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EXPANDING MORPHOLOGICAL KNOWLEDGE OF TWO NEMATODE SPECIES PARASITIZING SOUTHEASTERN BRAZILIAN MARINE FISHES

AMPLIACIÓN DEL CONOCIMIENTO MORFOLÓGICO DE DOS ESPECIES DE NEMATODOS PARÁSITOS DE PECES MARINOS DEL SURESTE DE BRASIL

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Running Head: Histological Data on Fish-Parasitic Nematodes
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ABSTRACT

Histological approaches have increasingly been incorporated into integrative taxonomy as complementary sources of morphological information. However, their application to parasitic nematodes remains limited. The present study provides a histological characterization of two nematode taxa recovered from marine fishes collected along the coast of the State of Rio de Janeiro, southeastern Brazil: larval specimens of *Anisakis* sp. from *Pogonias cromis* (Linnaeus, 1766) and adult specimens of *Dichelyne (Cucullanellus) sciaenidicola* Timi, Lanfranchi, Tavares & Luque, 2009 from *Micropogonias furnieri* (Desmarest, 1823). Recovered nematodes were fixed in neutral buffered formalin, processed using routine histological techniques, sectioned at 5 μm, and stained with hematoxylin and eosin and Gomori's trichrome. Histological examination of *Anisakis* sp. revealed a thick multilayered cuticle, well-developed somatic musculature, ventriculus, intestine, and boring tooth, corroborating the diagnostic morphology of anisakid larvae. In *D.* (*C.*) *sciaenidicola*, the esophagus exhibited a characteristic division into an anterior isthmus-like region and a posterior bulbous portion, while the intestine displayed small villus-like mucosal projections and a cuticular lining in the anterior digestive tract. The observations obtained were consistent with previous morphological descriptions and provided additional information on the internal organization of both species. The results demonstrate that histological analyses can contribute useful complementary characters for nematode taxonomy, while expanding knowledge of the morphofunctional organization of marine fish parasites from the Brazilian coast.

Keywords: Anisakidae – Cucullanidae – fish parasites – histology – integrative taxonomy – morphology

RESUMEN

Los enfoques histológicos se han incorporado cada vez más a la taxonomía integrativa como fuentes complementarias de información morfológica. Sin embargo, su aplicación al estudio de nematodos parásitos sigue siendo limitada. El presente estudio proporciona una caracterización histológica de dos taxones de nematodos recuperados de peces marinos recolectados a lo largo de la costa del estado de Río de Janeiro, en el sureste de Brasil: ejemplares larvarios de *Anisakis* sp., procedentes de *Pogonias cromis* (Linnaeus, 1766), y ejemplares adultos de *Dichelyne (Cucullanellus) sciaenidicola* Timi, Lanfranchi, Tavares & Luque, 2009, procedentes de *Micropogonias furnieri* (Desmarest, 1823). Los nematodos recuperados fueron fijados en formalina tamponada neutra, procesados mediante técnicas histológicas de rutina, seccionados a 5 μm y teñidos con hematoxilina-eosina y tricrómico de

Gomori. El examen histológico de *Anisakis* sp. reveló una cutícula gruesa y multilaminada, musculatura somática bien desarrollada, ventrículo, intestino y diente perforador, corroborando la morfología diagnóstica de las larvas anisákidas. En *D.* (*C.*) *sciaenidicola*, el esófago presentó una división característica en una región anterior semejante a un istmo y una porción posterior bulbosa, mientras que el intestino mostró pequeñas proyecciones mucosas similares a vellosidades y un revestimiento cuticular en la porción anterior del tracto digestivo. Las observaciones obtenidas fueron consistentes con las descripciones morfológicas previas y aportaron información adicional sobre la organización interna de ambas especies. Los resultados demuestran que los análisis histológicos pueden aportar caracteres complementarios útiles para la taxonomía de nematodos, además de ampliar el conocimiento sobre la organización morfofuncional de los parásitos de peces marinos de la costa brasileña.
Palabras clave: Anisakidae – Cucullanidae – histología – morfología – parásitos de peces – taxonomía integrativa

