

Severe spirorchidiasis associated with fibropapillomatosis in a juvenile green turtle (*Chelonia mydas*) from Alagoas, Brazil

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Abstract: Spirorchid blood flukes and fibropapillomatosis are important health concerns of sea turtles, particularly juvenile green turtles (*Chelonia mydas*). This report describes their concurrent occurrence in a free-ranging juvenile stranded alive on the coast of Alagoas, northeastern Brazil. The turtle had a carapace length of 37 cm, poor body condition, severe pallor, dehydration, ocular abnormalities, apathy, multiple cutaneous masses, and a 12-cm deep carapacial fracture. It died approximately four hours after admission. Necropsy revealed hemorrhagic coelomic fluid, multifocal dark visceral lesions, cardiac dilation, and numerous leaf-shaped trematodes in the cardiac chambers and mesenteric vessels, of which approximately 15 were collected for parasitological examination. Morphological features were consistent with *Learedius learedi*, although molecular confirmation was not performed. Histopathology demonstrated widespread egg-associated granulomas in the heart, lungs, liver, spleen, kidneys, and intestines. Most eggs were compatible with spirorchid type 1 morphology, whereas rare rounded, non-filamented eggs were suggestive, but not diagnostic, of *Neosporichis* spp. Cutaneous and eyelid masses showed epithelial and fibroblastic proliferation consistent with fibropapillomas. Acute traumatic injury with internal hemorrhage was considered the most likely immediate cause of death, while systemic spirorchidiasis, fibropapillomatosis, poor body condition, and chronic debilitation were considered significant pre-existing conditions. This case highlights the value of integrated necropsy, parasitology, and histopathology in sea turtle mortality investigations.

Keywords: Cheloniidae; Histopathology; *Learedius learedi*; *Neosporichis*; Testudines.

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1. Introduction

Sea turtle populations have declined markedly because of interacting anthropogenic, environmental, and infectious pressures. Green turtles (*Chelonia mydas*) are exposed to fisheries interactions, habitat degradation, marine pollution, trauma, and a wide range of parasitic and infectious diseases. Among the most consequential pathological conditions are spirorchidiasis, caused by blood flukes of the family Spirorchidae, and fibropapillomatosis, a neoplastic disease most frequently reported in juvenile green turtles [1–4].

Spirorchids are digenetic trematodes that inhabit the cardiovascular system of turtles. Adults occur in the heart and major vessels, where they produce eggs that dissemi-

nate through the circulation. Entrapped eggs can induce vasculitis, thrombosis, tissue necrosis, and granulomatous inflammation in multiple organs. Lesion severity varies from incidental microscopic findings to extensive multisystem disease associated with debilitation and death [5–8]. *Learedius learedi* is among the most frequently reported large spirorchids in green turtles, whereas *Neospororchis* species are smaller and may be difficult to recover during routine necropsy [4,9,10].

Fibropapillomatosis is characterized by single or multiple cutaneous, ocular, and occasionally visceral tumors. Its development is associated with Chelonid alphaherpesvirus 5, although disease expression is multifactorial and influenced by host immunity and environmental conditions. Tumor location and burden may impair vision, swimming, feeding, respiration, and overall fitness, while affected tissues can become sites of secondary infection [11–14].

Concurrent infectious, parasitic, and traumatic conditions can complicate the interpretation of sea turtle strandings. This report describes the clinical, macroscopic, parasitological, and histopathological findings in a juvenile green turtle with systemic spirorchidiasis, fibropapillomatosis, and severe carapacial trauma. To our knowledge, this is the first published report of this combination of findings from the coast of Alagoas, Brazil.

2. Case Report

A free-ranging juvenile female green turtle was found stranded alive on 29 November 2017 along the coast of Alagoas, northeastern Brazil, and transported to the Veterinary Medicine Teaching Clinic at Centro Universitário CESMAC. The turtle measured 37 cm in carapace length and had a deep fracture in the dorsal carapace. On admission, it was in poor body condition and showed markedly pale conjunctival mucosa, retracted globes, ocular opacity, severe apathy, and dehydration. Despite supportive clinical attention, the animal died approximately four hours after arrival and was submitted to the CESMAC Veterinary Necropsy Laboratory for post-mortem examination. No ante-mortem blood sample was collected for packed cell volume/total solids determination or blood-smear examination; consequently, the degree of anemia could not be objectively quantified, and circulating parasitic stages were not assessed.

