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Structure and mechanism of open-close brood pouch in male pipefish, *Trachyramphus bicoarctatus* at Lake Qarun, Egypt

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Abstract
The present study deals with the structure of brood pouch and its mechanism of open-close in male pipefish, *Trachyramphus bicoarctatus*. 241 fish samples were collected by seine net from Lake Qarun in El-Fayoum Governorate, Egypt. The wall of brood pouch consists of two lateral folds and is made up of three layers; perimetrium, myometrium and endometrium. The perimetrium is composed of simple squamous epithelium. This layer is increased in thickness towards the brood pouch opening for protection of embryo during closing and opening of the brood pouch. The myometrium is made up of two layers; outer thick layer of fibrous connective tissue is relaxed when brood pouch is opened**.** The inner thin layer of areolar connective tissue is gradually increased in thickness towards the dorsal side of brood pouch to make cushion to protect embryos and richly supplied with blood vessels for enrichment of embryos. The endometrium is made up of thin layer of simple squamous epithelium with large numerous mucous cells. This layer is protruded to form stopper like anchor between left and right lateral folds for close the opening of brood pouch. After finishing of young born, the ends of two folds are curved to the inside gradually until fused for closing the brood pouch.

Keywords: Brood pouch, Pipefish, *Trachyramphus bicoarctatus*, Lake Qarun

1. Introduction
Members of family: Syngnathidae typically have long tubular snouts and elongate bodies encased in rings of bony plates. It lives in coastal waters to depth of about 90 m and inhabited littoral pools, lagoons, estuaries and fresh water. There are approximately 200 species of pipefish [1]. Most pipefish are marine dwellers, usually 35–40 cm in length and generally inhabit sheltered areas in coral reefs; sea grass beds and sandy lagoons. Pipefish are zooplankton feeder and attract economic interest for their value as a live aquarium fish, curio trade, as ingredients in traditional Chinese medicine, as supplements in some specialized cuisine and as curios [2, 5].

Knowledge of the gonadal cycles and their functional mechanisms in pipefish is of the essential value for the successful management of either natural fisheries or fish farming. Syngnathid fish exhibit one of the most specialized forms of parental care, with females depositing eggs in a specialized incubating area, located either on the abdomen (Gastrophori) in the females or tail (Urophori) in the males. Even though male pregnancy is a wide spread characteristic feature in all syngnathids [6, 7].

The parental care in pipefish, *Trachyramphus bicoarctatus* is unusual. Male possesses a ventral brood pouch embedded in the skin in which the eggs are deposited. The brood pouch is located under the genital opening towards the caudal fin. The ovulated eggs in females are transported to male brood pouch after fertilization. Fertilized eggs were attached in two or three longitudinal strips inside the brood pouch. These eggs may have different developmental stages in the same brood pouch [8].

The structure of brood pouch in male syngnathid fish were described in *Syngnathus typhle* [9]; *Hippocampus erectus* [10]; *Syngnathus abaster* [11]; *Nerophis ophidion* [12]; *Sygnathus typhle* [13]; *Microphis brachyurus lineatus* [14]; *Nerophis lumbriciformis* [15] and *Syngnathus acus* [16]. However, the information on the structure of brood pouch in male pipefish, *T. bicoarctatus* are lacking. Therefore, the principal objective of the present work is to provide description on the structure and mechanism of open-close brood pouch in male pipefish, *T. bicoarctatus*.

2. Materials and methods

2.1. Specimens collection:

241 mature male Pipefish samples were monthly collected from South coast of Lake Qarun, Egypt, during the period from December 2013 to November 2014. Fish samples were collected by using a seine net of 5 meters x 1.5 meters with 5 mm mesh size. The net was dragged 5 times over an area of about 5x10 = 50 m^2 from the beach with an average depth of about 50 cm. Collected fish within the net were preserved in 10% formalin solution and transported to laboratory of Marine Biology, Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt for latter examination. In the laboratory, fish were identified according to Randall [3]. Fish were morphologically sexed by the absent of anal fin in males, and the presence of brood pouch in mature males in spawning season [8]. Mature males with brood pouch were separated and then used in the following studies.

