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Normal Stages of Development of the Axolotl, *Ambystoma mexicanum*

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Illustrations and descriptions of normal stages of the axolotl *Ambystoma mexicanum* are given. The time to reach each morphological stage is compared with similar stages in two other species, *Ambystoma maculatum* and *Taricha torosa*.

INTRODUCTION

The Mexican axolotl, *Ambystoma mexicanum* (also called *Siredon mexicanum*), has long been used in laboratories around the world. This convenient laboratory animal probably will be used increasingly for experimental studies of development. We give here a series of normal stages for this species and data on the timing of the stages.

We have tried to make these normal stages of the axolotl as comparable as possible to Harrison's (1969) staged series of *Ambystoma maculatum* (*Amblystoma punctatum*). The Twitty and Bodenstein (1962) staged series of *Taricha torosa* (*Triturus torosus*), which was also modeled after Harrison's series, served as a useful supplementary guide. Gastrulation and neurulation of the axolotl looks more like that of *T. torosa* than of *A. maculatum*, but the timing of stages is most similar in the two *Ambystoma* species.

Development is, of course, continuous and the designated stages gradually grade into one another. The stages are based on changing external morphological features. The amount of time between different stages varies. For example, neurula stages 14–19 follow one another rapidly while days may separate successive larval stages.

The time between the stages is illustrated in Fig. 1. The data for Fig. 1 were compiled by rearing embryos in a constant-temperature box (18° ± 0.5°C), inspecting frequently, and recording the time since stage 1 that each stage first appears. Even at constant temperature and using eggs from the same mating, there is variation in timing of stages. Differences in egg size may contribute to this timing variation.

We have timing data for the California newt *Taricha torosa* (staged by Twitty and Bodenstein, 1962), which we present here for comparison (Fig. 2). The *T. torosa* data is taken from time-lapse movies. The movies were made at an exposure interval of 1 min. The movies were analyzed using a stop-motion projector equipped with a frame counter. A movie was run forward and backward until a decision could be reached as to what frame was the midpoint of each stage. The time since stage 1 could then be read directly in minutes from the frame counter. The timing in different movies was similar except through neurula stages.

After cleavage stages, *T. torosa* lags

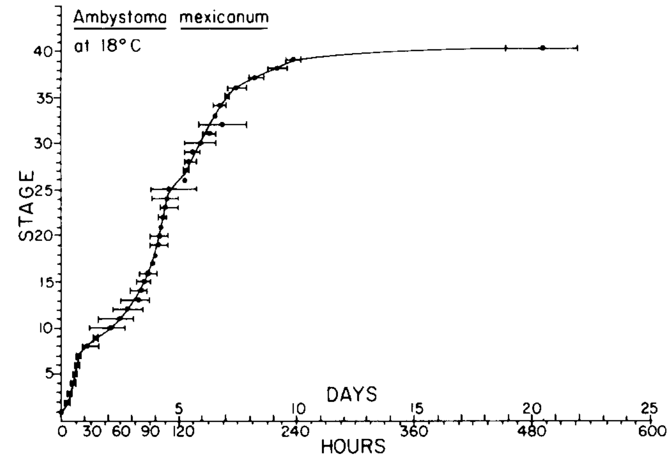

FIG. 1. Most points on this graph represent the average of several determinations. The horizontal lines through a point indicate the entire observed range of time that embryos reached that stage. The number of observations for each point varied from 1 to 22, the average is 7. Points without lines were single observations.

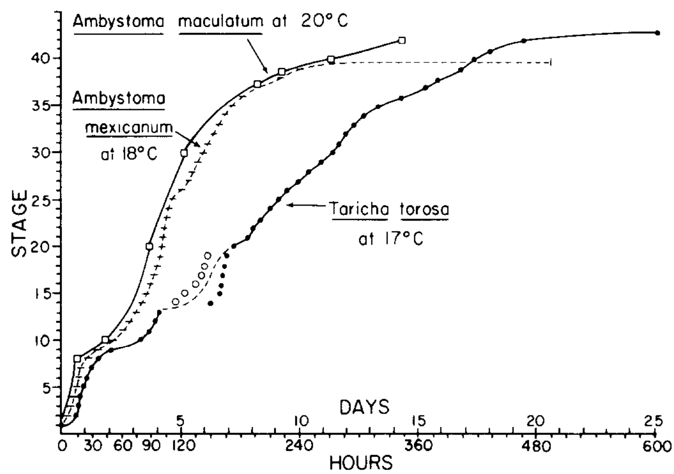

FIG. 2. *Taricha torosa* stage times were compiled from time-lapse movies of development of this species. The points represent the midpoint of each stage. Variation in time of stage is greatest during neurula stages and data from two films (open and closed circles) is given through this period. For comparison, we show our *A. mexicanum* time-stage curve, and Harrison's (1969) time-stage curve for *A. maculatum* (*Amblystoma punctatum*).

