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Morphological and histological analyses of ovary and pelvic fin structures in the deep-sea jellynose fish, *Guentherus katoi*

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Abstract

The deep-sea jellynose fish *Guentherus katoi* (Ateleopodidae) has rarely been studied since its description in 2008. We performed the morphological and histological analyses on the ovary and free pelvic fin rays (PFRs) of a female specimen (625.6 mm total length) caught in the Goto Islands, Japan. Histological analysis of the ovary revealed six stages of oocyte development, ranging from oogonia to migratory nucleus stage oocytes. The coexistence of multiple oocyte stages indicates that *G. katoi* may be a multiple spawner. Despite the low gonadosomatic index (0.35 %), the presence of migratory nucleus stage oocytes indicated that this female was close to spawning conditions. Immunohistochemical analysis revealed cell proliferation in oogonial nuclei and granulosa cells. The first PFR had a previously undescribed ventral longitudinal groove (~700 μm deep, 1.05 cm long) with central lepidotrichia surrounded by connective tissue, and no sensory receptor cell was observed. These findings provide new insights into the reproductive biology and appendage morphology of this rare deep-sea species.

Key words: deep-sea fish; oogenesis; pelvic fin; jellynose fish; *Guentherus katoi*; Ateleopodidae

Introduction

Guentherus katoi was first described in 2008 as a new species of jellynose fish belonging to the family Ateleopodidae (Senou et al. 2008), which consists of four genera (*Ateleopus*, *Guentherus*, *Ijimaia*, and *Parateleopus*) with 12 or 13 species (Nelson et al. 2016). Four ateleopodid species have been recorded in Japan: *Ateleopus japonicus*, *Ateleopus edentatus*, *G. katoi*, and *Ijimaia dofleini* (Fujiwara et al. 2019). The records of *G. katoi* are limited to several locations in waters off Japan, including at Kumano Nada (319.5–352.5 m depth) and near Kume Island and Uji Island in the Ryukyu Islands (612 m depth) (Senou et al. 2008; Fujiwara et al. 2019). This species reaches a maximum recorded standard length of 781.0 mm (total length: 840 mm) (Fujiwara et al. 2019).

Only two species (*G. altivela* and *G. katoi*) have been recognized in the genus *Guentherus*. Species of the other genera in the family exhibit elongated bodies with a single well-developed pelvic fin ray or one developed fin ray plus 0–3 rudimentary rays. On the other hand, species of the genus *Guentherus* are characteristic of having a stout body and well developed three pelvic fin rays (PFRs) (Senou et al. 2008). The tip of the first PFR (PFR1) of *G. katoi* is a large lanceolate, whereas that of the second PFR (PFR2) is thin and filamentous. The tip of the third PFR (PFR3) is also lanceolate but thinner than PFR1.

Deep-sea fishes are generally difficult to obtain as specimens or for studies (Priede 2017). Despite its large size, exceeding 700 mm in total length, only five specimens of *G. katoi* have hitherto been deposited in three museums worldwide, all located in Japan (Senou et al. 2008; Fujiwara et al. 2019). Therefore, basic biological information on this species is scarce. In particular, there is no information regarding its reproduction and maturation, including its spawning season and reproductive pattern. This lack of information hinders species conservation, stock assessment, and management planning (Roberts

2002).

In this study, we examined the ovarian histology of a female specimen collected from the East China Sea to gather basic information regarding the reproduction of this species. We examined the basic structure of the ovaries and the developmental stages of oocytes. The analysis included immunostaining with antibodies against proliferating cell nuclear antigen (PCNA), a cell proliferation marker, to investigate oogenesis (Maga and Hubscher 2003). In addition, we examined the internal structure of the distinctive pelvic fins of this species through histological observations.

