacent folds (Fig. 2B), indeed, the scolex caused mechanical displacement and epithelial losses adjacent to the scolex proper (not shown). In some sections, the scolex appeared as a long narrow structure which reaches the depth of the fold (Fig. 2C). Infrequently, the neck of cestode was separated from host tissue by a narrow space (Fig. 2B–D).

In sections of the infected intestine, the total number of mucous cells (65.0 ± 2.7, mean ± S.E.) was significantly higher in comparison to uninfected tissue (38.0 ± 2.5, mean ± S.E., Table 1). Mucous cells staining positively for acidic glycoconjugates (AB-positive) were present in abundance in infected chub (Fig. 2D–E) (46.9 ± 2.7, mean ± S.E.) and in chub with no cestode the number was (16.7 ± 1.7, mean ± S.E., Table 1) followed by those containing mixed glycoconjugates (AB/PAS-positive), in parasitized fish (15.1 ± 0.9, mean ± S.E.) and in uninfected conspecifics (12.6 ± 1.0, mean ± S.E., Table 1). The number of mucous cells staining positively for the presence of neutral muco-substances (PAS-positive) were less in infected fish (2.9 ± 0.3, mean ± S.E.) in comparison to chub with no tapeworm (8.7 ± 0.4, mean ± S.E., Table 1). Enhanced mucus secretion occurred near the worm's attachment site and close to the cestode neck, indeed, in depth of folds, around the *C. brachycollis* scolex apical region, less mucous cells and an absence of mucus layer were noticed in interface region between the apical surface of the scolex and intestinal epithelium and this aspect is visible in Fig. 2D–E and it is particularly remarkable in Fig. 2F. In both infected and uninfected intestines, mucous cells with acidic and mixed glycoconjugates were more abundant than PAS positive mucous cells (Fig. 2G–Table 1).

In many histological sections, release of amorphous-undefined substance into the lumen was only from the apical surface of the scolex and this material reacted positive to PAS and it assumed an intense magenta colour (Fig. 2D–F), and it was absent in interface regions of lateral sides of the scolex (Fig. 2D–F). Fig. 3A shows this amorphous-undefined substance in semithin section, nonetheless, ultrathin section of the same area is presented in Fig. 3B where the occurrence of a mixture of fibrillar and amorphous-undefined materials is more notable.

Transmission electron microscopy revealed, at the cestode attachment site, a well-developed interface region, where frequently mucous cells were discharging their contents (Fig. 3C). Moreover, occurrence of rodlet cells in the epithelium among the enterocytes, and several neutrophils and mast cells within the tunica propria-submucosa, below the site of scolex attachment were observed and was reported in Sayyaf Dezfuli et al. (2024). Interestingly, in the interface region, amorphous grey lipid droplets occurred as single droplets or as a fusion of several (Fig. 3D and 5A, F). TEM study of *C. brachycollis* revealed the presence of single lipid droplets in the distal tegumental cytoplasm, in cytoplasm of tegumental glands and sarcoplasmic processes of muscle cells (Fig. 3E and F).

With SEM, the anterior part of the scolex of C. *brachycollis* is slightly cone-shaped (Fig. 4A and B). Shallow semicircular grooves were observed on the surface of the scolex (Fig. 4B) and the scolex surface is covered by microtriches, the small gladiate spinitriches (see for terminology Chervy 2009) (Fig. 4C). With TEM, the apex of the scolex appears fringed due to the surface's folds which are tightly adjacent to each other; their height varies from 40 to 75 μm, and the width from 15 to about 23 μm (Fig. 4D). The scolex of adult specimens are characterized by the presence of a multicellular (syncytial) glandular complex (Fig. 4H), the so called tegumental (frontal) glands, which occupied the medullar region of the neck and are more extensive in their distribution in the *C. brachycollis* scolex. In the scolex, the processes of these syncytial glands are packed with secretory granules which are connected to the outer epithelial lining (= distal syncytial tegumental cytoplasm), transporting the secretory granules into the distal cytoplasm (Fig. 4E). At the scolex apex, the secretory granules occupied the whole thickness of the distal cytoplasm, besides usual tegumental inclusions, bacilliform electron-dense bodies and vesicles (Fig. 4F and J). Fully developed rod-shaped, membrane-bound, secretory granules of up to 2 μm in length and about 0.2 μm in width are present close to the surface membrane (Fig. 4F). The content of the secretory granules is fibrillar material (Fig. 4F and G). The multicellular glandular cytoplasmic matrix is filled with developed, sub-ovoid and rod-shaped secretory granules; the nascent rounded granules are located near Golgi complexes (Fig. G and H, insert, I). The electron-dense, amoeboid shaped nuclei of the syncytial glandular complex are surrounded by a thin, divaricate area of dense perinuclear cytoplasm (Fig. 4G and I).

