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HISTOLOGICAL DIAGNOSIS OF *LECITHOCHIRIUM* SPECIES
PARASITIZING *TRICHIURUS LEPTURUS* LINNAEUS, 1758 FROM
SOUTHEASTERN BRAZIL

DIAGNÓSTICO HISTOLÓGICO DE ESPECIES DE *LECITHOCHIRIUM*
PARASITANDO *TRICHIURUS LEPTURUS* LINNAEUS, 1758
DEL SUDESTE DE BRASIL

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ABSTRACT

Histological approaches remain underexplored in the taxonomy of marine digenean trematodes, despite their capacity to reveal internal morphological characters of diagnostic value. In this study, we provide the first detailed histological characterization and comparative analysis of two hemiurid species, *Lecithochirium microstomum* Chandler, 1935, and *Lecithochirium monticellii* (Linton, 1898), parasitizing the cutlassfish *Trichiurus lepturus* Linnaeus, 1758, from the southeastern Brazilian coast. Specimens were processed using classical histological techniques, enabling comprehensive examination of the tegumental, muscular, digestive, and reproductive systems. Both species exhibited a syncytial tegument covered by a thin glycocalyx, well-developed oral and ventral suckers with complex fibromuscular organization, and intestinal ceca lined by a simple epithelium. The reproductive system comprises testes, a compact ovary embedded in a fibroelastic stroma, and a distinct hermaphroditic duct formed by the junction of the ejaculatory duct and the terminal portion of the uterus. Histological differentiation between species was primarily based on cirrus morphology and posterior body organization. *Lecithochirium microstomum* exhibited a shorter and wider cirrus and a reduced, largely internalized ecsoma, whereas *L. monticellii* presented a longer, more slender cirrus and a well-developed ecsoma with a greater volume of connective tissue. By documenting previously undescribed histological characters, this study establishes histology as a robust and independent source of taxonomic evidence, reinforcing its value as a complementary tool in integrative taxonomy and evolutionary studies of Hemiuridae.

Keywords: digenean trematodes – Hemiuridae – histology – integrative taxonomy – *Trichiurus lepturus*

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RESUMEN

Los enfoques histológicos permanecen poco explorados en la taxonomía de los trematodos digéneos marinos, a pesar de su capacidad para revelar caracteres morfológicos internos de valor diagnóstico. En este estudio, presentamos la primera caracterización histológica detallada y un análisis comparativo de dos especies de hemiúridos, *Lecithochirium microstomum* Chandler, 1935 y *Lecithochirium monticellii* (Linton, 1898), parásitas del pez sable *Trichiurus lepturus* Linnaeus, 1758, procedentes de la costa sudeste de Brasil. Los especímenes fueron procesados mediante técnicas histológicas clásicas, lo que permitió un examen integral de los sistemas tegumentario, muscular, digestivo y reproductor. Ambas especies presentaron un tegumento sincitial recubierto por un glicocáliz delgado, ventosas oral y ventral bien desarrolladas con una organización fibromuscular compleja, y ciegos intestinales revestidos por un epitelio simple. El sistema reproductor comprendió testículos, un ovario compacto inmerso en un estroma fibroelástico y un conducto hermafrodita bien definido, formado por la unión del conducto eyaculador con la porción terminal del útero. La diferenciación histológica entre las especies se basó principalmente en la morfología del cirro y la organización de la región posterior del cuerpo. *Lecithochirium microstomum* presentó un cirro más corto y ancho y un ecsoma reducido y en gran parte internalizado, mientras que *L. monticellii* exhibió un cirro más largo y delgado y un ecsoma bien desarrollado, con mayor volumen de tejido conjuntivo. Al documentar caracteres histológicos previamente no descritos, este estudio establece la histología como una fuente robusta e independiente de evidencia taxonómica, reforzando su valor como herramienta complementaria en la taxonomía integrativa y los estudios evolutivos de Hemiuridae.

