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Health assessment of *Holothuria scabra* (Jaeger, 1938) from pond culture in Thailand: Size distribution and gonadal development

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ABSTRACT

The assessment of sea cucumber health in Thailand is incomplete, as it has not included *Holothuria scabra*, a potentially valuable species for aquaculture. In this study, we examined the health status and gonadal development of *H. scabra* in relation to its size distribution using morphological and histological methods, as well as the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. All sea cucumbers were obtained between July and August 2024 from pond cultures on Koh Yao Noi Island, Phang Nga Province, a coastal area on the Andaman Sea in Thailand. The specimens could be divided into three size classes: Group I (3.5–6.8 cm), Group II (16.5–19.5 cm), and Group III (20.5–23.5 cm). Specimens in Group I were sexually undifferentiated, whereas specimens in Groups II and III were predominantly sexually mature. The histological alteration index and average value of alteration showed a slight frequency of histopathological alterations in intestines, but the TUNEL assay strongly indicated the presence of apoptotic cells in the intestines, more evident in Group I specimens than other groups ($p < 0.05$).

Introduction

The sandfish, *Holothuria scabra* is a marine species of sea cucumber. The species is a highly nutritious food resource and is used for medicinal applications in China, Japan, Korea, Hong Kong, and Taiwan (Hamel et al., 2001). *H. scabra* is considered an important gelatinous marine resource that exhibits medicinal properties, including antioxidant (Nobsathian et al., 2017) and anti-inflammatory activities (Wargasetia et al., 2023). Furthermore, an earlier *in vivo* study revealed that scabraside D, a sulfated triterpene glycoside extracted from *H. scabra*, inhibited apoptosis, lymphangiogenesis, cancer cell invasion, and metastasis via the modulation of iNOS and STAT-3 (Assawasuparerk et al., 2016).

Wild populations of this sea cucumber have declined under the pressure of harvesting to meet increasing demand (Conand, 2004), causing it to be listed as endangered (EN), and placed on the International Union for Conservation of Nature (IUCN) Red List (Hamel & Mercier et al., 2013). In Thailand, a project to develop the commercial rearing of *H. scabra* has been established at the Prachuap Khiri Khan Coastal Fisheries Research and Development Center. Following the success of the development project (Sithisak et al., 2013), several reports have been published that address the aquaculture-related traits of this species. The reports have shed light on the co-culture with other species in captivity (Sithisak et al., 2013), population biology

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(Phattharapongphan et al., 2016), and population genetics (Ninwichian & Klinbunga, 2020). To protect wild stocks and promote the commercial aquaculture of the species in Thailand, we sought to build on the work of Tungse (2017) by providing additional data from the Thai population.

In their natural habitat, holothurians exhibit annual or biannual gonadal maturation (Cameron & Fankboner, 1985; Hamel & Mercier, 1995, 1996; Omar et al., 2013). The gonadal cycle comprises five sequential developmental stages: post-spawning, recovery, growth, advanced growth, and maturity (Foglietta et al., 2004). The reproductive cycles of sea cucumbers exhibit periodicity strongly influenced by environmental factors, including seawater temperature, food availability, and photoperiod (Dissanayake & Stefansson, 2010; Venâncio et al., 2022). Earlier studies of the sea cucumbers *Cucumaria frondosa* (Hamel & Mercier, 1996) and *H. sanctori* (Navarro et al., 2012) showed that the initiation of gonadal development was associated with the onset of low seawater temperatures and short days, while spawning occurred as the days lengthened and seawater temperatures peaked (Hamel & Mercier, 1997; Navarro et al., 2012; Venâncio et al., 2022). However, Foglietta et al. (2004) found that tubule development in holothurian gonads could occur synchronously or asynchronously, depending on the species.

Although gonadal maturation is regulated endogenously by several hormones, exogenous attentional cues are critical for activating the endogenous gonadal regulatory system in holothurians (Hamel & Mercier, 1997). Krishnan (1968) and Rosita et al. (1985) reported important data on the reproductive cycle of *H. scabra* in different geographical locations and environmental conditions. Morgan (2000) described the reproductive cycle of *H. scabra* at Stradbroke Island, Moreton Bay, Australia, observing that the gonad index was highest in November (summer) while the ovary was reabsorbed or spawned during or before the vitellogenic period, from September to November. Meanwhile, in the northern hemisphere at Gran Canaria, a summer-spawning *H. scabra* showed the highest gonad index in June–July (Navarro et al., 2012). However, the gonad index of *H. scabra* living in Mahout Bay, Oman, also in the northern hemisphere, reached its highest levels in April, coinciding with high temperatures and precipitation (Al-Rashdi & Claereboudt, 2018). Because the reproductive cycle and gonadal development of sea cucumbers largely depend on environmental conditions, as described above, reproductive data from their natural habitat are essential for conservation planning and management actions, and the condition of their habitat must be considered when assessing the impact on sea cucumber health.

Assessments of the health status of aquatic animals have used a wide range of biomarkers (National Research Council (NRC) (NRC) (NRC) (NRC), 1991). Quantitative histopathology is a rapid and sensitive tool for determining the health status of species under stressful conditions and in cases of environmental degradation (Wedderburn et al., 2000). Previous observations under both field and laboratory conditions have shown that histopathological alterations in various organs are associated with environmental contamination (Auró de & Ocampo, 1999). The severity of observed organ lesions exhibits a significant correlation with contaminant levels (DeCaprio, 1997; Teh et al., 1997). The spectrum of reported pathological manifestations includes circulatory disturbances, regressive alterations, progressive changes, inflammatory responses, and neoplastic development (Bernet et al., 1999). However, the relevant literature is dominated by studies focused on fish (Bernet et al., 1999; Bucher & Hofer, 1993; Mallatt, 1985; Myers et al., 1992; Teh et al., 1997), and only a few histopathological alterations have been reported in sea cucumbers (Jenzri et al., 2023).

The present study reports the health status and gonadal development in relation to the size of *H. scabra* from pond cultures in the Andaman Sea, Thailand. Morphological and histological methods were used, as well as the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.

Materials and method

Sea cucumber collection

Individual samples were randomly collected from pond cultures in the Andaman Sea near Koh Yao Noi Island, Phang Nga Province, Thailand, in July and August 2024. Sampling of *H. scabra* (n = 27 individuals) was conducted manually.

