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James T. Wetzel
Presbyterian College, South Carolina

John P. Wourms
Clemson University

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EMBRYOGENESIS IN THE DWARF SEAHORSE, *HIPPOCAMPUS ZOSTERAE* (SYNGNATHIDAE)

James T. Wetzel[1] and John P. Wourms[2]

[1]*Department of Biology, Presbyterian College, Clinton, SC 29325 USA*

[2]*Department of Biological Sciences, Clemson University, Clemson, SC 29634 USA*

ABSTRACT Embryogenesis of the dwarf seahorse, *Hippocampus zosterae*, was studied by scanning electron microscopy of a series of developmental stages. Stages ranged from initial cleavage of the egg through term embryos. Embryos hatch from their egg envelopes about midway through development, yet remain nestled in stromal chambers of vascularized epithelium within the male brood pouch until their yolk reserves are consumed. The difference in body shape between the pipefish and seahorse first becomes discernible during mid-development, just before hatching. At this stage, embryos begin to develop their characteristic prehensile tail in contrast to the straight body and typical caudal fin of most species of pipefish. Post-hatching, 'yolk-sac' larvae have a well developed head, that is set at a right angle to the body axis, and fully formed fins. As seahorse embryos approach term, lepidotrichia calcify, and the prehensile tail is capable of muscular contraction. Dermal scutes first appear at this stage and ossify in the term embryo. The dermal armor is then fully formed and functional. At term, the yolk reserves have been depleted, and the young are released from the brood pouch as free-swimming, free-feeding miniature versions of the adults.

INTRODUCTION

Fishes of the family Syngnathidae, which includes seahorses and pipefishes, display a number of specialized anatomical features that are characteristic of the group (Jungerson 1910, Khalil 1979, Fritzsche 1984). Within the Syngnathidae, seahorses are distinguished from pipefishes by the orientation of the head normal to the body axis and by a prehensile tail. It is, however, the peculiar reproductive mode of brooding their embryos on the skin of the male abdomen that collectively distinguishes the Syngnathidae from other syngnathiforme fishes (Nelson 1994). Developing eggs are either attached to the ventral surface of the male's body, or the eggs and free embryos are contained in a pouch that is formed from the epidermis of the abdomen.

In the putative ancestral form of male skin-brooding in pipefishes (Herald 1959), the female transfers eggs via her ovipositor to the caudal part of the male's body in a region posterior to the vent (*Solegnathus, Phyllopteryx,* and *Phycodurus*) or to the ventral abdominal region just anterior to the vent (*Syngnathoides* and some *Dunckerocampus*). The eggs adhere to the male and are brooded. In this primitive condition the eggs rest in depressions of modified epidermis or 'stromal tissue' that develops on the male abdomen. No further envelopment of the embryos occurs, and free-swimming young hatch directly from egg envelopes into the water. In the derived condition of skin-brooding (seahorses and nearly all pipefish species), eggs are oviposited into a male brood pouch that is formed from the epidermis along the ventro-abdominal surface. Depending on the degree of closure of the brood pouch, eggs may be partially sealed or completely sealed within this brood pouch. Eggs hatch within the brood pouch and are subsequently afforded some degree of paternal care prior to their release as free-swimming young. The brood pouch is lined with stromal tissue, a simple, cuboidal epithelium that becomes spongy in texture and heavily vascularized after oviposition (Wetzel and Wourms 1991). As development proceeds, the egg envelope of each embryo splits open. The hatched embryo is then nestled within the stromal tissue, where it remains until its yolk is absorbed.

The period of gestation within the male brood pouch is both temperature related and species specific. After a gestation of 30–50 d duration, young are released from the brood pouch either through a longitudinal fissure (pipefishes, with the exceptions of *Apterygocampus* and *Acentronura*) or through an antero-medial opening of the pouch (*Hippocampus, Apterygocampus,* and *Acentronura*). After parturition, the internal stromal tissue of the brood pouch returns to the normal pre-vascularized condition (Wetzel 1995). Free-swimming young have adult body form, except that males lack their characteristic brood pouch which will not be evident for an additional 3 to 4 weeks.

There are contemporary accounts on the anatomy and developmental morphology of the Syngnathidae (Sudarsan 1968, Blake 1976, Azzarello 1990). Although each of these studies was comprehensive in its own right, none provide an overview of the process of development from early cleavage through free-living juveniles. This study describes, using a series of scanning electron micrographs, embryogenesis of the dwarf seahorse, *H. zosterae*.

MATERIALS AND METHODS

Study specimens

Preserved museum specimens and newly collected live specimens were combined to assemble a series of seahorse embryos that represent the major stages of embryonic development. Museum specimens of the dwarf seahorse, *H. zosterae* Jordan and Gilbert, were obtained from the collections of the Gulf Coast Research Laboratory Museum (GCRL specimen #10560—collected Lee County, Florida, sta. 69-234, Wang/Jackson), Ocean Springs, Mississippi, and from the California Academy of Sciences (CAS specimen #1687—collected Key West, Florida, 4/18/71, D. Smith), San Francisco, California. Additional embryos of *H. zosterae* were the offspring of specimens maintained in aquaria.