2.2. The brood pouch histology

To study morphological and histological structure of brood pouch and the mechanism of opening and closing it in male pipe fish, the brood pouch area of the fish was cut into small pieces (5 mm thick). It was immediately preserved in Bouin`s fluid for at least 48 hours, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraplast wax (M.P.: 58°C). Transverse sections were cut by microtome at the thickness of 4-6 microns. Sections were stained with Harris`s haematoxylin and eosin solutions. Some sections were stained with Masson's trichrome for detection of connective tissue [17]. Finally, the slides were microscopically examined, then photographed by using camera mounted on light microscope and described.

3. Results

3.1. The structure of brood pouch in male *T. bicoarctatus*

3.1.1. Morphological structure of brood pouch

Male pipe fish, *T. bicoarctatus* possesses a ventral brood pouch, in which the eggs are deposited, is embedded in the skin. The brood pouch is located under the genital opening towards the caudal fin. It consists of two lateral folds which originated from the ventro-lateral sides of the body. The brood pouch opens longitudinally, when some embryo completed their development for born (Figure, 1). Inside the brood pouch, fertilized egg/embryo were arranged in two (mostly) or three (rarely) longitudinal strips. These strips are separated each other by longitudinal thin membrane which extended from the dorsal surface of the brood pouch to the internal edge of lateral folds (Fig. 2). The longitudinal thin membrane attached with edge of lateral folds and dorsal side of brood pouch by one or two short pointed protuberances in endothelial tissue (Fig. 3).

3.1.2. Histological structure of brood pouch:

The wall of brood pouch in male *T. bicoarctatus* is made up of three layers; Perimetrium, Myometrium and Endometrium (Fig. 4).

Perimetrium (Serosa): It is the first layer of the brood pouch wall. It composes of simple squamous epithelium in the outer layer, which covers and protects the outer surface (protective or covering epithelia), such as the epidermis of the skin. Towards the brood pouch opening, this serosa transformed to stratified squamous epithelium. Then, it gradually increases in thickness and reaches its maximum size at the edges of the brood pouch opening (Fig. 5).

Myometrium: This layer consists of connective tissue, and it has a large amount of intercellular substances. The myometrium is made up of two layers. The outer layer is fibrous connective tissue and the inner layer is areolar connective tissue. The outer layer of fibrous connective tissue is thick at the edges of the lateral folds and richly supplied with fibrocytes, small patches of muscle fibers and blood vessels. It gradually decreases in thickness towards the lateral sides of the brood pouch (Fig. 5 & 6). The upper part of this layer consists of bony plats which extended from the body covering with small and large vacuoles (Fig. 7). The inner layer of areolar connective tissue is thin at the edges of the lateral folds; and it gradually increases in thickness towards the lateral sides of the brood pouch (Fig. 5).

Endometrium: This third layer represents the inner layer of the brood pouch (maternal tissue). It is made up of thin layer of simple squamous epithelium with numerous large mucous cells. The later cells secrete a mucous material which surrounds the fertilized eggs⁄embryos. Blood vessels are found in the connective tissue beneath the epithelium. Blood capillaries are few in number and small in diameter before and after gestation, but they are bigger and more numerous during gestation (Fig. 8). This layer is protruded to form stopper like anchor between left and right lateral folds for close the opening of brood pouch (Fig. 9).

3.2. Mechanism of open-close brood pouch in male *T. bicoarctatus:*

The lateral folds of brood pouch originated from the ventro–lateral sides of the body and directed towards the ventral side. At this time, the brood pouch remains open. At coupling between male and female, the later make irregular movement (like squeezing), the ovulated eggs are transported to male brood pouch after fertilization. The articulation of the originated lateral folds takes place by circulating the articular head of bony base inside the articular cavity attached to the bony plates of the lateral folds. The upper part of myometrium consists of bony plats which extended from the body covering with large and small vacuoles. Accordingly, brood pouch gained flexibility of lateral folds by vacuoles to move towards the ventral side (Fig. 7).

