# Debunking an urban legend of the Deep Sea: The Queen of the Abyss and her contribution to Ceratioid Anglerfish biology.

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We inhabit a watery planet. The sea covers more than 70 % of the earth's surface. The average depth of the world's ocean is 3,800 m. So, if Mt. Fuji, with an elevation of 3,776 m, were placed on the bottom of the ocean, its summit would not break the sea surface at the ocean's average depth. The depth of 4,000 m is the divide between the bathypelagic and the abyssopelagic zone, so the major part of the world's ocean consists of the abyss, or deep sea, one of mankind's few remaining frontiers. This vast expanse of the earth's aquatic environment is filled with fish. About 29,000 species of fish are currently recognized, and they come in all sorts of shapes, sizes and colors (Nelson, 2006). Deep-sea fishes are no exception. Ask any boy or girl,'Do you know any deep sea fishes?' The chances are nine to one that they will answer 'Anglerfish!! They have lanterns that light up dangling right in front of their mouths! They use that lantern to attract and eat little fish!! Shrimp too!' Anglerfishes, Ceratioid Anglerfishes to be more precise, are by far THE most popular deep-sea organisms inhabiting the vast abyss of the world's oceans. Not only do Ceratioid Anglerfishes play an important role as high- ranking predators in the deep sea ecosystem, but also, their unique mode of reproduction, males adhering to the body surface of the female, is the sole example of such a mode of reproduction found among the vertebrates. This article is an account of my once-in-a-lifetime encounter with the Queen of the Abyss, which literally jump-started my ichthyological research on Ceratioid Anglerfish biology. It is by pure coincidence, a stroke of luck, that I now find myself looking into another human frontier, the abyss, through a unique looking glass, Ceratioid Anglerfishes. I consider myself a very lucky individual to be blessed with such an opportunity, and that is a gross understatement.

## *A short primer on Ceratioid Anglerfish biology.*

Among the 29,000 species of fish recognized to date, no group of Teleost stands out in its uniqueness, in both morphology and ecology, as the Ceratioid Anglerfishes. Currently 11 families, 35 genera and 162 species are recognized, making it one of the most specious groups of Teleosts in the deep sea. (Pietsch 2005. Pietsch and Orr, 2007). Attracting its prey with its bioluminescent light organ called the

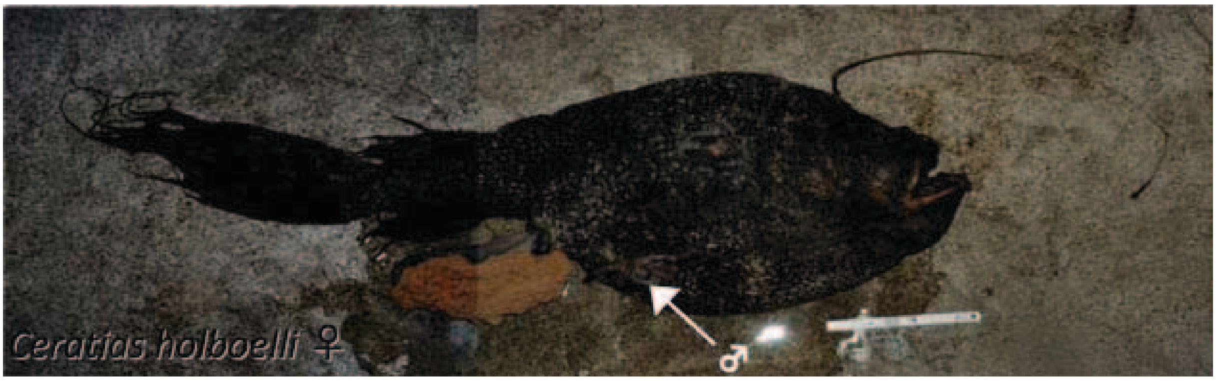


**Fig. 1**: Female *Ceratias holboelli*, Ceratiidae. HUMZ95300. Arrow indicates poiasitic male.

hanging from the tip of the illicium, a modified dorsal fin ray, Ceratioid Anglerfishes are the top ranking carnivore of the deep sea (Shimazaki and Nakaya, 2004) (Fig. 1). There even exists an interesting video taken from an underwater submersible showing a Gigantactinid Anglerfish fishing the ocean floor with its esca, swimming inverted (Moore, 2002). Not only is the esca important as an apparctus for predation, it is also the most important morphological diagnostic character of Ceratioid Anglerfishes.

