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## Embryonic development in alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)

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**The alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) is a common inhabitant of seagrass beds along Palk Bay, southeast coast of India. This primitive pipefish broods the embryos along the undersurface of its trunk. Eleven embryonic stages were sequenced based on morphological differences. The newborn resembles adults in form except for a slightly cylindrical body, shorter snout and brown colour. The gestation period was  $25 \pm 5$  days at a temperature range of  $28\text{--}32^\circ\text{C}$ .**

PIPEFISHES are amongst the most unusual and derive the name from their long and slender angular body. They belong to the family Syngnathidae, consisting of about 215 species in 52 genera. Pipefishes are cosmopolitan in distribution<sup>1</sup> between the north-south limits of latitudes  $71^\circ\text{N}$  and  $56^\circ\text{S}$ . They are commonly represented in fabrics and artwork and are sold dried or embedded in plastic as curios. Pipefishes are primarily sold for use in Traditional Chinese Medicine (TCM) and are credited with curing ailments ranging from asthma and arteriosclerosis to impotence and incontinence. They also provide remedies for skin ailments, high cholesterol levels, excess throat phlegm, goitre and lymph node disorders. They are reputed to facilitate parturition and also as powerful general tonic for the kidneys<sup>2</sup>. Apart from the inclusion in TCM, pipefishes are also exploited for aquarium-keeping for their peculiar and interesting habits<sup>3</sup>. Though as many as thirteen genera are reported to occur in Indian waters, not much is known about their reproduction. The present study has been undertaken for sequencing the embryonic development stages of the pipefish *Syng-*

*nathoides biaculeatus* for which no information is available so far.

The sampling was carried out in Thondi (lat  $945^\circ\text{N}$ ; long  $79^\circ 13'\text{E}$ ) along Palk Bay, Southeast coast of India. Live adult male and female fishes, caught as bycatches, were collected from country trawls and shore seines. They were transported and maintained in the laboratory. Males brood the embryos along the undersurface of their trunk. Embryos at different stages of development were randomly removed from the brooder using a canula and preserved in 5% neutral formalin or glycerol. The developmental stages were then observed under a binocular microscope and sequenced based on morphological features.

Three stages can be distinguished in the development of a bony fish: embryonic, larval and fry. The embryonic stage is divided into nine substages: activation of egg, cleavage, blastulation, gastrulation, neurulation, organogenesis, separation of tail region from the yolk sac, development of the embryonic circulatory system and development of gill and jaw apparatus. In the larval stages there are four substages: mixed feeding, complete external feeding and differentiation of the unpaired fin fold, development of rays in unpaired fins and development of rays in paired fins. The fry period is characterized by the development of scale cover<sup>4</sup>.

Eleven advanced stages of development were identified and sequenced in the present study (Table 1). In the first stage (Figure 1a), the fertilized egg is translucent and nearly round in shape, measuring  $1600\text{--}1635\ \mu\text{m}$  in diameter. Yolk contains many lipid granules. Small projections can also be seen all over the egg surface. The second stage is the organogenesis stage (Figure 1b). The egg becomes more pear-shaped and the embryo is just formed. The head region and the tail remain attached to the yolk. The yolk is still larger with many granules. During the third stage (Figure 1c), the head and the tail regions are separated from the yolk mass. The head is directed to the base of the egg attachment. Yolk is prominent and the space between the embryo and egg membrane contains large vacuoles. Slight elongation occurs and the egg measures 4 mm in length and 2 mm in width. The embryo during the fourth stage (Figure 1d) remains concentrated at one end of the egg. The other

**Table 1.** Stages of embryonic development of alligator pipefish

Number	Developmental stage
1	Activation – fertilized egg $1600\text{--}1635\ \mu\text{m}$ in diameter
2	Organogenesis – embryo just formed
3	Separation of tail from yolk sac
4	Development of embryonic circulatory system
5	Development of jaws
6	Differentiation of unpaired fins, embryo 10 mm
7	Development of rays in unpaired fins, dorsal fin – 13 to 15 rays
8	Development of snout, rays in paired fins, pectoral fin – 12 to 14 rays
9, 10	Development of bony plates, yolk in small quantities
11	Hatchling, yolk fully absorbed

