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Research article

Pulmonicola cochleotrema in dugongs from the Andaman Sea, Thailand — morphological and molecular characterization with pathological implications

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Abstract

Dugongs (*Dugong dugon*), the only extant herbivorous marine mammals, are vital to the maintenance of seagrass ecosystems but are currently listed as vulnerable on the IUCN Red List. In Thailand, the Andaman Sea supports the country's largest dugong population, yet mortality remains a serious concern, with parasites increasingly recognized as important natural threats. Respiratory trematodes such as *Pulmonicola cochleotrema* are known pathogens of sirenians, but their status in Thai waters has not been documented. Between January 2019 and May 2025, 166 stranded dugongs were systematically necropsied by veterinarians of the Department of Marine and Coastal Resources. Carcasses were examined for respiratory trematodes, and detailed records of parasite counts, anatomic distribution, and gross lesions were compiled. Representative adult worms were collected for morphological study, histopathology, and DNA-based phylogenetic analysis. *P. cochleotrema* infection was confirmed in 10 animals (6.02 %), comprising 40 % sub-adults, 40 % adults, and 20 % dependent calves, with no sex-related difference. Parasites were detected throughout the respiratory tract—nares (1/10), nasal cavity (2/10), trachea (3/10), bronchi (1/10), and pulmonary parenchyma (1/10). Gross lesions ranged from mild to moderate mucosal congestion and hemorrhage to severe verminous bronchopneumonia with suppurative exudation. Histopathology revealed severe chronic eosinophilic tracheobronchitis and bronchopneumonia. Morphologically, adult worms were ovoid and dorsoventrally flattened with a muscular fringe; molecular analysis confirmed their identity as *P. cochleotrema*. This study provides the first comprehensive evidence of *P. cochleotrema* infection in Thai dugongs, defining its occurrence, morphology, molecular characteristics, and pathological impact. These baseline data enhance understanding of parasite–host interactions and support future health management and conservation strategies for this threatened marine mammal.

Keywords: Dugong, Molecular phylogenetics, Morphology, Pathology, *Pulmonicola cochleotrema*

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INTRODUCTION

Dugongs (*Dugong dugon*), the only extant herbivorous marine mammals, are vital to the maintenance of seagrass ecosystems, promoting nutrient cycling and seagrass productivity and thereby supporting biodiversity. Their ecological importance underscores the global imperative for conservation (Marsh et al., 1992; Tol et al., 2016). In Thailand, dugongs are concentrated mainly in the Andaman Sea, with core populations in Trang, Satun, and Krabi provinces and more recent sightings in Phuket (Adulyanukosol, 2010; Cherdsukjai et al., 2014). Despite these strongholds, the Thai dugong population is declining and faces substantial health challenges (Eiamcharoen et al., 2025; Sukkarun et al., 2025; Daochai et al., 2024; Keawchana et al., 2026). Both anthropogenic pressures—such as ship collisions and interactions with fishing activities —and natural factors, including microbial and parasitic pathogens, contribute to elevated mortality and ongoing population reductions (Daochai et al., 2024).

Among the natural threats, respiratory trematodes have drawn increasing attention. *P. cochleotrema*, a digenean fluke of the family Opisthotrematidae, is known to infect the respiratory tract of sirenians (Blair, 1981). It has been reported in Antillean manatees (*Trichechus manatus manatus*) (Borges et al., 2017), West Indian manatees (*Trichechus manatus*) (Rivera-Pérez et al., 2024a) and dugong (Bonde et al., 2012). The infection has been linked to respiratory lesions ranging from serosanguinous, mucohemorrhagic, or suppurative exudation to pulmonary abscesses and eosinophilic bronchopneumonia (Rivera-Pérez et al., 2024b). In severe infections, these parasites can cause significant pulmonary compromise and increase susceptibility to secondary bacterial infections (Rivera-Pérez et al., 2024). In dugongs, *P. cochleotrema* has been reported only sporadically worldwide, and its pathological impact is generally considered mild (Woolford et al., 2015). However, data on the occurrence of this parasite in Thai dugong populations remain lacking.

Consequently, the present study was undertaken to investigate the occurrence, morphological features, phylogenetic relationships, and pathological impacts of *P. cochleotrema* in dugongs stranded along the Thai Andaman Sea. By integrating gross pathology, histopathology, and molecular analyses, these findings aim to establish baseline epidemiological and pathological data essential for understanding parasite–host interactions and for guiding future conservation and health-management strategies for this threatened marine mammal.

MATERIALS AND METHODS

Ethical approval and authorization for sample

This research was conducted using archived animal tissues in compliance with institutional guidelines and national regulations. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Prince of Songkla University (Approval No. MHESI 68014/1236), which classified the project as exempt under the category of Exempt Determination Research. Collection and use of tissue samples were authorized by the Department of Fisheries of Thailand (MOAC Document No. 0510.5/12652) in accordance with Section 73(1) of the Wildlife Preservation and Protection Act, B.E. 2562 (2019).

Sample collection and recording of respiratory trematode infections

From January 2019 to May 2025, carcasses of dugongs stranded along the Andaman Sea coast of southern Thailand—including Phang Nga, Trang, Satun, and Krabi provinces—were examined. Standard marine mammal necropsies were performed by trained veterinarians of the Department of Marine and Coastal Resources (DMCR), Andaman Coastal Research Center (Lower Andaman Sea).

Necropsies followed standard marine mammal procedures adapted from Pugliares et al. (2007) and Eros et al. (2007), including external examination, morphometrics, internal organ assessment, and tissue sampling for histopathology and molecular analysis. Carcass condition was assessed following Eros et al. (2007) and classified into five categories. Only carcasses in categories (1)–(3) were included to allow accurate pathological and parasitological evaluations, whereas those in advanced decomposition were excluded to maintain the reliability of gross and histopathological assessments.