Microscopy

Mature ova were removed from the germinal ridge, at the ovarian side of the oviducts excised from museum specimens of *H. zosterae*. To obtain embryos from live specimens, gravid males were anesthetized in a 1000 ppm solution of MS-222 (ethyl m-aminobenzoate methanesulfonate, Crescent Research Chemicals, Inc.) in seawater. Embryos were excised by a lateral incision through the brood pouch, then placed into a 2.5% glutaraldehyde-1.6% paraformaldehyde fixative in 0.1 M Sorensen's phosphate buffer (pH 7.4). Fixations were carried out at room temperature for 2 h, followed by two 10-min rinses in 0.1 M Sorensen's buffer. Fixed embryos that were used for descriptions of the developmental stages were manually dechorionated prior to dehydration. Specimens for SEM were dehydrated through absolute ethanol, critical point dried, and gold-coated for 400 sec. SEM observations were made at an accelerating voltage of 15kV with a JEOL 35cf SEM.

RESULTS

The stages of embryonic development

Description of the development of the dwarf seahorse, *H. zosterae*, is based on 8 different developmental stages that ranged from the 2-blastomere stage through term (Figure 1).

Unfertilized eggs: Mature infertile ova are slightly 'pear-shaped' (Hudson and Hardy 1975). They measure about 1100 µm in diameter along the long axis. Ova of other syngnathid species have been described as ellipsoidal or ovoid (Mito 1961). The chorion is transparent and its surface is devoid of any membrane projections (Wetzel and Wourms 1991). The egg envelope measures 0.86 µm thick and forms a homogeneous, non-fenestrated barrier around the egg. The individual eggs are transparent, vary in color from light amber to deep orange, and contain several oil droplets throughout the yolk (Gudger 1905). The vitelline membrane adheres closely to the ovum and is not readily discernible. No newly fertilized eggs were found.

Early cleavage

At the 2-cell stage, the blastodisc, yolk cytoplasmic layer, and deuteroplasm can be differentiated. The yolk cytoplasmic layer extends all around the surface, almost to the equator of the egg, covering the deuteroplasm. The lower half of the egg is formed of deuteroplasm and contains numerous vesicles, which are larger but less numerous than in the nonfertilized egg, as well as large oil globules. Cleavage is meroblastic, and the first division results in 2 equivalent sized blastomeres, each measuring about 180 µm in diameter (Figure 2a). Cleavage continues to form 16 blastomeres (Figure 2b) connected by cytoplasmic bridges along their cell boundaries (Figure 2c). Blastomeres at the 16-cell stage measure about 78 µm in diameter. They do not form the parallel rows that are often seen in typical teleostean early development (Armstrong and Child 1965). Rather, the margins of the cells form an irregular, ovoid shape on the surface of the yolk. An elaborate network of cytoplasmic processes (Figure 2d) appears along the outer edges of the blastoderm where individual blastomeres are in contact with the yolk cytoplasmic layer.

During late cleavage the blastoderm of *H. zosterae* (Figure 2e) is a discoidal mass that measures about 390 µm in diameter and lies on top of the deuteroplasm. Cleavage appears to proceed in a typical teleostean pattern, i.e., the blastomeres are about equal in size, somewhat flattened, and slightly raised above the surface of the blastoderm. Individual cells of the late-stage blastoderm measure about 15 µm in diameter (Figure 2f). The periphery of the blastoderm has a somewhat uneven outline, due to irregular contact of the blastomeres with the yolk cytoplasmic layer. No surface extensions were found on the individual cells that make up the blastoderm.

Tail-bud embryos

At the tail-bud stage of development (Figure 1b, 3a), the embryonic axis is about 1500 µm in length and is aligned along the midline of the embryonic shield. As embryogenesis proceeds, the embryo continues to elongate, yet remains coiled around the yolk mass (Figure 3d). The anterior end is slightly broadened and is clearly differentiated into the cephalic region. Regions of the brain are apparent, and there is a clear line of demarcation between