Gravimetric analysis, health assessment, and reproductive activity

Specimens were euthanized using a rapid cooling shock (Wilson et al., 2009) and measured for total length using a Vernier caliper. The internal organs were morphologically assessed according to the guidelines of Demeuldre & Eeckhaut, 2012 and Abdel-Razek et al. (2005), then resected and measured. Subsequently, all body samples were fixed in Davidson's fixative for approximately 48 h at room temperature and subjected to standard histological analysis (Suvarna et al., 2013; Presnell & Schreibman, 1997). Paraffin blocks were sectioned with a microtome at a thickness of 4 μm (Leica®, Wetzlar, Germany) and stained with Harris's hematoxylin and eosin (H&E). Gonadal development, structure, activity, and histopathology were examined using histological slides. Photographs of the slides were taken using the AmScope digital camera attached to the Ceti England microscope.

Reproductive differentiation was determined by the presence of gametes, as documented by Conand (1981) and Conand (1993) and Ramofafia, Battaglene, Bell, and Byrne (2000), who classified reproductive stages I-IV. The gonadal characteristics of each stage were recorded as percentages of the whole population (n = 27) and were correlated with size classes. All tissues were analyzed semi-quantitatively for histopathological changes and categorized into different levels of damage based on the histological alteration index (HAI) (Poleksic and Mitrovic-Tutundzic, 1994) and the average value of alteration (AVA) (Poleksic & Mitrovic-Tutundzic, 1994; Schwaiger et al., 1997). To compute the HAI, the degree of alteration in each tissue was assessed using the standard criteria for the stages of progressive tissue damage as outlined in previous studies (Paulo et al., 2012; Poleksic & Mitrovic-Tutundzic, 1994). The HAI was calculated using the formula: $HAI = 1 \times \sum I + 10 \times \sum II + 100 \times \sum III$, where I, II, and III represent alterations in stages I, II, and III, respectively. Based on the HAI, tissue damage was classified into five categories: 0–10 (normal organ/tissue function), 11–20 (slight alteration in the organ/tissue), 21–50 (moderate alteration in the organ/tissue), 51–100 (severe alteration in the organ/tissue), and values above 100 (irreparable alteration in the organ/tissue) (Poleksic and Mitrovic-Tutundzic, 1994).

The AVA was determined based on the frequency and severity of lesions, which were classified into three categories according to Poleksic and Mitrovic-Tutundzic, 1994: category 1 (no pathological alteration in the organ), category 2 (slight or mild pathological alterations in the organ), and category 3 (severe and extensive pathological alterations in the organ).

TUNEL assay

To detect apoptotic cells, sampled sections were analyzed using the TUNEL assay as described by Rojo and Gonzalez (1998). Consecutive sections from the histological study were deparaffinized, dehydrated, and blocked for 1 h with a solution containing 10% Normal Goat Serum (Vector Laboratories). They were then incubated with 4% bovine serum albumin (BSA) and subsequently immersed for 1 h in TdT buffer at 37°C. The sections were then scanned and examined using a 3DHISTECH panoramic viewer (3DHISTECH, Hungary) for high-resolution imaging to identify structural organizations and cell types. The apoptotic cells on the stained slides were counted using the ImageJ program after randomly selecting 200 cells at 40× magnification.

Data analysis

All data, including the density of apoptotic cells, were expressed as means ± standard deviation (SD). Normality was checked using the Shapiro–Wilk test and was compared among size classes using ANOVA with a significance level of $p < 0.05$. All data were performed using GraphPad Prism (version 8.0.1 for Windows).

Results

Physicochemical parameters

At the collection site, the water temperature was 28°C, salinity 29 ppt, pH 8.1, dissolved oxygen 6.5 mg/L, NH_3 0.0 mg/L, NO_2^- 0.0 mg/L, and transparency was about 80 cm.