Materials and Methods

Sample collection

A jellynose fish specimen was collected from a depth of approximately 200 m off Goto Islands, Nagasaki Prefecture, Japan, using bottom gill net fishing on 12 June 2023. Upon external examination, the specimen was identified as *Guentherus katoi* based on the diagnostic characteristics described by Senou et al. (2008): stout body with numerous dark brown spots, three characteristic free pelvic fin rays with white coloration, and the distinctive lanceolate tip of the first pelvic fin ray. The specimen was purchased from INDUKA Co. Ltd. at the Nagasaki fish market and transported to the laboratory in Nara, Japan, under refrigeration on 14 June 2023.

Upon arrival at the laboratory, the external morphology of the specimen was photographed, and the total length and body weight were measured. Standard length was also measured using Vernier calipers (CM60; Mitutoyo Corp., Kanagawa, Japan) to the nearest 0.1 mm. The gonads were dissected from the left ventral side. Based on gross morphological examination, the gonads appeared to be ovary, which was later confirmed through histological analysis. The gonadosomatic index (GSI) was calculated as (gonad weight/body weight) × 100. The left first pelvic fin ray (PFR1) was also collected. Both tissues were fixed in Bouin's solution overnight and then transferred to 70 % ethanol for storage. The whole fish specimen was fixed in 10 % formalin, preserved in 70 % ethanol, and deposited as a voucher specimen at the Fish Collection of the Faculty of Agriculture, Kindai University, Japan (voucher number: KUN-P 65942).

Histological analysis

Fixed ovaries and PFR1 were dehydrated through a graded ethanol series, cleared in Fast Solve (Pharma Inc., Tokyo, Japan), and embedded in paraffin. Tissue sections were sliced at a thickness of 5–7 µm using a rotary microtome and mounted on Superfrost slides (Matsunami Glass Inc., Osaka, Japan). After deparaffinization and rehydration, sections were stained with Carazzi's haematoxylin and 1 % eosin Y. The stained sections were dehydrated, cleared, and mounted in Eukitt (O. Kindler GmbH, Freiburg, Germany) for microscopic observation.

Immunohistochemistry of the ovaries

To examine cell proliferation in the ovaries, immunohistochemical analysis was performed following methods modified from Kobayashi et al. (2014). After the deparaffinization and rehydration of the ovarian sections, antigen retrieval was performed by incubation at 95 °C for 20 min in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3 % H_2O_2 for 30 min, and non-specific binding was blocked with 2 % normal horse serum for 20 min. Subsequently, the sections were incubated overnight at 4 °C with an anti-PCNA antibody (cat no: 10205-2-AP; Proteintech, Chicago, IL, USA) diluted to 1:2,000. After washing with TBST, the sections were incubated for 30 min at room temperature with ImmPRESS anti-rabbit IgG reagent (Vector Laboratories, Burlingame, CA, USA), and visualised using a diaminobenzidine substrate kit (Nakarai, Kyoto, Japan). The sections were dehydrated, cleared, and mounted in Eukitt for microscopic observation.

