

Histological Observations on the Ovaries of Two Species of Taeniosomatous Fishes (Lampridiformes) Caught in Waters Adjacent to the Japanese Main Island

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Abstract The ovaries of a large dealfish, *Trachipterus ishikawae*, 2.47 m in total length, caught off Kumano-Nada Sea, Pacific coast, and two medium-sized oarfishes, *Regalecus russelli*, 2.92 m and 2.65 m in total length, caught off Wakasa Bay, Sea of Japan, were examined histologically. The dealfish had mature ovaries, 2420 g in weight, those of the oarfishes being immature (180.2 g and 185.8 g). Microscopic examination revealed an ovarian cavity in the central axis of the tubular ovary in both the dealfish and oarfishes. In the former, in addition to ripe eggs, ovulation scars, atretic follicles, oocytes in the yolk vesicle, peripheral nucleoli and chromatin nucleolus stages were seen, whereas in the oarfishes, no ovulation scars were detected. The oocytes, in various developmental stages in both species, were attached to loose connective tissue, i. e., ovigerous lamellae, derived from a thick ovarian capsule of fibrous connective tissue, which was coated with thin mesothelium. Such ovarian architecture was classified as entovarian type, the developmental mode of the ovarian eggs being unequal.

Key words: ovarian histology, ovarian structure, taeniosomata, oarfish, ribbonfish,

Introduction

The gonadal histology of the rare and unusually-shaped-fishes belonging to Taeniosomi has been studied intermittently by the first author, taking advantage of availability from time to time, of comparatively fresh specimens of various species (Tamura and Honma, 1971, 1972, on *Trachipterus ishikawae*; Honma et al., 1973 on *T. trachipterus (=iris)*; Honma and Tsumura, 1980 on *Lophotus capellei*; Honma and Mizusawa, 1981 on *L. capellei*; Honma et al., 1999 on *Eumecichthys fiskii*, and 2002 on *Regalecus russelli*).

In many of the above cases, the gonads were unsuitable for histological study, since the specimens, although preserved in 10% formalin solution when made available, had been previously deep frozen for considerable period after capture and had therefore begun to deteriorate. Accordingly, the above listed reports had been unable to provide detailed histological pictures, although some understanding of the microscopic anatomy of the visceral organs, including gonads, had been possible.

Recently, we had an opportunity to examine three individual taeniosomatous fishes, one gravid dealfish and two immature oarfishes, all in comparatively good condition.

In order to further elucidate, therefore, the reproductive biology and life history of taeniosomatous fishes (order Lampridiformes), the present study was undertaken.

Materials and Methods

A large mature dealfish, *Trachipterus ishikawae*, 2.47 m in total length (without caudal fin), was caught at a depth of 500 m in the Kumano-Nada Sea (Pacific Ocean), off Tahara, Koza-cho, Wakayama Prefecture, during abyssal angling for bream (*Sebastes matsubarai*), on 15 January, 2002

(Fig. 1).

After capture, the live fish was transported immediately to the Kushimoto Marine Park Aquarium, Wakayama Prefecture, but died 30 minutes later. After the removal of the ripe ovaries, weight was measured and the eggs counted. The fish was then preserved in 10% formalin solution. It is likely that this individual represented the largest size recorded for dealfish.

Two oarfishes, *Regalecus russelli*, specimen A (2.92 m in total length and 15.32 kg in body weight) and specimen B (2.65 m and 11.00 kg), were caught in a set net installed off the coast of Hiruga, Wakasa Bay, Fukui Prefecture, Sea of Japan, on 11 January, 2003 (Fig. 2). The ovaries of both specimens were preserved in 10% formalin solution.

Three pieces (anteriormost tip, middle portion and posteriormost tip) were cut from each ovary, refixed in Bouin's solution and dehydrated through an alcohol series. After being embedded in paraffin, the blocks were cut at 5 μm , stained in hematoxylin-eosin (HE) double stain and Masson-Goldner (MG) associated with aldehyde fuchsin (AF) tetrachrome, and observed under a light microscope (Leitz Orthoplan).

The catch locations of both species are shown in Fig. 3.