Another remarkably unique feature of Ceratioid Anglerfish is its mode of reproduction. Males attach themselves to the female's body surface and become parasitic male, forming an extreme example of symbiosis in the abyss. (Fig. 2). 5 families, 10 genera and 23 species of Ceratioid Anglerfishes are known to possess this unique mode of reproduction (Pietsch, 2005). Pelagic males, when they find a female, bite onto her skin and make a permanent attachment to the female body. This unique and elaborate mode of reproduction was first reported by an Icelandic ichthyologist, Bjarni Saemundsson in 1922 (Saemundsson, 1922). Later, Regan and Trewavas (1932) made a first comprehensive review of Ceratioid Anglerfish biology, followed by Bertelsen (1951), and Pietsch (1976, 2005). In species whose males attach to the female, this attachment seems to be obligatory for the individual male's subsequent survival and successful reproduction**.** Males which have failed to find and attach to a female perish. For example, the largest known pelagic male *Cryptopsaras couseii* is 14.3 mm TL, smaller than most parasitic males reported (Bertelsen, 1951). A rather bizarre and extreme example of parasitic male is reported in Pietsch and Nafpaktitis (1971). A male *Melanocetus johnsoni* was found attached to a female *Centrophryne spimulosa*. The two species belong not only to different genera, but they are of different families, Melanocetidae and Centrophrynidae, respectively. Although parasitic male found in Ceratioid Anglerfish is well known, fundamental aspects of the unique mode of reproduction, such as how males find females in the abyss, how nutrients are passed over to the male from the female, how the immune system is suppressed so male tissue is not rejected by the female, are not known, and currently does not reach beyond


**Fig. 2**: Male attached to female *C. holboelli* of Fig.1. HUMZ95300.

the educated guess of researchers. The following is my small contribution to Ceratioid Anglerfish biology, conducted with the help of numerous friends and colleagues, as well as from the Queen of the Abyss and her eight little dwarfs.

### *The Queen of the Abyss enters the stage!*

My encounter with The Queen occurred unexpectedly. It was during research cruise KH00-2 of RV Hakuhou-maru. One of the graduate students carrying a large bucket containing the samples taken with 4m-frame trawl net exclaimed, 'There's something big in here!' Big and beautiful indeed. It was a truly beautiful specimen of *Cryptopsaras couseii*, 316.5 mm in standard length (SL)(Figs. 3,4) It was at that moment, there and then, that The Queen of the Abyss departed her abyssal palace and entered a new realm, that of mankind and more specifically, of ichthyology.


**Fig. 3**: The Queen of the Abyss. A female *Cryptopsaras couseii*, Ceratiidae, 316 mm SL. Right side of the body. Number indicate the number of male described in text.

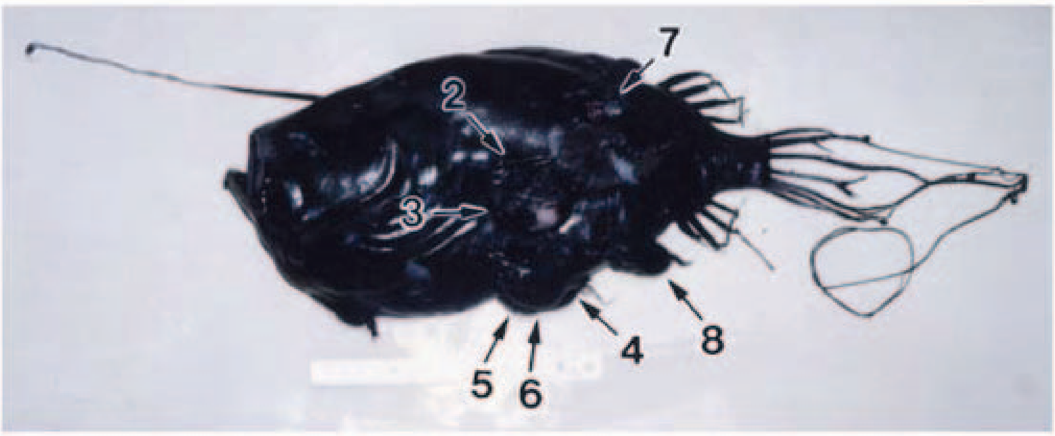


**Fig. 4**: The Queen of the Abyss. A female *C. couseii* 316 mm SL. Left side of the body. Number indicate the number of male described in text.