considerably in rate of development compared to *A. mexicanum*. Stage 28, for example, occurs about 5 days later in *T. torosa*. By stage 40, *T. torosa* has caught up.

Comparing the development of *A. mexicanum* to Harrison's (1969) time-stage graph for *A. maculatum* (Fig. 2), there is an almost exact parallel between the two species from stage 1 through stages 37–38. *A. maculatum* stages, measured at 20°C, are consistently a few hours earlier than *A. mexicanum* stages, measured at 18°C. *A. maculatum* reaches stage 37 after about 195 hr; *A. mexicanum* reaches stage 37 after 197 hr. After stage 37–38, *A. mexicanum* stages are very slow compared to *A. macalatum*. For example, *mexicanum* takes 488 hr to reach stage 40 while *maculatum* reaches stage 40 in just 265 hr.

We have illustrated the staged series

only through stage 40 when the larva hatches from the membranes and turns onto its belly. Older stages vary greatly depending on crowding, food, and other environmental factors.

Limb development of the axolotl is very much retarded compared to *A. maculatum*. Forelimb development of the stage 40 axolotl compares to that of the stage 36 *A. maculatum*. Hindlimbs first appear in *A. maculatum* at stage 43 after 17 days development (at 20°C). Hindlimbs first appear in the axolotl on the 56th day of development (at 18°C). The limb development of *A. maculatum* seen at stage 46 (22 days at 20°C) is attained in the axolotl at 18°C after 64 days of development (Fig. 3). The hind limbs of the axolotl larva are well developed with toes after 84 days of development (Fig. 3).

A discussion of the care and breeding of axolotls is presented by Fankhauser (1967).