During necropsy, the presence of respiratory tract trematodes was systematically recorded on standardized postmortem data sheets. For each animal, veterinarians documented (i) the number and precise anatomical location of trematodes within the respiratory tract, (ii) the age class of the host based on established morphological criteria (Marsh, 1980), and (iii) any associated gross lesions such as mucosal congestion, hemorrhage, or purulent exudate. In addition, basic biological and necropsy data were recorded for each individual, including age class, sex, presumptive cause of death, and body condition score (BCS), with the latter adjusted according to the criteria modified by Khumraksa et al. (2025). The infection intensity was determined following the criteria proposed by Sornying et al. (2025), whereby infection levels of trematodes were categorized as mild (1–50 trematodes), moderate (51–100 trematodes), and severe (>101 trematodes). This classification provides a standardized framework for quantifying parasitic burden across individuals. These data were subsequently used to calculate the occurrence of *P. cochleotrema* infection by age group and to describe the distribution of gross lesions.

Tissue and parasite sampling for pathological and molecular analyses

Respiratory tissues showing trematode infection, including trachea and lung tissue, were collected for detailed pathological evaluation. Representative samples were trimmed to approximately 1.0 cm thickness to ensure optimal fixative penetration and immediately preserved in 10 % neutral buffered formalin for histopathology (Sornying et al., 2025; Suyapoh et al., 2025). Adult trematodes were carefully retrieved from the nasal cavity and trachea using sterile forceps. To prepare the worm specimens for downstream analyses, each was meticulously rinsed three successive times in sterile normal saline to ensure complete removal of adherent debris and potential contaminants (Suyapoh et al., 2021). In total, 10 dugongs were confirmed to be infected with *P. cochleotrema*. Approximately 10–15 intact adult worms were collected from all infected dugongs combined for detailed morphological examination. For molecular analysis, 2 representative specimens were randomly selected from two dugongs originating from different locations. Fixed specimens were processed for morphometric evaluation as described below.

Histopathological examination

Formalin-fixed tissues (trachea, bronchi, and lung) were routinely processed following established methods (Jantrakajorn et al., 2024), embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). Slides were examined microscopically using a Nikon ECLIPSE Ni-U advanced upright microscope equipped with a high-resolution digital video camera (Nikon, Japan) to characterize host responses and lesion patterns associated with *P. cochleotrema* infection, including epithelial disruption, inflammatory cell infiltration, vascular congestion, hemorrhage, and secondary changes such as bronchopneumonia. The characterization of normal histological cell and tissue structures of the dugong followed the descriptions of Kaewmong et al. (Kaewmong et al., 2023). Lesion severity and distribution were recorded and photographed for documentation.

Parasitic DNA Extraction

Genomic DNA was extracted from trematodes recovered from the nasal cavity (TRDU-91-10) and trachea (TRDU-91-13) following previously described protocols with minor modifications (Suyapoh et al., 2024), using the PureLink™ Genomic DNA Kit (Invitrogen™ , MA, USA) according to the manufacturer's instructions. Briefly, specimens preserved in absolute ethanol were homogenized with a tissue grinder and digested with proteinase K (Invitrogen™). DNA was then separated using the kit's extraction buffer, precipitated with ethanol, and quantified with a NanoDrop® ND-1000 UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Parasite identification

Microscopic examination

Unstained, formalin-fixed specimens were mounted as whole-mount slides on glass. Morphological identification followed established taxonomic keys and previous descriptions (Rivera-Pérez et al., 2024a), with emphasis on body shape, size, and the arrangement of internal organs. Diagnostic characters included an ovoid, dorsoventrally flattened body with a prominent muscular fringe, a ventrally positioned oral sucker, branched intestinal ceca, paired lobed testes, a lobate ovary, and vitellaria distributed throughout the posterior region. Morphological measurements were conducted using a Nikon ECLIPSE Ni-U advanced upright microscope equipped with a high-resolution digital video camera (Nikon, Japan). Image capture and analysis were performed using NIS-Elements Imaging Software (Nikon). Illustrations were generated from the captured micrographs and digitally processed to enhance clarity and detail for taxonomic documentation.

Molecular detection

To identify the species of trematode, the 18S ribosomal RNA gene were amplified by PCR using the primers OP-18-F (5'-ACAGAACCAACCGGATGCAG-3') and OP-18-r2 (5'-ACTGCCCGTGAGGCCAATAGTG-3') (Rivera-Pérez et al., 2024b). Each PCR reaction was performed in a final volume of 25 µL, contained a maximum of 50 ng of template DNA in 4 µL, along with 0.2 µmol each of forward and reverse primer, and 12.5 µl of OnePCR Ultra Master Mix (Bio-Helix, New Taipei City, Taiwan). PCR reactions were amplified in a PCR thermal cycler (Eppendorf, Hamburg, Germany) under the conditions: an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 7 min. DNA extracted from a previously confirmed *Pulmonicola* spp. specimen, identified taxonomically by the Parasitology Laboratory of the Faculty of Veterinary Science, Prince of Songkla University, was used as the positive control, while distilled water served as the negative control.

PCR products obtained from all samples were visualized by electrophoresis on a 1.5% agarose gel (Invitrogen™) and observed under UV transillumination (E-Box VXII, Vilber, Marne-la-Vallée, France). The PCR positive products were purified utilizing the GenepHlow™ Gel/PCR kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) and subjected to sequencing using the Big Dye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems 3730 DNA Analyzer instrument (ATCG, Bangkok, Thailand). Nucleotide sequences were analysed using the BioEdit v.7.2 software and were cross-referenced with available sequences in the GenBank database using the BLASTN tool provided by the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify their nucleotide identity.

Phylogenetic analysis

Our nucleotide sequences were multiple aligned with additional sequences published in GenBank, including 15 other trematodes from several families within the superfamily Pronocephaloidea, using Clustal W algorithm of the MEGA 11 software (Saitou and Nei, 1987). A phylogenetic tree was constructed using the Maximum Likelihood (ML) method with the Hasegawa–Kishino–Yano (HKY) substitution model (Hasegawa et al., 1985) including estimates of invariant sites (I) and gamma distribution. The tree was resampled by 1,000 bootstrap replicates to evaluate the reliability of the groups (Tamura et al., 2021).