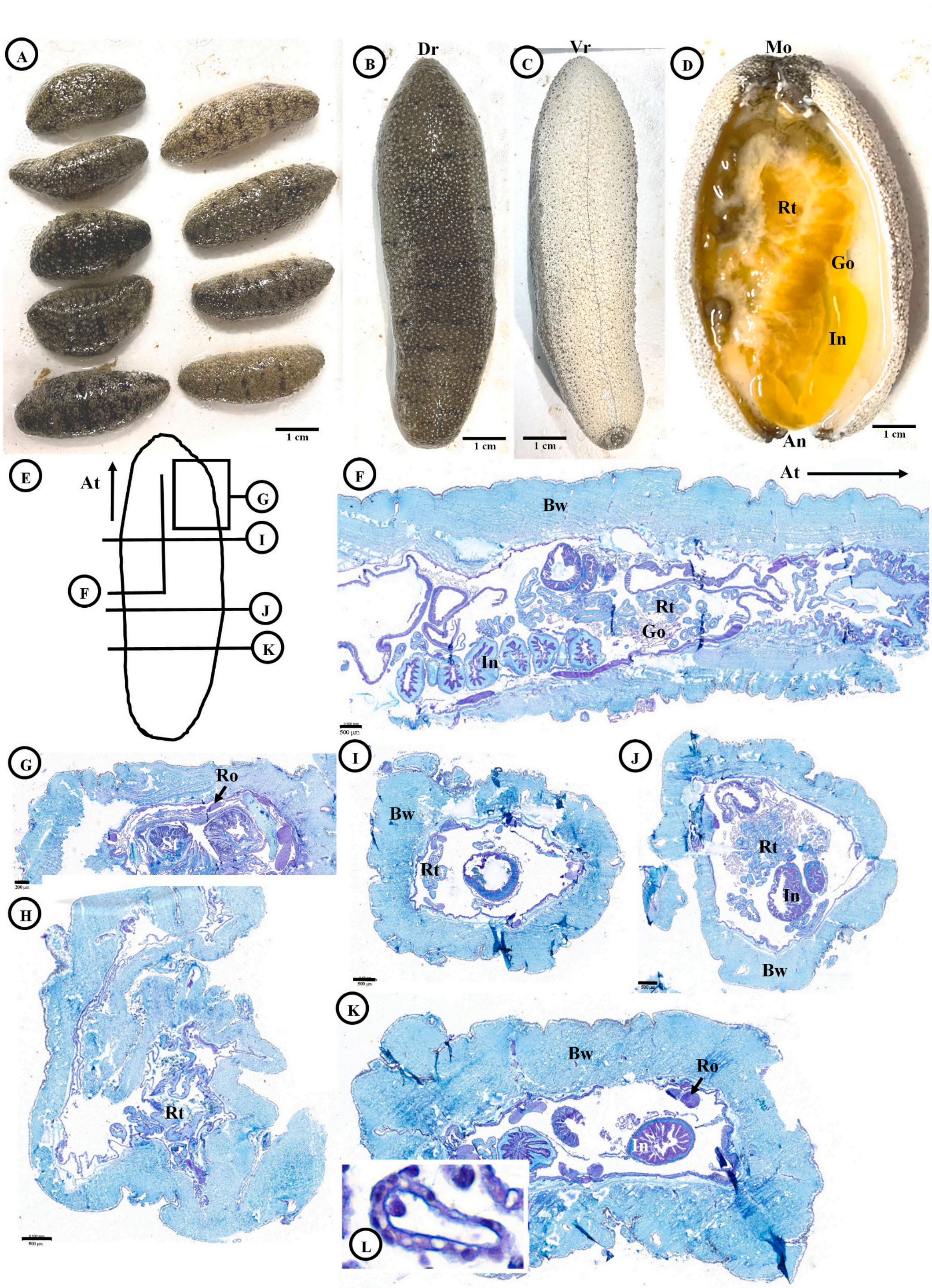

Fig. 1. External morphology of *Holothuria scabra* at the light microscopic level. A: Small *H. scabra* specimens. B and C: A large *H. scabra* specimen in dorsal (Dr) and ventral (Vr) views. D: A view of the internal morphology shows the mouth (Mo), respiratory tree (Rt), gonad (Go), intestine (In), and anus (An). E: Several plans via the body. Longitudinal (F-G) and cross (H-K) sections show body wall (Bw), respiratory tree (Rt), radial organs (Ro), and gonad (Go). L: Undifferentiated cell in the gonad. Staining methods: F–L = alcian blue–periodic acid–Schiff (AB-PAS).

Size distribution and external morphohistology

H. scabra could be divided into three size classes: Group I (3.5–6.8 cm), Group II (16.5–19.5 cm), and Group III (20.5–23.5 cm) (n = 9 individuals per size class).

The common external morphology of Group I *H. scabra* was an elongated tubular body (Fig. 1A–C). The dorsal region of these specimens had a dark brown coloration with tiny conical pinnules and white spots (Fig. 1B). In contrast, its ventral region was white with black spots (Fig. 1C). The mouth was located at the anterior end of the body, and the anus at the posterior end (Fig. 1D). The plane of a section in both a longitudinal and cross-sectional views of a dissected *H. scabra* in Fig. 1E-L shows the main composition of the body wall, intestine, respiratory tree, radial organ and gonads.

Gonadal development in relation to size

All the stages of gonadal development, including undifferentiated sex, mature, spawning, and post-spawning stages, were represented among the sampled *H. scabra* (Figs. 1L and 2). Group I specimens were of undifferentiated sex (100%), the gonads exhibiting several small tubules and undifferentiated cells (Fig. 1L). Groups II and III mostly presented mature ovaries with oocytes (Fig. 2A-E). The highest proportion of mature ovaries (88.89%) was noted in Group II; however, only *H. scabra*