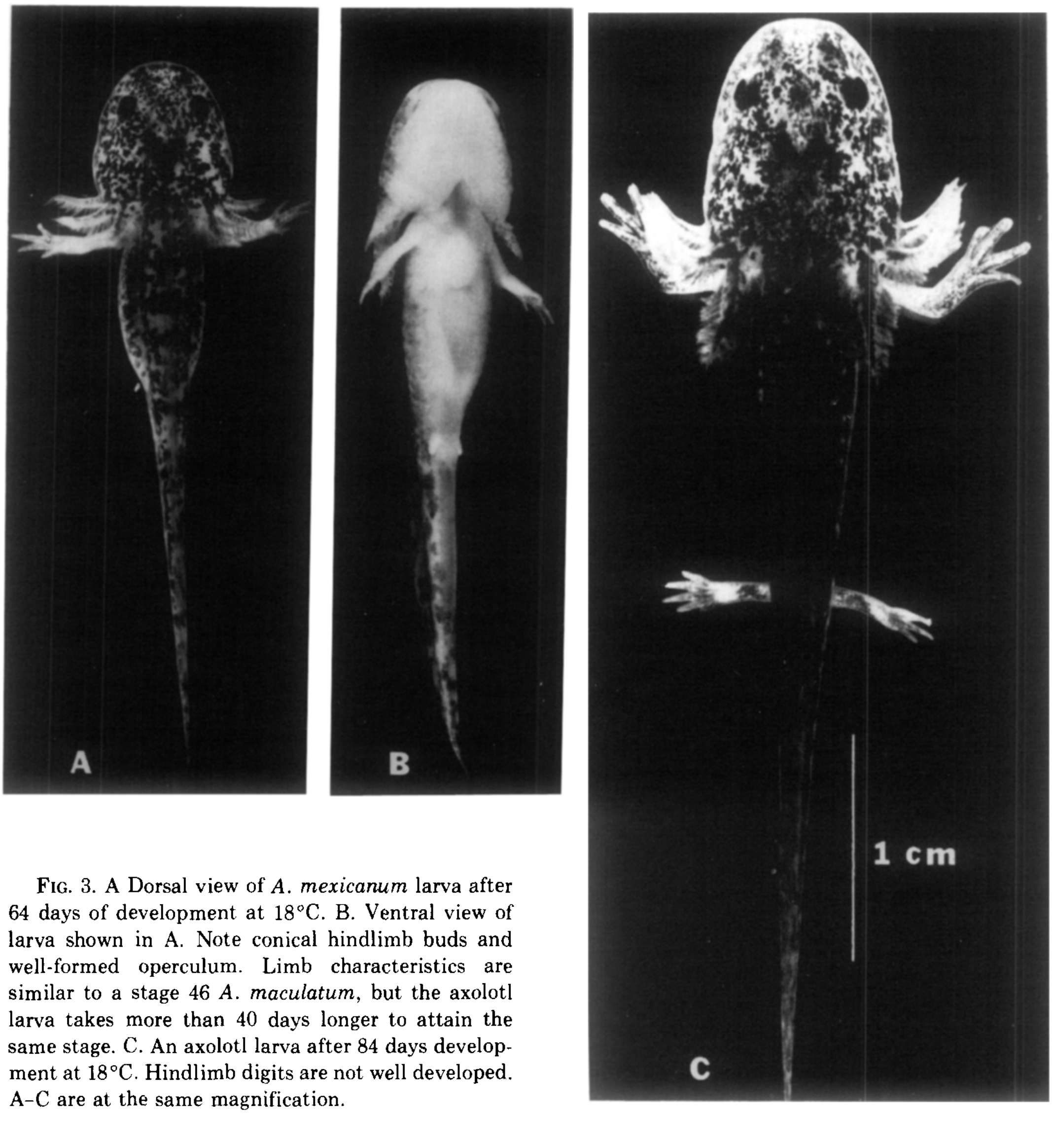

Fig. 3. A Dorsal view of *A. mexicanum* larva after 64 days of development at 18°C. B. Ventral view of larva shown in A. Note conical hindlimb buds and well-formed operculum. Limb characteristics are similar to a stage 46 *A. maculatum*, but the axolotl larva takes more than 40 days longer to attain the same stage. C. An axolotl larva after 84 days development at 18°C. Hindlimb digits are not well developed. A–C are at the same magnification.

The cleaving axolotl egg has been studied in detail (Skoblina, 1965; Meinertz, 1970; Signoret and Lesfresne, 1971; Hara, 1971; Rott, 1973). Ignat'eva (1968) described axolotl gastrulation and compares it to the sturgeon. C.-O. Jacobson (1959, 1962) described and mapped axolotl neurulation.

The illustrations of stages that follow (Fig. 4A–C) are photographs of living embryos, all at the same magnification. Except as noted below, all embryos are viewed from above as they naturally orient themselves when they are removed from the jelly capsules and placed unrestrained in a dish of water. The only exceptions are stages 9, 10, and 11 whose diagnostic feature, the forming blastopore, is visible only from beneath. These three embryos were photographed through an inverted microscope. The view of them is of their vegetal hemispheres.

Through cleavage stages (1–7), blastula stages (7–9), and early gastrula stages (9–11), the embryos rest with their animal poles uppermost. Between the end of stage 11 and the beginning of late gastrula stage 12, the embryo rotates, turning the future dorsal side uppermost. This change in orientation coincides with the formation of the archenteron and the disappearance of the blastocoel. The embryo retains this orientation from gastrula stage 12 through neurula stages 13–19. As neural tube formation approaches completion at stage 19, the embryo begins to lie on its side, is partially on its side at stage 20, and completely on its side from stage 21 through 39. Stage 39 lasts for many days and gradually grades into stage 40. At stage 40, larvae have turned onto their bellies. They hatch from the membranes about this time.

In the list that follows, each stage is briefly characterized to call attention to diagnostic features in staging. Note the progressive increase in length of the embryo from stages 25–40.

Stage 1. Uncleaved, single-celled egg. Cytoplasmic rearrangements occur during this stage. Polar bodies may often be seen near the animal pole.

Stage 2. Two cells. The cleavage furrow extends from pole to pole.

Stage 3. Four cells. The second cleavage furrow also extends from pole to pole at right angles to the first.

Stage 4. Eight cells. The third cleavage furrow forms along a latitude above the equator. The animal hemisphere quartet of cells is thus smaller than the quartet below.

Stage 5. Sixteen cells. Stage 5 animal hemisphere cells appear about half the size of stage 4 cells.