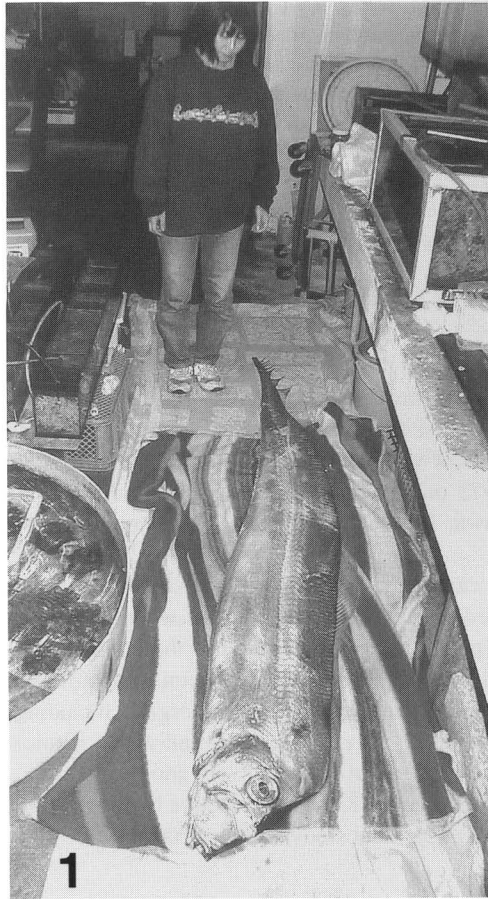


Fig. 1. A gigantic dealfish, *Trachipterus ishikawae*, caught by abyssal angling off Kumano-Nada Sea, Pacific coast, on 15 January, 2002, 2.47 m TL. (Photographed by M. Masumoto, a reporter of Kii-minpo Press)



Fig. 2. Two medium-sized oarfishes, *Regalecus russelli*, caught by a set net off Hiruga, Wakasa Bay, Sea of Japan, on 11 January, 2003, 2.92 m and 2.65 m TL.



Fig. 3. Map of Japan showing collection localities of dealfish (arrow) and oarfish (arrow head).

Results

Macroscopy

Trachipterus ishikawae

Unfortunately, size measurements of elongate ovary were not taken, although the weight of the ovary (2420 g) and diameter of ovarian eggs which had passed through the cloaca were recorded. The anterior portion, corresponding approximately half of the ovary, was bifurcated.

The eggs, separate and transparent without oil globules or droplets, seemed to be pelagic, the diameter of larger eggs ranging from 2.28 to 2.43 (mean 2.41) mm.

The number of eggs isolated from the ovigerous lamellae and discharged toward the cloaca was roughly estimated as 100,000 grains, those remaining attached to the lamellae being about 200,000. In addition, smaller eggs (oocytes) less than 1mm in diameter were prominent.

Regalecus russellii

The ovary of specimen A was slender and cylindrical, the anterior half being bifurcated. The posterior unified proper portion was 28 cm long (3 cm wide), with 2 cm wide lobes of 33 cm (left lobe) and 30 cm (right lobe) length. The weight of ovary was 180.2 g.

Morphological features of specimen B were similar to those in A, the length of the posterior portion being 33 cm, with the bifurcate lobes being 33 cm (left lobe) and 31 cm (right lobe). The width of the posterior portion was 3 cm, the left lobe being 2 cm and the right lobe 1.3 cm. The weight of ovary was 185.8 g.

Microscopy

Trachipterus ishikawae

The ovary was entirely covered by a comparatively thick ovarian capsule, being suspended by a delicate mesovarium from the dome of the body cavity and attached to the dorsal surface of a blind sac from the stomach. The capsule consisted of superficial mesothelium, collagenous connective tissue and few smooth muscle fibers. Numerous ovigerous lamellae of loose connective tissue were derived from this capsule including a number of ovarian eggs (oocytes) (Fig. 4a). Although various developmental stages of oocytes (chromatin nucleolus, peripheral nucleolus, yolk vesicle and yolk globule stages) were detected in the ovary (Fig. 4b), it proved impossible to obtain good preparations of ripe eggs owing to technical difficulties.

Accordingly, the most developed oocytes observed were the later yolk globule stage and more mature oocytes. In these, theca, granulosa, thick zona radiata and peripherally-shifted yolk globules changing into homogeneous yolk platelets or fine foamy ooplasm were recognized (Fig. 4c). It was noted that younger oocytes had massed to gather (Fig. 4d), sometimes, being disposed in a line along the ovigerous lamellae. Notably, besides atretic oocytes (Fig. 4e), ovulation scars were sporadically encountered, the scars consisting of hypertrophied follicular cells and their remnants arranged in irregular rings. Masses of follicular cells occurred in a vesicular structure (Fig. 4f).

An entovarian type was inferred for the existence of an ovarian cavity in the core of the ovary (Fig. 4a).