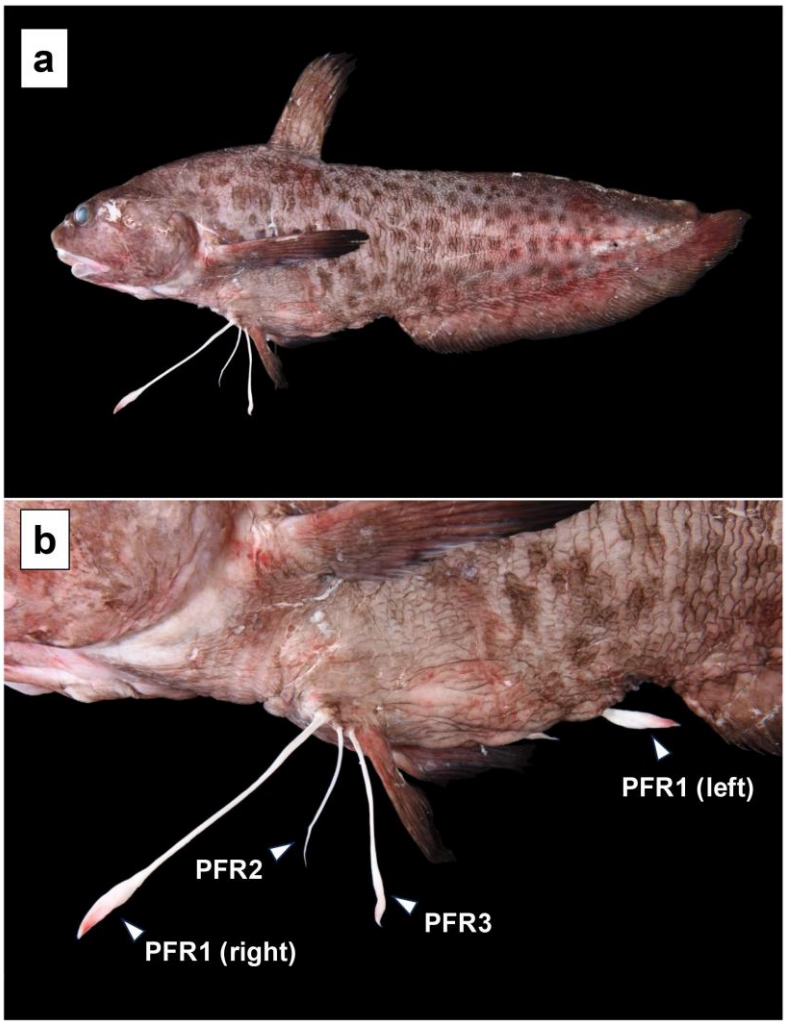

Fig. 1. Female specimen of *Guentherus katoi* examined in this study (total length: 625.6 mm; standard length: 566.6 mm; body weight: 2.20 kg; voucher number: KUN-P 65942). a: Left lateral view. b: Enlarged view of pelvic fin. Abbreviations: PFR1, first free pelvic fin ray; PFR2, second free pelvic fin ray; PFR3, third free pelvic fin ray.

Results

General description

The examined female specimen of *G. katoi* was 625.6 mm in total length, 566.6 mm in standard length, and 2.20 kg in weight (Fig. 1). Its body surface was extremely soft and lacked scales and lateral lines. The mouth was large but without prominent teeth. The body exhibited a dark brownish base color with distinctive black leopard-like spots distributed across the surface (Fig. 1a). The pelvic fins were well developed with three anterior free rays. PFR1 was the thickest and longest, PFR2 was thin and elongated, and PFR3 exhibited an intermediate thickness (Fig. 1b).

Ovarian morphology and histological observations

Ovaries were removed from the left lateral side of the specimen (Fig. 2). No apparent difference in the external structure was observed between the left and right ovarian lobes (Fig. 2a). Ovarian weight was 7.8 g, with a gonadosomatic index (GSI) of 0.35 %. The results of the histological analysis revealed an ovarian cavity in the centre (Fig. 2b). Various developmental stages of oocytes were observed (Fig. 2b–g), which were classified into six categories following Brown-Peterson et al. (2011): oogonia, large nucleus with minimal cytoplasm and associated undifferentiated germ somatic cells (Fig. 2c); primary growth stage