Stage 6. About 32 cells around a central cavity.

Stage 7. The upper cells appear about half the size of stage 6. Cleavage is becoming asynchronous even among the animal hemisphere cells. Stage 7 may be considered the end of cleavage stages and the beginning of blastula stages.

Stage 8. Blastula stage. The upper cells are about half the size of those at stage 7 at the beginning of stage 8. Stage 8 lasts 9–16 hr and the cells continue to divide, becoming too small to distinguish individually without magnification (Stage 8+).

Stage 9. Vegetal pole view. The first signs of gastrulation are pigment concentration (indicative of cell elongation perpendicular to the surface) and invagination at the midline of the future dorsal lip of the blastopore. An animal pole view does not appear much different from stage 8+.

Stage 10. Vegetal pole view. The dorsal lip of the blastopore is fully formed as a crescent and involution has begun.

Stage 11. Vegetal pole view. The lateral lips have formed making the blastopore into a semicircle.

Stage 12. Dorsal view. As the archenteron forms and the blastocoel recedes, the embryo rotates until it rests dorsal side up. This brings the blastopore into view from above. The ventral lip has formed to make the blastopore a complete circle surround-

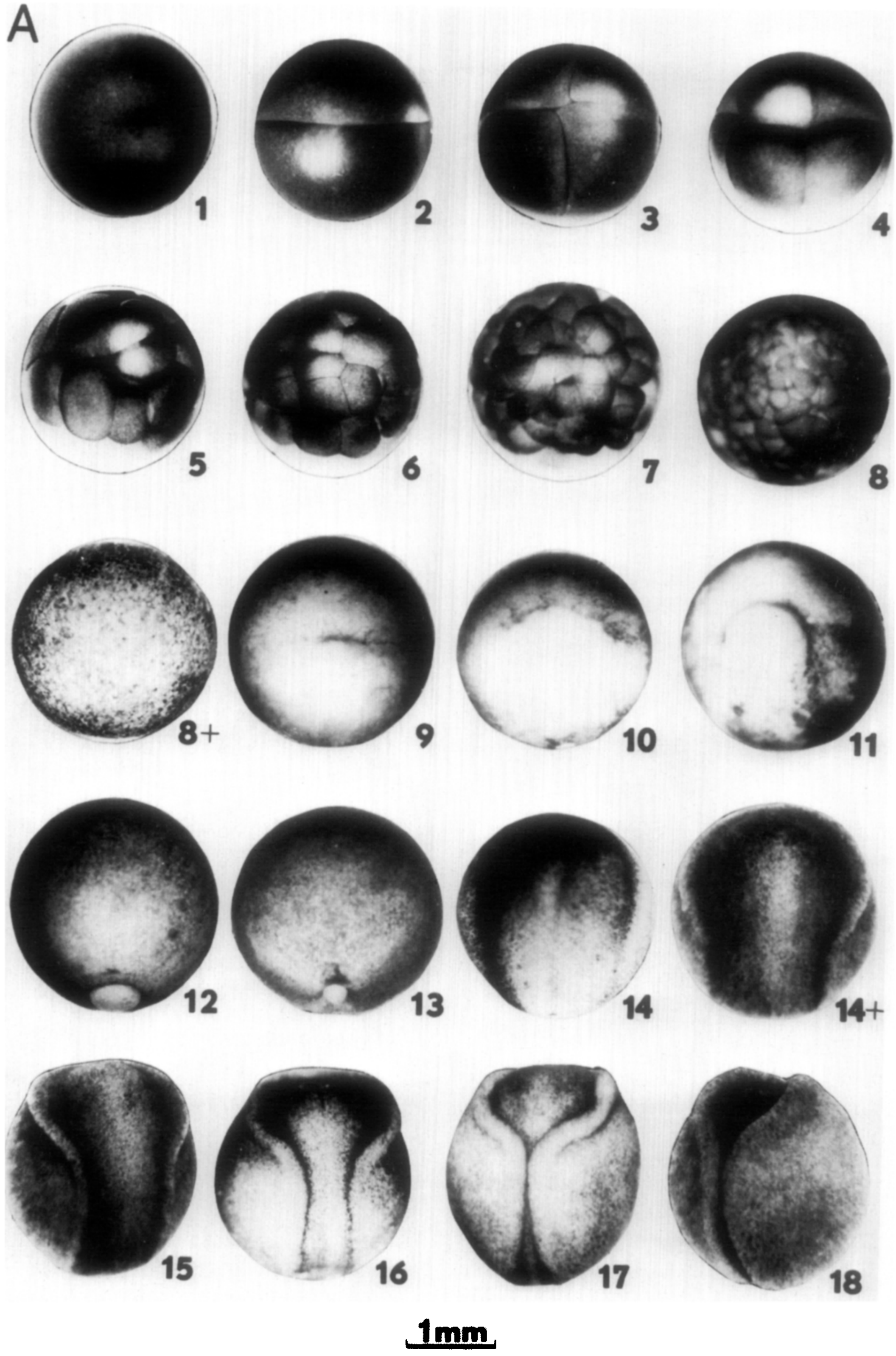

FIG. 4 A

FIG. 4. Cleavage, blastula, gastrula, and neurula stages (see text for descriptions). B. Late neurula and tail-bud stages (see text for descriptions). C. Late tail-bud and early larval stages (see text for descriptions).