INTRODUCTION

With the advent of new techniques and technologies, an increasing number of tools have been incorporated into species descriptions and redescriptions. Within the framework of integrative taxonomy, multiple complementary approaches, including diverse morphological analyses, molecular methods, ecological data, histology, and scanning electron microscopy, have been applied concurrently in the study of parasitic species (Dayrat, 2005; De León & Poulin, 2018; Machado *et al.,* 2024ab). Recent studies have demonstrated that the integration of multiple sources of morphological and molecular evidence provides a more comprehensive understanding of parasite morphology and contributes to more robust species diagnoses and redescriptions (Machado *et al.,* 2024ab), while histological analyses have emerged as an additional complementary source of taxonomic information. This methodological integration allows a more comprehensive assessment of taxonomic characters at both species and higher taxonomic levels, while promoting the exchange of knowledge among distinct research fields. Furthermore, it may facilitate the recognition of novel taxonomically informative characters through the application of methodologies that have been rarely or never previously employed in the study of a given group, as well as support evolutionary interpretations (Schlick-Steiner *et al.,* 2010; Rojas *et al.,* 2025).

In this context, histological analyses may expand the range of descriptive characters available for parasitic species by incorporating a cellular and tissue-level perspective into morphological studies, thereby enabling functional interpretations of organs and structures.

Although histological approaches have occasionally been employed in taxonomic studies of parasites, their use has only recently received renewed attention (Machado *et al.,* 2024ab, 2026; Silva De Oliveira *et al.,* 2026). However, most of these investigations have focused on platyhelminths, leaving substantial gaps in knowledge regarding the histological organization of other parasitic groups. In addition, a large proportion of parasitological studies employing histological techniques have been directed toward the assessment of host-parasite interactions and associated pathological alterations rather than the intrinsic structural characterization of the parasites themselves (Dezfuli *et al.,* 2005; Magdálek *et al.,* 2021; Ramdani *et al.,* 2022).

Accordingly, the present study provides a histological characterization of two nematode taxa, *Anisakis* sp. and *Dichelyne (Cucullanellus) sciaenidicola* Timi, Lanfranchi, Tavares & Luque, 2009, recovered from the marine fishes *Pogonias cromis* (Linnaeus, 1766) (Black drum) and *Micropogonias furnieri* (Desmarest, 1823) (Whitemouth croaker), respectively, collected along the coast of the State of Rio de Janeiro, southeastern Brazil. *Anisakis* sp. was selected because of its worldwide distribution, zoonotic importance, and the limited histological information available for its larval stages, whereas *D. (C.) sciaenidicola* represents a common parasite of sciaenid fishes and provides an opportunity for comparative histological analysis between distinct nematode taxa. By complementing the existing morphological knowledge of these taxa, this study seeks to encourage the incorporation of histological approaches into nematode taxonomy, contributing additional sources of descriptive information and broadening the scope of future taxonomic and comparative investigations.

MATERIAL AND METHODS

Host Necropsy and Parasite Collection

A total of eight specimens each of *Micropogonias furnieri* (Desmarest, 1823) and *Pogonias cromis* (Linnaeus, 1766) were obtained from local fishermen at the Central de Abastecimento do Estado do Rio de Janeiro (CEASA), Rio de Janeiro State, Brazil. The fish were maintained under refrigeration and transported to the Laboratório de Parasitologia de Peixes e Mamíferos Silvestres e Sinantrópicos, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brasil, where necropsies were performed. Host identification followed Menezes & Figueiredo (1985).

The digestive tract of each fish was removed, opened longitudinally, and washed under running water through a 150 µm mesh sieve. The retained material was transferred to Petri dishes and examined under a stereomicroscope for the presence of parasites. Recovered specimens were collected and fixed in 10% neutral buffered formalin for subsequent

histological processing. All procedures involving formalin were carried out in a chemical fume hood using appropriate personal protective equipment, in accordance with institutional biosafety guidelines for the handling and disposal of hazardous chemical substances. Preliminary identification was based on the available taxonomic literature. Larvae of *Anisakis* sp. were identified following Berland (1961), Smith (1983), and Mattiucci & Nascetti (2008), whereas specimens of *Dichelyne (Cucullanellus) sciaenidicola* were identified according to the original description by Timi *et al.* (2009) and subsequent taxonomic revisions.