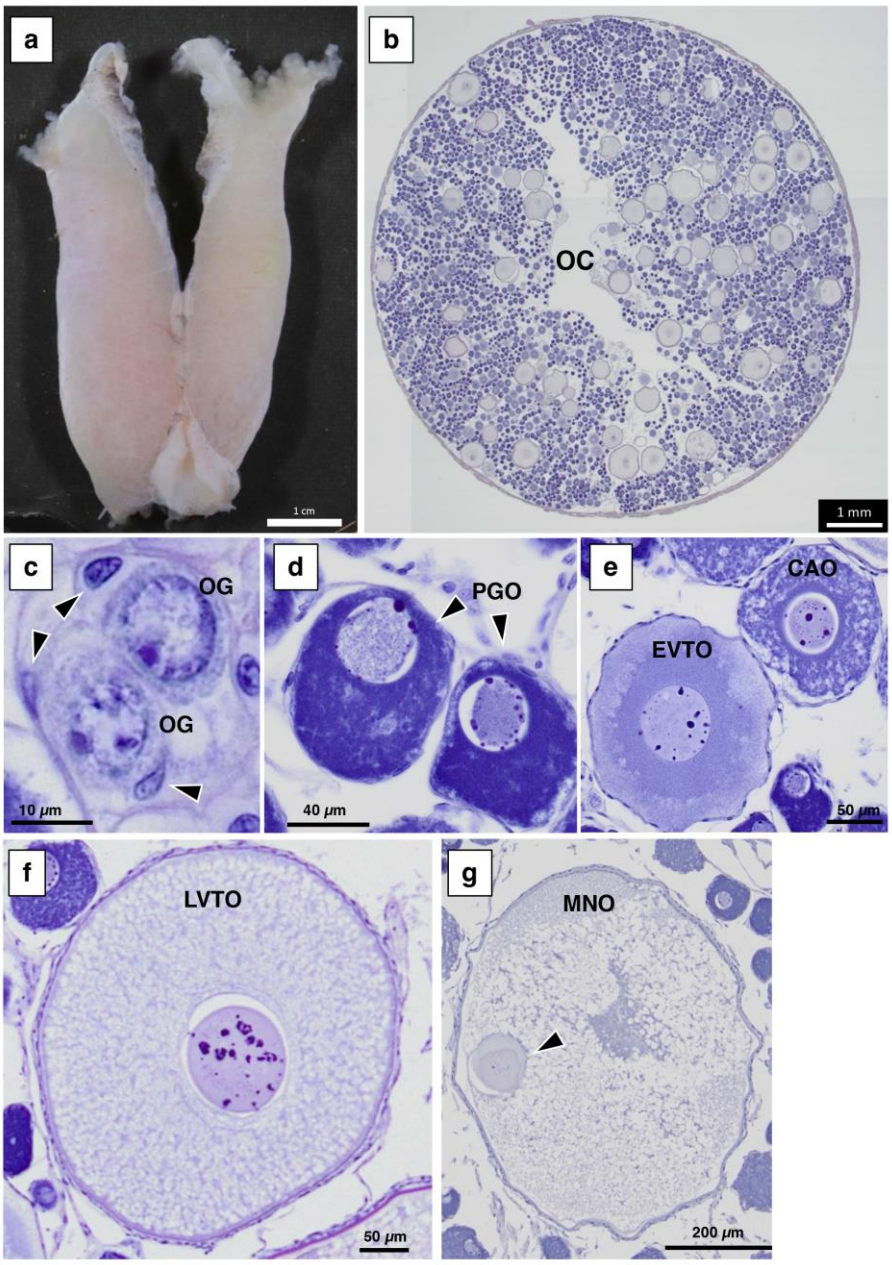

Fig. 2. Images showing the structure of the ovaries and various developmental stages of oocytes in *Guentherus katoi*. a: Macroscopic view. b: Low magnification micrograph of ovarian tissue showing oocytes at various developmental stages and central ovarian cavity (OC). c: Oogonia (OG) and undifferentiated somatic cells (arrowheads). d: Primary growth stage oocytes (PGO) (arrowheads). e: Cortical alveolar oocytes (CAO) and early vitellogenic oocytes (EVTOs). f: Late vitellogenic oocytes (LVTOs). g: Migratory nucleus stage oocytes (MNOs).

oocytes, basophilic oocyte cytoplasm with multiple spherical nucleoli in the nucleus (Fig. 2d); cortical alveolar oocytes, oil droplets present in cytoplasm (Fig. 2e); early vitellogenic oocytes, yolk accumulation initiated with yolk globules appearing in the peripheral cytoplasm (Fig. 2e); late vitellogenic oocytes, yolk globules filling the entire cytoplasm at maximum diameter (Fig. 2f); and migratory nucleus stage oocytes (MNOs), germinal vesicle migrated from central to peripheral position (Fig. 2g). No germinal vesicle breakdown (GVBD) oocytes, hydrated oocytes, atretic oocytes, or post-ovulatory follicles were observed.