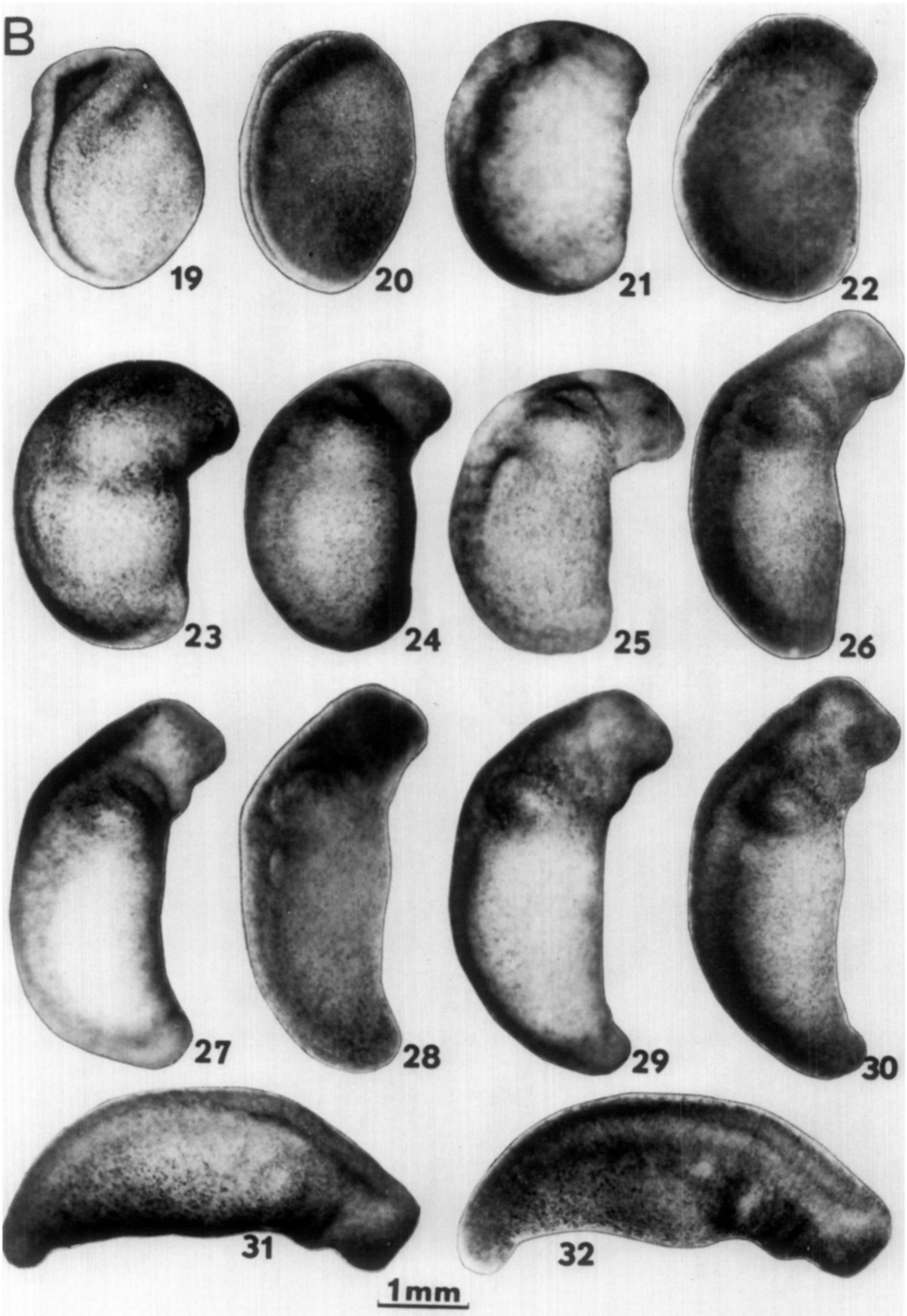

FIG. 4 B