Palabras clave: Hemiuridae – histología – taxonomía integradora – trematodos digéneos – *Trichiurus lepturus*

INTRODUCTION

The genus *Lecithochirium* Lühe, 1901 (Digenea: Hemiuridae) comprises digenean parasites of marine fishes and is characterized by a smooth tegument, a prominent or weakly developed ecsoma, a tubular pars prostatica, and vitelline follicles arranged in two lateral fields. The type species is *Lecithochirium rufoviride* (Rudolphi, 1819) Lühe, 1901 (Gibson & Bray, 1979; Gibson *et al.*, 2002). Currently, 132 species are recognized within the genus (WoRMS, 2025).

Lecithochirium microstomum Chandler, 1935 was originally described from *Trichiurus lepturus* Linnaeus, 1758 (Actinopterygii: Trichiuridae) in the United States (Chandler, 1935). In Brazil, this species has been reported from a broad range of marine fish hosts, reflecting its wide ecological distribution (Kohn *et al.*, 2007; Pantoja & Kudlai, 2022). *Lecithochirium monticellii* (Linton, 1898) Skrjabin & Guschanskaja, 1955, originally described as *Distomum monticellii* from *Remora remora* (Linnaeus, 1758) in the United States (Linton, 1898), has also been recorded from several marine fishes along the Brazilian coast (Kohn *et al.*, 2007; França *et al.*, 2020; Fonseca *et al.*, 2022).

Despite the taxonomic relevance of internal morphology, descriptive histological approaches remain underutilized

in helminth systematics. Histology enables direct access to internal structural organization and tissue differentiation, providing diagnostic characters that are often unavailable through whole mounts or surface-based techniques, including scanning electron microscopy. When integrated with conventional morphological analyses, histological data offer independent and complementary evidence, expanding the resolution of taxonomic interpretations (Machado *et al.*, 2024a; Machado *et al.*, 2024b).

In this context, the present study aims to provide the first detailed histological description and comparison of *L. microstomum* and *L. monticellii* collected from *T. lepturus* off the coast of Rio de Janeiro State, southeastern Brazil. By documenting previously unexplored histological characters, this work highlights the taxonomic value of histology and contributes to the advancement of integrative approaches in the study of marine digenean trematodes.

MATERIALS AND METHODS

Host and Parasite Collection:

A total of 13 specimens of *T. lepturus* were obtained from artisanal fishers at local markets in the state of

Rio de Janeiro, southeastern Brazil, between 2024 and 2025. This sample size was considered representative for histological analyses, given the consistency of internal morphological features observed among specimens and the objective of detailed tissue-level characterization. Fish were taxonomically identified according to Menezes & Figueiredo (1985).

Digestive tracts were carefully removed, opened longitudinally, and washed in saline solution. The contents were rinsed through a 150 µm mesh sieve, and the retained material was examined under a stereomicroscope for parasite recovery. Digenean trematodes and nematodes were collected and fixed immediately in 10% neutral buffered formalin, ensuring adequate preservation of internal tissues prior to histological processing.

Histological Processing and Analysis:

Helminths were processed following standard histological protocols, allowing detailed assessment of internal morphology complementary to classical whole-mount and surface-based analyses. Fixed specimens were dehydrated through a graded ethanol series (70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin. Longitudinal sections, 5 µm thick, were obtained using a rotary microtome (Bancroft & Gamble, 2013).

Histological sections were stained with hematoxylin–eosin (H&E) for general tissue architecture, Gomori's trichrome (GT) to differentiate connective and muscular components, and orcein to evidence elastic fibers (Abrahamsohn, 2016). Slides were examined and photomicrographed using an Olympus BX51 light microscope equipped with Capture 2.4 image analysis software, enabling detailed documentation of tegumental, muscular, digestive, and reproductive structures.

Ethic aspects: All applicable institutional, national, and international guidelines for the care and use of animals were strictly followed.

RESULTS

Based on 13 specimens of *L. microstomum* (Fig. 1) and 18 specimens of *L. monticellii* (Fig. 2), both recovered from *T. lepturus*, the two species exhibited a largely conserved histological organization, while presenting consistent characters that allow reliable interspecific differentiation.