Regalecus russellii

In cross section, an ovarian cavity in the core of the cylindrical ovary (i. e., entovarian type) was evident in both oarfish specimens, the development of ovarian eggs being at a relatively earlier stage than in the dealfish (Fig. 5a). As shown in Fig. 5b, ramifications of ovigerous lamellae from the ovarian capsule were also prominent. The ovarian eggs (oocytes), in various developmental stages, attached to and along the ascending lamellae were covered with a delicate loose connective tissue.

The most developed oocytes had attained the yolk vesicle stage (Fig. 5c). Whereas neither oil droplets nor globules were seen in the oocytes, AF- positive yolk vesicles and globules were situated in the peripheral zone (Figs. 5d, e). Numerous interdigitated processes existed between the exterior of

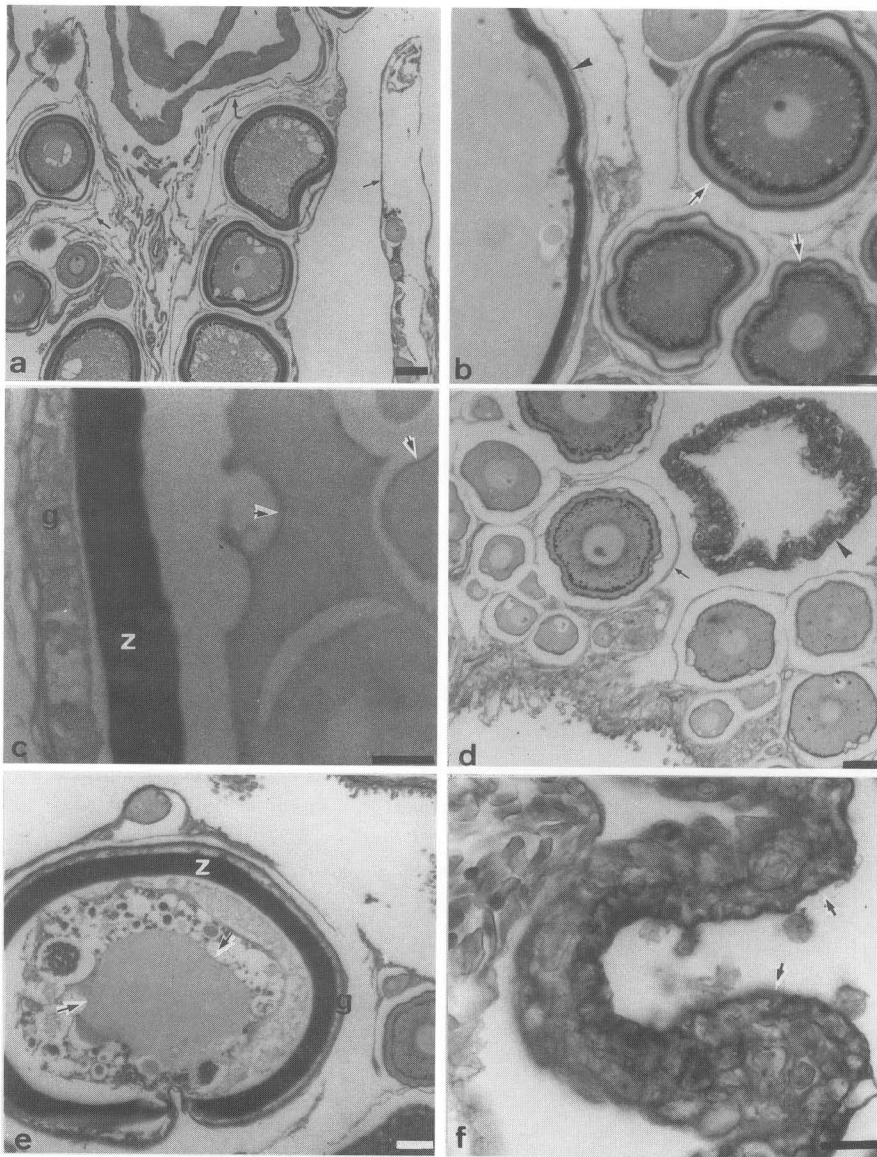


Fig. 4. a-f. Section of dealfish ovary.