RESULTS

Occurrence and gross pathology of Pulmonicola cochleotrema infection in dugongs

Among 166 stranded dugongs examined between January 2019 and May 2025, *P. cochleotrema* infection was confirmed in 10 individuals, giving an overall occurrence of 6.02%. All infected dugongs were carcasses recovered from stranding events, with no individuals rescued alive prior to death. Within the infected subset (n = 10), the age distribution comprised 40% sub-adults (4/10), 40% adults (4/10), and 20% dependent calves (2/10). Both sexes were affected—seven males and three females—indicating no appreciable difference in occurrence between genders. The body condition scores (BCS) of the infected animals ranged from 1 to 3, including one emaciated case (BCS = 1; 10%), four with moderate condition (BCS = 2; 40%), and five with good condition (BCS = 3; 50%), suggesting that respiratory trematodiasis was not consistently associated with poor nutritional status. The detailed distribution of infection by age, sex, and anatomical region is summarized in Table 1.

Table 1 Distribution of Pulmonicola cochleotrema infection among infected dugongs (n = 10)

Category	Group	No. infected (n)	Occurrence within infected group (%)	Remarks / Observation
Age class	Dependent calves	2	20.0	Mild infection in nasal cavity and
	Subadult	4	40.0	Mild infection in trachea and bronchi
	Adult	4	40.0	Mild to severe lesions involving bronchi and lungs
Sex	Male	7	70.0	Slightly higher infection rate
	Female	3	30.0	Lower occurrence
Anatomical region	External nares	1	10.0	Surface congestion and mucus
	Nasal cavity	2	20.0	Worms attached to mucosa
	Trachea	3	30.0	Eosinophilic tracheitis and mucosal thickening
	Bronchi	1	10.0	Bronchial congestion and mucus exudation
	Lung parenchyma	1	10.0	Chronic eosinophilic bronchopneumonia
Total		**10**	**100.0**	

At necropsy, *P. cochleotrema* was detected throughout the respiratory tract, including the external nares (1/10), nasal cavity (2/10), trachea (3/10), bronchi (1/10), and pulmonary parenchyma (1/10). No *P. cochleotrema* were found in other organs (recorded as not applicable), and all recovered specimens were mature. Notably, every dugong infected with respiratory trematodes also harbored gastrointestinal roundworms. Grossly, *P. cochleotrema* infection produced a wide spectrum of lesions involving both the upper and lower respiratory tracts. Adult trematodes were localized mainly in the external nares (Figure 1a–c) and nasal cavity (Figure 1d–e), occasionally extending into deeper portions of the trachea and bronchi (Figure 1g–h). Purulent nasal discharge was frequently observed at the external nares (Figure 1c). In light infections, single or small clusters of trematodes were embedded within seromucous tracheal exudates (Figure 1d, g), with only mild mucosal congestion. In contrast, heavy infections were marked by large clusters of

worms, severe mucosal congestion, luminal hemorrhage with blood clots (Figure 1e), and extensive suppurative exudation (Figure 1i), often culminating in pneumonia.

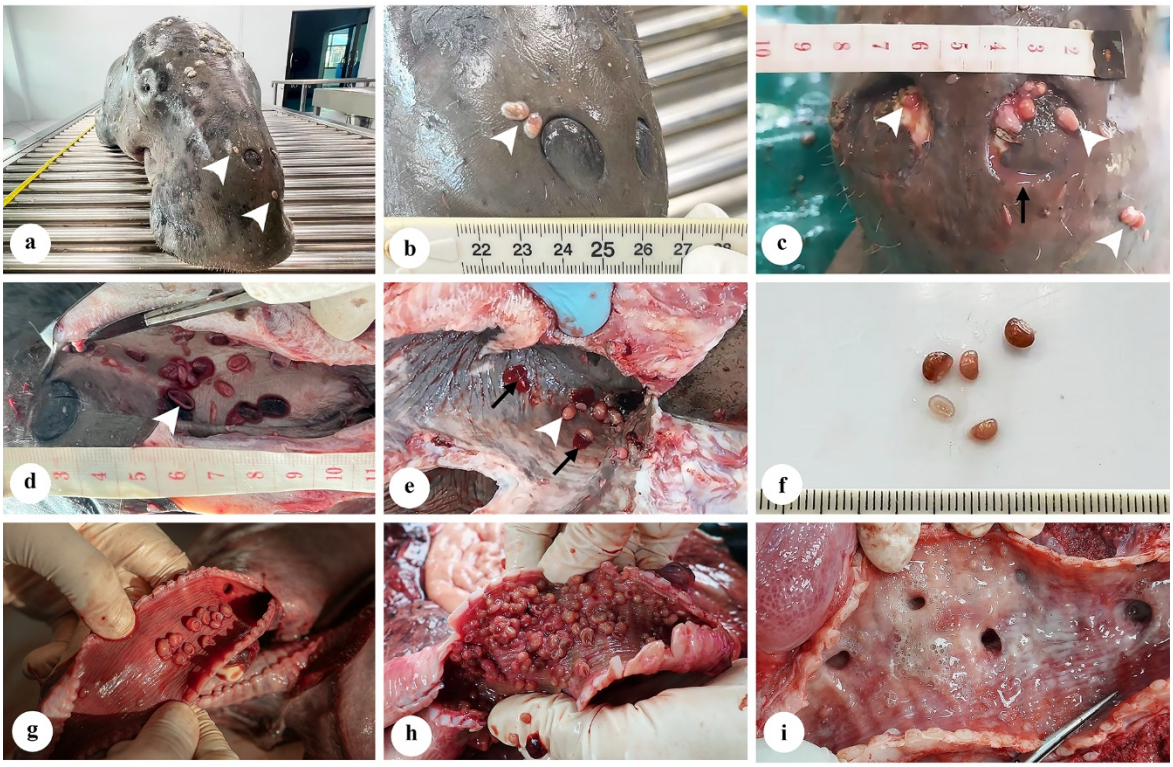

Figure 1 Gross pathological findings associated with *Pulmonicola cochleotrema* infection in the respiratory tract of a dugong. (a) Rostral region of the carcass showing trematodes attached within the external nares (white arrowhead). (b) Closer view of the infected external nares. (c) Heavy infestation accompanied by abundant suppurative exudate (white arrowhead, black arrow). (d) Lesions within the nasal cavity showing mucosal damage and hemorrhage. (e) Blood clot formation and mucosal congestion in the nasal cavity. (f) Adult trematodes collected from the nasal cavity. (g) Light infection within the trachea. (h) Severe tracheal involvement with extensive mucosal injury, active congestion, and hemorrhage. (i) Suppurative tracheal exudate within the conductive respiratory system.