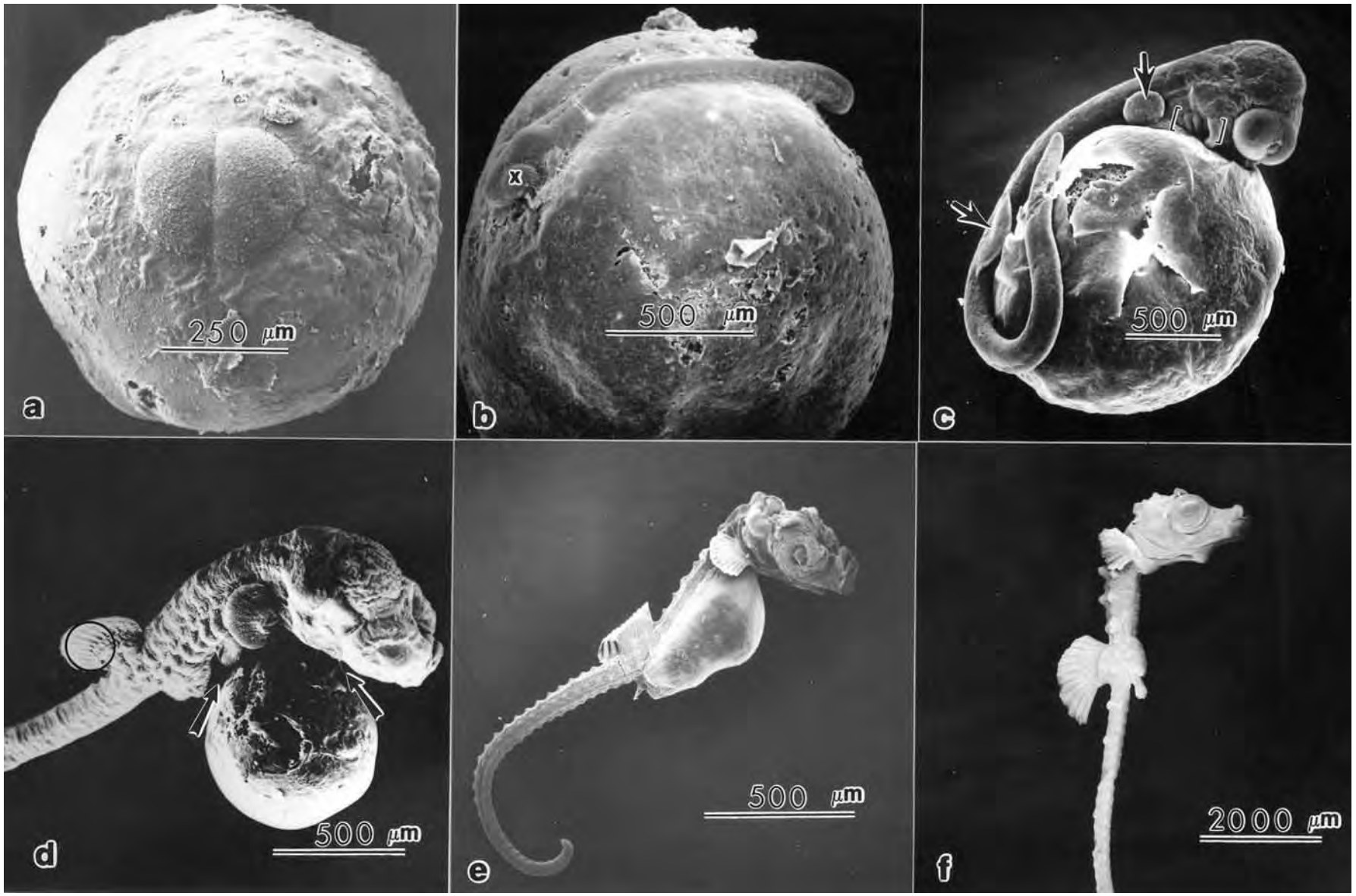

Figure 1. Major developmental events in the pre-hatching (a, b, and c) and post hatching (d, e, and f) *Hippocampus* embryos. a) Cleavage of the *Hippocampus* ovum, 89X. b) The tail-bud embryo optic vesicles (x), 52X. c) The dorsal-fin bud (posterior arrow), pectoral-fin bud (anterior arrow), branchial arches (brackets), and the mandibular arch differentiating into the lower jaw, 36X. d) 'Yolk-sac' larva with lepidotrichia (circle), 47X. e) The near-term embryo, 57X. f) The term neonate, 11X.