In both taxa, the tegument was composed of flattened cells arranged in a single layer, supported by a thin underlying connective tissue layer (Figs. 1c, 2c). Subtegmentary musculature consisted of small bundles of striated muscle fibers, more pronounced at the anterior and posterior extremities, resulting in localized thickening of the body wall (Figs. 1a, 2d). The oral and ventral suckers exhibited a complex muscular architecture, formed by large longitudinal muscle bundles interspersed with collagen and elastic fibers and externally covered by a thin epithelial layer (Figs. 1a, 1b, 2a). A shallow tegumentary invagination between the genital pore and the ventral sucker was consistently observed in *L. monticellii* (Fig. 2a), whereas it was less conspicuous in *L. microstomum* (Fig. 1b). In both species, the intestinal ceca were lined by flattened cells arranged in a single layer and exhibited a uniformly smooth luminal surface (Figs. 1e, 2c).

The reproductive system was well developed in both species. In *L. microstomum*, the testes were arranged along the longitudinal axis of the body (Figs. 1c, 1d, 1f), whereas the ovary consisted of germ cells at different developmental stages embedded in a fibroelastic stroma with associated muscle cells (Fig. 2c). The seminal vesicle, positioned in close proximity to the ventral sucker, was enclosed by fibromuscular stroma and contained abundant spermatozoa (Figs. 1a, 2a, 2b). The cirrus was enclosed within a cirrus sac lined by an epithelium slightly thicker than that of the copulatory organ itself and surrounded by well-developed musculature, reflecting the structural specialization of the male copulatory apparatus (Fig. 2b). The junction between the ejaculatory duct and the terminal portion of the uterus formed a distinct hermaphroditic duct. The uterus was lined by a simple squamous epithelium and contained eggs embedded in a matrix with modest elastic content (Figs. 1c, 1e).

Histochemical analysis using Gomori's trichrome enabled clear differentiation between connective and muscular components, with collagen fibers staining green and musculature staining red (Fig. 1a). In contrast, hematoxylin and eosin staining highlighted intensely basophilic nuclei and eosinophilic cytoplasm, facilitating detailed assessment of tissue organization and cellular architecture (Figs. 1b–f, 2a–d).

Comparative synthesis:

Although the overall histological organization was conserved between species, marked interspecific differences were observed in the posterior body region and male copulatory structures, which are of clear taxonomic relevance.