The perimetrium is increased in thickness towards the brood pouch opening for protecting the embryos through closing and opening the pouch. The outer thick layer of myometrium (fibrous connective tissue) is relaxed when the brood pouch is opened (Fig. 4). The inner thin layer of myometrium (areolar connective tissue) gradually increases in thickness towards the dorsal side of the brood pouch to make cushion for protection of the embryos; and richly supplied with blood vessels for enrichment of embryos (Fig. 8). In pregnancy period, the inner layer of brood pouch (maternal tissue) is protruded to form stopper like anchor between left and right lateral folds for close the opening of brood pouch (Fig. 9).

When the embryos complete their development, the male makes irregular movement (squeezing) for birth of young. The margins of lateral folds are out opened for the young's release (Fig. 10). After finishing of young born, the ends of two folds are curved to the inside gradually until fused for closing the brood pouch by highly articulation and contraction of fibrous connective tissue in the myometrium (Fig. 11).

Fig 1: Photomicrograph of T.S. in tail region of *T. bicoarctatus*, showing the brood pouch (BP), right lateral fold (RLF), left lateral fold (LLF) and brood pouch opening (BPo), (Hx-E x 40).

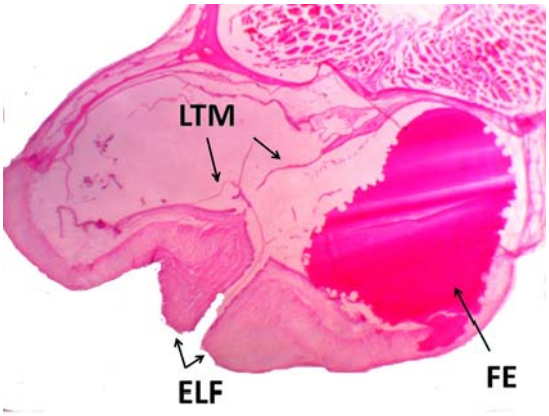

Fig 2: Photomicrograph of T.S. in tail region of *T. bicoarctatus*, showing the brood pouch containing longitudinal thin membrane (LTM), fertilized egg (FE) and edges of lateral folds (ELF) (Hx-E x 40).

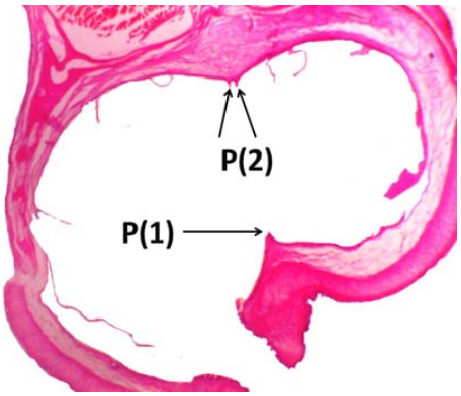

Fig 3: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing the longitudinal thin membrane attached with edge of lateral folds and dorsal side of brood pouch by one or two short pointed Protuberances (P) of endothelial tissue (Hx-E x 40).

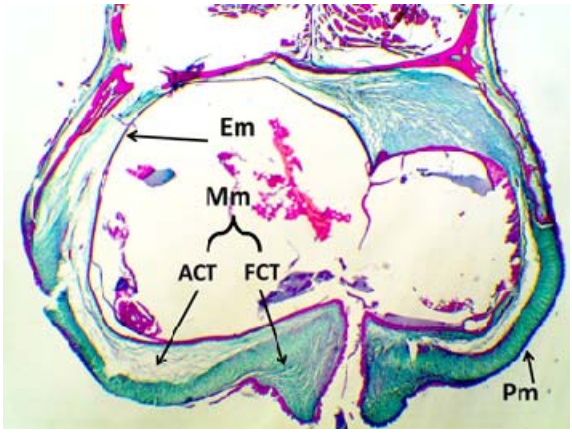

Fig 4: Photomicrograph of T.S. in the wall brood pouch of *T. bicoarctatus*, showing the Perimetrium (Pm), Myometrium (Mm), Endometrium (En), fibrous connective tissue (FCT) and areolar connective tissue (ACT) (Masson's trichrome x 40).