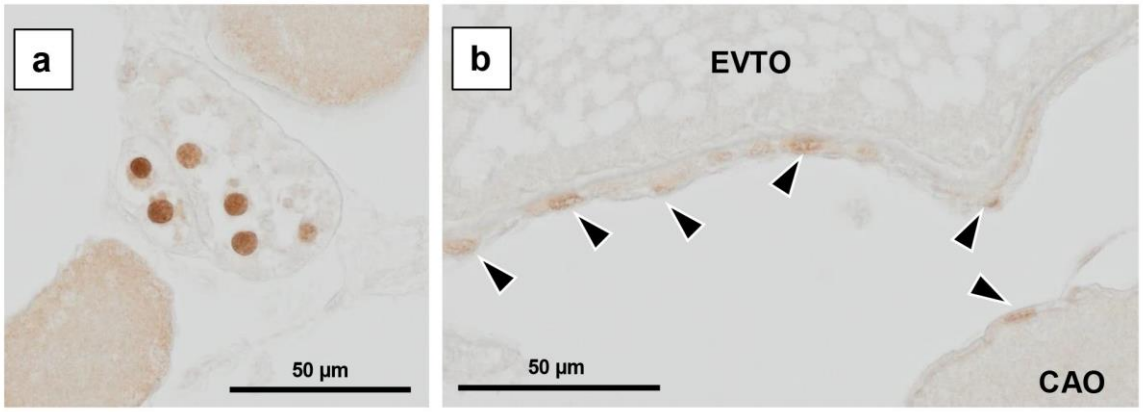

Fig. 3. Immunohistochemical staining of ovarian tissue using anti-proliferating cell nuclear antigen (PCNA) antibody. a: Strong immunopositive reaction in the nuclei of oogonia (OG). b: Immunopositive reaction in certain granulosa cells of vitellogenic oocytes.

Immunohistochemistry of the ovaries

To examine proliferating cells within the ovary, immunostaining was performed using primary antibodies against PCNA (Fig. 3). Strong immune-positivity was observed in oogonial nuclei (Fig. 3a). Additionally, positive staining was detected in a few granulosa cells surrounding the vitellogenic oocytes (Fig. 3b). However, no PCNA-positive reactions were observed in the nuclei of oocytes starting from the primary growth stage through the migratory nucleus stage.

Morphological and histological analyses of PFR1

The tip of PFR1 appeared flattened and broad like a spearhead (lanceolate), with its ventral surface showing a red colouration (Fig. 4a). The widest part of this spearhead-shaped structure measured approximately 0.75 cm in diameter, and the length of the terminal expansion was approximately 3.7 cm. Histological observation revealed a single eosinophilic lepidotrichium in the centre of the tip of PFR1 (Fig. 4b), which branched towards the base of PFR1 (Fig. 4c). A longitudinal groove was observed on the ventral surface of PFR1, extending approximately 1.05 cm along its proximodistal axis at a depth of approximately 700 µm (Fig. 4a, d). Further towards the base of PFR1, this groove closed to form a cavity (Fig. 4e). No such groove was observed in PFR3, despite its similar spearhead-shaped tip (data not shown). PFR1 contained one to several lepidotrichia in its centre, surrounded by connective tissue and enclosed by a subdermal sheath (SDS) (Fig. 4f). Multiple gaps along with cellular degradation were observed in the SDS beneath the skin (Fig. 4f). No sensory receptor cells, such as taste buds, were observed in the SDS.

Discussion

In this study, we report the first detailed morphological and histological examination of ovarian and pelvic fin structures in *G. katoi*, a deep-sea fish species. The examination revealed two significant findings regarding its reproductive biology and specialized appendage morphology.