### *The Queen of the Abyss in all her charm.*

Gleaming in black, 'The Queen' was the most beautiful and intact specimen of an anglerfish one would ever see, in an almost pristine condition, A rarity in deed considering the fact that most Ceratioid Anglerfish are caught with torn and ruptured skin. Her spinulous skin was intact, not ruptured and torn anywhere. A closer examination of this black beauty revealed even more surprising findings.

When touched, the three knob like appendages located at the anterior base of the dorsal fin secreted light bluish fluid. At first, these knob-like appendages and fluid were thought to be parasitic males and sperm, but were later confirmed to be carancules and bioluminescent fluid. But the largest surprise(s?) were to follow. A total of EIGHT! males were attached to her body. Yes, not one, two or three, but eight. One male on the right side of her body, and seven more males on her left side! Until then, the maximum number of males attached to a female Ceratioid Anglerfish was six, so she was indeed The Queen of the Abyss, having eight little dwarfs attached to her body. One for the Guinness Book of World Records!

### *The eight little dwarfs.*

A brief description of each parasitic male is provided below. Size of attached males ranged from 35.0 to 56.0 mm in total length (TL). It should be noted that eye, dorsal, anal, pectoral and caudal fin rays, and mouth opening were still present, and also, the degree of development of the skin flap varied between males. The skin flap is part of the skin around the snout of males, which expand after attaching to a female.

Male No. 1: 46.5 mm TL. Sole male attached to the right side of the female, in the branchial region. (Fig. 5) Skin flap, where the male adheres to the female body was round in shape.

Male No. 2: 35.0 mm TL. One of two males attached to the base of the left pectoral fin. This individual is located more dorsally, above male No. 3 (Fig. 6). This individual is attached with an upright body orientation. Both upper and lower skin flaps are well developed, fused to each other as well as to that of male No. 3. But an opening still exists between the two skin flaps. One notable feature of how this male attaches to the female is that the skin flap extending from the lower jaw is located above the upper jaw skin flap of male No. 3.

Male No. 3: 56.0 mm TL. Attached to the left side of the female, below male No. 2 (Fig. 6). This male is the largest of the eight attached males. This male is also attached with an upright body orientation. Upper-jaw skin flap is partially covered by lower-jaw skin flap of male No. 2.


**Fig. 5**: Male No.1. A male *C. couseii* attached to the right side of the female. 46.5 mmTL.

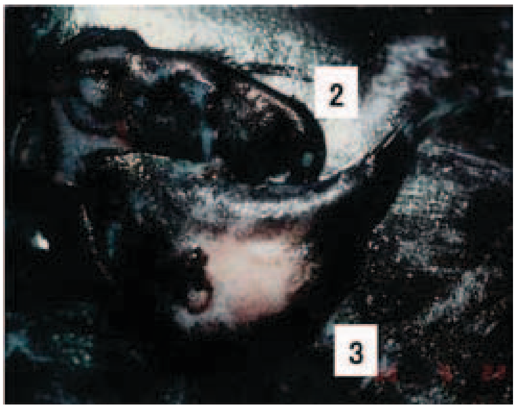


**Fig. 6**: Males No. 2 and 3, attached to the left side of the female. 35.0, 56.0 mm TL respectively.

lower-jaw skin flap of male No. 2.

Male No. 4: Attached to the female below the left pectoral fin, and below male No.3 (Fig.7). In Fig. 9, caudal fin of male No. 4 reaches the cephalic region of male No. 8. Upper and lower jaw skin flaps are not fused to each other, and an opening exist between the two skin flaps.

Male No. 5: 47.0 mm TL. Attached below male No.4, on the left abdominal region of the female (Fig. 7). Upper jaw skin flap is well developed. Mouth opening is still present. This male is attached with an upright body orientation. After initial observation, this male was surgically removed from the female and was fixed in 10 % buffered formalin. It swam in the formalin, vigorously moving its whole body for a few seconds.