Based on the Department of Marine and Coastal Resources (DMCR) necropsy records, one infected dugong died from anthropogenic trauma caused by a boat propeller, while the remaining nine were classified as natural deaths. Among the latter, three individuals exhibited severe pneumonia with frothy tracheal exudate, three presented with systemic septicemia, and three showed hemorrhage of internal organs including the liver, heart, and kidney. The pneumonia cases corresponded to severe *P. cochleotrema* infections with heavy parasite burdens, suggesting that respiratory trematodiasis acted as a primary or contributing cause of death in these individuals, whereas other cases were regarded as incidental infections without evidence of fatal respiratory compromise.

Histopathology of *Pulmonicola cochleotrema* infection in dugongs

Microscopic examination of the conductive respiratory system, including the trachea and primary bronchi, revealed lesions consistent with *P. cochleotrema* infection. The bronchial lumina were partially to completely occluded by eosinophil-rich exudate admixed with degenerating inflammatory cells and cellular debris

(Figure 2a, e). Mucosal inflammation was evident along the exudative surface (Figure 2a). At higher magnification, the bronchial mucosa showed severe, chronic, eosinophil-dominated inflammation, accompanied by marked infiltration of macrophages, lymphocytes, and plasma cells that extended through the mucosal and submucosal layers (Figure 2b–d).

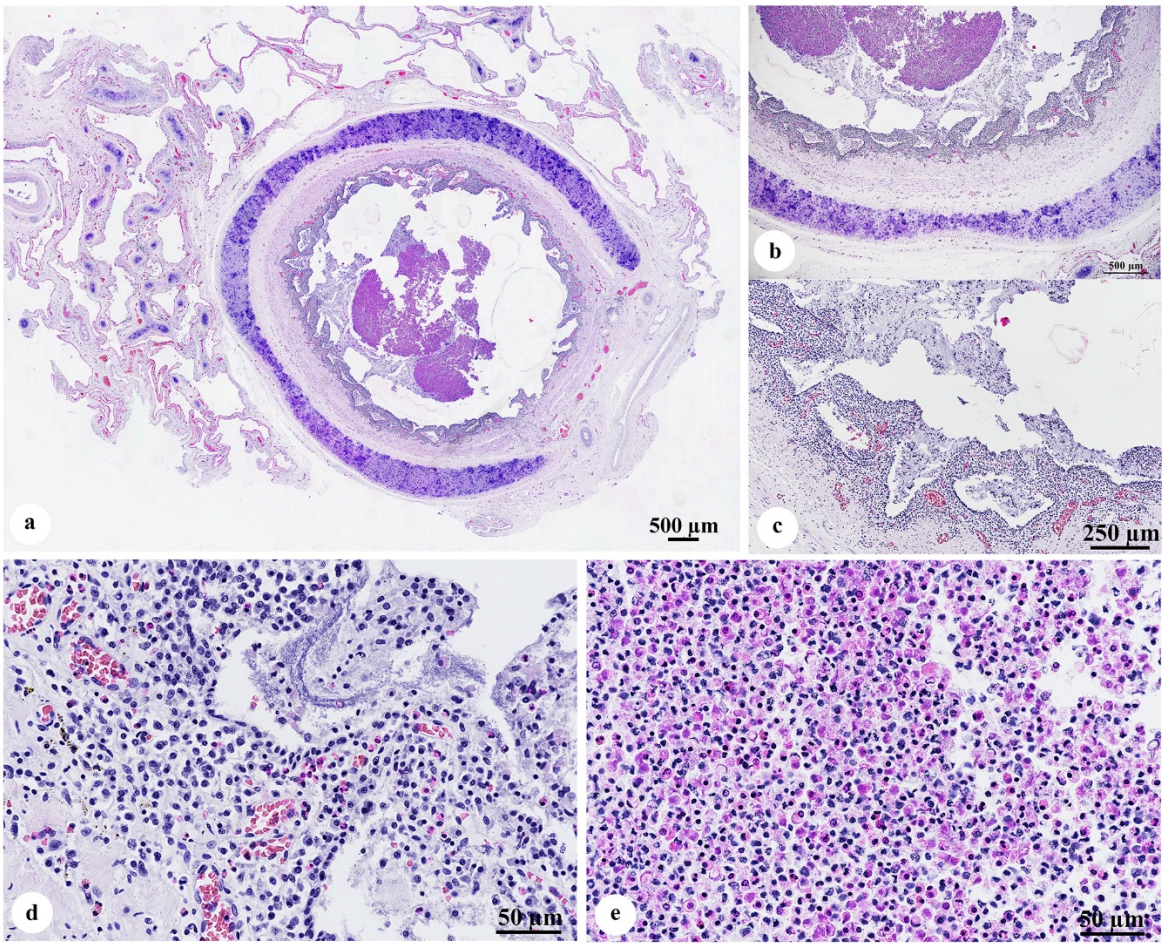

Figure 2 Histopathological findings of *Pulmonicola cochleotrema* infection in the conductive system of a dugong. (a) Chronic bronchitis with an eosinophil-rich exudate filling the bronchial lumen. (b) Low-magnification view showing partial to complete occlusion of the bronchial lumen by eosinophil-rich exudate and chronic inflammatory infiltration of the submucosa. (c) Higher magnification highlighting areas of active vascular congestion and inflammatory cell infiltration. (d) Detailed view of severe, chronic, eosinophil-dominated inflammation with marked infiltration of macrophages, lymphocytes, and plasma cells extending through the mucosal and submucosal layers. (e) Close-up of eosinophil-rich exudate admixed with degenerative inflammatory cells and cellular debris. (a–e = H&E stain; a, subgross image, scale bar = 500 μm; b, original magnification ×4, scale bar = 500 μm; c, original magnification ×10, scale bar = 250 μm; d–e, original magnification ×40, scale bar = 50 μm.)