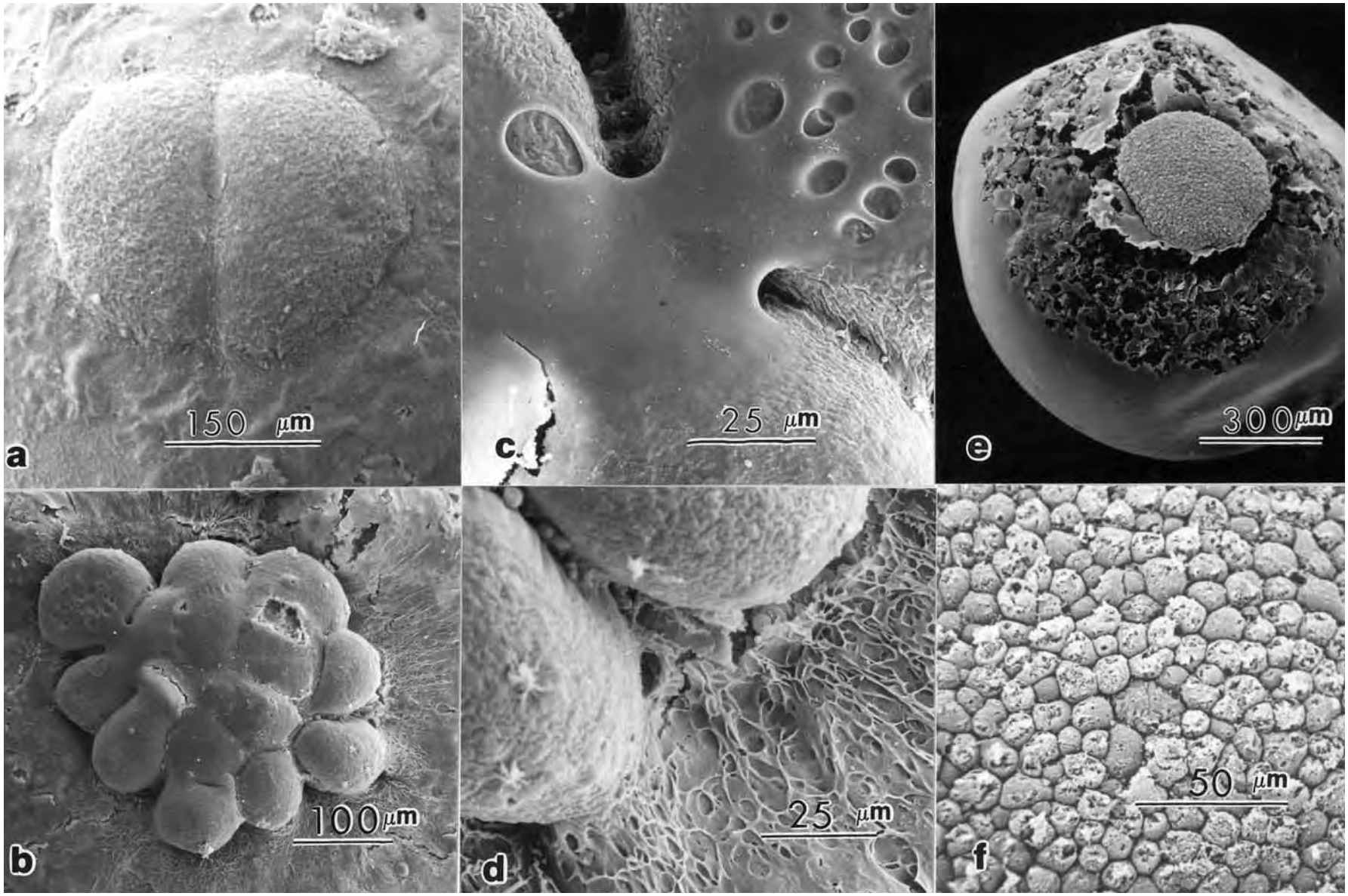

Figure 2. Cleavage of the *Hippocampus* ovum: Two blastomeres (2a) through formation of the blastoderm (2e). a) Individual blastomeres at the two-cell stage, 167X. b) Blastomeres at the 16-cell stage, 160X. c) Each cell is connected by a protoplasmic bridge (2c) along its membrane, 800X. d) The periblast where cells contact the yolk cytoplasmic layer, 720X. e) The discoidal blastoderm (2f) that rests on the deuteroplasm, 68X. f) 473X.