The secretory granules produced by syncytial tegumental glands of *C. brachycollis* discharged their secretion onto the host-parasite interface by means of apocrine and merocrine secretion (see Fig. 5). Along the apical surface of the scolex there are different sizes of the tegumental protrusions filled with secretory granules, some of which may reach significant lengths (Fig. 5A–F, I). Such long growths originated between tegumental folds (Fig. 2B). Isolated small cytoplasmic fragments with secretory granules occur in the interface region. These fragments are realized by separation of the small, cytoplasmic protrusions (the apocrine secretion) (Fig. 5F). TEM observation of the apical part of the

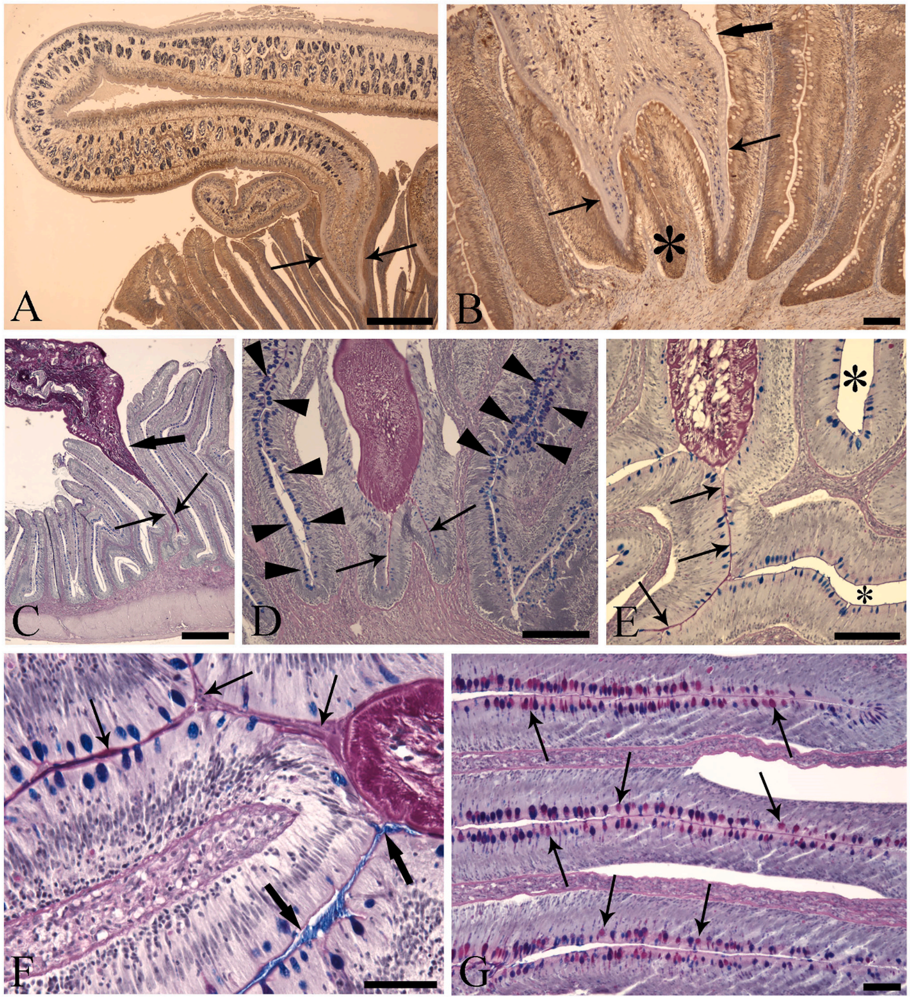

Fig. 2. Histological sections of infected/uninfected intestine of chub *S. tenellus.* (**A**). An adult specimen of *C. brachycollis* with strobila in lumen and scolex within the intestinal folds, note strict contact with epithelia (arrows), Grimelius stain with haematoxylin counterstained, scale bar = 500 μm. (**B**). Scolex folder anterior margin grasped some villi (asterisk) and made intimate contact with adjacent folds (arrows), sometimes between neck and epithelium a narrow white space was visible (thick arrow), Grimelius stain with haematoxylin counterstained, scale bar = 100 μm. (**C**). Scolex appeared as a long narrow structure and reached the depth of the fold and maintains strict attachment with the epithelia (arrows), white space (thick arrow) near the neck, Alcian Blue/PAS staining, scale bar = 500 μm. (**D**). Micrograph shows release of amorphous-undefined substance PAS positive (magenta) from the apical part of the scolex toward the end of two folds (arrows). Note occurrence of numerous AB (blue) and AB/PAS (violet) positive mucous cells (arrow heads) in folds around the site of attachment of tapeworm and lack of amorphous-undefined substance in these folds, Alcian Blue/PAS staining, scale bar = 200 μm. (**E**). Release of amorphous-undefined substance PAS positive (arrows) from apical surface of the scolex to the depth of fold. This substance is lacked in folds far from the site of parasite attachment (asterisks), Alcian Blue/PAS staining, scale bar = 100 μm. (**F**). High magnification of apical surface of the scolex, amorphous-undefined substance PAS positive (magenta) occupied luminal space of folds (arrows), mucous cells of epithelia beside the scolex produce an evident blue mucus layer (thick arrows), Alcian Blue/PAS staining, scale bar = 50 μm. (**G**). Section of uninfected intestine, note the presence of numerous mucous cells with PAS positive (magenta) glycoconjugates (arrows), Alcian Blue/PAS staining, scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