The bronchial epithelium was multifocally infiltrated and disrupted, and the inflammatory reaction extended into the surrounding interstitium and adjacent alveolar spaces, where mild septal thickening and scattered eosinophils were observed (Figure 2d). Importantly, the inflammatory process did not extend extensively into the supporting bone (Figure 2b). Vascular changes included diffuse active congestion and multifocal hemorrhage, but no thrombus formation was detected (Figure 2a–d). No granulomatous reaction, overt necrosis, or intralesional parasitic structures were identified in the examined sections, suggesting that the primary pathogenic mechanism involved an intense allergic or hypersensitivity-type

response to the trematodes. Collectively, these histological alterations are consistent with chronic eosinophilic tracheobronchitis and bronchiolitis, reflecting the host's prolonged inflammatory reaction to parasitic infestation. The severity of the inflammatory response appeared to correlate with infection intensity. Lightly infected individuals showed only mild mucosal congestion with minimal tissue reaction, whereas heavily infected dugongs exhibited marked eosinophilic tracheobronchitis and bronchopneumonia, consistent with higher parasite burdens.

The pulmonary parenchyma exhibited chronic eosinophilic bronchopneumonia characterized by dense infiltration of eosinophils extending from the bronchioles into the alveolar septa and interstitial spaces (Figure 3a). Numerous alveoli were partially to completely filled with eosinophils, intermingled with degenerating inflammatory cells and cellular debris (Figure 3b, c). Multifocally, the interstitium also contained lymphoplasmacytic infiltrates (Figure 3b). Areas of necrosis and interstitial edema were variably present. In regions adjacent to these lesions, the lung tissue showed marked vascular changes, including diffuse active congestion, multifocal pulmonary hemorrhage, and pulmonary edema (Figure 3d).

Pulmonicola cochleotrema identification based on morphology

The morphology of *P. cochleotrema* was characterized as ovoid and dorsoventrally flattened, with a convex dorsal surface and a concave ventral surface. A prominent muscular fringe encircled the body margin. Microscopic examination of fresh specimens revealed a body length of 6.09 ± 0.14 mm. and a width of 4.61 ± 0.03 mm. (mean ± SD). At the anterior end, a ventrally positioned oral sucker measured approximately 0.74 ± 0.02 mm. in length and 0.98 ± 0.01 mm. in width. The intestinal ceca extended posteriorly toward the distal extremity of the body. Reproductive organs included a pair of lobed, flower-like testes and a single oval, lobate ovary situated between the testes and the intestinal ceca, while vitellaria were distributed throughout the posterior region (Figure 4a-b).

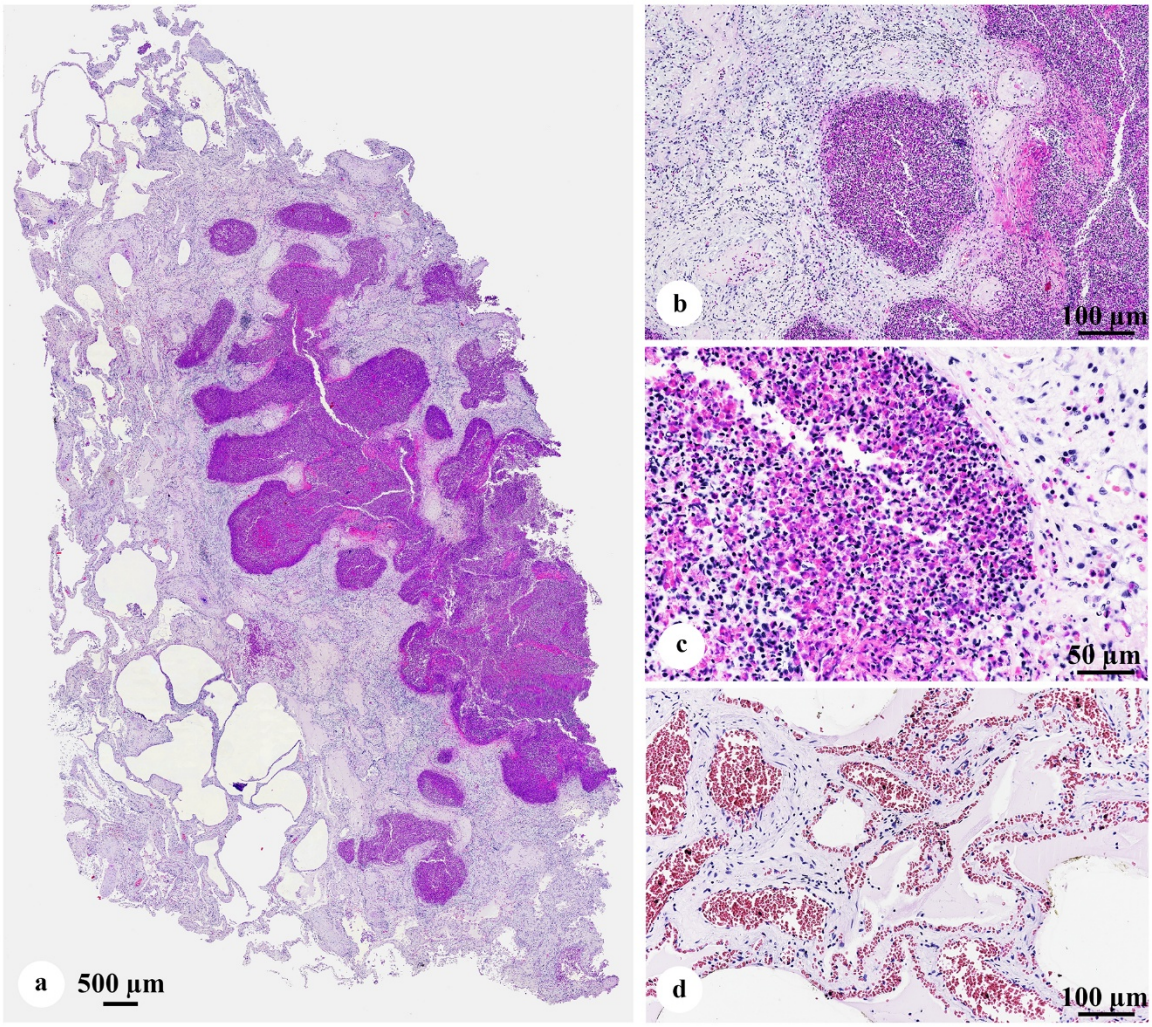

Figure 3 Histopathological findings of *Pulmonicola cochleotrema* infection in the pulmonary parenchyma of a dugong. (a) Subgross section of lung showing eosinophilic bronchopneumonia. (b) Low-magnification view demonstrating dense eosinophilic infiltration extending from bronchioles into the alveolar septa and interstitial spaces. (c) Higher magnification detailing the exudate, composed predominantly of eosinophils with admixed cellular debris. (d) Lung parenchyma adjacent to the lesions exhibiting marked vascular changes, including diffuse congestion, multifocal pulmonary hemorrhage, and pulmonary edema. (a–d = H&E stain; a, subgross image; b and d, original magnification ×20, scale bar = 100 µm; c, original magnification ×40, scale bar = 50 µm.)