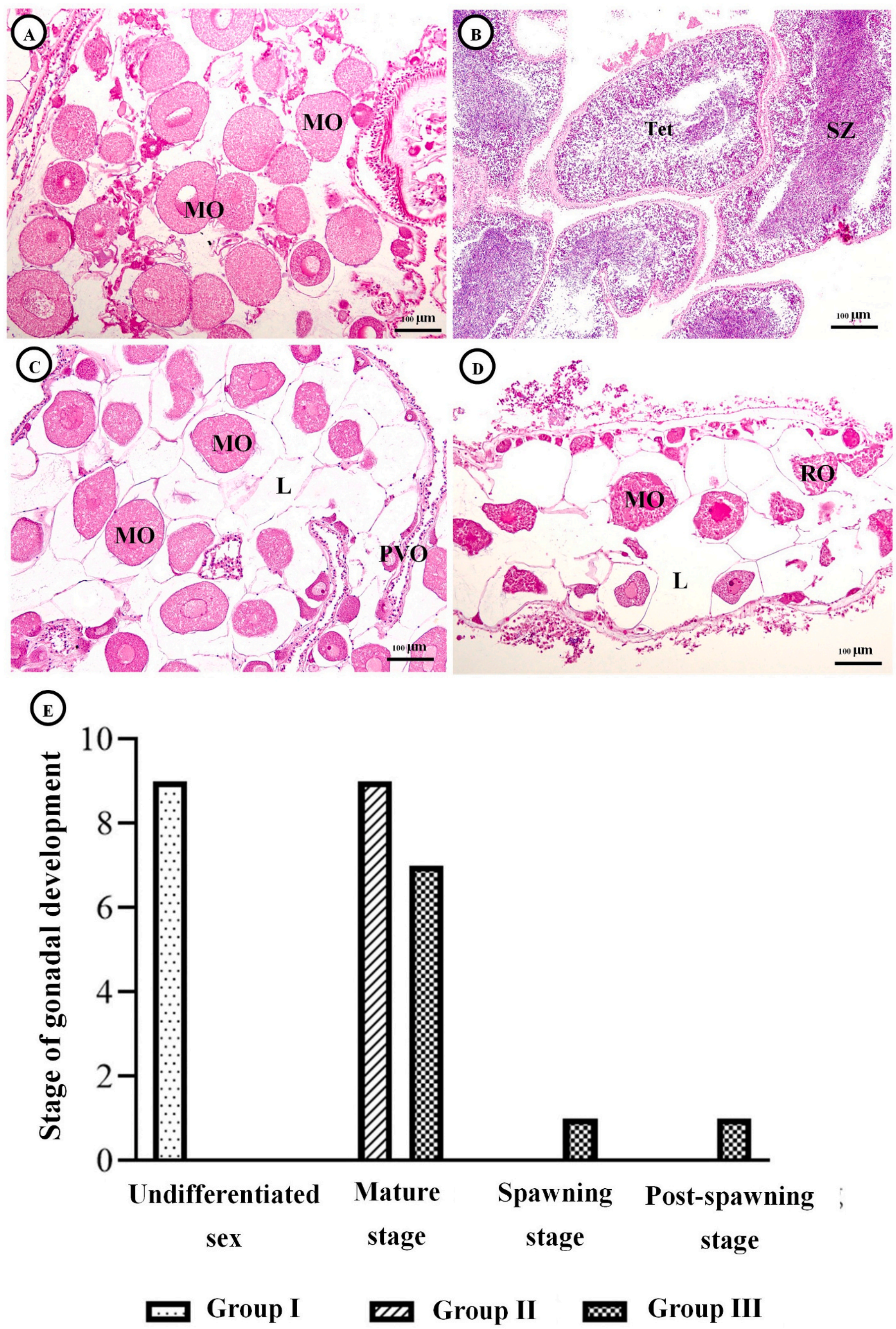

Fig. 2. The gonads at various developmental stages at the light microscopic level and a chart of gonadal development among groups. A: Ovary in the mature stage. B: Testis in the mature stage. C: Spawning stage. D: Post-spawning stage. E: The percentage of gonadal development in each size group. Abbreviations: L = gonadal lumen, MO = mature oocyte, PVO = previtellogenic oocyte, Tet = testicular tubule, RO = relict oocyte, SZ = spermatozoa. Staining methods: A-D = Harris's hematoxylin and eosin (H&E).

in the mature stage presented spermatozoa in the testicular tubules (11.11%, Fig. 2B and 2E). Group III comprised specimens in the mature stage (77.79%), spawning stage (11.11%), and post-spawning stage (11.11%) of ovarian development (Fig. 2C–E).

Histology of important organs and their TUNEL assays

We examined specimens in Group I, in which the body wall was easily observed (Figs. 1F and 3A). The body wall of specimens in this group presented three distinct layers. From outside to inside, these layers were the epidermis with epithelial cells, the superficial dermis, and the dermis (Fig. 3B–C). Collagen fibers in the dermis were observed with alcian blue–periodic acid–Schiff (AB-PAS) staining (Fig. 3D). All juvenile and adult stages examined showed no damage and no occurrence of apoptotic cells on the body wall (Fig. 3E). Additionally, a large cluster of brown cells was identified in the dermis, but the cells did not react positively in the TUNEL assay (Fig. 3F-G).

Tubular projections, or podia, formed a wall of connective tissue (Fig. 3H). The podial disc was associated with the podial channel (Fig. 3I). There was no histological evidence of apoptotic cells in this feature (Figs. 3I-J), nor in the radial nerve cord (Fig. 4A-B).