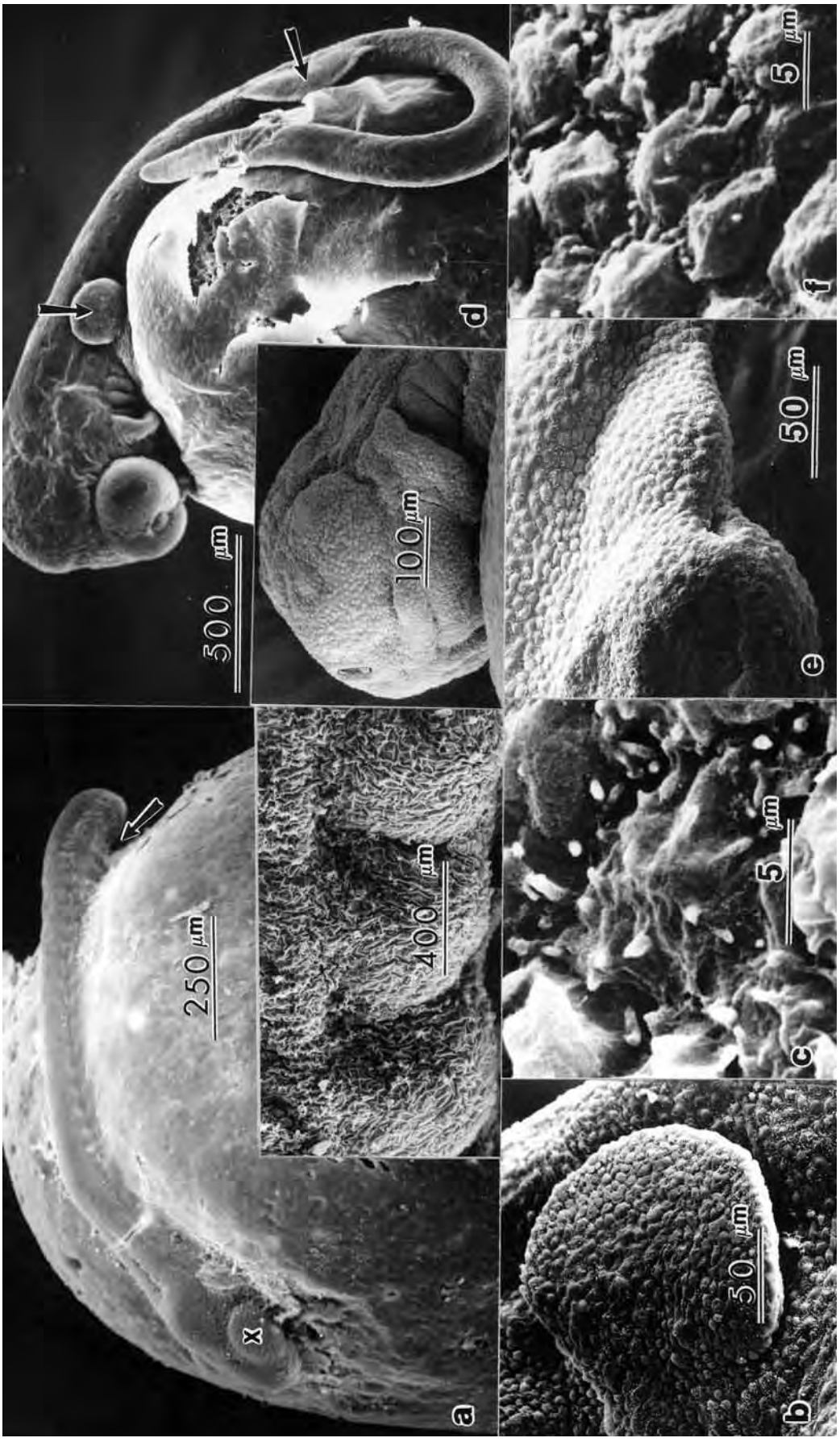

Figure 3. a) The tail-bud: The caudal region is raised from the surface of the deuteroplasm (arrow). Myomeres (inset) developing in the lateral body, 86X; inset 400X. b) Pectoral-fin buds have emerged, 320X. c) Surface of the fins, 4400X. d) The lens (inset) has differentiated from the optic vesicle, 58X; inset 120X. e) The anal fin emerging as a swelling posterior to the vent, 400X. f) Surface of the developing anal fin, 3600X.

the embryonic diencephalon and metencephalon. Optic vesicles (Figures 1c, 3a (x)) are present on each side of the diencephalon but the lens placodes are not yet developed. Branchial arches have emerged (Figure 1c, brackets) and the anteriormost arch has begun differentiating into the mandibular arch that will form part of the jaw. Pectoral-fin buds are present (Figure 3b) but lepidotrichia within the fins have not yet developed. No surface amplifications are present on the fins (Figure 3c). A series of 12 to 14 myomeres are aligned along the lateral body region (Figure 3a inset). The posterior end of the body is slightly raised and laterally flattened to form the caudal-fin bud. The caudal-fin bud is slightly elevated from the surface of the yolk mass (Figure 3a, arrow). Caudal fins that are characteristic of almost all pipefishes do not develop from the fin bud. Instead, the caudal bud eventually elongates into a prehensile tail. The anal fin first appears as a swelling posterior to the vent (Figure 3e). No surface amplifications are seen on the emerging anal fin (Figure 3f). Embryos of *H. zosterae* are 5300 µm in length during mid-development (Figures 1c, 3d) and still reside in their egg envelopes. At this phase, a distinct dorsal-fin bud is present (Figure 1c, posterior arrow). Paired pectoral-fin buds also appear (Figure 1c, anterior arrows). Although the head region is clearly delineated, the rostrum has not yet emerged and the head is connected to the deuteroplasm (Figure 3d inset). The mesencephalon is greatly enlarged relative to the diencephalon and metencephalon brain regions. The eye lens vesicles reside in the center of the greatly expanded optic cups and the eye is pigmented. Auditory placodes have developed lateral to the metencephalon. The anterior arch is differentiating into the buccal apparatus.