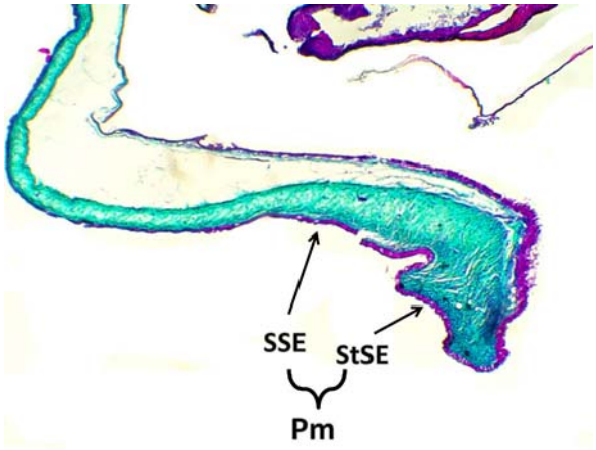

Fig 5: Photomicrograph of T.S. in the Perimetrium (Pm) brood pouch of *T. bicoarctatus*, showing the serosa (S.); composed of simple squamous epithelium (S.S.E.) and convert to stratified squamous epithelium (St.S.E.) towards the edge of lateral folds (Masson's trichrome x 100).

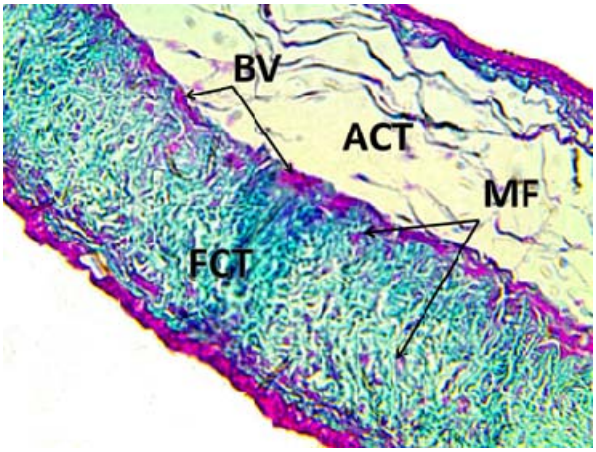

Fig 6: Enlarged portion of T.S. in myometrium brood pouch of *T. bicoarctatus*, showing blood vessels (BV) and small patches of muscle fibrous (MF) are distributed in fibrous connective tissue (FCT) and areolar connective tissue (ACT) (Masson's trichrome x 400).

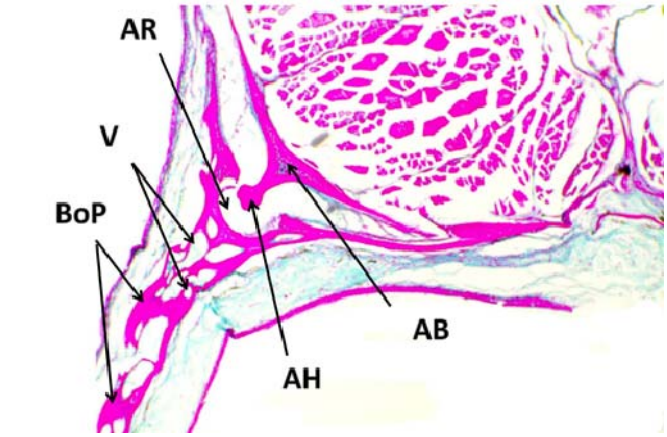

Fig 7: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing the articulation between protruding bony plates (Bo.P.), articulation base (AB), articulation region (AR), articular head (AH), bony plate (BoP) and vacuoles (V) (Masson's trichrome x 100).