scolex of *C. brachycollis* shows that the secretory granules located close to the surface membrane was aligned with it and the granule's limiting membrane of granules and surface plasma membrane fuse (Fig. 5C–E, H). As a result of such connection, granules release their content according to the merocrine secretion, that is exocytosis (Fig. 5C–E, H). The granular fibrillar content most probably become diffuse before extrusion onto the body surface (Fig. 5E and H). Fibrillar granular material is persistent outside the body, it is often observed between tegumental folds (Fig. 5D and G).

4. Discussion

In fish, enteric helminths commonly provoke gut inflammation and elicit host immune reactions manifested by recruitment of several immune cell types like mast cells, macrophages, neutrophils, epithelioid cells, rodlet cells and mucous cells in the epithelium (see Sayyaf Dezfuli et al., 2021, 2023; Williams et al., 2023). Concerning the pathogenesis of tapeworms in fishes, it is rare for cestodes to reach the host's intestinal muscle layer (Kanaris and Taraschewski, 1993; Morley and Hoole, 1995; Molnár et al., 2003; Barčák et al., 2021). Records on cestodes pathogenicity in fishes appeared in Borucinska and Caira (2006), Bosi et al. (2017b), Sayyaf Dezfuli et al. (2021) and Scholz et al. (2021). Among Caryophyllidea species, in which the scolex is armed with developed holdfast organs, it induces little harm to the epithelium (Mackiewicz et al., 1972), conversely, other species that lack specialized structures elicit a pronounced host reaction as shallow ulcers or pronounced nodules (Mackiewicz et al., 1972; Dezfuli et al., 2011; Williams et al., 2011). *Caryophyllaeus laticeps* is a parasite of three cyprinid species, bream, roach and chub, and only in the intestine of bream it induced conspicuous granulomas (Karanis and Taraschewski, 1993). *Caryophyllaeus brachycollis* is indigenous to the Lake Blidinje and infects chub *Squalius tenellus* and we did not find any granulomas in the host's

Table 1
Mean number of mucous cells (± standard error) per 100,000 μm^2 of epithelial area in the intestine of *Squalius tenellus* containing acidic (Alcian blue-positive, AB), neutral (periodic acid Schiff-positive, PAS), and mixed (AB/PAS-positive) mucins. Mucous cells were counted in 5 fields (40× objective), from 2 slides for each of the 5 uninfected and 5 infected chub (50 + 50 microscopic fields). Mann–Whitney test and probability values are reported.

Mucous cells positive to	Fish		Mann-Whitney test		p
	Uninfected	Infected	U	z	
AB	16.7 ± 1.7	46.9 ± 2.7	200	−7.265	<0.0001
PAS	8.7 ± 0.4	2.9 ± 0.3	107	−7.879	<0.0001
AB/PAS	12.6 ± 1.0	15.1 ± 0.9	896	−2.437	**0.0148**
Total	38.0 ± 2.5	65.0 ± 2.7	346.5	−6.225	<0.0001

gut, indeed, the occurrence of an immune response with cellular involvement was described in Sayyaf Dezfuli et al. (2024) where the mucous cells in infected chub were mentioned briefly. The present study was undertaken in order to record data concerning the statistical analysis between number and type of mucous cells in infected/uninfected intestines.