Located close to the gonads, the respiratory tree presented three

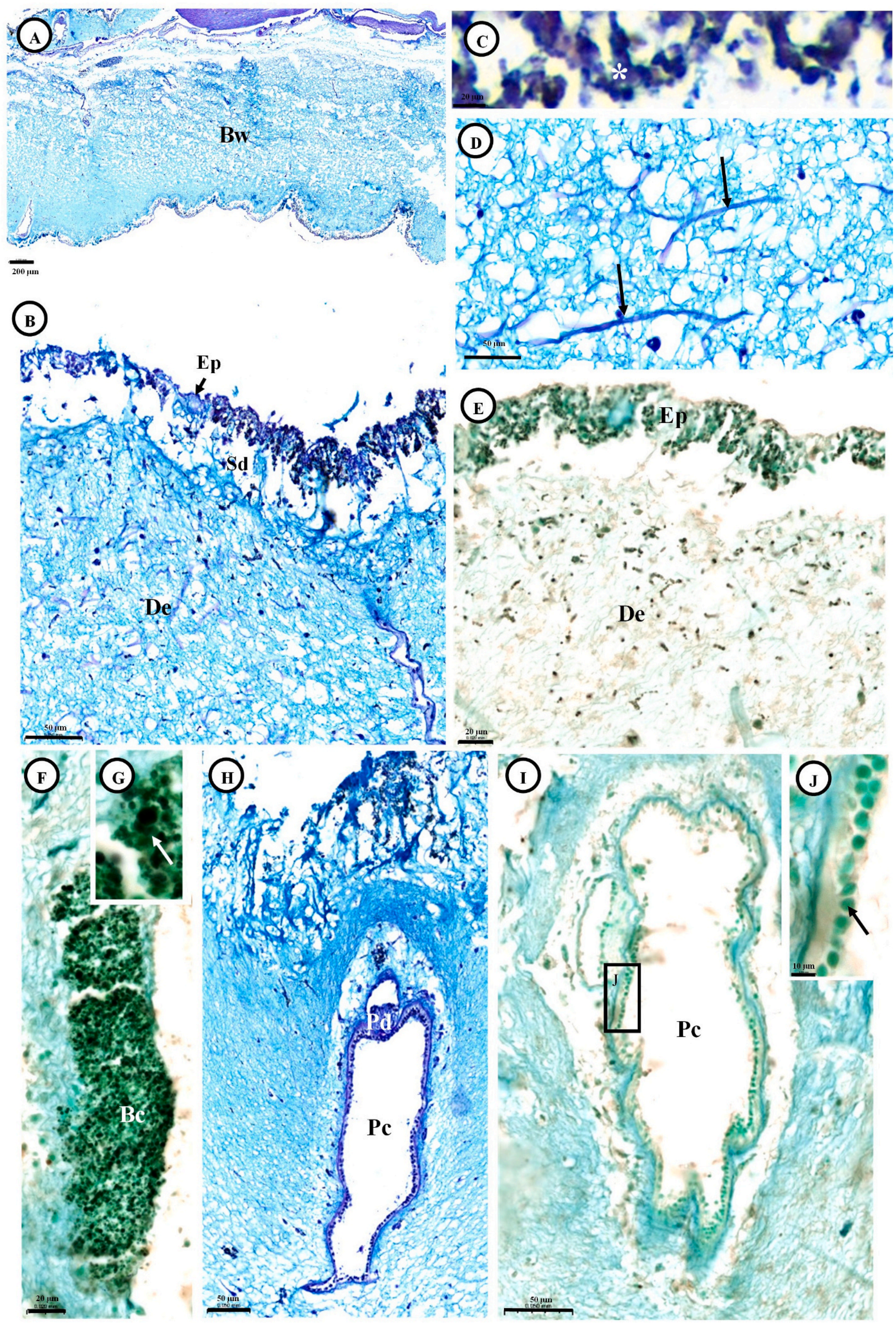

Fig. 3. The body wall of *Holothuria scabra* at the light microscopic level. A: A clear localization of the body wall (Bw). B: The body wall includes the epidermis (Ep), subdermis (Sd), and dermis (De). C: A high magnification image shows an epidermal cell (asterisk) in the epidermis. D: Collagen fibers (arrows) in the dermis. E: Apoptotic cells were not observed in the dermis (De) and epidermis (Ep). F and G: Brown cells (Bc) in the body wall. H: One of the podia (Pd) with its podial canal (Pc). I and J: No apoptotic cells were observed in the podia. Staining methods: A–D, H = alcian blue–periodic acid–Schiff (AB-PAS) method, and E–G, I, J = TUNEL assay.

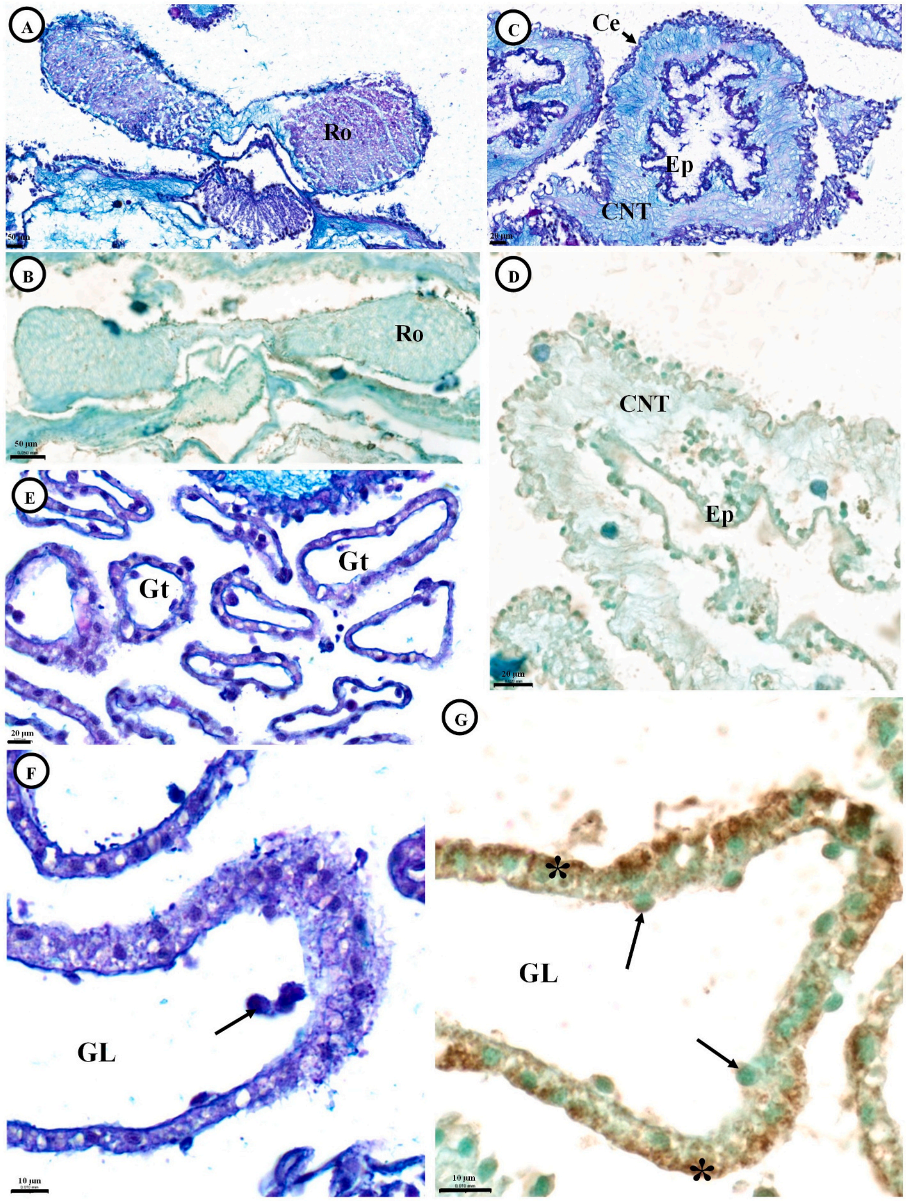

Fig. 4. The radial organs, the respiratory tree, and the undifferentiated sex of *Holothuria scabra* at the light microscopic level. A: The radial organs (Ro) without apoptotic cells (see B). C: The cross-section shows the respiratory tree divided into the luminal cuboidal epithelium (Ep), the thick connective tissue (CNT), and the slender coelomic epithelium (Ce). D: The respiratory tree without apoptotic cells. E: Gonadal tubules (Gt) in the gonad. F: A high magnification image reveals small gametes (arrow) in the gonad. G: The TUNEL-positive area of a layer of connective tissue (asterisk) close to the gametes (arrows). Abbreviations: GL = gonadal lumen. Staining methods: A, C, E, F = alcian blue–periodic acid–Schiff (AB-PAS), B, D, G = TUNEL assay.

layers in cross-section: luminal cuboidal epithelium, thick connective tissue, and slender coelomic epithelium (Fig. 4C). The TUNEL assay revealed an absence of apoptotic cells in the respiratory tree (Fig. 4D).

The cross-sectional microarchitecture of the reproductive apparatus of *H. scabra* consisted of several gonadal tubules (Fig. 4E). Each tubule was composed of germinal epithelium with small gametes in the lumen close to a layer of connective tissue (Fig. 4F). However, this tissue was only positive in the TUNEL assay (Fig. 4G). Mature oocytes showed no apoptotic signs (Fig. 5A-B), but spermatocytes showed signs of degeneration (Fig. 5C-D and HAI in Fig. 5E). Relict oocytes, follicular atresia, and degeneration of the gonadal wall were observed, which gave HAI scores of 10–20, respectively, and positive reactions in the TUNEL assay (Fig. 5F-G).

