

# ARTIFICIALLY METAMORPHOSED NEOTENIC CAVE SALAMANDERS

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## ABSTRACT

Five specimens of *Gyrinophilus pallescens* McCrady, collected from a cave in Franklin County, Tennessee in 1954, were subjected to powdered thyroid and three were used as controls. Those treated metamorphosed into salamanders, which after comparisons, were found to be distinct from species of *Gyrinophilus*, *Pseudotriton* and *Eurycea* in external appearances and dermal skull bones. One treated specimen is illustrated before metamorphosis, at nine months after metamorphosis, and at nine years, seven months after beginning metamorphosis. Controls failed to metamorphose in three years of captivity.

## INTRODUCTION

Neoteny refers to the development of functional gonads in animals which are structurally larval or immature. This condition is generally due to mutations and therefore can be inherited by descendants of neotenic animals. Neoteny may be an important aid to evolution. Specialized species of animals may, through their offspring, revert to a simple larval type. This neotenic animal's descendants can then more easily evolve through mutations to an animal entirely different from its specialized ancestor. There is evidence that the worm-like Hemichordates such as *Balanoglossus* evolved through neoteny from simple echinoderm larvae.

The classic example of neoteny is the Mexican axolotl salamander which has a larval external appearance (including gills) and a larval type skull, but breeds and is maintained in this condition throughout life unless treated with thyroxine from the thyroid gland of any animal. Then it will metamorphose and become a normal *Ambystoma tigrinum* adult salamander.

Dr. Edward McCrady (1954) published a detailed description of a new species of Plethodontid salamander, *Gyrinophilus pallescens*, from Sinking Cove Cave, Franklin County, Tennessee. Although achieving a larger size (up to 155 mm total length) than most larvae of Plethodontid salamanders, this animal was definitely larval in appearance and was considered to be neotenic by Dr. McCrady. He collected 31 specimens from caves in the region and kept some in aquaria up to one and a half years without any animals showing metamorphosis. In addition, the oviducts of the females were well-developed and some ovaries contained eggs 0.5 mm in diameter. As is usual in obligate cave vertebrates, they had reduced eye size and lacked pigment except for haemoglobin.

Twelve of these salamanders were collected by Dr. Albert P. Blair, Dr. Frederick R. Whitesell, and the author from Sinking Cove Cave on 25 August 1954, less than a month after McCrady's published description. Dr. Blair began subjecting two of these salamanders to drops of iodine-potassium iodide solution in a battery jar of water in his laboratory at the University of Tulsa, and the author began thyroxine experimentation with eight selected salamanders: half from the August collection and half from five collected

on 9 September 1954 by the author and Dr. McCrady from the same cave. One male in the August collection extruded a spermatophore from its cloaca upon fixation with formalin. This spermatophore was sectioned and its cap was found to contain sperm. It is shown in the paper by Lazell and Brandon (1962). Its occurrence gives conclusive evidence that at least some specimens are neotenic.

Blair's iodine treatment resulted in one completely metamorphosed salamander within three months and the other unaffected even after three more months of treatment (Blair 1961). Dent, Kirby-Smith and Craig (1955) published a short paper on the metamorphosis of 2 *Gyrinophilus pallescens* treated with thyroxine and another injected with thyroid stimulating hormone. In a later paper, Dent and Kirby-Smith (1963) gave the results of extensive experiments with thyroxine treatments, thyroid stimulating hormone injections, frog pituitary gland implants, and radioactive sodium iodide injections in metamorphosis induction studies. Changes in morphology during metamorphosis were described. They did not publish detailed studies of the skull bones nor did they attempt treatments on the larvae of normally-metamorphosing species of salamanders to determine if treatments produce abnormal adults.

Grobman (1959) studied the anterior cranial elements of *Pseudotriton* and *Gyrinophilus* and concluded that due to the close resemblance of their skulls, they should be congeneric and bear the older name *Pseudotriton*. Blair (1961) briefly described the cranial elements of his single metamorphosed *Gyrinophilus pallescens* which he considered *Pseudotriton pallescens*, following Grobman's opinion. Martof and Rose (1962) studied the anterior cranial elements of *Gyrinophilus* and *Pseudotriton* and concluded that the genus *Gyrinophilus* is valid. They did not include the skull of any artificially-metamorphosed *Gyrinophilus pallescens* in their study.