Mucous cells are considered a specific type of innate immune cell (Johansson and Hansson, 2014; Salinas, 2015; Wu et al., 2016; da Silva et al., 2017; Cabillon and Lazado, 2019; Bosi et al., 2022; Sayyaf Dezfuli et al., 2023) and produce mucus which has an essential role in defence (Bosi et al., 2017a, 2022; Schröeder, 2019; Souza et al., 2019; Sayyaf Dezfuli et al., 2021, 2024). In interaction between gastrointestinal helminths and mucus, the concept that mucus is just a simple barrier is no longer tenable (Sharpe et al., 2018). The mucus layer contains molecules like lectins, antimicrobial peptides, immunoglobulins, lysozymes and mucins (Ellis, 2001; Sharpe et al., 2018). Mucins are proteins with high molecular weight and constitute the principal components of vertebrate mucus, due to type of enteric pathogen, the expression of the mucins also will change (Linden et al., 2008; Pérez-Sánchez et al., 2013; Bosi et al., 2020; Riera-Ferrer et al., 2024). In this study, excessive mucus secretion near the site of the worm's attachment and close to the cestode neck was noticed, moreover, the number of mucous cells and those staining positively for acid glycoconjugates were significantly higher compared to the number of mucous cells found in the intestines of uninfected conspecifics. A similar finding was reported for two different tapeworm species infecting freshwater fishes in Italy (Dezfuli et al., 2010, 2011). General increase in the number of mucous cells was observed for neutral and mixed glycoconjugates in several fish host-parasite systems (Bosi et al., 2017a). In the chub infected intestine, the number of mucous cells secreting neutral glycoconjugates was lower in comparison with uninfected specimens, and no difference was observed in the number of mucous cells secreting mixed glycoconjugates between infected and uninfected chub. These different trends could be a peculiarity of the *S. tenellus*-*C. brachycollis* system. In the pit of the folds, around the *C. brachycollis* scolex, less mucous cells were noticed and surprisingly there was lack of mucus layer in the interface region between the apical surface of the scolex and the intestinal epithelium.

Tapeworms expel extracellular vesicles, these particles have an important role in communication between worm and host and modulate the host response (Ancarola et al., 2017; Mazanec et al., 2021). In this study, the occurrence of single or fused lipid droplets were documented within the interface region. The presence of lipid droplets in the tegumental glandular cells was reported in caryophyllidean cestodes like *C. laticeps* (Richards and Arme, 1981) and in *C. brachycollis* (present study). Moreover, our study revealed the presence of single lipid droplets in the outer layer of the distal tegumental cytoplasm of *C. brachycollis* and their gathering in the sarcoplasmic processes of the muscle cells. Occasionally, lipids were encountered in the chub