The intestine of *H. scabra* could be classified into two regions: the anterior and posterior intestines (Fig. 6A–J). Structurally, both areas shared three tissue layers: a mucosa (luminal epithelium), a muscular layer, and a coelomic epithelium (Fig. 6A–H). In several views, the anterior intestine was clearly differentiated from the posterior intestine by deep longitudinal folds in the lumen (Fig. 6A and D). The intestinal luminal epithelium in all size classes was characterized by a typical epithelial cell architecture, and reacted positively in the TUNEL assay (Fig. 6B-C and E-F). This feature was common to the posterior intestine of specimens in all size classes (Fig. 6G–I).

The intestinal epithelium showed slight degeneration, resulting in an HAI of 20 (Fig. 6K). Although the density of apoptotic cells in the intestine varied, the difference was statistically significant only between Group II and Group III ($p < 0.6$, Fig. 6L).

Discussion

Several studies have documented the morphohistology of various organ systems of *H. scabra* from different regions of the world. These

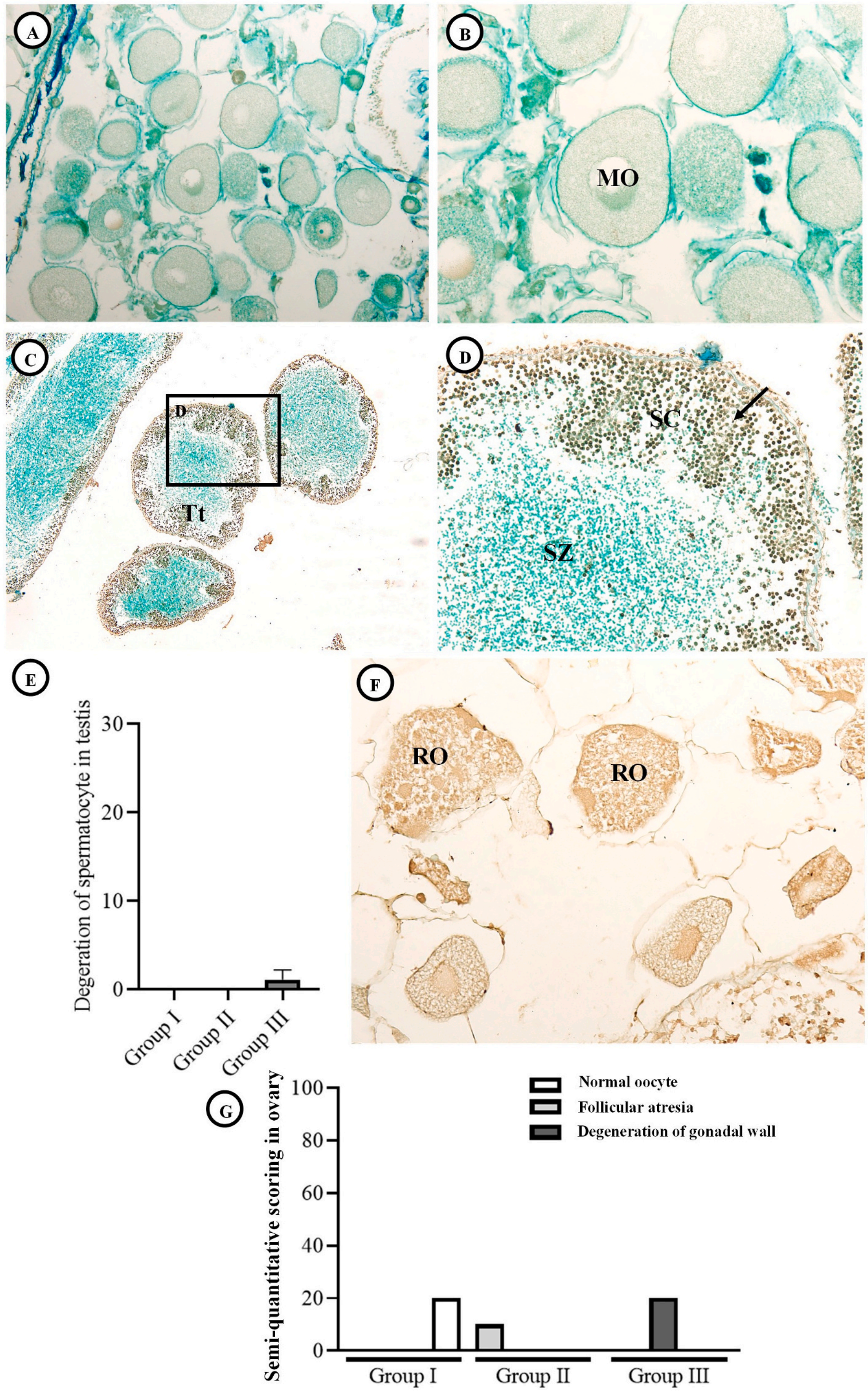

Fig. 5. Light microscopic level observation of apoptotic cells in *Holothuria scabra* in size groups II and III. A-B: Mature oocytes in the mature ovary. C-D: Degeneration of spermatocytes (arrows) and TUNEL-positive spermatocytes in the mature testis. E: The chart shows the degeneration of spermatocytes in the testis by size group. F: TUNEL-positive relict oocytes (RO). G: The mean of the histological alteration index in the ovary by size groups. Abbreviations: MO = mature oocyte, SZ = spermatozoa, Sc = spermatocytes, Tt = testis. Staining methods: A-D, F = TUNEL assay.