Ovarian histological observations revealed strong PCNA immunoreactivity in the oogonial nuclei, suggesting continuous production of new oocytes. The presence of PCNA-positive granulosa cells surrounding vitellogenic oocytes supports their role in oocyte growth (Nagahama 1994). The presence of oocytes at different developmental stages suggests that *G. katoi* may have the capacity for multiple spawning events within a spawning season, as observed in several other teleost species (Brown-Peterson et al. 2011). Particularly noteworthy was the presence of MNOs despite an extremely low GSI

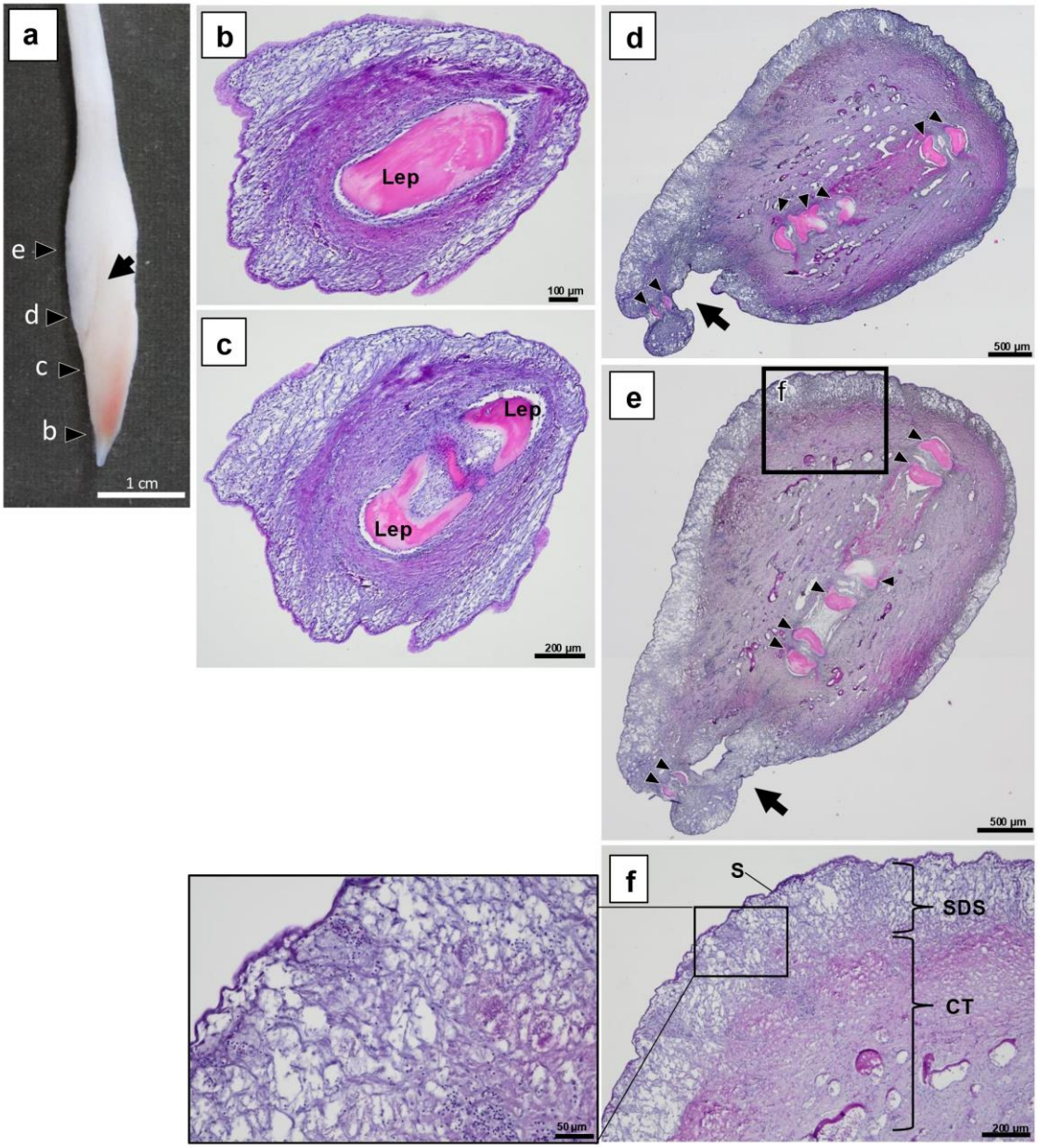

Fig. 4. Morphological and histological characteristics of the first free pelvic fin ray (PFR1) in *Guentherus katoi*. a: Ventral view showing red-colored tip with longitudinal groove (arrow). Arrowheads indicate the approximate locations of histological sections (b–e). b: Tip of PFR1 showing the single central lepidotrichium (Lep). c: Multiple lepidotrichia are visible towards the base of PFR1. d: Multiple lepidotrichia (arrowheads) and the ventral longitudinal groove (arrow) are evident. e: Closed longitudinal groove (arrow) and multiple lepidotrichia (arrowheads) are observed towards the base of PFR1. f: Higher magnification of the boxed area in e showing central lepidotrichia surrounded by connective tissue (CT), subdermal sheath (SDS) exhibiting cells with abundant intercellular spaces, and the skin (S).

value of 0.35 %. We carefully examined the ovarian tissues but found no post-ovulatory follicles, suggesting this specimen had not yet spawned. In most deep-sea demersal fishes, benthic anglerfish (*Lophiomus setigerus*) show GSI values exceeding 15 % (Yoneda et al. 2001), while deep-sea rattail (*Coryphaenoides acrolepis*) and orange roughy (*Hoplostethus atlanticus*) show GSI values of 12 and 8–10 %, respectively (Stein and Pearcy 1982; Pankhurst et al. 1987). The remarkably low GSI observed in this specimen, despite exhibiting MNOs, suggests two possibilities: (1) this individual was