Histological Processing

Histological processing of the nematodes followed the protocol described by Abrahamsohn (2016). Specimens were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Longitudinal and transverse sections, 5 μm thick, were obtained using an automatic microtome. Sections were stained with hematoxylin-eosin (HE) and Gomori's trichrome (GT). Permanent slides were mounted using Entellan® mounting medium. Histological preparations were examined and photographed using an Olympus BX51 light microscope equipped with the Capture 2.4 image analysis software. Voucher specimens (histological slides) of both specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro (Brazil), under deposit number xxxxxx.

Ethical Aspects

All applicable institutional, national, and international guidelines for the care and use of animals were followed. As no live animals were used and all fish specimens were obtained from a local fish market, approval by an animal ethics committee was not required, in accordance with Article 3, Item III, of Brazilian Federal Law No. 11.794.

RESULTS

Anisakis sp. (L_3, third-stage larvae)

Based on 06 specimens.

Host: *Pogonias cromis* (Linnaeus, 1766), black drum.

Site of infection: Gut.

Prevalence: 25%.

Deposited material: deposit number to be provided.

Acellular tegument covered by a thick multilaminar cuticle composed of regular transverse striations (Fig. 1a–e). Subcuticular region formed by extensive bundles of

transversely striated muscle fibers arranged longitudinally (fig. 1a–e). Interior of pseudocoelomic cavity composed of stroma consisting of loose connective tissue and free acidophilic cells with prominent nucleus (fig. 1a–c). In transverse section, two large lateral muscle groups are observed, whose dorsal and ventral junctions appear associated with attachment sites for internal structures and the subcuticular region (fig. 1a). Esophagus lined by columnar epithelium (fig. 1d). Ventriculus lined by epithelium of variable thickness (fig. 1a, b). Intestine lined by columnar epithelium (fig. 1c, e). The digestive tract contains luminal content throughout its extension (fig. 1a, c, e). Boring tooth consisting of cuticular projection (fig. 1b). Latero-terminal anus represented by region of cuticular invagination (fig. 1e).

Dichelyne (Cucullanellus) sciaenidicola Timi, Lanfranchi, Tavares & Luque 2009

Based on 27 adult specimens

Host: *Micropogonias furnieri* (Desmarest, 1823), whitemouth croaker.

Site of infection: Gut.

Prevalence: 37.5%.

Deposited material: deposit number to be provided.

Thin syncytial tegument covered by thin acidophilic cuticle (fig. 2a–h). Subepidermal region composed of loose connective tissue (Fig. 2a–h). Supporting musculature not evident. Interior of pseudocoelomic cavity composed of stroma consisting predominantly of loose connective tissue and small free cells (Fig. 2a–h). Large acidophilic coelomocytes with prominent, apparently lobulated nucleus and cytoplasmic granules observed in region lateral to intestine (fig. 2a). In transverse section, two discrete lateral muscle groups observed, whose dorsal and ventral junctions appear associated with attachment points of filling stroma and internal structures (fig. 2a). Cephalic structures consisting of small projection of thin lining epithelium and underlying stroma (fig. 2b, c). Mouth terminally located in the anterior region, with the presence of small cells embedded in surrounding connective tissue (fig. 2b, c). Pseudobuccal cavity composed of two lateral groups of small longitudinal muscle bundles, basophilic cells, and mucous glands, with a hollow cavity present in the most anterior portion of the structure (fig. 2b, c). Esophagus elongated, divided into two portions, anterior portion isthmus-shaped and posterior portion bulb-shaped; esophageal lined by acidophilic cuticle, with directly underlying region formed by acidophilic glandular cells arranged as tubular structures (fig. 2b–e). Intestine lined by cuboidal epithelium composed of small cells of variable thickness; in transverse section, small mucosal projections similar to villi are observed (fig. 2a, e–h). The digestive tract contains luminal content throughout its extension (fig. 2e–h).

DISCUSSION

This study provides a histological characterization of two nematode species recovered from marine fishes: larval specimens of *Anisakis* sp. and adult specimens of *D.* (*Cucullanellus*) *sciaenidicola*. Histological techniques have traditionally been employed in helminthological studies primarily to assess the pathological effects of parasites on their hosts (Dezfuli *et al.,* 2005; Magdálek *et al.,* 2021; Ramdani *et al.,* 2022). More recently, however, these approaches have also been incorporated into integrative taxonomic studies to provide morphofunctional information that complements conventional morphological descriptions (Machado *et al.,* 2024ab, 2026; Silva De Oliveira *et al.,* 2026).