Yolk sac 'larva'

Although hatching from the egg envelope occurs at the 'yolk-sac larva' phase (Hardy 1978) (Figures 1d, 4a), the embryo will remain nestled within the stromal tissue of the brood pouch until the yolk reserves are exhausted. This terminal phase of development is characterized by morphogenesis of the jaw, growth of the dorsal and pectoral fins, and decrease in size of the yolk sac as the yolk reserves are utilized. Pectoral and dorsal fins are broadened and flattened, and their lepidotrichia are differentiated (Figure 4b, circle). Contact of the embryo with the yolk sac is limited to its thoraco-abdominal region. The entire prehensile tail and the anterior half of the head are free. In embryos, at this stage, that were removed from the brood pouch the tail did not respond to mechanical stimulation. The anal fin begins to develop (Figure 3e). No surface amplifications are found on the emerging anal fin (Figure 3f). The vent posterior to the anal fin is closed. Two transverse cartilages form from the anterior arches and represent the initial formation of the upper (maxillary) and lower (mandibular) jaws (Gill 1904). The upper jaw is straight, whereas the lower jaw, which is broadly triangular in shape, curves upward to partially cover the median portion of the upper jaw. The branchial arches are covered by an operculum.

Near-term embryos

In the near-term embryo (Figure 1e, 4c), the head is completely free from the surface of the yolk sac, and the jaws elongate because of growth of the ethmoid and quadrate cartilages (Azzarello 1990). The characteristic posture of the seahorse head, i.e. oriented at about 90 degrees to the body axis, is apparent at this stage. This differs from pipefishes, wherein the angle of the head remains in line with the elongate body axis. The anal fin is formed (Figure 4d) and the vent is open. Microvilli are present on the anal fin (Figure 4e). Lepidotrichia within the dorsal and paired pectoral fins, as well as the anal fin, are fully formed, although not yet fully calcified (Wetzel 1995). The prehensile tail is capable of muscular contraction. Yolk reserves are nearly consumed, but the extraembryonic yolk sac is still evident as a slight swelling from the abdominal region. Chromatophores are developed along the lateral body surface, except for the fins. Dermal armour, characteristic of *Hippocampus* and comprised of a series of interlocking epidermal plates, first appears at this stage as small scutes along the lateral body (Figure 4c inset). The largest scute measures about 190 µm in diameter at the base and they are spaced about 240 µm apart. Each dermal scute is covered by an epidermal layer.

Term embryos

In the term embryo (Figure 1f, 4f) internal yolk reserves are completely exhausted and there is no notable protrusion of the extraembryonic yolk sac. The young are able to feed freely upon release from the brood pouch. The prehensile tail is fully capable of adhering to the substrate and the extrinsic eye musculature is fully functional. Following parturition, the lepidotrichia within the fins become fully calcified and rigid (Wetzel 1995). The dermal scutes of the body armour are now greatly enlarged, 360 + 38 µm, and are in contact with adjacent scutes along their bases. The calcified dermal crowns erupt through the epidermis and appear as a series of spines along the lateral body and down the tail (Figure 4f inset). Neuromasts (Figure 4g) develop along the rostrum. Pigmentation increases considerably beyond that of the near-term embryo, except for a region of the abdomen near the vent. The characteristic male brood pouch is not yet evident, but