include studies of the respiratory system (Shukhairi et al., 2025), reproductive system (Ramofafia, Byrne, & Battaglene, 2003), nervous system and ovary (Chaiyamoon et al., 2018), and integumentary structure (Delroisse et al., 2020). Among health concerns in *H. scabra* populations, skin ulceration disease (SKUD) has been recognized as a recurring issue (Eeckhaut et al., 2019; Delroisse et al., 2020). Other reported health threats have included bacterial infections caused by *Vibrio owensii*, *V. azureus*, and *V. fortis,* which have been associated with skin lesions and high mortality in juveniles and broodstock (Tangestani & Kunzmann, 2019). Environmental stressors, such as temperature fluctuations and low dissolved oxygen levels, have further exacerbated disease outbreaks in pond systems. Moreover, chronic exposure to microplastics has been shown to cause epithelial erosion, mucosal thinning, and inflammatory infiltration in juvenile *H. scabra*, particularly in intestinal and respiratory tissues (Delroisse et al., 2023). In our study, all *H. scabra* individuals in every size group were initially

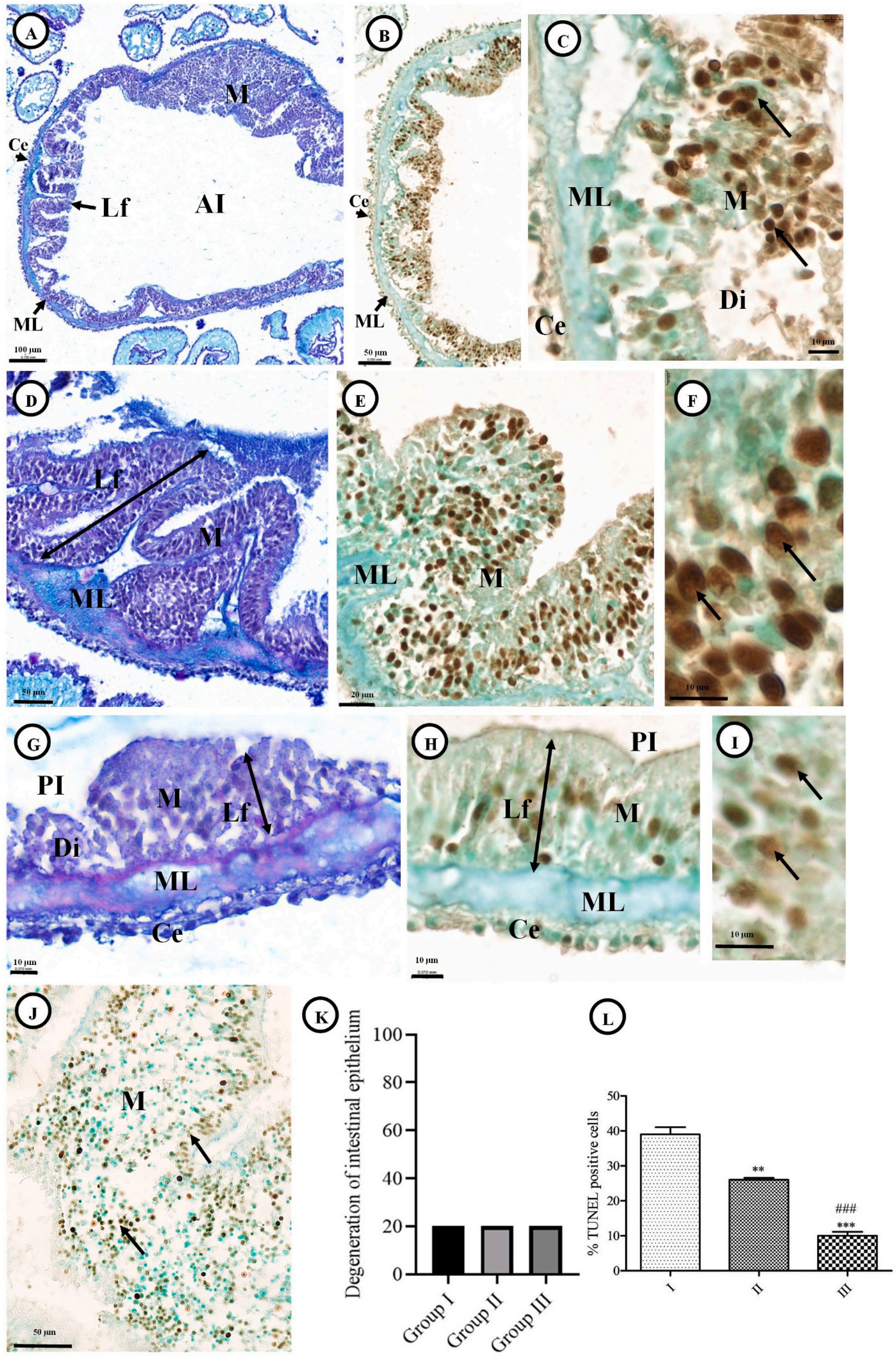

Fig. 6. Light microscopic level observation of the intestine in *Holothuria scabra*. A: A cross-section of the anterior intestine (AI) in Group I shows the mucosa (M), muscular layer (ML) and coelomic epithelium (Ce). The longitudinal fold (Lf) of the anterior intestine was observed. B-C: Apoptotic cells (arrows) were present in the mucosal epithelium. D: Other areas of the longitudinal fold (Lf) in the anterior intestine with the prominent muscular layer (ML). E-F: Apoptotic cells seen in figures B and C at higher magnification. G: The short longitudinal fold (Lf) of the posterior intestine (PI) and a slight degeneration of intestinal epithelium (Di). H-I: Apoptotic cells were present in the nucleic epithelial layer (arrows) of the mucosa. J: Apoptotic cells were present in the mucosa specimens from Group III. K: The density of apoptotic cells was used to calculate the degeneration of the intestinal epithelium. L: Asterisks indicate statistically significant differences among sites ($^{**}p < 0.01$, $^{***}p < 0.001$, $^{###}$ $p < 0.0001$). Staining methods: A, D, G = alcian blue–periodic acid–Schiff (AB-PAS), B-C, E-F, H-J = TUNEL assay.

considered unhealthy due to the presence of apoptotic cells in intestinal and gonadal tissues. This observation has not been previously reported. In the following paragraphs, we provide a detailed assessment of the health status of the studied sea cucumbers, which is particularly important given the negative impact of disease on the performance of *H. scabra* in pond culture systems in Thailand.