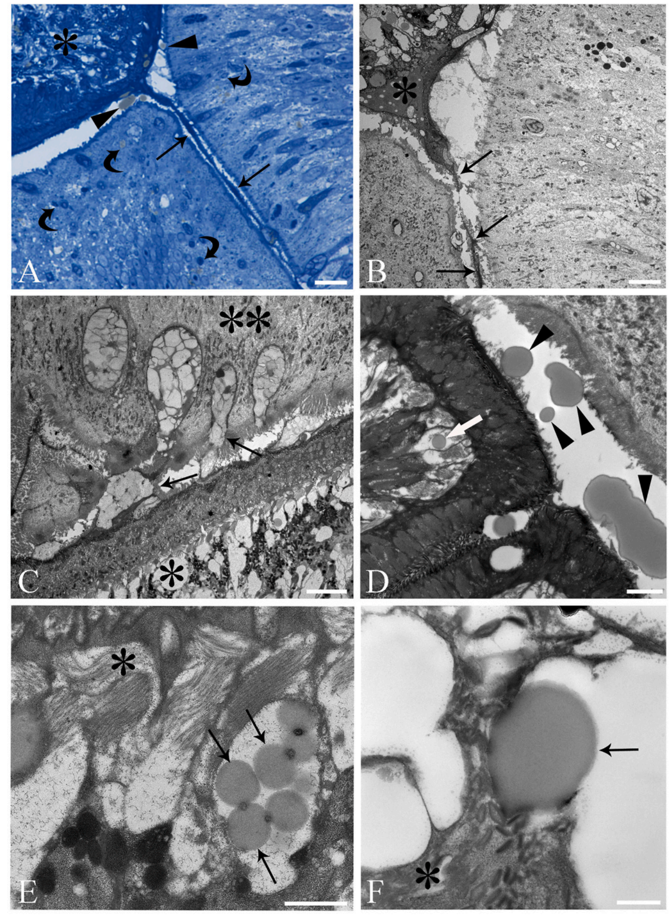

Fig. 3. Light and Transmission electron micrographs of interface region between intestine of *S. tenellus* and *C. brachycollis*. **(A)**. Semithin section of interface region, note release of amorphous-undefined substance (arrows) from apical surface of the tapeworm scolex (asterisk), lipid droplets in enterocytes (curved arrows) and in interface region (arrow head) are notable, toluidine blue staining, scale bar = 10 μm. **(B)**. Ultrathin section of the interface region showed in Fig. 3A, fibrillar aspect of amorphous-undefined substance is appreciable (arrows), some lipid droplets (arrow heads) in tegument of the scolex apical region (asterisk) can be seen, scale bar = 5 μm. **(C)**. Release of mucus (arrows) from the mucous cells in interface region between chub intestine (two asterisks) and cestode tegument (asterisk) is visible, scale bar = 5 μm. **(D)**. Micrograph shows lipid droplet (thick arrow) within the tapeworm tegument and in interface region (arrow heads), scale bar = 2 μm. **(E)**. Some lipid droplets (arrows) within the tegument (asterisk) of *C. brachycollis*, scale bar = 1 μm. **(F)**. Image or some lipid droplet (arrow) on the outer surface of the tapeworm tegument (asterisk), single droplet appears round-shaped and filled with grey amorphous material, scale bar = 0.5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

enterocytes. Concerning the lipid droplets (volatile fatty acids) in cestodes, usually, they are considered to be the end product of anaerobic fermentations with phosphorylation of the cestode's glycogen during carbohydrate metabolism (Soprunov, 1987). The lipid utilization in caryophyllidean tapeworms will be the object of our future investigation.

Exocrine scolex glands are well-developed in adult tapeworms (see Whittington and Cribb, 2001) especially in those caryophyllidean cestodes that have poorly developed or totally lack mechanical attachment apparatus (see Hayunga, 1979b; Richards and Arme, 1981; Davydov and Poddubnaya, 1988; Karanis and Taraschewski, 1993; Williams et al., 2011, present study). Usually, cestode glands are unicellular and giving rise to specialized ducts strengthened by longitudinal microtubules for