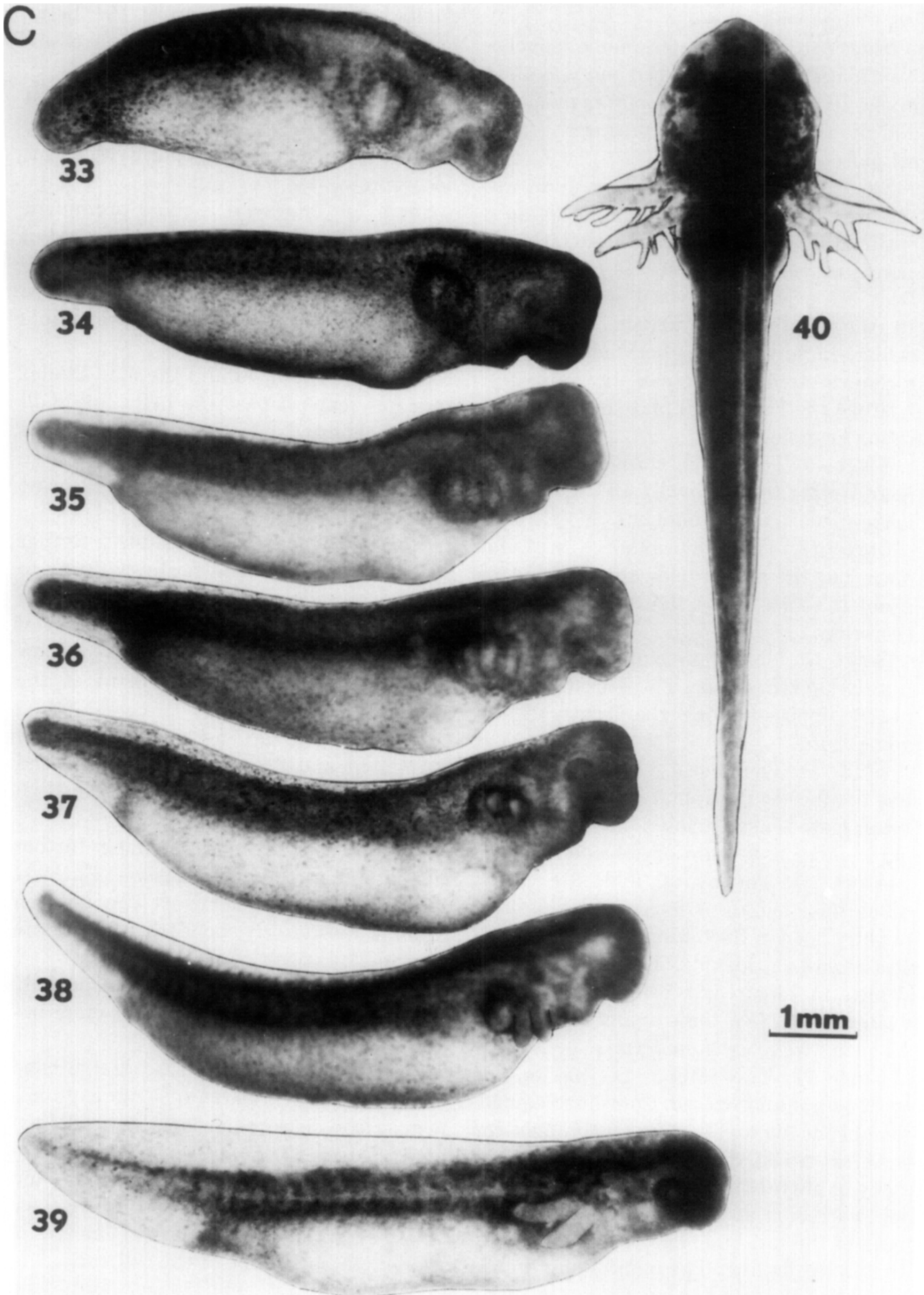

FIG. 4 C

ing a large yolk plug.