Previous reports have shown that juveniles develop their gonads during ontogenesis until they become mature specimens capable of reproduction (Ramofafia, Battaglene, & Byrne, 2001). The structure of the gonadal tubules of various adult sea cucumber species is well understood through the works of Davis (1971), Atwood (1973), and Smiley and Cloney (1985). Our research revealed three concentric layers that delimit a central lumen containing germ cells. The layers comprised an outer peritoneal layer, a connective tissue compartment, and an inner epithelium that contained germ cells. On the other hand, in sexually undifferentiated juveniles in Group I, the anatomy of the gonads could not be well distinguished. It is possible that the same three tissue layers present in adults are also found in small juveniles, but their histological structures did not seem identical, despite their size range from 3.5 to 6.8 cm. In specimens longer than 16.5 cm, mature gonads were present in the gonadal tubules, suggesting that these specimens were in the adult stage, as previously reported for *H. scabra* with a total length of 16.8 cm (Kithakeni & Ndaro, 2002). It is possible that various hormones within the organism primarily regulate size during gonadal maturation, although external environmental cues such as seawater temperature, food availability, and photoperiod are essential for triggering the internal system (Dissanayake & Stefansson, 2010; Gaudron et al., 2008; Hamel & Mercier, 1997; Venâncio et al., 2022).

In Group II exclusively, we observed that gonadal maturation in both male and female *H. scabra* occurred synchronously, a finding similar to previous reports for *H. scabra* (Kithakeni & Ndaro, 2002; Tuwo, 1999). The gonadal maturity of both sexes was therefore considered to have occurred at the same time during the sampling period, suggesting the possibility of more readily procuring a supply of mature broodstock for breeding purposes. In this way, the limitation imposed on the species by its restricted reproductive cycle and spawning could be addressed by enabling mass spawning using numerous small broodstocks.

The HAI scores indicated slight alterations in the intestines, but a prominent feature observed across all *H. scabra* size classes was the presence of apoptotic cells within the intestinal epithelium. While a certain level of epithelial cell turnover is essential for maintaining intestinal homeostasis, particularly in organisms with high exposure to environmental stimuli (Blander, 2018), excessive apoptosis may indicate pathological conditions. In our study, the observed epithelial destruction suggests that this apoptotic activity may be linked to disease processes, potentially mediated by inflammatory signaling pathways involving NF-κB activation and caspase-8–dependent mechanisms (Blander, 2018; Ikeda et al., 1998; Ramachandran et al., 2000). Furthermore, the presence of apoptosis may be associated with exposure to external toxicants or stressors, such as heavy metals or microplastics, which are known to induce cell death via oxidative stress, mitochondrial disruption, and the activation of caspase and apoptosis-inducing factor (AIF) pathways (Liu et al., 2022; Salamone et al., 2025).

Given the essential role of the intestine in nutrient and water absorption, these histological lesions may compromise digestive efficiency and overall physiological performance (Artis, 2008). To better understand the mechanism of apoptosis in *H. scabra*, future investigations should focus on the activity of key apoptotic markers, including the initiator caspases (caspase-8 and caspase-9), the effector caspase (caspase-3), the activation state of NF-κB, and reactive oxygen species levels. Such studies would help clarify whether the observed damage was caused by inflammation, oxidative stress, or both. Notably, apoptotic areas of relict oocytes in the ovary were commonly observed in Group III specimens, suggesting a developmental or biological process (Reyes-Rivera et al., 2024) or a survival mechanism (Reviewed by Tilly, 2001). Although arguably, the study of germ cell death in holothurians is still in its early stages (Reyes-Rivera et al., 2024), this is the first report of lesions in the ovary of *H. scabra*. These lesions could be caused by reduced oocyte production, which would disadvantage the initial performance of developing *H. scabra* in the sampled pond culture. Indeed, this continuous loss of oocytes in Groups I through III was, according to Morita and Tilly (1999) and Reyes-Rivera et al. (2024), thought to indicate an unhealthy state of the female reproductive system.

Taken together, our findings underscore the significance of apoptosis as a key histopathological marker of *H. scabra* health status. While the presence of apoptotic cells in the intestine may reflect pathological responses to environmental stressors or infectious conditions, the detection of apoptosis in the ovary across all size classes poses a more fundamental biological implication. Given the central role of the ovary in reproductive output, the presence of apoptotic lesions—especially involving oocytes from early to advanced developmental stages—points to compromised reproductive capacity and potential long-term impacts on the sustainability of cultured populations. These observations are consistent with previous studies that have identified gonadal tissues as highly sensitive to both internal physiological cues and external environmental stressors (Eckelbarger & Hodgson, 2021; Głód et al., 2025). Therefore, our examination supports the recommendation that the intestine of *H. scabra* should be considered a preferred target organ for histological monitoring and early detection of health deterioration in aquaculture settings.

Conclusion

Our research of the literature revealed that the detailed morphohistology of *Holothuria scabra*, the most intensively studied holothurian, was not available. However, using the TUNEL assay, we detected the presence of apoptotic cells in the gonads and intestines. We suggest that apoptotic cells in relict oocytes are typical of the reproductive physiology of *H. scabra*, but that apoptotic cells present in the intestine (according to HAI and apoptotic cell density) may indicate digestive dysfunction. All the specimens studied contained mature oocytes and the total length at first maturity of the sampled *H. scabra* was estimated to be under 16.5 cm.

Ethical clearance for the use of animals in scientific research

All experimental procedures were conducted in accordance with institutional guidelines for animal research and approved by the Animal Care and Use Committee of Prince of Songkla University, Thailand (approval ID: 2024-VET01-001 and Ref. AQ094/2024).

CRediT authorship contribution statement

Wikit Phinrub: Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Narit Thaochan:** Writing – review & editing, Methodology. **Atsuo Iida:** Writing – review & editing, Investigation. **Peerapon Sornying:** Writing – review & editing, Methodology, Investigation. **Wichaya Tongtako:** Writing – review & editing, Methodology, Investigation. **Natthawut Charoenphon:** Writing – review & editing, Methodology, Investigation. **Gen Kaneko:** Writing – review & editing, Investigation, Formal analysis. **Kitipong Angsujinda:** Writing – review & editing, Validation, Investigation. **Suwat Tanyaros:** Writing – review & editing, Investigation. **Sinlapachai Senarat:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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