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end is transparent with a clear space. Formation of eye can be observed and a rudimentary heart can also be seen above the yolk sac and behind the head. A small dorsal fin bud is visible. Chromatophores are found distributed along the entire body of the embryo. Stage five (Figure 1e) is characterized by further development of eye and eye lens. Development of jaw apparatus begins at this stage. A small dorsal fin is also visible. The embryo measures about 9 mm when stretched. Development of mouth progresses during the sixth stage (Figure 1f). A small anal fin bud and a pec-

toral fin can be seen at this stage. Dorsal fin is transparent and longer. Projections of bony plates can be seen and the embryo is transparent with chromatophores distributed in the body. Embryo measures 10 mm when stretched. In the seventh stage (Figure 1g), the head is more developed with two small spine-like projections. Pectoral fin is further enlarged, but without rays. Bony plates begin to develop along the trunk region. Dorsal fin consists of 13–15 rudimentary rays. Anal fin bud is prominent, but without rays. Embryo measures 1.0–1.2 cm when stretched. Snout starts

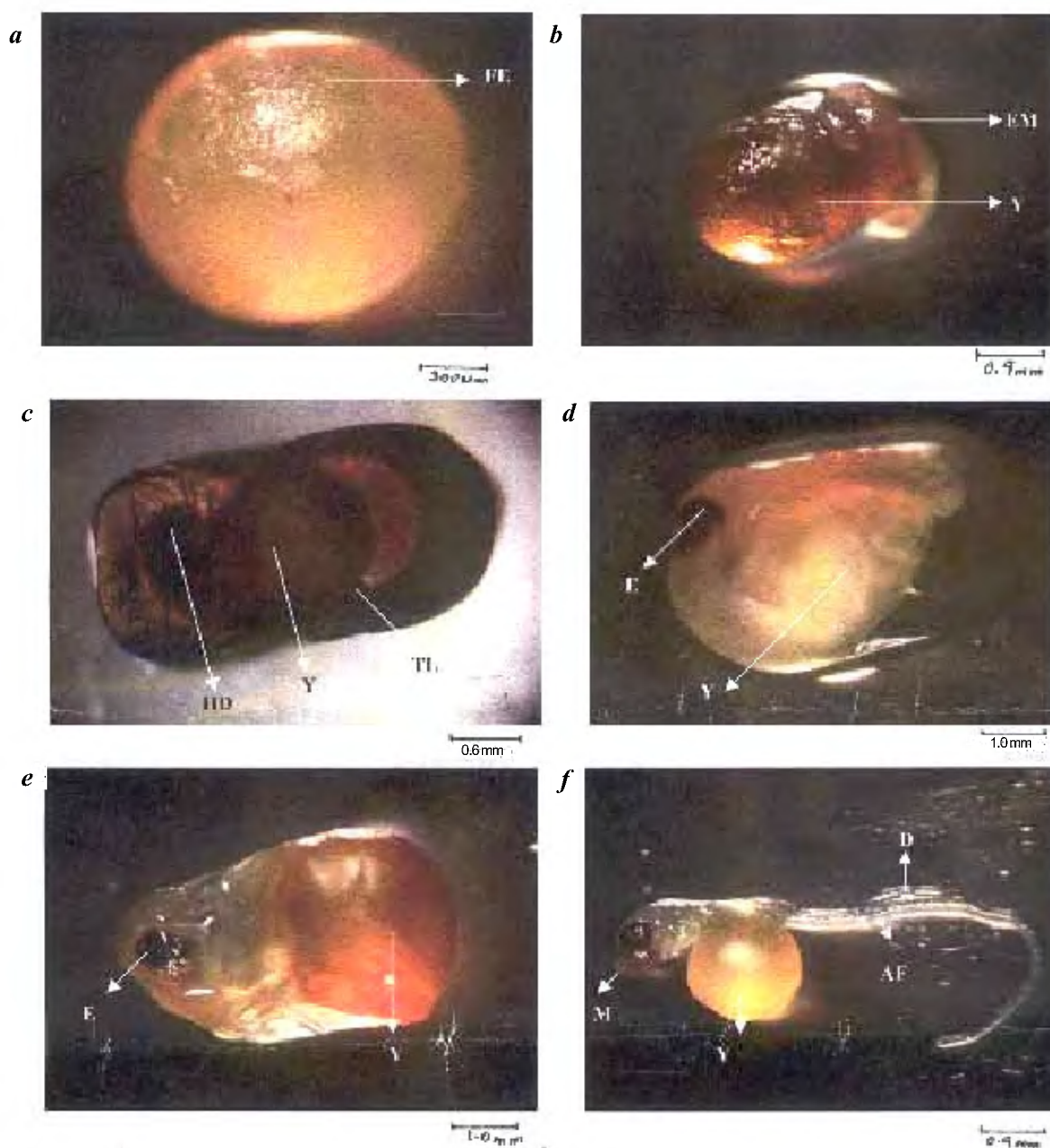
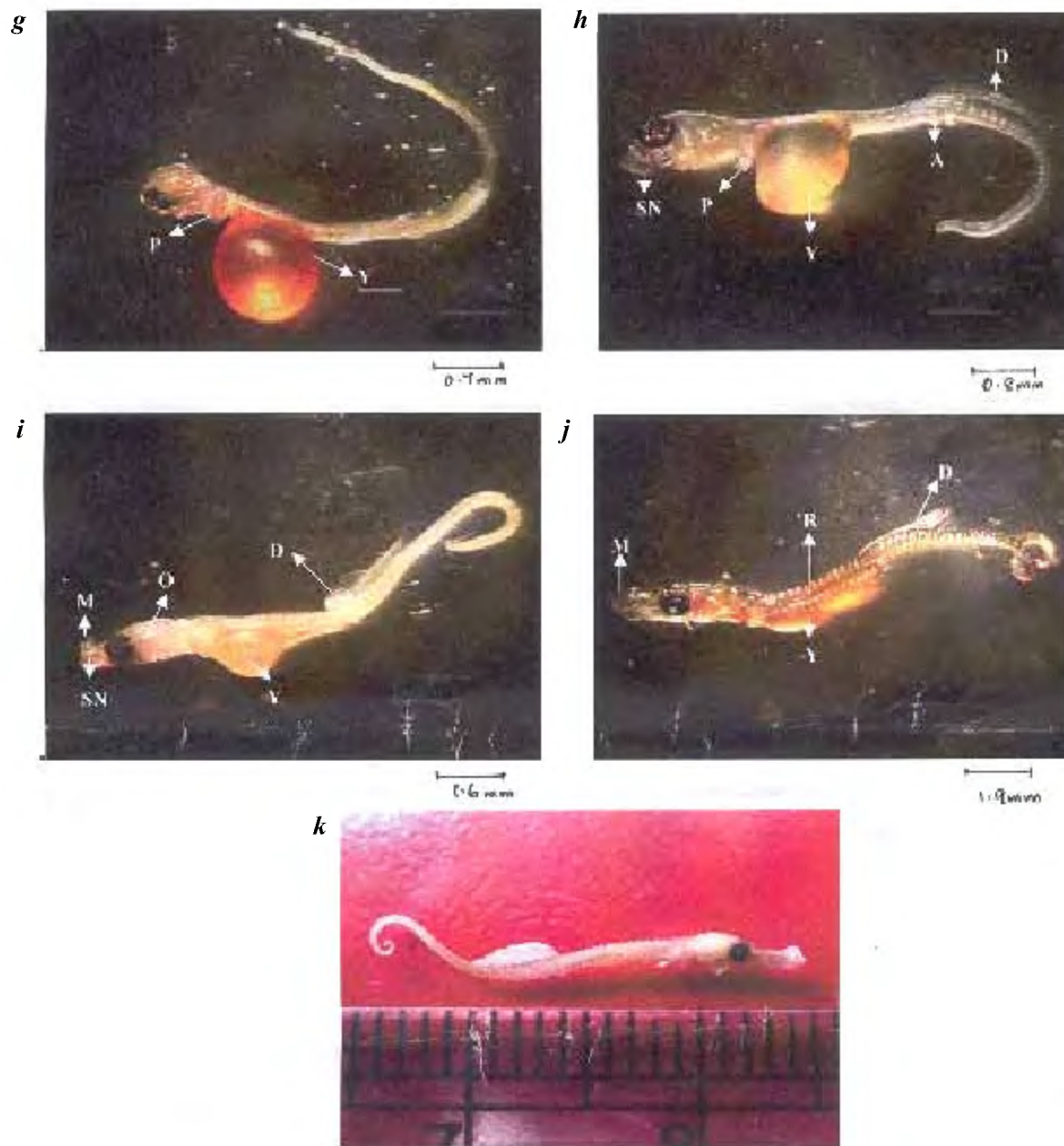


Figure 1. (Continued)



**Figure 1.** *a-k*, Embryonic development of *Syngnathoides biaculeatus* (stages one to eleven); A, Anus; AF, Anal fin bud; D, Dorsal fin; E, Eye; EM, Embryo; FE, Fertilized egg; HD, Head; M, Mouth; O, Opercle; P, Pectoral fin; R, Body ring; SN, Snout; TL, Tail; Y, Yolk.