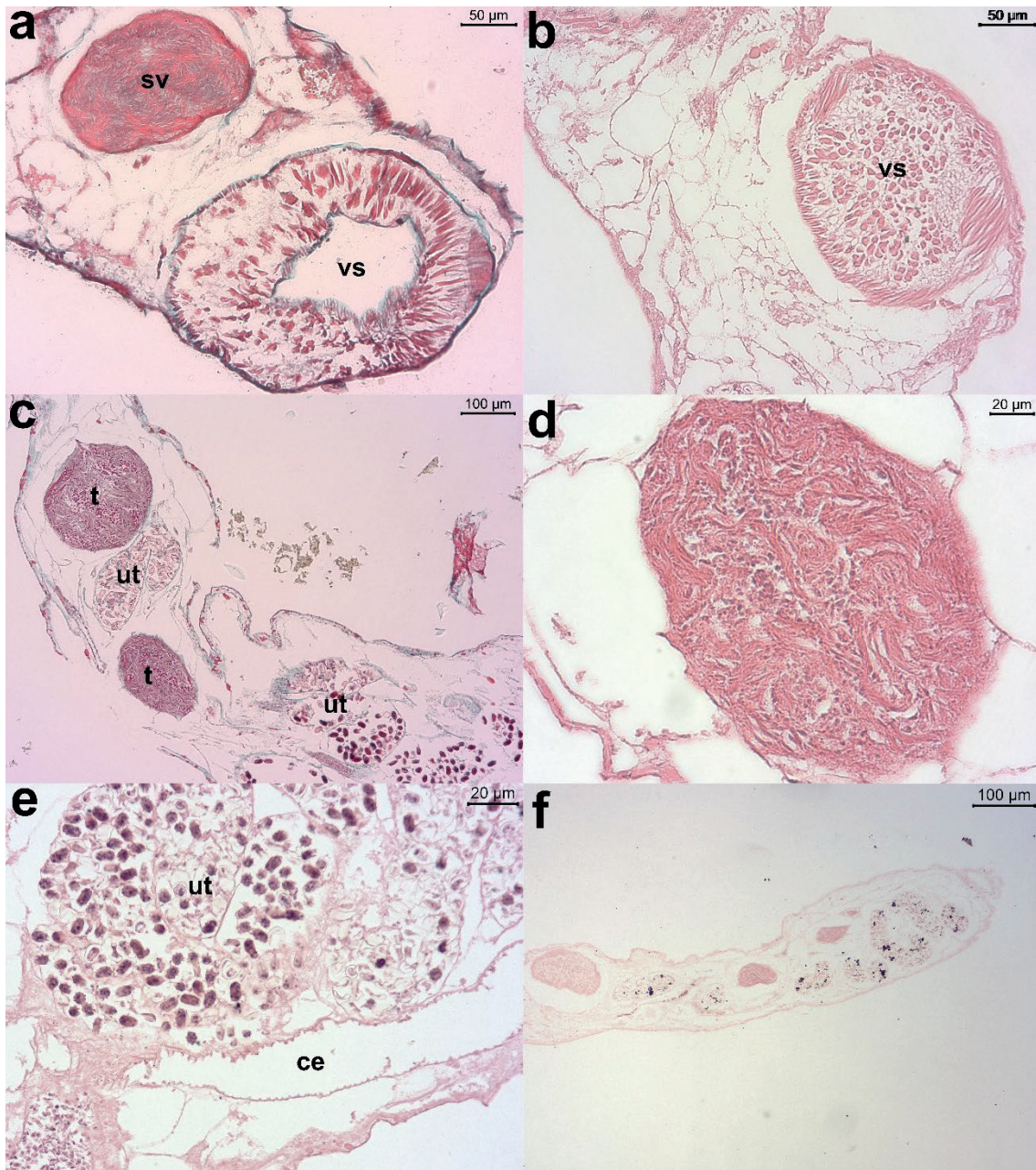


Figure 1. Histological longitudinal sections of *Lecithochirium microstomum*. Figure 1a shows the ventral sucker stained with Gomori's trichrome. Figures 1b–f corresponds to sections stained with hematoxylin and eosin (H&E), illustrating the ventral sucker at a deeper tissue level (Fig. 1b), reproductive structures (Fig. 1c), the seminal vesicle in detail (Fig. 1d), the intestinal ceca in detail (Fig. 1e), and the posterior extremity (Fig. 1f). Abbreviations: vs, ventral sucker; sv, seminal vesicle; t, testicle; ut, uterus filled with eggs; ce, ceca.

Lecithochirium microstomum exhibited a shorter and more robust body, with a reduced and partially internalized ecsoma (Fig. 1f), whereas *L. monticellii* displayed a well-developed ecsoma, representing a diagnostically informative character (Fig. 2d). No evidence of histological sexual dimorphism was detected beyond the expected differentiation of reproductive structures, indicating

that the observed variations primarily reflect species-level morphological divergence rather than intraspecific variation.