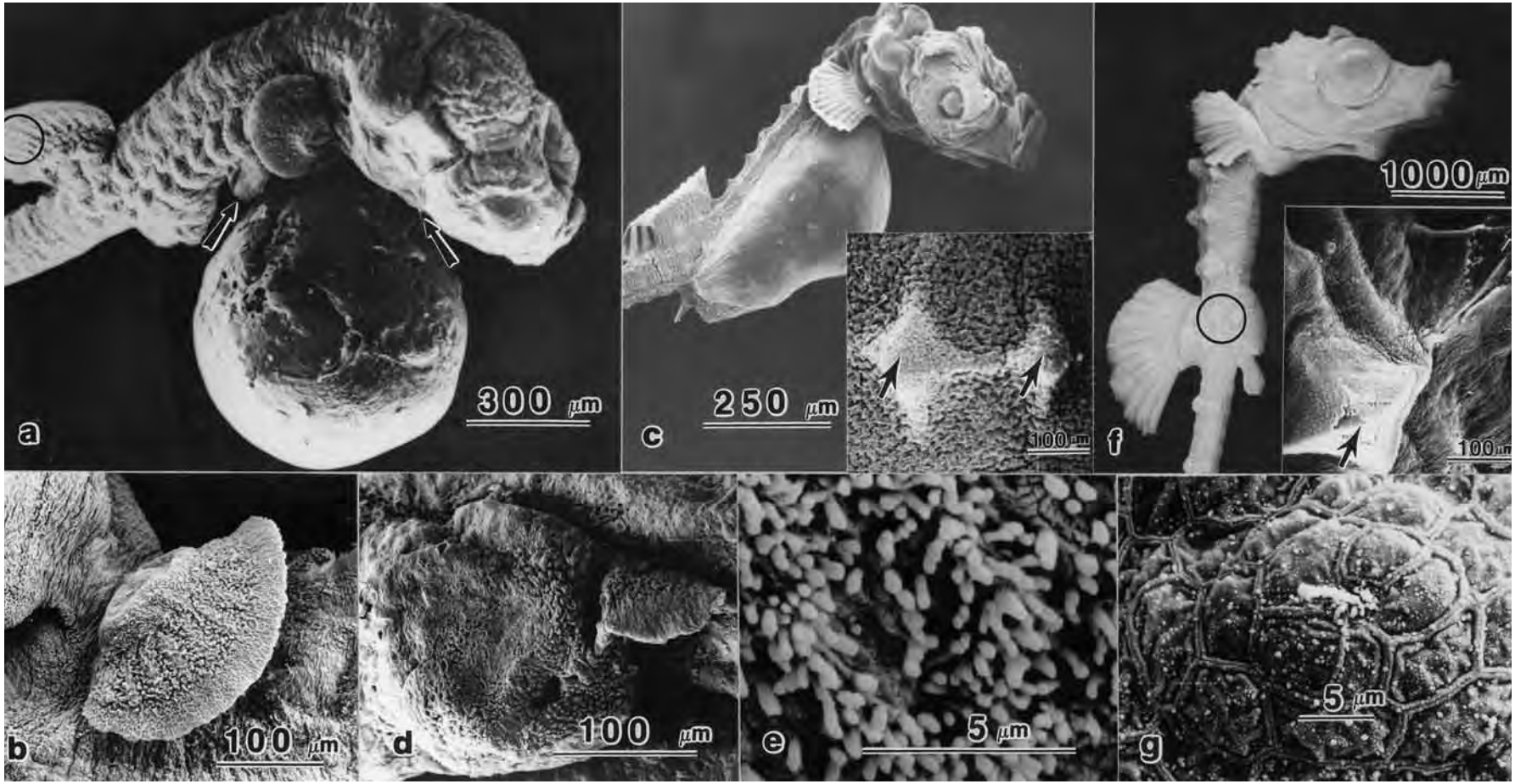

Figure 4. a) The yolk-sac larva: The abdominal and head regions are free from the surface of the yolk (arrows), 74X. b) Lepidotrichia (4a circle) within the dorsal fin, 180X. c) Dermal scutes (arrows) along the lateral surface of the tail, 93X; c inset 107X. d) The anal fin and the open vent, 260X. e) Microvilli on the anal fin, 7800X. f) Fully formed scutes (circle and inset) in the term neonate, 20X; inset 116X. g) Neuromasts along the rostrum of the neonate, 2600X.

within 3 to 4 weeks it will emerge in what is now a lightly pigmented region of the abdomen (Bellomy 1969). Upon release from the adult brood pouch, term embryos measure about 8600 + 158 µm.

DISCUSSION

Comparison of seahorse and pipefish development

Scanning electron micrographs of 6 distinct stages in the development of *H. zosterae* provide a basis for comparing embryogenesis of seahorses with that of other syngnathid fishes. Based on our observations and previous reports on pipefishes (Gudger 1905), there are no significant morphological differences in early development, i.e. cleavage through tail-bud embryos, between seahorse and pipefish embryos. Embryos of *Hippocampus* can first be distinguished from other syngnathid embryos midway through development, i.e., just prior to hatching. At hatching, yolk-sac larvae have a well developed head and fully formed pectoral fins. At this stage, a developing caudal fin is evident in pipefish embryos, whereas seahorse embryos lack any indication of a caudal-fin fold. In the more advanced yolk-sac larval stage, which we refer to as the near-term embryo, the prehensile tail of the seahorse is fully formed and capable of muscular contraction. Orientation of the head of the seahorse embryo at a 90º angle normal to the body axis also occurs at near-term whereas the head of pipefishes continues to develop in line with the body axis. In seahorses, their characteristic dermal armor has completely formed prior to release from the brood pouch (vida infra). In summary, major morphological differences between seahorses and pipefishes become evident after hatching from the egg envelopes. In the case of the seahorse, the subsequent sojourn in the brood pouch not only is the time period during which these differences are established, but it also allows the young seahorse to develop to a morphologically more advanced state than the newly hatched pipefish, i.e., seahorse young are precocial.