The histological characterization of the *Anisakis* sp. larvae recovered from *P. cromis* revealed an internal organization consistent with the diagnostic morphology traditionally recognized for the genus, despite the absence of species-level identification (Bicudo *et al.,* 2005). The presence of a thick, acellular, multilayered cuticle associated with well-developed somatic musculature represents a characteristic feature of anisakid nematodes, as described by Moravec (1998). In addition, the visualization of the boring tooth and ventriculus in longitudinal sections provided anatomical evidence supporting generic identification, highlighting the value of histological preparations for the examination of structures that may be obscured in whole-mounted larval specimens. For *D. (Cucullanellus) sciaenidicola*, histological examination allowed a detailed assessment of the esophageal morphology, including an anterior isthmus-like region and a posterior bulbous portion, in agreement with the diagnostic features reported by Timi *et al*. (2009). An additional feature documented in the present study was the occurrence of villus-like projections of the intestinal mucosa, a character rarely emphasized in descriptions based exclusively on external morphology or cleared specimens.

The agreement between the present observations and the descriptions provided by Moravec (1998), Bicudo *et al.* (2005), and Timi *et al.* (2009) demonstrates that histological data can provide a valuable source of complementary taxonomic information. Beyond corroborating species identity, the examination of internal tissue organization may reveal additional characters that contribute to comparative morphological studies and broaden the range of traits available for integrative taxonomic analyses.

The muscular organization of nematodes has been investigated since the early development of nematode systematics, including attempts to classify taxa according to the arrangement of muscle cells and fibers (Chitwood, 1931). In the present study, the somatic

musculature exhibited morphological similarities to striated muscle tissue described in vertebrates. Comparable observations have also been reported in other parasitic invertebrates (Landman *et al.,* 2023; Machado *et al.,* 2024a; Machado *et al.,* 2024b; Machado *et al.,* 2026; Silva De Oliveira *et al.,* 2026). Although these similarities are based solely on histological appearance, they may indicate the conservation of certain structural features among metazoans. Further studies integrating histological, molecular, and physiological approaches are necessary to determine whether these similarities extend beyond morphology.

Regarding muscular development, larval specimens of *Anisakis* sp. exhibited proportionally more developed somatic musculature than adult specimens of *D. (C.) sciaenidicola*. This difference may reflect distinct functional demands associated with different life stages and ecological strategies. However, previous studies on *Ascaris* Linnaeus, 1758 have demonstrated substantial changes in musculature throughout ontogenetic development (Stretton, 1976). Consequently, comparative investigations involving multiple developmental stages of the same species are required to determine whether the differences observed here are primarily related to ontogeny or represent species-specific traits.

In *Dichelyne* (*Cucullanellus*) *sciaenidicola*, small projections of the intestinal mucosa extending toward the lumen were observed. Similar structures have previously been reported in a digenean trematode (Machado *et al*., 2024a), where they were interpreted as increasing the absorptive surface of the digestive tract. Although functional studies are lacking, the structures observed in the present material may perform a comparable role. Furthermore, the cuticular lining observed in the anterior portion of the digestive tract agrees with previous descriptions for other nematode taxa (Basyoni & Rizk, 2016).

The histological analyses performed on *Anisakis* sp. larvae and *D.* (*Cucullanellus*) *sciaenidicola* adults demonstrated that the internal organization of these nematodes is consistent with diagnostic features previously established through classical morphological studies. The visualization of structures such as the cuticle, somatic musculature, and digestive tract provided complementary evidence for taxonomic characterization while revealing anatomical details that are often difficult to observe in conventional whole-mounted specimens. Although restricted to two species, the present study highlights the usefulness of histological approaches as a complementary tool in nematode taxonomy and morphology, expanding the morphofunctional knowledge available for marine fish parasites from the Brazilian coast and providing a comparative basis for future integrative studies involving related taxa.