A necropsy was performed. Samples of the eyelid and cutaneous nodules, heart, lungs, liver, spleen, kidneys, stomach, and intestines were fixed in 10% neutral-buffered formalin. After approximately 48 hours of fixation, tissues were routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light-microscopic evaluation.

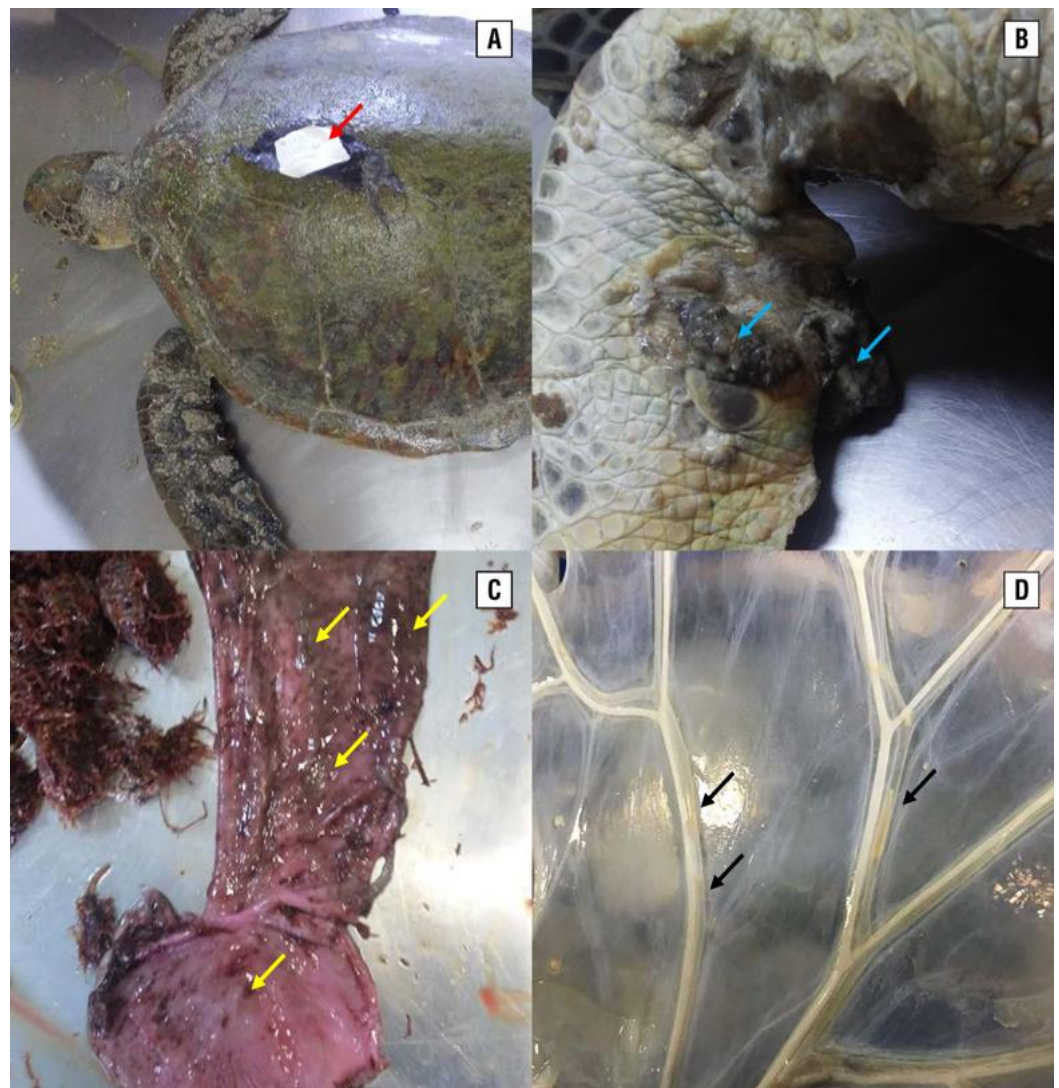
Approximately 15 representative cardiovascular trematodes were collected from the atria, ventricle, and mesenteric vessels, preserved in 70% ethanol, and submitted to the CESMAC Laboratory of Parasitic Diseases. Additional trematodes were observed but not collected; therefore, the number of specimens examined parasitologically should not be interpreted as an estimate of the total parasite burden. Specimens were measured, clarified in Petri dishes, and examined by light microscopy. Identification was based on body form and internal anatomical features described for spirorchiid trematodes, including the criteria proposed by [15].