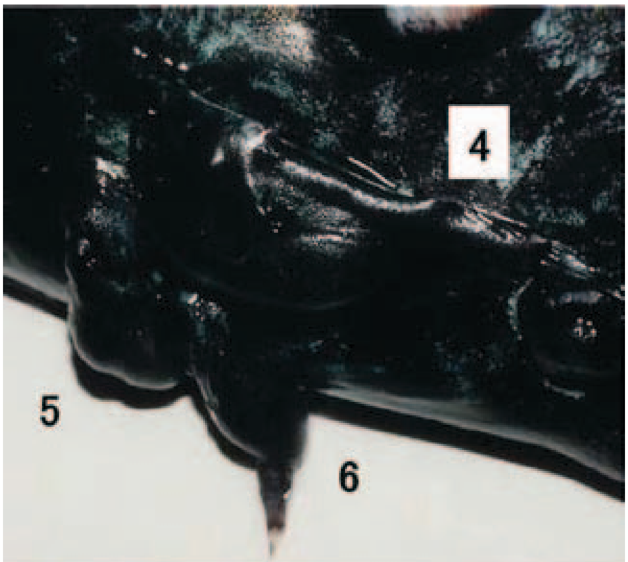


**Fig. 7**: Males No. 4, 5, and 6 attached to the left side of the female. Male 5 and 6 are 47.0, 43.3 mm TL respectively.

Male No. 6: 43.3 mm TL. Attached to the female below male No. 4 and behind male No. 5 (Fig.7). This male is attached to the female with an upright body orientation.

Male No. 7: 37.4 mm TL. Attached to the left dorsal region of the female, below the carancules, anterior to the dorsal fin origin (Fig. 8). Upper and lower jaw skin flaps are well developed, fused to each other. However, an opening still exists between the upper and lower skin flaps.


**Fig. 8**: Male No. 7, attached to the left side of the female. 37.4 mm TL.

Male No. 8: 44.0 mm TL. Attached to the left abdominal region of the female, posterior to males Nos. 4, 5, 6 and below male No. 7 (Fig. 9). Caudal fin of male No. 4 reaches the cephalic region of male No.8. Skin flaps of both upper and lower jaws are well developed, the two fused to each other anteriory. However, an opening still exists between the two skin flaps. This male is attached to the female with an upright body orientation.


**Fig. 9**: Male No. 8, attached ot the left side of the female. 44.0 mm TL.

After making initial observations and photographing the specimen, I put my head to work in full gear, deciding what to do to make the best possible use of this specimen(s?). Fortunately, I had with me on board RV Hakuhou-maru a JEOL rapid freezing apparatus and two thermos containers full of liquid nitrogen. Vials, ethanol and formalin were abundant. So, decided to make some samples for histological study using the rapid-freezing apparatus for freeze-substitution fixation, as well as to preserve tissue samples from the males and female for molecular study. Sibling analysis and electron microscope observations were later made utilizing these samples.

***Sibling analysis between female and parasitic males.***

Muscle tissues were taken from the dorsal region of female and male Nos. 2, 3, 5, 6 and 8 and were preserved in absolute ethanol. Sibling analysis was conducted by RAPD-PCR fingerprinting using Operon 10-mer kit A set. Clear bands were obtained from primer nos. 3, 4, 5, 7, 9, 11, 13, 15 and 17 (Fig. 10). The band patterns obtained denied sibling relationships between female and attached males as well as between males (Saruwatari et al., 2001). The queen and the dwarfs did not share a common bloodline.