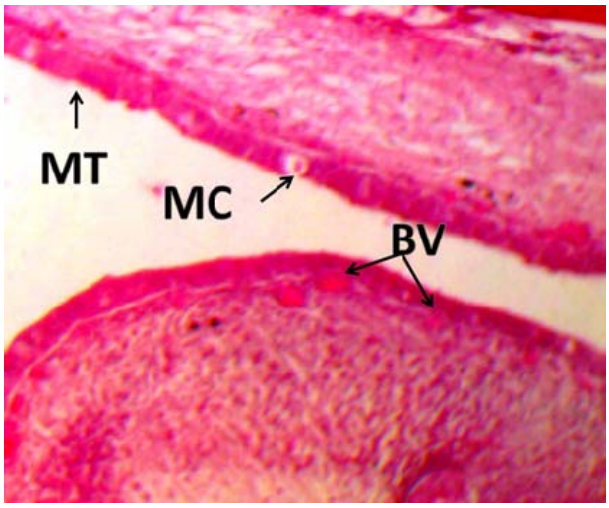

Fig 8: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing endometrium structure; the maternal tissue (MT) contain mucus cells (MC) and many large blood vessels (BV) in myometrium (Hx-E x 400).

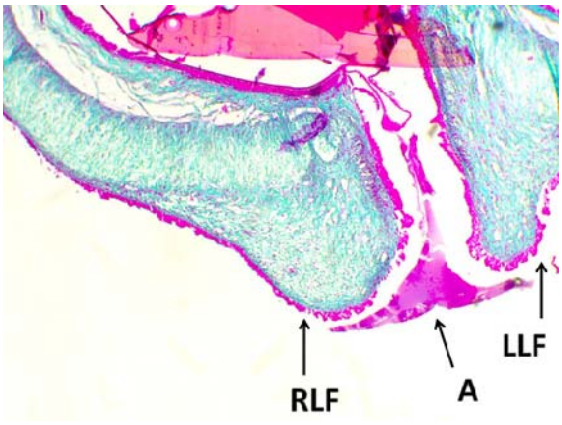

Fig 9: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing that, the maternal tissue is protruded to form anchor (A) between left and right lateral folds (RLF & LLF) for close the opening of brood pouch (Masson's trichrome x 400).

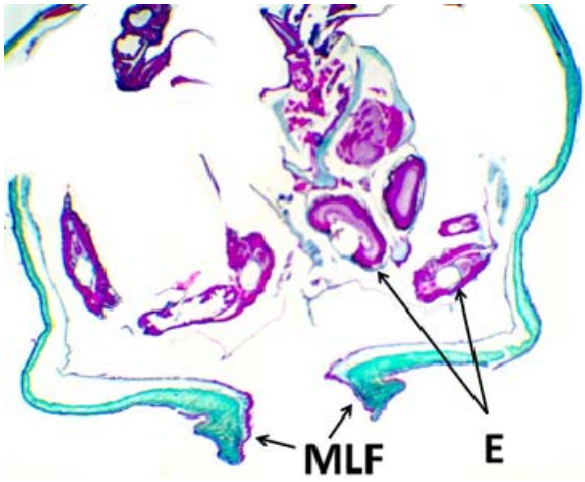

Fig 10: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing the embryos (E) and margins of lateral folds (MLF) are out opened for young's release (Masson's trichrome x 100).

Fig 11: Photomicrograph of T.S. in brood pouch of *T. bicoarctatus*, showing curvature the margins of lateral folds (MLF) towards the inside for closing the brood pouch (Masson's trichrome x 100).

4. Discussion

The present study revealed that, the examined fish specimens of T. bicoarctatus exhibit sexual dimorphism. Sex identification is possible externally; the determination of sex was identified by presence of anal fin in female and absence in male. Male have a brood pouch on ventral embedded in the skin in the spawning season (secondary character between male and female). This is a common feature in few teleost species. Similar results were detected at the same species [8].