Parasite recovery was based on gross collection of visible trematodes; intravascular flushing and gastrointestinal vascular or mucosal scraping were not performed. The brain was not sampled because the skull was required to remain intact for subsequent taxidermy. The spinal cord was also not collected during the original necropsy. External examination revealed a deep fracture measuring approximately 12 cm in the central dorsal carapace (Figure 1A). Approximately 13 papilliform to cauliflower-like masses, ranging from 0.5 to 13 cm, were distributed primarily around the eyes, neck, and both foreflippers (Figure 1B).

The coelomic cavity contained a moderate volume of viscous hemorrhagic fluid. Multifocal dark punctate lesions were distributed over several visceral surfaces (Figure

1C), and the skeletal musculature was diffusely pale with little blood released on sectioning. The heart was moderately dilated and had thinned myocardium, gelatinous degeneration of pericardial fat, and multifocal myocardial pallor. Numerous leaf-shaped trematodes were present in both atria and the ventricle (Figure 1D), and similar parasites were dispersed within mesenteric veins and arteries. The stomach and intestines were pale and contained a small amount of green plant material compatible with marine algae. Gonadal examination confirmed that the turtle was female.

Figure 1. Gross findings. (A) Deep fracture measuring approximately 12 cm in the center of the dorsal carapace. (B) Papilliform nodules of variable size on a foreflipper. (C) Multifocal dark punctate lesions on visceral surfaces. (D) Leaf-shaped trematodes in cardiovascular vessels.