preparing for its first spawning event with relatively few mature oocytes, or (2) this deep-sea fish has evolved a unique reproductive strategy involving lower energy investment in each spawning event. To our knowledge, there are no published studies on the reproductive biology of any Ateleopodid species that might allow direct comparison. Further examination of additional specimens at different reproductive stages would be necessary to distinguish between these possibilities.

The pelvic fins of teleosts show considerable morphological variations reflecting diverse environmental adaptations (Yamanoue et al. 2010). Our histological examination revealed a distinctive ventral longitudinal groove in PFR1, measuring approximately 700 μm in depth. This specialized structure was characteristic of PFR1, which became progressively flatter towards its distal end. While we could not directly compare our findings with previously collected specimens, the external morphology of the pelvic fins in our specimen matched the descriptions provided by Senou et al. (2008) and Fujiwara et al. (2019). Although sea robins (*Prionotus carolinus*) possess highly specialized sensory systems in their free fin rays (Allard et al. 2024), and other teleosts show specialized sensory structures in their fins (Kiyohara et al. 2002; King and Hale 2014), no sensory organs were observed in the examined sections of PFR1. Further research is needed to determine potential sensory functions in unexamined regions of the fin ray and to investigate its innervation patterns.

Although we examined only a single specimen, our findings provide fundamental insights into the reproductive strategy and specialized appendage morphology of this rarely encountered species. Such basic biological knowledge of deep-sea species is becoming increasingly urgent as these environments face growing anthropogenic impacts (Koslow et al. 2000; Roberts 2002).

It should be noted that literature on the reproductive biology of jellynose fishes (Ateleopodidae) is extremely scarce. Despite our extensive literature search, we could not find any previous studies describing the gonadal histology or reproductive cycles of any species within this family. The present study therefore provides the first detailed documentation of ovarian structure and oocyte development in an ateleopodid species, serving as a baseline for future comparative studies within this poorly studied deep-sea fish family.

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