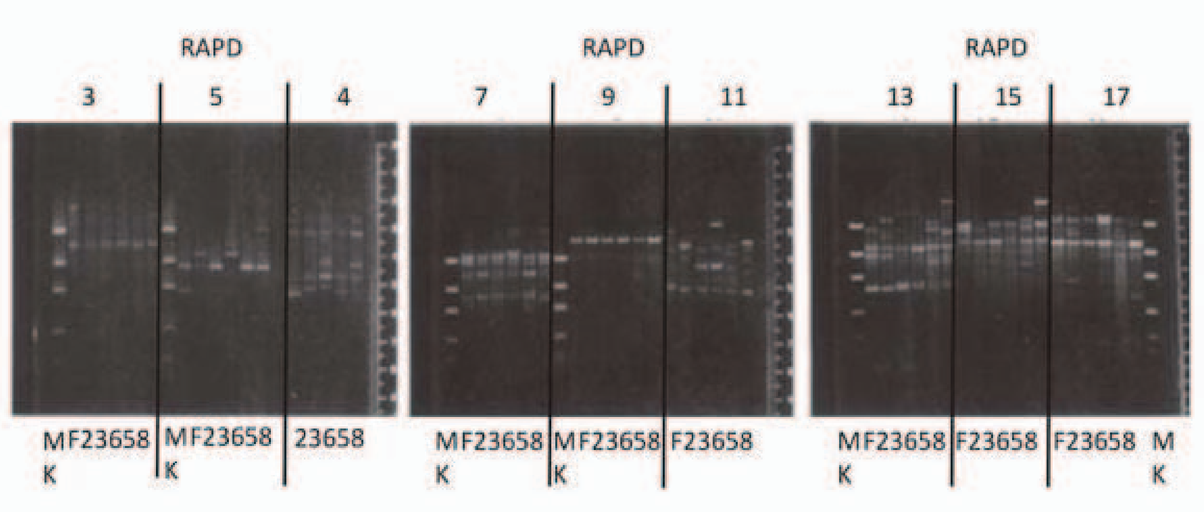


**Fig. 10**: Results of RAPD-PCR fingerprint method. Numbers above each image depict primer No. of Operon 10-mer kit A. Letters and numbers below are, MK: marker. F: female. Other numbers represent male numbers described in text. Modified from Saruwatari et al., 2001.

After hatching, males of *C. couseii* have a pelagic larval stage in the sea prior to attaching to a female. Therefore, individuals undergo a great deal of oceanic dispersal. causing the mixing of the gene pool in the sea, resulting in the lack of sibling relationships between the female and the attached males as well as between the attached males. Denial of sibling relationships reinforces another question on parasitic males. If male and female do not possess sibling relationships, then why are males not rejected from the female body surface? It there some type of mechanism to suppress the female and male immune system? Answers to these questions will have to wait, until an immunologist shows interest in Ceratioid Anglerfishes and goes out to sea to collect new and fresh material, another Ceratioid Anglerfish female with male(s) attached to her body. An immunologial study on male attachment has great medical and pharmaceutical potential. It may be possible to apply the results of such studies to human organ transplant research, to aid in development of immune suppression agents.

### *Ultra microstructure of sperm.*

After initial examination of the queen and her eight little dwarfs, testes and seminal fluid were sampled from males Nos. 5 and 6. Samples were frozen rapidly using JEOL rapid freezing apparatus and were preserved in liquid nitrogen. Samples were then transferred into freeze substitution fluid consisting of 2% osmium in acetone, and then into acetone frozen with dry-ice for 3 days at -79□ , -20□ for 3 days and preserved at 4□ . After freeze substitution, samples were embedded in Epon resin. Ultra-thin sections were prepared using a Nanotome diamond knife attached to a LKB-V Ultra microtome and observed with a Philips CM-10TEM. This is the first TEM observation made on the sperm of a Ceratioid anglerfish to date. The spermatozoon possessed a large spherical head, numerous mitochondria and a single flagellum (Fig.11). Transverse section of the tail revealed a 9 + 2 flagellar pattern.