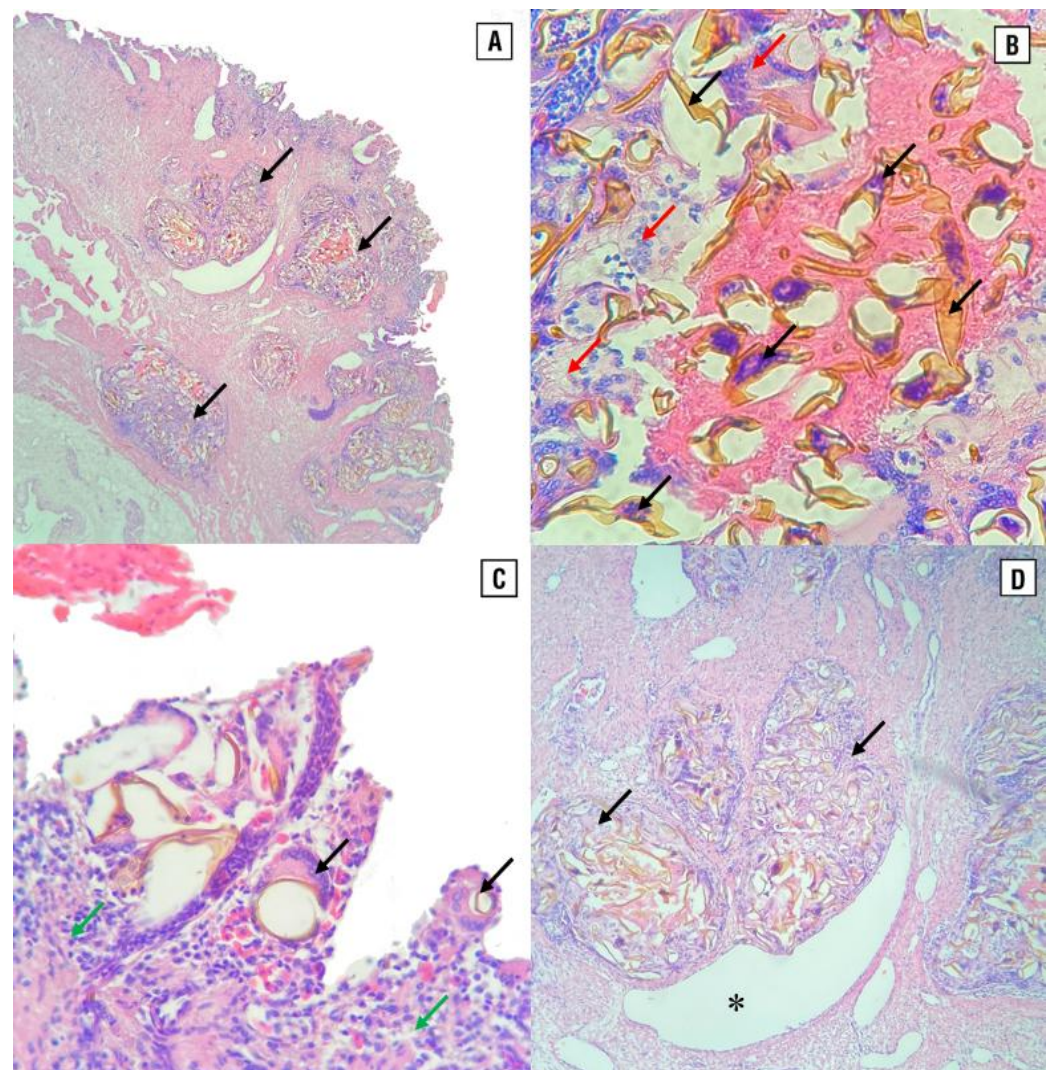


Based on the gross findings, the severe carapacial fracture and associated hemorrhagic coelomic fluid were interpreted as the principal acute lesions related to death. Systemic spirorchidiasis, fibropapillomatosis, poor body condition, and chronic debilitation were considered significant pre-existing conditions that may have reduced physiological reserve. The recovered flukes measured approximately 0.4 cm long and 0.1 cm wide. They had an elongated, dorsoventrally flattened body with rounded extremities, a slight constriction near the acetabulum, and a long, narrow, sinuous esophagus ending in a bulb. The combination of morphology and cardiovascular location was consistent with

Learedius learedi. Molecular confirmation was not performed; therefore, the identification should be interpreted as morphology-based rather than definitive.

The myocardium contained numerous multifocal to coalescing, non-encapsulated granulomas between myocardial fibers (Figure 2A). Their centers contained yellow-brown, thick-walled eggs that were fusiform to rounded and commonly had two polar filaments (Figure 2B). These structures were surrounded by multinucleated giant cells, macrophages, and lymphocytes (Figures 2B and 2C).