- a. Ovigerous lamellae (arrows) comprising oocytes in various developmental stages. Note a part of ovarian cavity. Masson-Goldner-aldehyde fuchsin (MG-AF) stain.
- b. Maturing oocytes at yolk vesicle stage (arrows) and a ripe oocyte (arrow head). MG-AF.
- c. Part of a ripe oocyte showing yolk platelets (arrows). g, granulosa cell; z, zona radiata. Hematoxylin-eosin (HE) stain.
- d. Developing oocytes enveloped by lamellae (arrow) showing younger aggregated oocytes. Note ovulation scar (arrow head). MG-AF.
- e. Atretic egg showing heavy oolysis. arrow, yolk platelet; g, granulosa cells; z, zona radiata. HE.
- f. Enlarged view of an ovulation scar showing active state of granulosa cells (arrows). MG-AF. Scale bar: a, b, d, e=50 μ m; c, f=25 μ m.

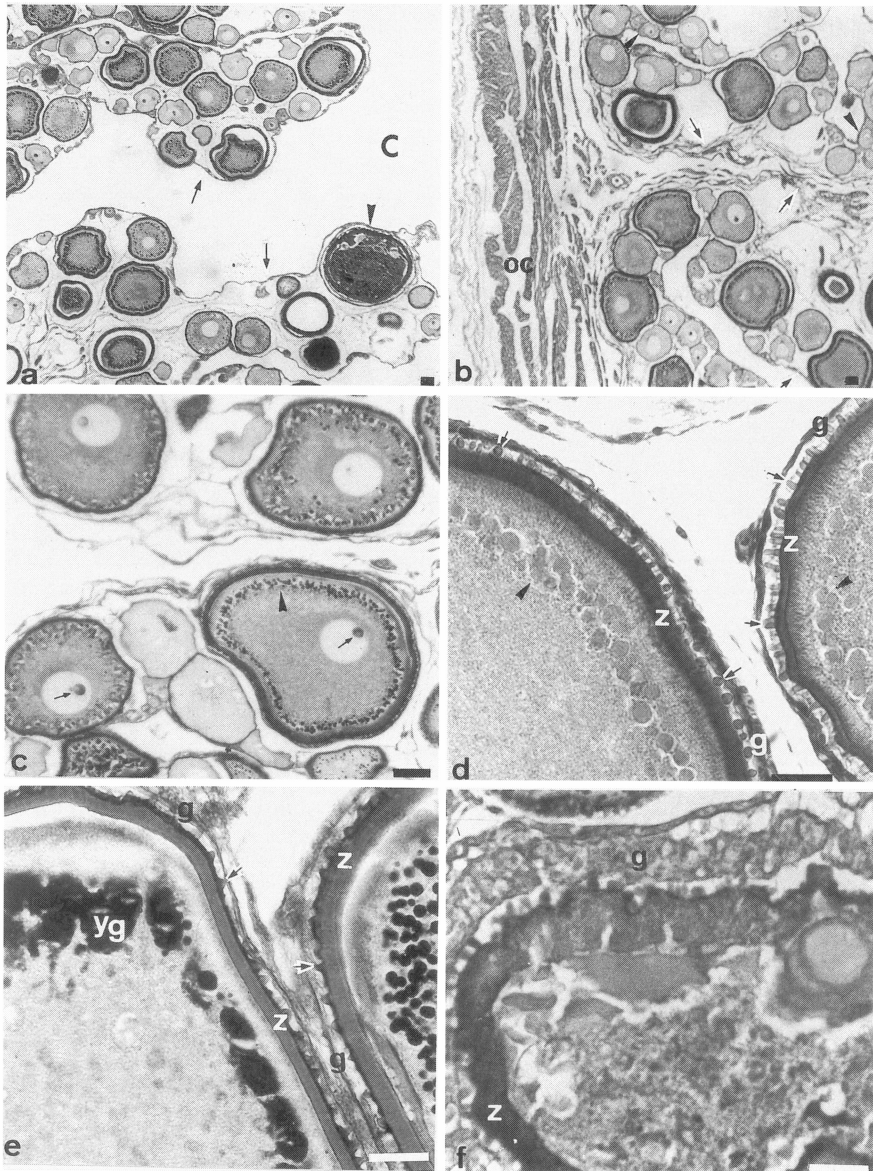


Fig. 5. a-f. Section of oarfish ovaries.