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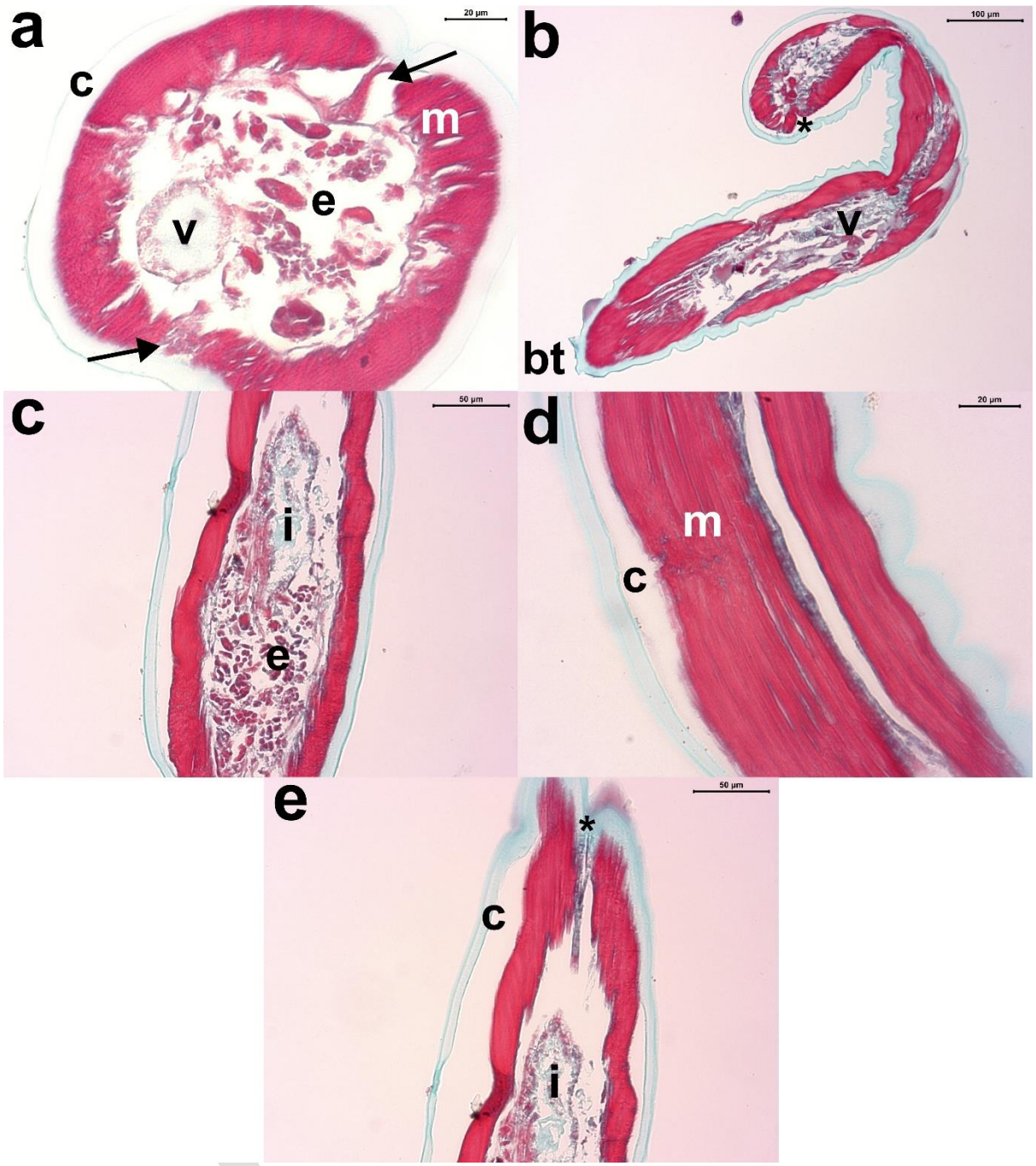

Figure 1. Histological transversal (a) and longitudinal sections (b–e) of *Anisakis* sp., stained with Gomori's trichrome (GT). **a** middle body, **b** entire body, **c** middle body, **d** esophagus in detail, and **e** terminal portion in detail. Abbreviations: **c** cuticle, **m** musculature, **e** stroma, **v** ventriculus, **bt** boring tooth, **i** intestine, and * indicates anus.