Mitchell and Redell (1965) described a new neotenic salamander *Eurycea tridentifera*, which is apparently intermediate between two genera, *Eurycea* and *Typhlomolge*, now supposed to be distinct. Further study on the problem posed by *E. tridentifera* may result in these genera being combined into one—*Eurycea*. A further question is whether or not the artificially-metamorphosed *G. pallescens* helps bridge the structural gap between *Pseudotriton* and *Gyrinophilus*. Since there is a fairly close relationship of *Eurycea* to *Gyrinophilus*, these genera are compared throughout this report.

This paper is a brief review of metamorphosis and a study of the anterior dermal bones of larval skulls and skulls in different stages of metamorphosis of *Gyrinophilus pallescens* for comparisons with body structures and skulls of certain species of normally meta-

morphosing *Gyrinophilus*, *Pseudotriton* and *Eurycea*. It is an attempt to answer the questions:

1. Does the skull and external appearance of metamorphosed *G. pallescens* help in clearing up the *Pseudotriton-Gyrinophilus* taxonomic controversy?
2. Is the skull of *G. pallescens* sufficiently distinctive to warrant making a new genus to accommodate it or should it be left in the genus *Gyrinophilus*?
3. Does the skull of a recently-metamorphosed *G. pallescens* undergo changes in bone shapes during subsequent years of life of the salamander?
4. Do thyroxine treatments result in abnormal skulls and abnormal external morphology in salamanders, whether neotenic or not?

#### MATERIAL AND METHODS

All eight neotenic salamanders (124.5 to 154 mm total length) mentioned above were placed in large fingerbowls containing small rocks and one inch of spring water. Bowls were kept at temperatures between 14 and 18 C in subdued light. All salamanders were fed earthworms every 2 to 4 weeks and occasionally small epigeal salamanders (*Eurycea*). A weekly application of a small pinch of powdered beef thyroid was made to the water surrounding five of the salamanders, leaving three untreated controls for this experiment.

Controls were preserved after three years. The five treated animals were killed with dilute alcohol and preserved as follows: 1 after 3 months of treatment; 2 after 9 months; and 1 after 3 years. The last remaining treated salamander died after 9 years and 7 months in captivity. Figures 1. through 4. show it before and after metamorphosis.

Mr. J. C. Nicholls, Jr. of Murphy, North Carolina furnished the author with three unmetamorphosed larvae of *Gyrinophilus danielsi dunnii* Mittleman and Jopson. These were subjected to powdered thyroid to determine if this treatment at the intervals and amounts given to *G. pallescens* produces abnormal effects such as a single premaxilla in the adult, etc. These were killed and preserved after metamorphosis.

The following species and their collecting data were used for comparisons with *G. pallescens*: 2 old adult *Gyrinophilus porphyriticus porphyriticus* (Green), Afton Cave, Greene County, Tennessee, 25 July 1946, sent by the late Professor Mike Wright to Dr. McCrady; 1 young adult *Gyrinophilus porphyriticus duryi* (Weller) [whose larval form was described by Rafinesque in 1832 as *Triturus lutescens* and reclassified by Mittleman in 1942 as *Gyrinophilus lutescens*], Carter Caves State Park, Carter County, Kentucky, 23 April 1960, given by James D. Lazell; 25 adult and 2 larval *Gyrinophilus danielsi dunnii* Mittleman and Jopson, Jacks River, Georgia, November 1961, sent by J. C. Nicholls, Jr.; 5 adult *Pseudotriton ruber ruber* (Latreille), Sewanee, Tennessee, June 1953; 1 adult *Pseudotriton ruber schenki* (Brimley), Macon County, North Carolina, 24 June 1947; and 2 adult *Eurycea lucifuga* (Rafinesque), Columbia, Tennessee, June 1952.