Stage 13. The yolk plug is much reduced. Neurulation has commenced and is evident in the flattening of the dorsal hemisphere and the thickening of the prospective neural plate.

Stage 14. The yolk plug is withdrawn; the blastopore is a slit. The neural plate is outlined by pigmentation concentration in elongated cells. By stage 14+, the neural folds are evident. Between stages 14 and 19, the length of the nervous system increases rapidly pushing both head and tail regions around the curve of the embryo.

Stage 15. The neural plate has distorted into a keyhole shape.

Stage 16. The spinal cord region of the neural plate has narrowed and the neural folds appear more prominent.

Stage 17. The brain plate is still flat open, but the neural folds are touching, or about to touch, where the spinal cord joins the brain.

Stage 18. The brain plate is still open, but rolling into a tube. The neural folds are nearly apposed along the length of the spinal cord.

Stage 19. The neural folds are touching one another in the spinal cord region. The brain plate is nearly closed into a tube. The embryo begins to turn on its side.

Stage 20. The neural folds are closed along their length. A few embryos may be slightly open in the brain area. The embryo lies almost on its side. The epidermis has drawn in along and below the nervous system so the first three or four somites can be seen through the epidermis.

Stage 21. The embryo lies on its side with neural folds closed. Continued elongation of the nervous system has brought the anterior tip of the head in line with the belly or slightly extending beyond. A similar protrusion at the tail end is the beginning of the tail bud. The optic vesicles are becoming distinct. The mandibular arch is apparent as a band below the brain.

Stage 22. The head is larger and protrudes more past the line of the belly. Further nervous system elongation is apparent.

Stage 23. The head protrudes well past the line of the belly. The tail end is slightly more developed. The pronephric swelling is becoming visible.

Stage 24. The head is more prominent and more clearly separated from the rest of the embryo by grooves anterior and posterior to the mandibular arch. The gill bulge is beginning. About 9 somites are visible through the epidermis.

Stage 25. The head and the mandibular arch now extend past the belly line. Gill and pronephric bulges are prominent. This stage is made quite distinctive by the large head protruding almost at right angles past the belly line.

Stage 26. The nervous system is further elongated and the head region is lifting to become more in line with the long axis of the rest of the embryo. The smooth curve of the top of the head of stage 25 is now interrupted by the abrupt bend of the cephalic flexure.

Stage 27. The tail end has become quite prominent and flexes ventrally. The head has further straightened and the branchial grooves appear more distinct.

Stage 28. The nervous system has further elongated and the head appears relatively larger. The pronephric duct can be seen running posteriorly beneath the somite margin.

Stage 29. The gill bulge is especially prominent. The tail end is also more noticeably distinct.

Stage 30. The head has straightened noticeably. The tail end is more prominent. Head features become more delineated. Stages 30–34 show a continued increase in nervous system length and straightening of the head and tail regions. These stages also show marked changes in the length and shape of the tail end.

Stage 31. Tail end longer and head straighter.

Stage 32. Tail end is longer, head is straighter. The gill bulge is more circumscribed.

Stage 33. Tail and head both straighter. Groove posterior to gill bulge more prominent. At this stage the body muscles become responsive. If pricked in the side, the animal bends toward the tormentor.

Stage 34. The nervous system is a straight line from tip of the tail to cephalic flexure. The heart begins to beat. Three divisions of the gills are first apparent.

Stage 35. The three gills are more prominent. Fins appear on tail and back. A few pigment cells make their appearance on the flank.

Stage 36. The three gill buds and the nasal pit lie in an almost straight line. Fins and tail have enlarged.

Stage 37. Pigmentation is more extensive. The gills protrude farther. Tail and fins are larger.

Stage 38. Gills are longer, finger-like, and project ventrally.

Stage 39. Gills are longer, point more horizontally and posteriorly, and begin to branch. Flanks and eye are much more pigmented. Fins are very extensive. The cloaca is more distinct. This stage lasts a long time and during it all these features change, especially the size and elaboration of the gills.

Stage 40. The distinguishing feature is the turning of the larva onto its belly. Hatching from the membranes occurs at this time. The gills are long and branched. Pigmentation is very extensive. Forelimbs are just beginning to become visible as bulges behind the gills.

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