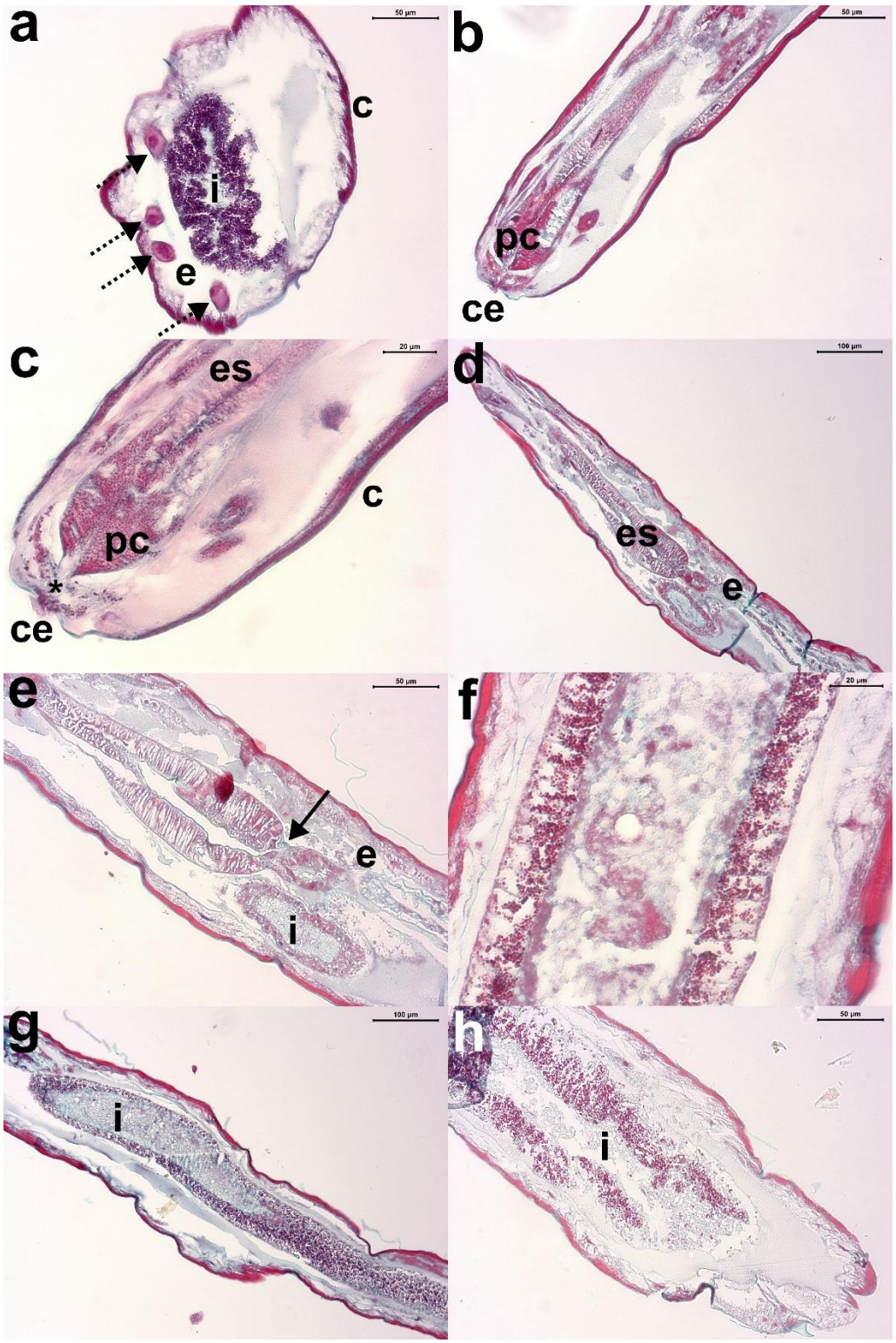

Figure 2. Histological transversal (a) and longitudinal sections (b–h) of *Dichelyne (Cucullanellus) sciaenidicola*, stained with hematoxylin and eosin (HE). **a** middle body, **b** anterior portion, **c** anterior portion in detail, **d** forebody, **e** digestive structures in detail, **f** intestine in detail, **g** posterior body section, and **h** terminal portion in detail. **Abbreviations: c** cuticle, **e** stroma, **i** intestine, **pc** pseudobucal cavity, **es** esophagus, **ce** cephalic structures, and * indicates mouth.