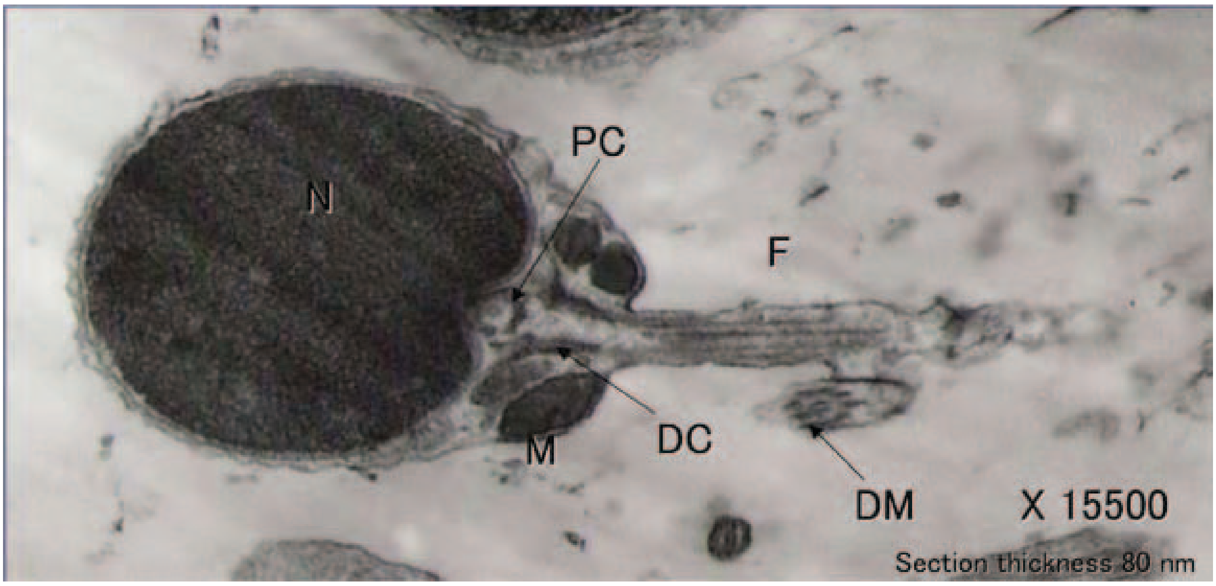


**Fig. 11**: Electron micrograph of *C. couseii* spermatozoon. F: flagellum; M: mitochondria; N:nucleus; PC: proximal centriole. Modified from Saruwatari and Sakai, 2006.

### *Debunking an urban legend of the abyss.*

There exist an urban legend of the abyss. It has been believed for some time now, that after attaching to a female, male Ceratioid Anglerfish lose their fins, are absorbed by the female and turn into a simple knob like structure. And, that the male testes is the only remaining organ which mature and become functional! Apparently, this is not the case, as the queen and her little dwarfs have shown. The sizes of attached males ranged from 35 to 56.0 mm TL. Differences in the sizes of parasitic males are indicative of different and multiple incidences of attachment to the female at different times. As mentioned earlier, the largest known pelagic male *C. couseii* is 14.3 mm TL (Bertelsen, 1951). Males that fail to find a female and attach to her body perish. Male No.3 at 56.0 mm TL still possesses fin rays on all the fins, as did all other males. Degree of somatic development of the attached males is more advanced than pelagic male larvae prior to attachment. When removed from the female and placed in a vial filled with formalin, male No.5 swam in the preservation fluid. This shows that males are able to move their muscles to some extent, even after attaching to a female. These observations show that after attaching to a female, somatic growth and development of parasitic males continue, utilizing nutrients received from the female for growth instead of consuming internal nutritional reserves or through self-degeneration and absorption of their own tissue. How nutrients are transferred from the female to the attached male is yet to be revealed.

Debunking a long standing urban legend of the deep, that male Ceratioid Anglerfish degenerate and turn into a knob-like appendage after attaching to a female, is perhaps the greatest contribution of the Queen of the Abyss and her eight little dwarfs to ichthyology so far.

My studies on Ceratioid Anglerfish biology are still ongoing, with aid from the Queen as well as from museum specimens. Efforts are being made to obtain fresh new material from the sea by going out on research cruises…a vast frontier of research in Ceratioid anglerfish biology still remains to be explored!

***Acknowledgements.***

I am greatly indebted to Senzo Uchida, Keiichi Sato and Minoru Toda, as well as the curators and staff of the National Okinawa Churaumi Aquarium for providing me with this opportunity to present a talk at their International Symposium as well as providing me with the opportunity to present this manuscript. Sincere thanks goes to researchers and crew on board cruise KH-00-2 of RC Hakuhou-maru, especially Teruhisa Komatsu, Kenichi Ishida, Tadashi Inagaki and Chiaki Igarashi. Thanks are also extended to Theodore W. Pietsch, Andrew Shedlock, Yoshiro Watanabe, Takanori Kobayashi and Toshio Sakai for their help in the experiments and advice.

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