developing in the succeeding stage (Figure 1*h*). Lower jaw protrudes slightly from the upper jaw. Dorsal fin rays are 36–38 in number. Anal projection is quite distinct. Trunk and tail rings (16–17 + 25–27) are more visible. Bony rings along the trunk and tail are more pronounced. About 12–14 pectoral fin rays are present. Embryo measures 1.1–1.2 cm in length when stretched. In the next stage (Figure 1*i*), snout becomes longer but mouth remains closed and

protrudes in front of the eye. Dorsal fin is well expanded and possesses 40–42 rays. Four rays are visible in the anal fin. Pectoral fins possess 17–18 rays. The numbers of body rings are 17–18 + 44–45 and the ridges are clearly visible. Opercle is also clearly visible. Chromatophores distributed all over the body are concentrated along the head, yolk and anus. Embryo measures 1.5–1.6 cm in length. In the tenth stage (Figure 1*j*), the jaw apparatus is fully developed

and mouth is perforated. Eye spine and opercular ridge are well formed. Dorsal fin rays are 44–46 in number. Pectoral fin rays are 18–22 in number and ring count 16–18 + 46–53. Yolk is present in small quantities. Embryo measures 1.7–1.9 cm in length. The new born (Figure 1k) is a miniature form of the adult. Dorsal fin rays are 40–48 in number, pectoral fin rays 20–24 and rings 16–18 + 46–53 in number. Spine-like projections are prominent along the body ridges of each body ring. Yolk is fully absorbed and the young one measures 1.7–2.0 cm in length.

There have been a few reports on the embryonic stages of different pipefishes. Segmentation in *Siphostoma floridae* eggs<sup>5</sup> and eggs and larvae of *Syngnathus schlegelii*<sup>6</sup> has been reported. However, the information presented was poor and fragmentary. A more detailed study on the developmental stages of *Syngnathus acusimilis* was published later<sup>7</sup>. In Indian waters, studies on early life history of pipefishes are scanty and limited, except for a few descriptions of embryonic stages from the pipefishes *Solenostomus cyanopterus*<sup>8</sup>, *Syngnathus cyanospilos*<sup>9</sup> and *Micrognathus brevisrostris*<sup>10</sup>. In this backdrop, the present study sequences the advanced stages of embryonic development of the pipefish, *S. biaculeatus*.

The embryonic period begins with activation and is characterized by exclusively endogenous nutrition from the yolk. The larval period commences with the transition to exogenous feeding and lasts until metamorphosis. But a larval period is generally absent in fishes which develop definitive organs throughout embryogenesis. In the pipefish, the end result is thus a free-swimming juvenile. It is also apparent that the yolk sac is retained during the whole embryonic development, disappearing just before birth. This also prevents the splitting of the period into the prelarvae, which extends from the hatching from egg envelopes to disappearance of yolk sac, and larvae, which extends from complete external feeding to formation of rays in unpaired fins. In pipefishes, there is also an overlap in the stages. The rays in unpaired and paired fins develop well before the complete development of the mouth apparatus<sup>11</sup>.

Brood pouches in pipefishes vary from simple ventral gluing of eggs to completely enclosed pouches<sup>12</sup>. In advanced pipefishes such as *S. acusimilis*, the embryos are released from the egg membrane into the brood pouch when they are 8 mm in length<sup>7</sup>. The embryos continue to feed on their yolk sac before being released from the brood pouch. However, in *S. biaculeatus*, a primitive pipefish without a brood pouch, the embryos are released at an advanced stage of development. There has been an incorrect description that the new born *S. biaculeatus* measures 8.1 mm in length and contains yolk mass<sup>13</sup>. This observation was criticized, stating that the yolk sac was completely absorbed in the hatchling<sup>14</sup>. In the present study, the stage suspected as

hatchling by the former worker was observed to emerge from the egg membrane on few occasions. This was noticed when the parent shook its body vigorously or rubbed against any hard substrate, which resulted in the accidental rupture of the egg membrane. From the present study it is apparent that the yolk sac is retained during the whole embryonic development and the hatchling is a free-swimming juvenile. Temperature shows a pronounced influence on the brooding period<sup>15</sup>. In the present study, the gestation period of the fish was found to be  $25 \pm 5$  days at the temperature range of 28–32°C.

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