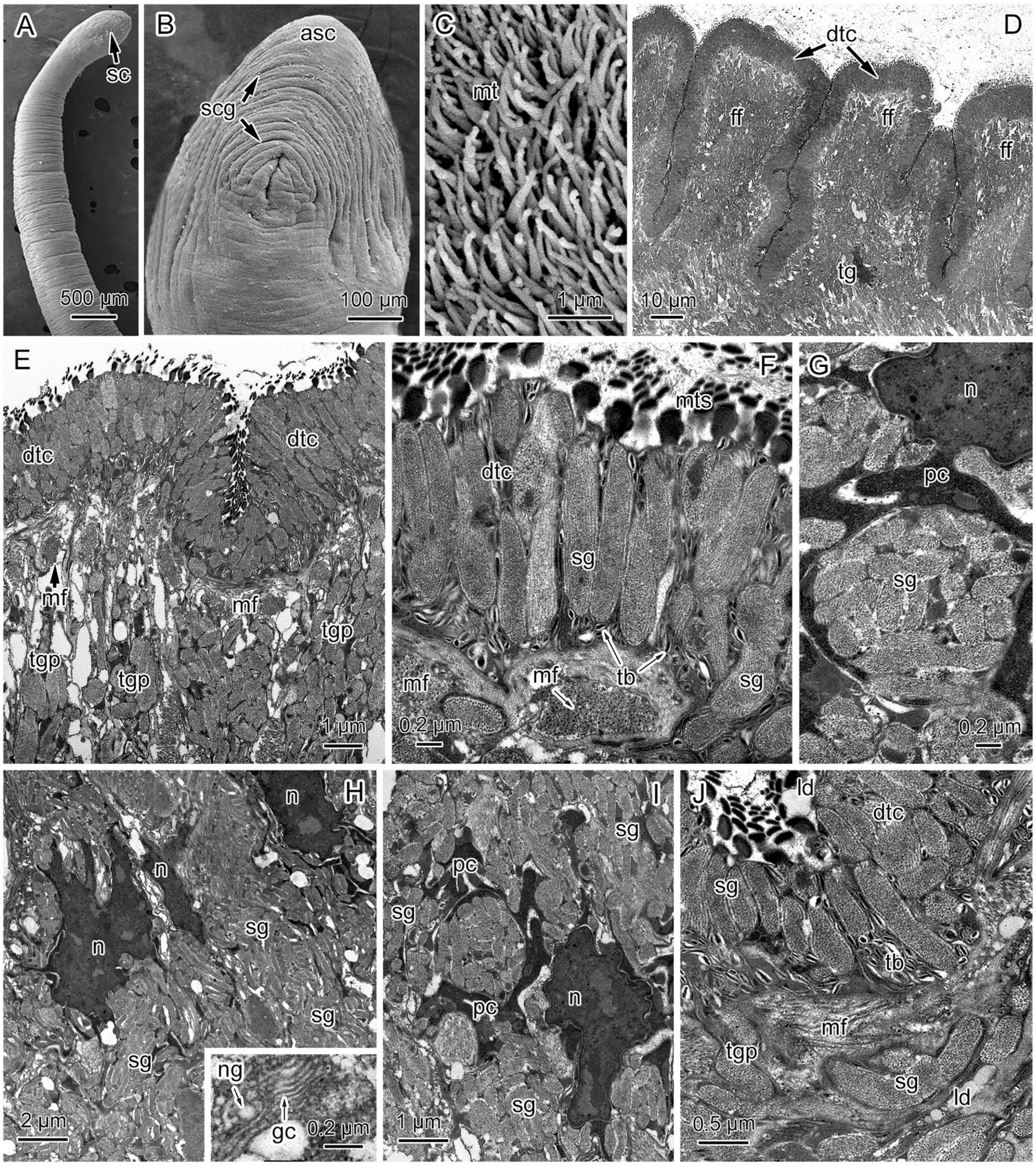

Fig. 4. Scolex region and syncytial frontal (tegumental) glands of *C. brachycollis.* **(A)**. SEM, anterior haft of the worm. **(B)**. SEM, scolex view, note semicircular grooves. **(C)**. SEM, microtriches covered with scolex surface. **(D)**. TEM, fringed surface of the apical scolex, note isolated gland in the tegumental layer. **(E)**. Secretory processes filled with secretory granules directed towards distal tegumental cytoplasm. **(F, J)**. Mixture of secretory granules and tegumental bodies within distal tegumental cytoplasm. **(G, I)**. Area of glandular syncytial cells, note electron-dense perinuclear cytoplasm. **(H)**. Part of the syncytial glandular complex, note three nuclei, the cytoplasm around which filled with tightly packed moderately dense secretory granules. *Abbreviations:* asc, apical scolex; dtc, distal tegumental cytoplasm; ff, fold of the fringed surface; gc, Golgi complex; ld, lipid droplet; mf, muscle fibres; mt, microtriches; mts microthrix sections; n, nucleus; ng, nascent granule; pc, perinuclear cytoplasm; sc, scolex; scg, scolex surface grooves; sg, secretory granules; tb, tegumental bodies; tg, tegumental gland; tgp, tegumental glandular processes.

the transport and release of their secretions to the environment via eccrine mechanism (Kuperman and Davydov, 1982; Poddubnaya et al., 2008). Besides unicellular glands, there are also multicellular (syncytial) glands, which are specific feature of adult specimens of the order Caryophyllidea (Hayunga, 1979b; Richards and Arme, 1981; Davydov and Poddubnaya, 1988; Karanis and Taraschewski, 1993; Williams et al., 2011, current study). These multicellular glands are considered to be modified tegumental cells of the anterior part of the cestode's body because they produce both secretory granules and tegumental structures, typically components of the caryophyllidean tegument. Like in the tegumental cells, the processes of caryophyllidean glands are connected to scolex epithelium (distal tegumental cytoplasm) by cytoplasmic extensions (Richards and Arme, 1981; Kuperman and Davydov, 1982; Davydov and Poddubnaya, 1988, present survey). In this order of tapeworms, the secretory granules of the multicellular glands release the contents out of the body by merocrine and apocrine mechanisms (Richards and Arme, 1981; Davydov and Poddubnaya, 1988, current investigation).