- a. Ovigerous lamellae (arrows) comprising oocytes in various developmental stages. Each lamellar coat projected into a wide ovarian cavity (c) indicative of entovarian type. Note atretic follicle (arrow head). MG-AF.
 - b. Section showing ramification of ovigerous lamellae (arrows). Younger oocytes corresponding to chromatin nucleolus stage nested together (arrow head). oc, ovarian capsule. MG-AF.
 - c. Oocytes in yolk vesicle stage. Yolk vesicles (arrow head) positive to AF situated in peripheral zone. Note prominent nucleolus in the light nucleus (arrows). MG-AF.
 - d. Enlarged view of oocytes at yolk vesicle stage. Yolk vesicles (arrow heads) situated in peripheral zone. Note digital processes (see text) (arrows) between zona radiata (z) and granulosa (g) of follicular epithelium. HE.
 - e. Enlarged view of oocytes in yolk vesicle to yolk globule (yg) stages. Digital processes (arrows) between zona radiata (z) and granulosa cell (g). MG-AF.
 - f. Enlarged view of an atretic oocyte showing oolysis. z, zona radiata; g, granulosa cells. MG-AF.
- Scale bar: a, b=150 μ m; c=50 μ m; d, e, f=25 μ m.

the incomplete zona radiata and the granulosa cells of the follicular epithelium (Figs. 13, 14). In the early peripheral nucleolus stage oocytes a yolk nucleus and nucleolus were prominent. Less developed oocytes at the chromatin nucleolus stage were nested together (Fig. 5b).

Atretic oocytes with severe oolysis and multilayered follicular epithelium were seldom seen (Figs. 5a, f). Active invasion of follicular epithelial cells into the oolytic material was found in several atretic follicles. However, no ovulation scars were encountered.

Discussion

Okiyama (1979, 1988) reported the diameter of pelagic eggs (without oil globules) of deal fish, collected during May-June, 1971 and 1975 in the Sea of Japan, as ranging from 2.83-3.14 mm, and speculated that the spawning season of dealfish ranged from December to June, citing a record of gravid females collected from January to May around Izu Oshima Island in the Pacific (Hirose et al., 1970). Although the present deal fish was caught off Kumano-Nada Sea, in January, the eggs were just prior to spawning. The reason for the diameter of the latter (2.28-2.43 mm), as initially reported by Misaki (2002), being smaller than described by Okiyama (1979) may be due to environmental conditions. Upon release from the body cavity, eggs in the process of embryogenesis while floating in the sea, would absorb sea water naturally, thereby increasing in size.

Egg diameters of three specimens of scalloped ribbon fish, *Zu cristatus* (identified following Walters and Fitch, 1960), collected by plankton net from the mid-Atlantic Ocean, ranged from 2.17 to 2.27 mm, with the notochord length in a dechorionated later embryo, being 5.13 mm (Olney and Naplin, 1980). Sardou (1966) observed a series of embryonic development of *Trachipterus taenia*, including epiboly to hatched larva, and emphasized that the size of eggs with rich yolk, 3.0-3.5 mm, are the largest amongst the marine teleosts.

General histology notes already published on female taeniosomatous fishes, including 3 deal fishes (*T. ishikawae*, including the present specimen), one crestfish (*Lophotus capellei*), one unicornfish (*Eumecichthys fiskii*) and one oarfish (*R. russelli*), were all made on specimens in spent condition, whereas the present two oarfishes were immature (Tamura and Honma, 1971, 1972; Honma and Tsumura, 1980; Honma et al., 1999, 2000, 2002). In addition to the above female specimens, a young male crestfish has also been examined (Honma and Mizusawa, 1981).

Notes on the dates of collection, body sizes and gonad condition of the above specimens are given in Table 1. Among the 10 examples, comprising 8 females, one male and one sex unknown, only 3 immature examples (2 females and one male) are included. Although the collection dates of spent fishes occurred from December to June/July, the likely breeding season based on histological criteria was coincident with this extended period. The present conjecture on the breeding season of dealfish is similar to an opinion expressed by Okiyama (1979).

The possibility of two or more spawning times undergone by a single female during an extended breeding season was indicated by the present and previous histological criteria, based on the existence of various stages of ovarian eggs. In addition, the entovarian type of ovary and unequal developmental mode of ovarian eggs have already been reported by Tamura and Honma (1971, 1972), Honma and Tsumura (1980) and Honma et al. (1999, 2002). Kobayashi et al. (1996) also discussed these ovarian structures in a preliminary oral paper.

Previously, we considered the possibility that dealfish die after spawning (Tamura and Honma, 1971, 1972). However, the very large size of the present gravid specimen, nearly twice that of previously-reported specimens, suggests that dealfish are able to spawn on multiple occasions. Since no larvae of dealfish and/or other taeniosomatous species have been recorded from the Sea of Japan, it is unknown whether or not the dealfish or allied species recorded represent established populations.