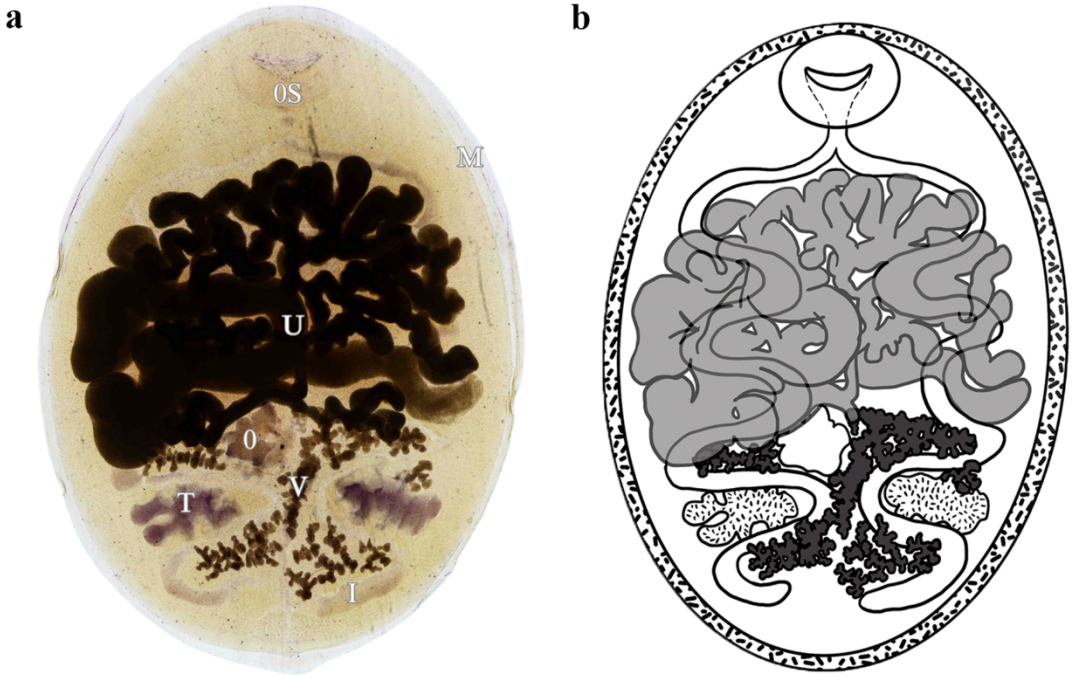

Figure 4 Microscopic morphology of *Pulmonicola cochleotrema*. (a) Fresh specimen collected from a stranded dependent-calf dugong in the Lower Andaman Sea, Thailand.
(b) Illustrative diagram highlighting key anatomical features: oral sucker (OS), muscular fringe (M), uterus (U), ovary (O), testes (T), vitellaria (V), and intestinal ceca (I).

Molecular identification and phylogenetic analysis of *Pulmonicola cochleotrema*

Both TRDU-91-10 and TRDU-91-13 were obtained from the same stranded dugong (specimen code TRDU-91) recovered from Kantang, Trang province. TRDU-91-10 was collected from the nasal cavity, and TRDU-91-13 from the trachea. Nucleotide sequences of the partial 18S rDNA gene (~900 bp) from the Pulmonicola specimens in this study were deposited in GenBank under the accession numbers PX091137 and PX091138, respectively. In BLASTN analysis, these sequences showed 99.79% and 99.78% identity with *P. cochleotrema* (OR428573) isolated from West Indian manatee (*Trichechus manatus*) in Puerto Rico, respectively. The ML tree demonstrated that 6 families within the superfamily Pronocephaloidea were distinctly separated. In the *P. cochleotrema* clade, two new sequences (TRDU-91-10 and TRDU-91-13) from dugong in Thailand clustered closely with manatee isolates in Puerto Rico and USA (accession numbers OR428573–OR428574), with high bootstrap support (94%). The tree demonstrated low genetic divergence among isolates from these different host species (manatee vs dugong). Moreover, these sequences were placed in the same clade of the family Opisthotrematidae with *Opisthotrema dujonis* (AY222117) and *Lankatrema mannarense* (AY222116) isolated from dugong in Australia (Figure 5). The tree root includes distant trematode species like *P. cochlear* from sea turtles and *P. cornu* from bird, used as outgroups to provide phylogenetic context.