Extensive literature on the fine structure of the scolex tegumental glands in adults of other caryophyllidean species mentioned above supported us to improve our understanding of the features of these glands. In *C. brachycollis*, the secretory granules are moderately-dense with fibrillar heterogeneous content whereas other species of the order possess electron-dense homogenous contents. Furthermore, in this species the apical scolex surface has the ability to form the finger-like protrusions of significant length originating between tegumental folds.

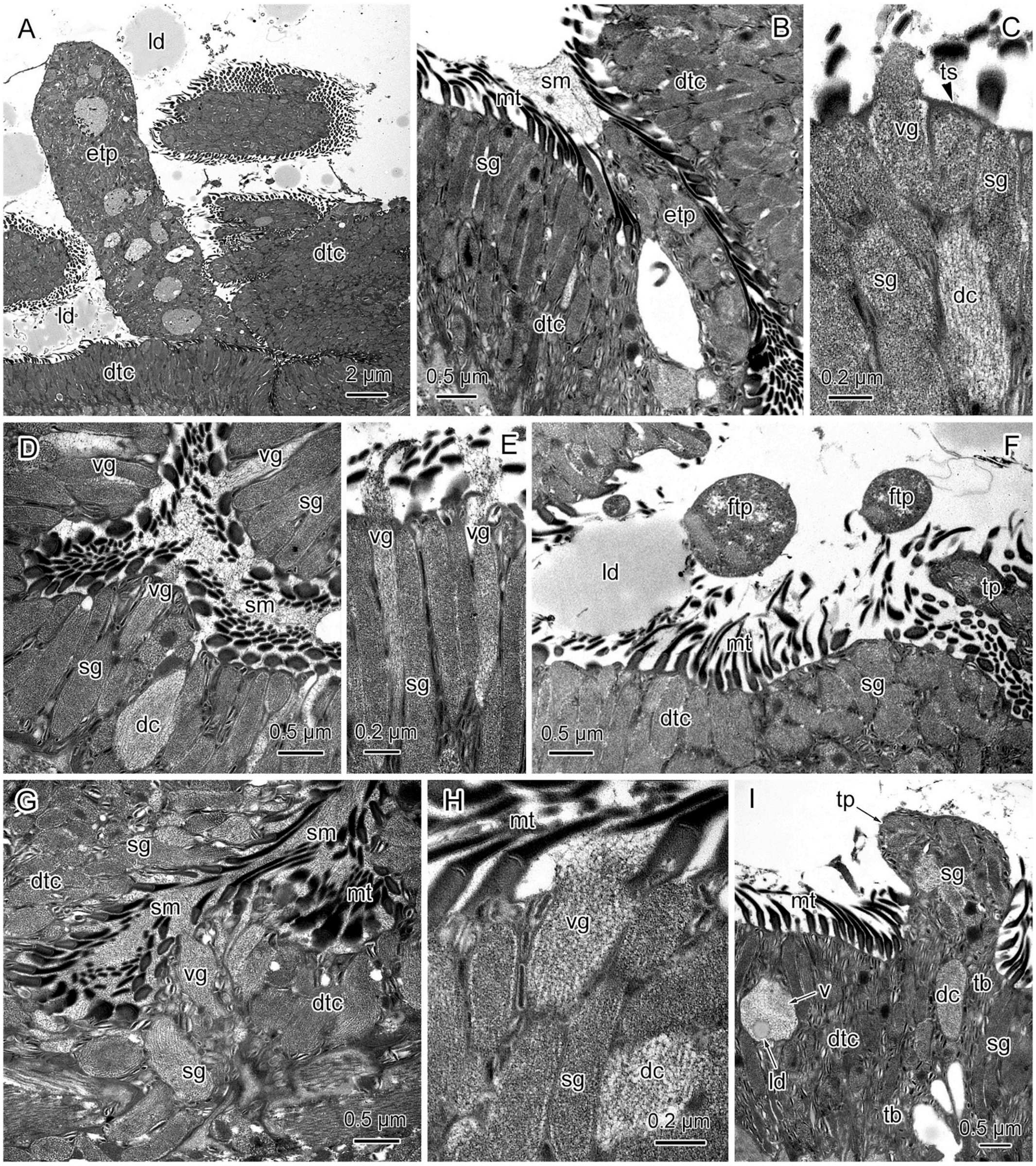

Fig. 5. The ways of the releasing of secretory product onto host-parasite interface in *C. brachycollis.* **(A)**. Extended surface tegumental finger-like protrusion filled with secretory granules, note lipid droplets in interface area. **(B)**. Formation of the tegumental protrusion filled with secretory granules in the tegumental fold. **(C)**. Voiding of the content of individual secretory granule by exocytosis (merocrine secretion). **(D)**. Space between tegumental folds filled with secretory material, note some granules discharge their content into space. **(E)**. Elongated granules within distal cytoplasm localized perpendicular to the surface membrane, note merocrine secretion of fibrillar granule content by two granules. **(F)**. Free cytoplasmic fragments outside worm as result of apocrine secretion by means of the separation of small tegumental protrusions, note lipid droplet in interface area. **(G)** Space between folds filled with secretory fibrillar material showing the similarity of fibrillar material in the space and granule content. **(H)**. The voiding granule, note different granule content. **(I)**. Surface tegumental protrusion filled with secretory granules, note lipid droplet in tegumental vacuole. *Abbreviations:* dc, diffuse content of granule; dtc, distal tegumental cytoplasm; etp, extended tegumental protrusion; ftp, free tegumental protrusion; ld, lipid droplet; mt, microtriches; sg, secretory granule; sm, secretory material; tb, tegumental bodies; tp, tegumental protrusion; ts, tegumental surface; v, vacuole; vg, voiding granule.

Light and electron microscope observations of the interface region between *C. brachycollis* and the intestinal epithelium revealed that the scolex came into close contact with the chub intestine. Although, in some instances, the neck of cestode was separated from host tissue by an electron lucent space-layer, similar to what was reported for other cestodes in fish (Hayunga, 1979a; Morley and Hoole, 1995).