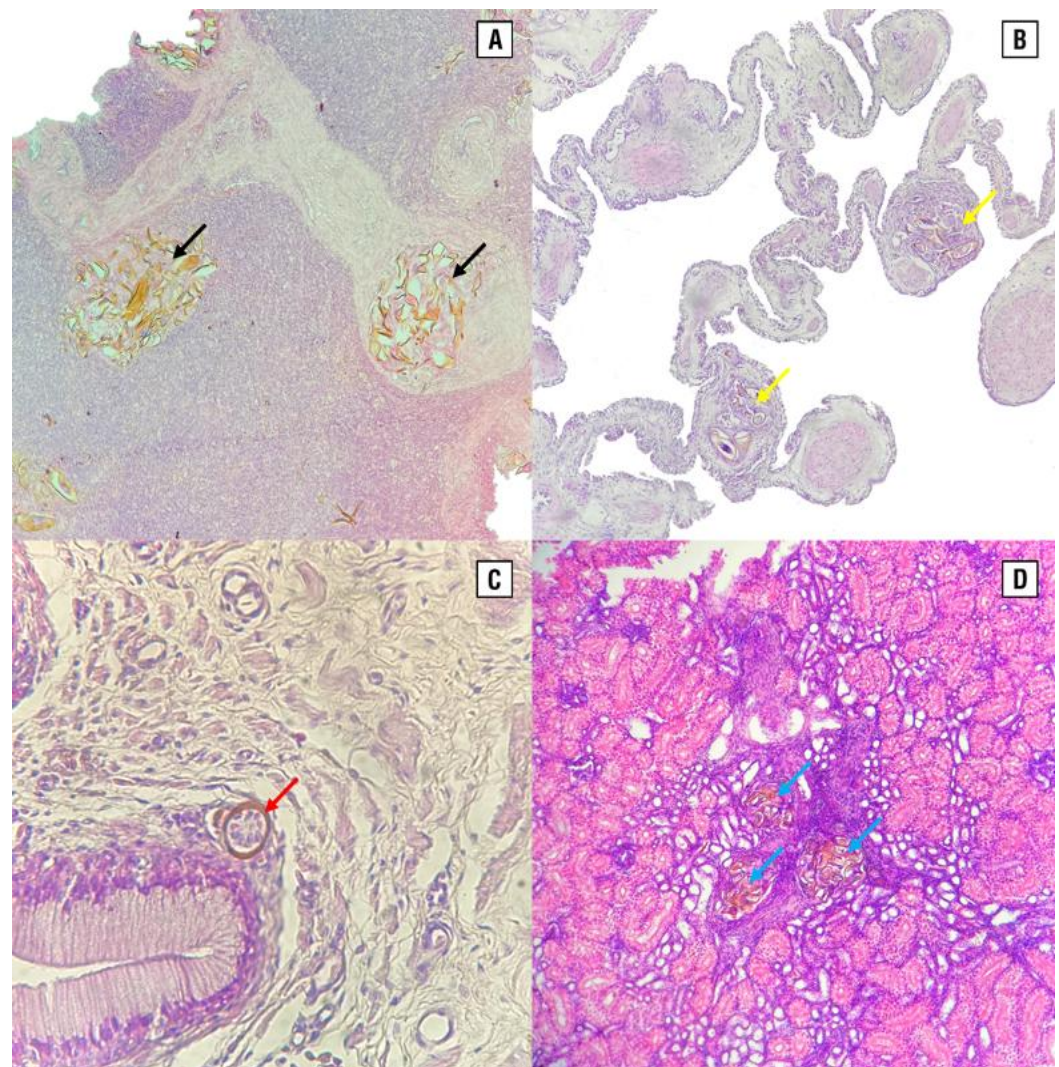
Figure 2. Cardiac histopathology. (A) Multiple parasitic granulomas between myocardial fibers (arrow), 4× objective. (B) Yellow-brown, thick-walled eggs with cellular contents (black arrow) and multinucleated giant cells (red arrow), 40× objective. (C) Macrophages surrounding parasitic structures (black arrow) with mononuclear inflammation (green arrow), 40× objective. (D) Granulomatous inflammation closely associated with a myocardial blood vessel (*), 10× objective. Hematoxylin and eosin.



Granulomatous inflammation was closely associated with myocardial vascular profiles, with apparent luminal narrowing in some sections (Figure 2D). The vascular wall architecture was not clearly discernible in the routine hematoxylin-and-eosin sections, precluding definitive confirmation of vascular wall invasion. Additional cardiac lesions included congestion, hemorrhage, degeneration and fibrosis of myocardial fibers, interstitial mononuclear inflammation, and endocardial inflammation associated with parasitic structures.

Egg-associated granulomas were also present in the pulmonary interstitium adjacent to respiratory bronchioles (Figure 3A), portal regions of the liver, splenic red and white pulp and splenic arterial walls (Figure 3B), renal interstitium (Figure 3C), and intestinal lamina propria. The liver additionally showed moderate hemosiderosis and mild hepatocellular vacuolation. The kidneys had mild diffuse tubular vacuolar degeneration and multifocal lymphoplasmacytic interstitial inflammation. In the intestines, eggs occurred in the lamina propria and within crypt lumina, accompanied by moderate lymphoplasmacytic inflammation.

Figure 3. Multiorgan histopathology. (A) Granulomas in the pulmonary interstitium adjacent to bronchioles (yellow arrow), 10× objective. (B) Parasitic granuloma in splenic parenchyma and stroma (arrow), 10× objective. (C) Parasitic granuloma in the renal interstitium (blue arrow), 40× objective. (D) Rounded, non-filamented egg profile in the intestinal lamina propria (red arrow), suggestive but not diagnostic of type 3 spirorchiid morphology, 40× objective. Hematoxylin and eosin.