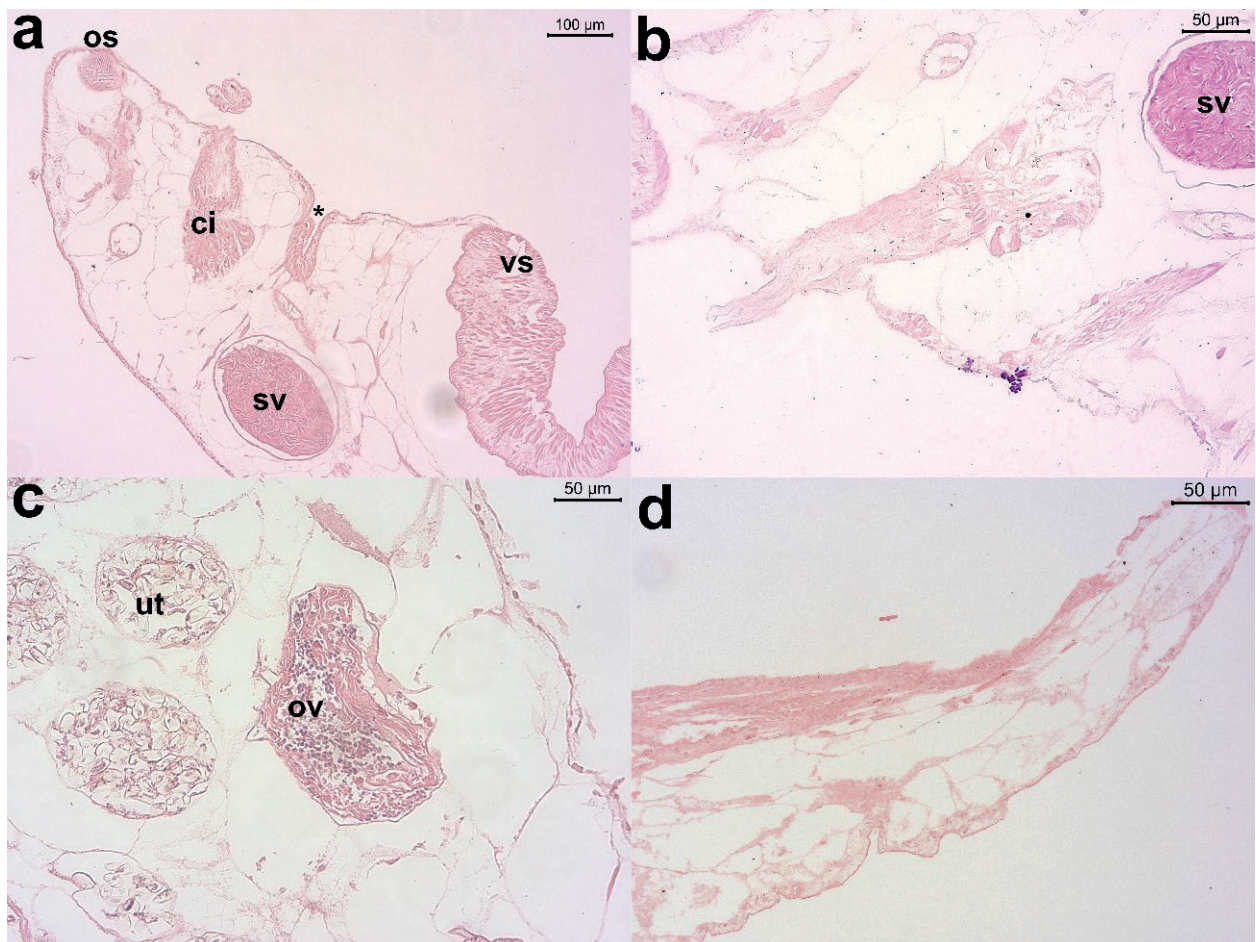


Figure 2. Histological longitudinal sections of *Lecithochirium monticellii*. (a) Anterior extremity. (b) Cirrus and associated structures in detail. (c) Female reproductive structures and intestinal ceca in detail. (d) Posterior extremity. Abbreviations: os, oral sucker; vs, ventral sucker; sv, seminal vesicle; ci, cirrus; ut, uterus filled with eggs; ov, ovary. Note: An asterisk (*) indicates a small tegumentary invagination.

DISCUSSION

- Corroboration of previous knowledge

Histological analysis contributes substantially to the understanding of helminth morphology and physiology, refining taxonomic descriptions and supporting functional interpretations. The histological features observed in *L. microstomum* and *L. monticellii* corroborate classical descriptions based on external morphology and morphometry (Chandler, 1935; Linton, 1898; Fonseca *et al.*, 2022; Pantoja & Kudlai, 2022), while providing internal anatomical confirmation through a methodological approach not previously applied to these taxa.