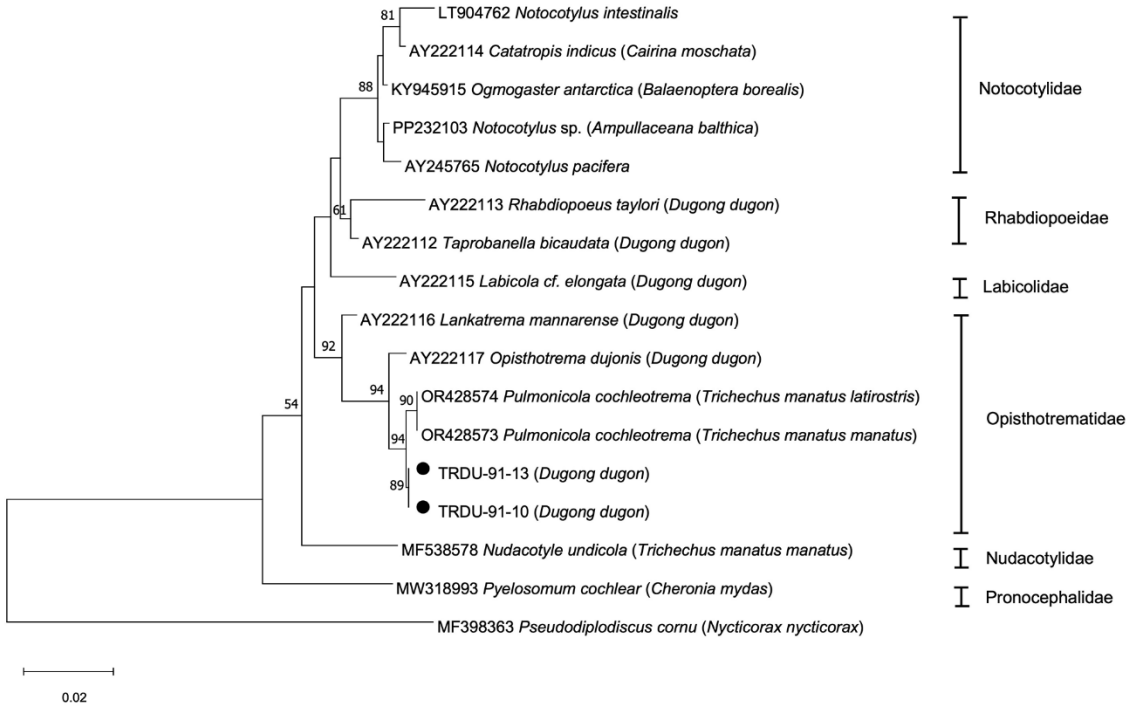

Figure 5 Maximum-likelihood phylogenetic tree based on the partial 18S rRNA gene of the *Pulmonicola cochleotrema* specimens obtained from stranded dugongs in Thailand (represented with black circle bullets), along with the most closely related trematode species deposited in GenBank. Thai isolates TRDU-91-10 and TRDU-91-13 clustered with manatee-derived sequences (OR428573 and OR428574). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown below the branches. Scale bar represents distance value.

DISCUSSION

The dugong (*Dugong dugon*) is currently listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List and has become a focal species for marine conservation efforts worldwide (IUCN, 2025). In Thailand, the Andaman Sea supports one of the largest and most important habitats for dugong populations (Panyawai and Prathep, 2022). Nevertheless, mortality events remain a serious concern. Between 2018 and 2023, 125 dugong deaths were officially recorded in Thailand, with the majority attributed to natural causes, including infectious and parasitic diseases (Daochai et al., 2024). Parasitic infections have long been implicated in the morbidity and mortality of marine mammals and are increasingly recognized as important contributors to stranding events worldwide (Arbelo et al., 2013; Díaz-Delgado et al., 2018). High endoparasite prevalence has been reported in stranded dolphins and dugongs (Angsinco-Jimenez et al., 2013; Terracciano et al., 2020; Sornying et al., 2025), suggesting a significant role in the stranding process. Within this context, respiratory trematodes such as *P. cochleotrema* are notable. These parasites are established pathogens of sirenians and have been documented in both dugongs and manatees (Carvalho et al., 2009; Bonde et al., 2012; Borges et al., 2017). In the present study, *P. cochleotrema* infection was detected in 10 of 166 Thai dugongs (6.02 %), a markedly lower occurrence than that reported in Brazilian manatees (26.7 %, 4/15) (Carvalho et al., 2009). This discrepancy may reflect regional differences in endemic intermediate hosts, parasite establishment rates (Froelick et al., 2021), or contrasts

in food-web structure and host behavior between manatees and dugongs (Wang et al., 2025). The lower prevalence of *P. cochleotrema* in Thai dugongs may reflect ecological and geographic variation influencing transmission, including differences in intermediate-host distribution, seagrass habitats, and environmental conditions that affect parasite life cycles and exposure risk. Our findings further demonstrate that the parasites inhabit both upper and lower respiratory tracts—including the external nares, nasal cavity, trachea, bronchi, and lungs—in agreement with previous observations (Rivera-Pérez et al., 2024a). Age analysis indicated that sub-adults and adults each accounted for 40 % of infections, suggesting these groups may serve as the main parasite reservoirs, a pattern consistent with reports of parasitism in cetaceans (Suárez-González et al., 2024) and likely reflecting the cumulative exposure to parasites that wildlife experiences throughout life (Kołodziej-Sobocińska, 2019).

In sirenians, two common respiratory trematodes have been documented. The first, *Cochleotrema indicum*, was originally identified in the nasal passages of dugongs (Eduardo, Yaptinchay and Lim, 1998) Subsequent studies reported *P. cochleotrema* occupying the same region of the respiratory tract (Carvalho et al., 2009; Borges et al., 2017). *P. cochleotrema* is characterized by a distinctive monostome structure that enables firm attachment to the host's respiratory mucosa (Carvalho et al., 2009). In the present investigation, detailed morphological examination of the recovered trematodes confirmed their identity as *P. cochleotrema*, with diagnostic traits consistent with earlier descriptions of this species (Carvalho et al., 2009).