Herein we documented the release of fibrillar and amorphous-undefined substance from the apical part of the scolex into the intestinal folds. There is a widely-held belief that the above substance in caryophyllidean is produced by the scolex glands (Mackiewicz et al., 1972; Hayunga, 1979b; Richards and Arme, 1981; Kanaris and Taraschewski, 1993; Morley and Hoole, 1995; Williams et al., 2011). However, contrasting points of view have been proposed on its function, such as that amorphous-undefined substance is a kind of lytic secretion, which helps the tapeworm in penetration into the host intestine (Stirewalt, 1963). Thereafter, Mackiewicz et al. (1972) speculated that it is a nutritive source for the parasite and it was called "mucoid layer", successively it was considered as an excretory product of the worm (Hayunga, 1979b). Nevertheless, the vast majority of the experts concur that the amorphous-undefined substance has an adhesive function (Richards and Arme, 1981; Kanaris and Taraschewski, 1993; Morley and Hoole, 1995; Stoitsova et al., 1997; Williams et al., 2011). Like most caryophyllidean species, *C. brachycollis* lacks any specialized adhesive structures and from our experience we do agree with the proponents that the above substance provides firm attachment of the worm to the fish mucosal tissue. Herein, one of unexpected findings was that where the amorphous-undefined substance occurred there was a lack of a mucus layer (Figs. 2A and 3A-B), it is a function of this layer to protect adequately the intestinal folds from parasite mechanical action (Bosi et al., 2020). Thus, until further evidence emerges, a reasonable conclusion might be that tapeworm frontal glands production might interfere with mucus secretion at the interface region between the apex of the scolex and the intestinal folds. This survey appears to be the first to document by TEM the presence of scolex product in the interface region. It is hoped that the data provided by this study will spur further investigation in search of the precise functions of scolex glands secretion.

Note

Until 2016, in all his articles Bahram Dezfuli appeared as Dezfuli B. In 2016 another author with the same surname (Dezfuli) and initial (B) working on human cancer started to publish as Dezfuli B. To avoid this homonymy and problems in bibliometric analyses, since 2016, the first part of the surname (Sayyaf) was added to Dezfuli thus in all subsequent articles, Bahram Sayyaf Dezfuli appears as Sayyaf Dezfuli B.

CRediT authorship contribution statement

Bahram Sayyaf Dezfuli: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Massimo Lorenzoni:** Funding acquisition, Data curation. **Antonella Carosi:** Data curation. **Giampaolo Bosi:** Data curation. **Emanuela Franchella:** Data curation. **Larisa G. Poddubnaya:** Writing – review & editing, Writing – original draft, Funding acquisition.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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