Both species exhibited a syncytial tegument covered by a glycocalyx, robust oral and ventral suckers, and a well-developed reproductive system, consistent with histological

patterns reported for other digenean trematodes (Halton & Dermott, 1967; Machado *et al.*, 2024a, b). The dense subtegumentary fibrous network likely contributes to protection against host digestive enzymes and provides mechanical resistance, supporting earlier functional interpretations of the tegument. Similarly, the muscular architecture of the suckers, characterized by densely packed longitudinal fibers, corroborates their role in firm attachment within the host intestine, where peristaltic forces are intense (Bosi *et al.*, 2022).

The organization of the testes, ovary, and seminal vesicle also aligns with histological descriptions reported for other digeneans (Hanna, 2015; Silva *et al.*, 2005; Panyarachun *et al.*, 2013), reinforcing the presence of conserved morphofunctional traits within Hemiuridae.

- Novel histological findings

Beyond corroboration, the present study provides previously undocumented histological data for *L. microstomum* and *L. monticellii*. The combined use of hematoxylin–eosin, Gomori's trichrome, and orcein staining allowed a detailed assessment of tissue composition and spatial organization that is inaccessible through whole mounts or surface-based techniques alone.

The thin, uniformly organized intestinal ceca, lined by flattened cells arranged in a single layer, were shown to be continuous along the body, refining earlier anatomical descriptions and confirming patterns observed in other digeneans (Lakshmi & Rao, 1978). The ovary, embedded in a fibroelastic stroma, exhibited a compact histological organization, adding novel information on its internal composition and mechanical support during gametogenesis.

Most notably, the present study documented clear histological differences in the copulatory apparatus between the two species. The cirrus of *L. microstomum* was shorter and wider, whereas that of *L. monticellii* was longer and narrower, differences that had been previously inferred from external morphology but not confirmed histologically. This internal differentiation provides independent evidence supporting species-level divergence.

- Taxonomic and evolutionary implications

The histological differences identified here have direct taxonomic relevance. The morphology of the copulatory organ and the organization of the posterior body region, particularly the presence of a well-developed ecsoma in *L. monticellii* and its reduction or absence in *L. microstomum*, represent reliable diagnostic characters. The histological confirmation of these traits strengthens species delimitation and reduces ambiguity in the identification of closely related taxa.

Comparative analysis with other hemiurids, such as *Aponurus laguncula* (Looss, 1907) (Machado *et al.*, 2024b), and with digeneans from other families, including *Prosozonotrema bilabiatum* (Vigueras, 1940) (Machado *et al.*, 2024a), suggests the existence of conserved histological patterns within Digenea. These patterns likely reflect evolutionary constraints associated with a shared intestinal niche, where efficient attachment, nutrient absorption, and resistance to host digestive processes are essential.

At the same time, the subtle but consistent histological differences between *L. microstomum* and *L. monticellii* indicate that evolutionary divergence within *Lecithochirium*

is expressed not only externally but also in internal anatomical organization. Such differences may reflect adaptive responses to microhabitat variation or host-related selective pressures, as previously suggested by ecological studies (Carvalho & Luque, 2011; França *et al.*, 2020).

By integrating histology with traditional morphology, ecology, ultrastructure, and molecular approaches (Pantoja & Kudlai, 2022), the present study demonstrates that histological characters constitute robust, independent taxonomic markers. This integrative perspective enhances taxonomic resolution, deepens understanding of parasite functional biology, and provides a solid morphological framework for future evolutionary and phylogenetic studies of *Lecithochirium* and related hemiurid trematodes.

The histological evidence presented here demonstrates that tissue-level characters provide reliable diagnostic information that complements traditional morphological approaches and enhances species delimitation. By documenting the internal organization of key structures—particularly the tegument, musculature, and reproductive and digestive systems—this study highlights histology as a robust and independent source of taxonomic evidence. These findings reinforce the role of histological analysis within an integrative taxonomic framework and underscore its relevance for understanding morphological differentiation and evolutionary patterns in Hemiuridae.

- Methodological limitations

The limitations of the present study are mainly related to the inherent difficulties associated with the histological processing of small-bodied digenean platyhelminths of the genus *Lecithochirium*, whose reduced size, delicate tegument, and highly compact internal organization impose technical challenges during fixation, embedding, sectioning, and staining.

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