The brood pouch and dermal calcification

Embryos of syngnathid fishes that are not enclosed within a brood pouch, such as the pipefishes *Syngnathoides*, *Phycodurus*, *Phyllopteryx*, and *Solegnathus*, hatch and immediately enter the surrounding water as functional, free swimming young. In contrast, embryos of species that retain their eggs within a pouch, hatch from their egg envelopes prior to the completion of incubation and then continue to develop for an additional 6 to 10 days within the male's pouch (Takai and Mizokami 1959). During this time, additional materials not provided during oogenesis can be sequestered from the pouch fluid by the young. Embryos of *H. zosterae* hatch from their egg envelopes before embryogenesis is complete and remain nestled in stromal chambers of vascularized epithelium within the male brood pouch until their yolk reserves are consumed. Because supplemental nutrients are not transferred to the developing embryo by the male (Wetzel and Wourms 1991), maintenance of the embryos within the brood pouch would appear to serve a different function. In an earlier study (Wetzel and Wourms 1991), we noted a marked and progressive decline in organic weight during development, representing the catabolism of yolk reserves. In contrast, while inorganic weight also declines steadily to the hatching stage, there is an increase in weight during the post-hatching, terminal phase of development, so marked that it results in a net increase in total weight of the term embryo. Elevated levels of calcium ions have been found in *Hippocampus* brood pouch fluid (Linton and Soloff 1964). Furthermore, the emergence of the dermal skeleton of the seahorse embryo is associated with the accumulation of calcium within the brood pouch during the post-hatching phase of development (Wetzel 1995). Dermal scutes that first appear in the near-term embryo become fully ossified in the term embryo so that the dermal armor is completely formed prior to 'birth.' Calcification of the dermal skeleton while in the pouch confers a subsequent advantage of protection to the young seahorse, because they will be heavily armored upon their release from the brood pouch.

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LITERATURE CITED

Armstrong, P.B. and J.S. Child. 1965. Stages in the normal development of *Fundulus heteroclitus*. Biological Bulletin 128:143–168.

Azzarello, M.Y. 1990. A comparative study of the developmental osteology of *Syngnathus scovelli* and *Hippocampus zosterae* (Pisces: Syngnathidae) and its phylogenetic implications. Evolutionary Monographs 12:1–90.

Bellomy, M.D. 1969. Encyclopedia of Sea Horses. T.F.H. Publications, Inc., Neptune City, NJ, USA, 192 p.

Blake, R.W. 1976. On seahorse locomotion. Journal of the Marine Biological Association of the United Kingdom 56:939–949.

Fritzsche, R.A. 1984. Gasterosteiformes: Development and relationships, In: H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall Jr., and S.L. Richardson, eds. Ontogeny and Systematics of Fishes. Special Publication No.1, American Society of Ichthyologists and Herpetologists. Allen Press, Lawrence, KS, USA, p. 398–405.

Gill, T. 1905. The life history of the sea-horses (Hippocampids). Proceedings of the United States National Museum 28:805–814.

Gudger, E.W. 1906. The breeding habits and the segmentation of the eggs of the pipefish, *Siphostoma floridae*. Proceedings of the United States National Museum 29: 447–500.

Hardy Jr., J.D. 1978. Development of Fishes of the Mid-atlantic Bight—an atlas of egg, larval and juvenile stages, Vol. 2: Anguillidae through Syngnathidae. Fish and Wildlife Service, US Dept. of Interior, Washington, DC, USA, 455p.

Herald, E.S. 1959. From pipefish to seahorse—a study of phylogenetic relationships. Proceedings of the California Academy of Sciences 29(13):465–473.

Hudson, L.L. and J.D. Hardy. 1975. Eggs and larvae of the Atlantic seahorse, *Hippocampus hudsonius*. University of Maryland Publication, CEES, Ref. 75-12CBL, 4 p.

Jungersen, H.F.E. 1910. Ichthyotomical contributions. II. The structure of aulostomidae, syngnathidae and solenostomidae. Kongelige Danske Videnskabernes Selskabs Skrifter. Naturvidenskabelig og Mathematisk Afdelin. Series VIII, 7:268–364.

Khalil, R.M. 1979. Histological Studies on the kidneys of the Red Sea syngnathiformes fishes. Bulletin of the Zoological Society of Egypt 29:28–36.

Linton, J.R. and B.L. Soloff. 1964. The physiology of the brood pouch of the male seahorse *Hippocampus erectus*. Bulletin of Marine Science 14:45–61.

Mito, S. 1961. Pelagic fish eggs from Japanese waters. I. Clupeina, Chanina, Stomiatina, Myctophida, Anguillida, Belonidae, and Syngnathida. Science Bulletin of the Faculty in Agriculture of Kyushu University 18: 285–310.

Nelson, J.S. 1994. Fishes of the World, 3rd. ed., John Wiley & Sons, NY, USA, 600 p.

Sudarsan, D. 1966[1968]. On the early development of the pipefish *Syngnathoides biaculeatus* (Bloch). Journal of the Marine Biological Association of India 8:222–224.

Takai, T. and A. Mizokami. 1959. On the reproduction, eggs and larvae of pipe-fish *Syngnathus schlegeli* Kaup. Journal of the Shimonoseki College of Fisheries 8:85–89. (in Japanese with English summary)

Wetzel, J.T. and Wourms, J.P. 1991. Parental-embryonic relationships in pipefishes and seahorses (Syngnathidae). American Zoologist 31:83A. (Abstract)

Wetzel, J.T. 1995. On the functional morphology of epidermal modifications in the skin-brooding fishes: *Platystacus*, *Solenostomus*, and *Hippocampus*. Ph.D. Dissertation, Clemson University, Clemson, SC, USA, 230 p.