In the present study, the male *T. bicoarctatus* exhibits one of the most specialized forms of parental care, depositing eggs in a specialized incubating area, located either on the tail (Urophori). Similar results were detected at the same species [1, 3] and in *Syngnathus abaster* [7]. The syngnathid species were depositing eggs in a specialized incubating area, located either on the abdomen (Gastrophori) in the females or tail (Urophori) in the males [6].

The male pipefish bear embryos in a particular brood chamber or pouch. The pouch is rather multifunctional; it's a mechanical protection of eggs and embryos and osmoregulatory function. The unusual parental care supposes that, some specific features should exist in the osmoregulatory organ for developing embryos. As evidence for the former

conclusion, the fact that, the eggs and embryos of seahorses did not develop after they had been removed from the pouch was put forth. The brood pouch of the seahorse is a chamber of osmotic assimilation for embryos: the eggs and early embryos are protected against the hyperosmotic pressure of the environment [12]. This was also recorded in the pipe fish, *T. bicoarctatus.*

In the present study, the wall of brood pouch in male *T. bicoarctatus* is made up of three layers; perimetrium, myometrium and endometrium. The perimetrium (serosa) is composed of simple squamous epithelium (protective or covering epithelia) in the outer layer which, cover and protect an outer surface, such as the epidermis of the skin. The perimetrium is increased in thickness towards the brood pouch opening for protection of embryo through closed and opened pouch. In the myometrium, the outer thick layer of fibrous connective tissue is relaxated, when the brood pouch is opened. The inner thin layer of areolar connective tissue is more in thickness gradually towards the dorsal side of the brood pouch to make cushion for protection of the embryos and richly supplied with blood vessels for enrichment of embryos.

In the present study, the articulation of the originated lateral folds takes place by circulating the articular head of bony base inside the articular cavity attached to the bony plates of the lateral folds. The upper part of myometrium consists of bony plats which extended from the body covering with large and small vacuoles. Accordingly, brood pouch gained flexibility of lateral folds by vacuoles to move towards the ventral side**.**

The brood pouch becomes vascularized to facilitate nourishment of the eggs as well as provide oxygen and protection [1]. Vascularization in the syngnathid pouch wall indicated a nutritional exchange between father and embryos [18]. The yolk sac was the only source of nutriment for the developing embryos [10]. The present study suggested that, the vacuoles are effective in flexibility of lateral folds. The blood vessels in myometrium and yolk sac in embryo were the main sources for enrichment of embryos.

In the present study, the endometrium is made up of thin layer of simple squamous epithelium and represents the inner layer of the brood pouch (maternal tissue) with large numerous mucous cells. The later secrete a mucous material which surrounds the eggs. Blood vessels are found in the connective tissue beneath the epithelium. Capillaries were bigger and more numerous during gestation for enrichment of embryos.

The internal wall of the brood pouch in *Syngnathus abaster* has round deep egg imprints and the chamber is filled with a mucous material which surrounds the eggs. The brood pouch conspicuous changes occur in the brood pouch wall of the syngnathid, *Syngnathus abaster* during different functional stages (before, during, and after egg incubation). One of the most evident changes occurred at the stage of egg incubation and consisted of an increase in vascularization of the connective tissue beneath the epithelium. Capillaries were few and small in diameter before and after gestation, but were bigger and more numerous during gestation [11]. This was also evident in the present study.

In the present study, the endometrium is protruded to form stopper like anchor between left and right lateral folds for close the opening of brood pouch in pregnancy period**.** Similar results were detected in *Syngnathus abaster* [11]. In the present study, when the embryos complete their development, the male makes irregular movement (squeezing) for birth of young. The margins of lateral folds are out opened for the young's release. After finishing of young born, the ends of two folds are curved to the inside for closing the brood pouch.

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