Table 1. Data of taeniosomatous fishes examined histologically by the senior author (see text).

Name	Date of collection	Total length (m)	Sex	Gonadal condition
dealfish (<i>T. ishikawae</i>)	15 June, 1969	1.50	♀	spent
dealfish	24 May, 1970	1.12	♀	spent
king-of-the-salmon (<i>T. trachipterus</i>)	7 July, 1970	0.41	unknown	
crestfish (<i>L. capellei</i>)	26 Dec., 1978	1.80	♀	spent
crestfish	3 Jan., 1980	1.15	♂	immature
unicornfish (<i>E. fiskii</i>)	16 Dec., 1996	1.05	♀	spent
oarfish (<i>R. russelli</i>)	10 Jan., 2001	3.49	♀	spent
dealfish	15 Jan., 2002	2.47	♀	spawning
oarfish	11 Jan., 2003	2.92	♀	immature
oarfish	11 Jan., 2003	2.65	♀	immature

References

- Hirose, I., Tsubokawa, S. and Kubota, Y. 1970. Marine products of Izu Islands. II. A curious fish, *Trachipterus ishikawae* Jordan et Snyder. Collecting and Breeding., 32, 50-51 (in Japanese).
- Honma, Y. and Mizusawa, R. 1981. A crestfish (Lophotidae, Lampridiformes) collected on the coast of Itoigawa City, facing the Sea of Japan, with special regard to its ink production and ejection. Bulletin of Niigata Prefectural Biological Society for of Education, 16, 21-26 (in Japanese).
- Honma, Y. and Tsumura, K. 1980. Notes on the crestfish (Lophotidae, Lampridiformes) caught off Sado Island in the Sea of Japan. Report of Sado Marine Biological Station, Niigata University, 10, 11-16.
- Honma, Y., Yasuda, T. and Kon, T. 1973. A record the young of King- of- the- Herring, *Trachipterus iris*, from Tsuruga Bay, the Japan Sea. Report of Sado Marine Biological Station, Niigata University, 3, 1-10.
- Honma, Y., Ushiki, T. and Takeda, M. 1999. Histology of the ink tube and its associated organs in a unicornfish, *Eumecichthys fiskii* (Lampridiformes). Ichthyological Research, 46, 19-25.
- Honma, Y., Ushiki, T. and Takeda, M. 2000. Histological studies on the internal organs of the inkfishes and ribbonfishes stranded on the coast of Niigata, Sea of Japan. International Symposium on Diversity of Fishes-Tenth Anniversary His Majesty the Emperor's Enthronement. (abstract) P-15.
- Honma, Y., Ushiki, T., Takeda, M. and Ishibashi, T. 2002. Comparative anatomy and histology of the visceral organs of an oarfish, *Regalecus russelli*, and a razorback scabbardfish, *Assurger anzac*, caught off the coast of Fukui Prefecture, Sea of Japan. Bulletin of Kashiwazaki City Museum, 16, 71-86.
- Kobayashi, K., Suzuki, K. and Shiobara, Y. 1996. Histological observations on the gonads of taeniosomous fishes, especially oarfish. 41st Meeting of Aquarists of Japanese Association of Zoological Gardens and Aquariums, (abstract) 22, (in Japanese).
- Misaki, H. 2002. A gravid dealfish from the Kumano-Nada Sea, the Pacific. Marine Pavillion, 31(5), 3 (in Japanese).
- Okiyama, M. 1978. Successful spawning of some holoepipelagic fishes in the Sea of Japan and zoogeographical implications. Proceeding of the 7th Japan-Soviet Joint Symposium on Aquaculture, 223-233.
- Okiyama, M. (ed.) 1988. An Atlas of the Early Stage Fishes in Japan. Tokai University Press, Tokyo 1154pp. (in Japanese).
- Olney, J. E. and Naplin, A. 1980. Eggs of the scalloped ribbonfish, *Zu cristus*, (Pisces: Trachipteridae) in the western North Atlantic. Copeia, 1980, 1, 165-166.
- Sardou, J. 1966. Oeuf et developpement embryonnaire de *Trachipterus taenia* Bloch (Ordre des Lampridiformes Allotriognathes famille des Trachipteridae). Vie et Milieu, Series A, 17, 99-215, 4pls.
- Walters, V. and Fitch, J. E. 1960. The families and genera of the lampridiform (Allotriognath) suborder Trachipteroidei. California Fish and Game, 46, 441-451.

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