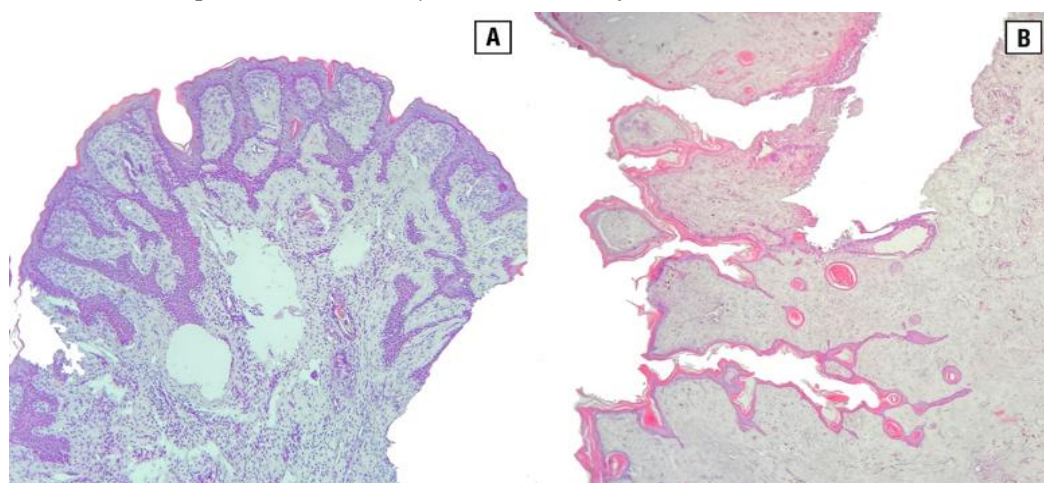


Most eggs corresponded to the type 1 pattern described for spirorchids: yellow-brown, fusiform eggs with bipolar filaments, compatible with *Learedius*, *Hapalotrema*, *Monticellius*, or *Amphiorchis*. Rare completely rounded, non-filamented egg profiles, including one in the intestinal lamina propria (Figure 3D), were also observed in the heart, liver, stomach, and intestines. These profiles were compatible with the type 3 morphology described for *Neospororchis* spp.; however, a tangentially sectioned type 1 egg could not be

excluded on morphology alone. Adult *Neospororchis* specimens were not recovered. Accordingly, these findings were considered suggestive, but not diagnostic, of concurrent *Neospororchis* infection.

The eyelid mass showed exophytic epidermal hyperplasia with trabecular projections into the underlying connective tissue, vacuolar degeneration of epithelial cells, and marked dermal fibroblast proliferation (Figure 4A). Comparable epithelial and fibroblastic proliferation was present in the cutaneous masses (Figure 4B). These features were consistent with fibropapillomas. Spirorchiid eggs were also present within the dermis of affected tissues.

Figure 4. Fibropapillomatosis. (A) Exophytic epidermal proliferation of the eyelid, consistent with fibropapilloma, 4× objective. (B) Cutaneous fibropapilloma with epithelial and fibroblastic proliferation, 4× objective. Hematoxylin and eosin.



3. Discussion and Conclusion

The principal chronic pathological finding in this turtle was severe multisystem spirorchiidiasis occurring concurrently with histologically confirmed fibropapillomatosis and a major acute traumatic injury. The distribution of adult flukes in the cardiac chambers and mesenteric vessels, together with their morphological features, was consistent with *Learedius learedi*. The widespread type 1 eggs and associated granulomatous lesions further supported infection by a large cardiovascular spirorchiid. However, molecular studies have demonstrated cryptic diversity and close morphological overlap among related spirorchiids [9]. The species-level identification in this case should therefore be regarded as morphology-based rather than definitive. Comparable adult and egg-associated lesions have been documented in green turtles from Brazil and other tropical and subtropical regions [2,4,6,7,9].

The dark punctate visceral lesions observed grossly likely represented foci of egg deposition, necrosis, and chronic inflammation. Histologically, parasitic granulomas affected the heart, lungs, liver, spleen, kidneys, and intestines, demonstrating extensive hematogenous dissemination. Granulomatous inflammation associated with myocardial vascular profiles, together with myocardial degeneration, hemorrhage, and fibrosis, could have impaired cardiovascular function. The vascular wall architecture was not clearly resolved in routine sections, limiting interpretation of Figure 2D and precluding definitive confirmation of mural invasion or true intravascular occlusion. The turtle's pallor, weakness, poor body condition, and lethargy were compatible with severe systemic disease, although these nonspecific findings could also reflect acute blood loss, hypovolemia, and chronic debilitation.