The 18S rRNA gene was selected for phylogenetic analysis in this study because it is a highly conserved nuclear marker widely used for taxonomic identification and phylogenetic reconstruction of digenean trematodes (Blair and Barker, 1993; Tkach et al., 2000), including members of the family Opisthotrematidae (Rivera-Pérez et al., 2024). This gene provides sufficient resolution to elucidate relationships at the genus and family levels and enables comparison with many reference sequences available in GenBank. Although other ribosomal DNA regions, particularly ITS, and mitochondrial genes such as COI can offer higher discriminatory power (Nolan and Cribb, 2005; Bray et al., 2022), sequence data for *P. cochleotrema* and closely related taxa are scarce or unavailable in public databases, thereby limiting their suitability for comparative phylogenetic analysis. Phylogenetic analysis placed *P. cochleotrema* in a well-supported, distinct clade within the family Opisthotrematidae, clustering with other genera known to infect dugongs. This finding agrees with the work of Rivera-Pérez et al. (2024b), who demonstrated that *P. cochleotrema* forms a monophyletic group sharing a common ancestor with *Opisthotrema dujonis*. Moreover, the Thai isolates showed high genetic similarity to *P. cochleotrema* previously reported from West Indian manatees. Although *P. pulmonalis* has been described in Australian dugongs (Lehnert et al., 2019), direct genetic comparison was not possible because 18S rDNA sequence data for *P. pulmonalis* are currently unavailable in GenBank. Morphological examination of the recovered specimens from all sampled dugongs revealed consistent characteristic features corresponding to *P. cochleotrema*. These morphological traits supported species-level identification. However, the molecular analysis in this study was based on a small sample size (n = 2), which may limit the representativeness of the obtained 18S rDNA sequences and the robustness of the phylogenetic interpretation. Although the generated sequences provided preliminary insights into the genetic relationships of the parasites, further studies incorporating a larger number of specimens and longer gene fragments are required to confirm species identification and clarify the taxonomic relationships among *P. cochleotrema* infecting dugongs and other host species. *P. cochleotrema* infection in sirenians has been associated with a wide range of pathological outcomes, from subclinical infections with no visible lesions to severe changes directly linked to mortality (Norina, 2018). In the present study, lesions consistent with previous reports were observed, including mucosal congestion,

hemorrhage, suppurative exudation, and severe chronic eosinophilic tracheobronchitis and bronchopneumonia at parasite attachment sites. However, in some dugongs exhibiting eosinophilic bronchopneumonia, neither trematode sections nor parasite eggs were detected within the pulmonary parenchyma. This finding suggests that the pulmonary lesions may arise secondarily from airway inflammation extending into adjacent lung tissue (Salahuddin et al., 2023), or from systemic hypersensitivity reactions associated with heavy parasitic infection, analogous to hypereosinophilic syndrome (HES) or chronic eosinophilic pneumonia (CEP) described in humans (Faridah et al., 2026). Alternatively, eosinophilic pneumonitis without intralesional parasites may represent an immunologically mediated hypersensitivity response similar to canine eosinophilic bronchopneumopathy (EBP) (Clercx and Peeters, 2007). Although HES-like, CEP-like, and EBP-like lesions have not been reported in dugongs to date, this possibility warrants further investigation to elucidate the immunopathological mechanisms and differential diagnoses relevant to dugong respiratory diseases. The pneumonia detected in three cases strongly implicates *P. cochleotrema* as a primary or contributing cause of death. Variation in clinical severity among infected dugongs is likely influenced by several factors, such as the anatomical location of parasites, parasite burden and activity, helminth-derived metabolic products, and host immune and inflammatory responses (Wakelin, 1996). Within the family Opisthotrematidae, four principal mechanisms of pathogenesis have been described (Sripa et al., 2018; Suyapoh et al., 2021a; Suyapoh et al., 2021b) : (1) mechanical damage caused by feeding parasites; (2) immunopathology associated with reactive oxygen intermediates and nitric oxide; (3) direct effects of parasite-secreted proteins; and (4) the action of bacterial pathogens harbored within the parasites. Research on *Opisthorchis viverrini* has shown that secreted proteases, antioxidants, oncogenic proteins, and exosome-like extracellular vesicles are key mediators of tissue inflammation and immunomodulation (Ninlawan et al., 2010; Sripa et al., 2018; Suttiprapa et al., 2018). Similarly, *Opisthorchis felineus* excretory and secretory antigens play a crucial role in disease induction (Lvova et al., 2012). However, the detailed disease mechanisms of other opisthotrematid trematodes in sirenians—including *C. indicum*, *O. dujonis*, *P. pulmonalis*, and particularly *P. cochleotrema*—remain poorly understood and require further investigation. Such studies will provide deeper insight into host–parasite interactions and the pathogenic processes underlying trematode infections in these threatened marine mammals. Despite these important pathological findings, caution is warranted in interpreting their population-level significance. The relatively low prevalence of *P. cochleotrema* infection (6.02%) observed in this study suggests that these data should be regarded as baseline epidemiological information for future health monitoring and conservation assessments rather than as conclusive evidence of a direct impact on dugong population decline. Continued longitudinal surveillance incorporating larger sample sizes and broader geographic coverage will be necessary to clarify potential epidemiological trends and ecological risk factors associated with respiratory trematodiasis in dugongs.

CONCLUSIONS

This study establishes *P. cochleotrema* as a notable respiratory parasite of dugongs in Thailand and provides essential baseline data on its morphology, pathology, and phylogenetic position. These findings underscore the importance of integrating parasitological surveillance into dugong health monitoring and highlight the need for further research on host–parasite interactions and disease mechanisms within sirenians. Although the infection prevalence was low, the associated respiratory lesions indicate that *P. cochleotrema* can impair lung function and potentially contribute to mortality. These findings highlight the need for continued parasitological surveillance to support dugong health and

conservation efforts. This study also contributes to clarifying the taxonomic history of this parasite, originally described as *O. pulmonale* (Blair, 1981) and later reclassified as *P. pulmonalis* and *P. cochleotrema*, thereby supporting accurate species identification and future parasitological research in sirenians.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Domechai Kaewnoi: Conceptualization (equal); data curation (equal); formal analysis (lead); methodology (lead); writing – original draft (supporting).
Sasibha Jantrakajorn: formal analysis (lead); Methodology (equal).
Peerapon Sornying: Conceptualization (equal); data curation (equal); formal analysis (lead); methodology (lead).
Narissara Keawchana: Methodology (supporting); writing – review & editing (supporting). **Piyarat Khumraksa**: Data curation (supporting); formal analysis (lead); methodology (equal); resources (equal).
Sattaya Ruangpoon: Data curation (supporting); formal analysis (lead); methodology (equal); resources (equal). Santi Ninwat: Resources (lead).
Najyamee Inthacho: writing – original draft preparation (supporting).
Chayanis Daochai: writing – original draft preparation (supporting).
Watcharapol Suyapoh: Conceptualization (lead); data curation (lead); formal analysis (supporting); funding acquisition (lead); investigation (lead); methodology (supporting); project administration (lead); software (lead); supervision (lead); validation(lead); visualization (lead); writing – original draft preparation (lead); writing – review & editing (lead)

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