Rare rounded, non-filamented egg profiles were suggestive of the type 3 morphology described for *Neospororchis* spp. Adult *Neospororchis* can be difficult to recover because of their small size and predilection for narrow vessels. Their absence at necropsy does not

exclude infection. Nevertheless, rounded egg morphology is not pathognomonic, and tangential sectioning of type 1 eggs may produce similar profiles. In the absence of recovered adults or molecular confirmation, these findings should therefore be interpreted only as suggestive of concurrent *Neosporichis* infection. The inability to examine the brain and spinal cord further prevented assessment of possible neurospirorchidiasis.

The fibropapillomas were distributed around the eyes, neck, and foreflippers, locations commonly affected in juvenile green turtles. Histological examination demonstrated combined epithelial and fibroblastic proliferation, supporting the diagnosis of fibropapillomatosis. No molecular testing for Chelonid alphaherpesvirus 5 was performed; therefore, viral infection should not be presented as laboratory-confirmed in this case. Nevertheless, ocular and periocular tumor growth could have reduced vision, while the overall tumor burden may have impaired mobility and contributed to chronic physiological stress [3,11,12].

A possible biological interaction between chronic spirorchidiasis and fibropapillomatosis is plausible but cannot be resolved from this single case. Previous pathological work has documented concurrent spirorchidiasis and fibropapillomatosis and proposed that their combined effects may act synergistically as debilitating disease processes [16]. In addition, altered immune parameters have been reported in green turtles with fibropapillomatosis [13]. These observations raise competing, non-exclusive hypotheses: chronic parasitic disease could reduce physiological and immunological reserve in a manner that favors tumor expression or persistence, whereas the energetic and physiological costs associated with fibropapillomatosis could increase susceptibility to severe parasitic disease.

The presence of spirorchid eggs within the dermis of fibropapillomas was a noteworthy histological observation. The available findings, nevertheless, do not establish that egg deposition altered the tumor microenvironment or accelerated lesion growth. Without targeted quantification, serial evaluation, or other evidence linking egg-associated inflammation to tumor architecture, this observation should be interpreted cautiously as concurrent parasitic involvement of neoplastic tissue.

The severe carapacial fracture and hemorrhagic coelomic effusion indicate substantial acute trauma. The precise cause of the injury could not be established. Vessel strike, interaction with fishing activities, predation, or collision with a hard structure are possible mechanisms, but none can be assigned without direct evidence. Acute traumatic injury with internal hemorrhage was considered the most likely immediate cause of death. Systemic spirorchidiasis, fibropapillomatosis, poor body condition, and chronic debilitation were considered important pre-existing conditions that likely reduced physiological reserve and may have limited the turtle's capacity to compensate for acute blood loss.

The pathological significance of concurrent disease nevertheless deserves attention. Chronic parasitism and fibropapillomatosis can each reduce physiological reserve, and their coexistence may increase susceptibility to additional injuries and infections. However, a single case cannot establish an epidemiological association or estimate disease frequency in Alagoas. Broader surveillance using standardized necropsy, histopathology, parasite collection, and molecular assays would be required to determine prevalence and clarify whether this co-occurrence is common in local green turtles.

Ante-mortem diagnosis of spirorchidiasis remains challenging in free-ranging turtles. Copromicroscopy may detect eggs in captive animals, while serological and molecular approaches have potential but are not routinely available. In this case, the lack of ante-mortem hematology and blood-smear examination prevented objective assessment of anemia and evaluation for circulating parasitic stages. Consequently, integrated post-mortem examination remains essential for detecting adult flukes, characterizing egg-associated lesions, and distinguishing parasitic disease from other causes of morbidity. Collection of parasites for both morphological and molecular analysis, paired with systematic sampling of the central nervous system, would strengthen future investigations.

In conclusion, this case documents severe disseminated spirorchidiasis associated with trematodes morphologically consistent with *L. learedi*, together with histological findings suggestive, but not diagnostic, of *Neosporichis* spp. and concurrent fibropapillomatosis in a juvenile green turtle from Alagoas, Brazil. The animal also sustained a severe carapacial fracture with internal hemorrhage, which was considered the most likely immediate cause of death. Systemic spirorchidiasis, fibropapillomatosis, poor body condition, and chronic debilitation were interpreted as significant pre-existing conditions. The findings emphasize the importance of integrating traumatic, parasitic, and neoplastic findings within a pathological hierarchy when investigating sea turtle mortality. Continued pathological surveillance is warranted to define the distribution and conservation relevance of these diseases along the northeastern Brazilian coast.

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Conflicts of Interest: All other authors declare no conflicts of interest.

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