All salamanders, including the non-metamorphosing controls, were preserved in 10% formalin for at least 2 weeks, then cleared in 2% potassium hydroxide, stained in alizarin red S, destained in 3% potassium hydroxide,

and cleared in glycerine (Evans 1948). This enabled studies of all bones, particularly the anterior dermal bones of the skull.

Skulls were drawn with the aid of a camera lucida and dissecting microscope illuminated by both reflected and transmitted light. Skull lengths were measured from the anterior edge of the premaxillae to an imaginary line connecting the posterior ends of the occipital condyles. Skull widths were measured at the widest divergence of the pterygoids in the larvae and the maxillae in the adults.

#### RESULTS

The three untreated *G. pallescens* controls remained unchanged except for moderate size growth during the three years of captivity. Their skulls were indistinguishable from those freshly captured from Sinking Cove Cave (Figs. 9-10).

The five treated *G. pallescens* specimens showed much absorption of the caudal fin elevation after one week, and sloughing of skin and development of some orange pigmentation and hazy spots after two weeks. After two months, the gills were disappearing and by three months external metamorphosis was accomplished. The animals were frequently found out of water upon the small rocks for stays up to 24 hours.

In these treated animals, metamorphosis of the skull proceeded as would be expected, with the development of nasals, septomaxillaries, maxillaries, and prefrontals and the gradual loss of pterygoids. Parasphenoid teeth were slowly acquired and then lost in old age (Fig. 16). Nasal processes of the premaxillaries were always separate as in other species of the genus *Gyrinophilus* (Figs. 9-16).

The premaxilla of Plethodontid salamanders probably arises as a single center of ossification which in some species becomes separated anteriorly by bone-destroying cells. In *G. pallescens* the premaxilla is a single bone in all stages, from larvae with snout to vent length of 20 mm to old artificially metamorphosed adults.

Externally and internally the artificially metamorphosed *G. pallescens* differs considerably from normally metamorphosed species in every genus. *G. pallescens* lacks the light line from eye to nostril so conspicuous in naturally metamorphosing species of *Gyrinophilus* (Figs. 4 and 6). Its eyes are much less conspicuous and its skull is much more slender, being 1.68 to 1.72 times longer than the width at ends of maxillae in 4 specimens and 1.52 in the very old specimen. Width into length was 1.41 in the single *G. porphyriticus duryi* specimen, 1.31 to 1.36 in 25 *G. danielsi dunnii* specimens, and 1.34 to 1.36 in 2 *G. porphyriticus* specimens. The premaxillae are always single in metamorphosed *G. pallescens*, whereas they are 2 separate bones in adult *G. porphyriticus* and *G. danielsi* and their subspecies. The prefrontals border on the nares dorsally in *G. pallescens*, and this is also true in young adults of *G. porphyriticus*, but old adults of the latter species generally

have enlarged nasals which cover the anterior part of the prefrontals (Fig. 24).

Examination of additional specimens of cleared *G. palleucus* showed 25 out of 26 had 18 ribs. 25 specimens of *G. danieli dunnii* and 2 of *G. p. porphyriticus* had 19 ribs (Figs. 5 and 7). Rib number does not change during metamorphosis.

When the three larvae of *Gyrinophilus danieli dunnii* (which would have metamorphosed without treatment) were treated with thyroxine, they metamorphosed into adults indistinguishable from untreated metamorphosing examples of that subspecies. This shows that artificially administered thyroxine is not responsible for the differences between metamorphosed *G. palleucus* and other species of *Gyrinophilus*.

Although differing from Cope's original description of the genus *Gyrinophilus* in which two premaxillaries are specified, it differs more radically from species in the genera *Pseudotriton* and *Eurycea* which are closely related to the genus *Gyrinophilus*. *Pseudotriton* has a stout, well-rounded body (Fig. 8) and *Eurycea* has a slender body (Fig. 8) and *Gyrinophilus* a moderately stout body (Figs. 4 and 6). *Pseudotriton* has a single premaxilla in the skull, but the nasal processes of the premaxilla are fused and its nasal bones either come in contact or almost do so dorsal to these processes (Figs. 28, 30, 32). Grobman (1959) indicated that the nasal bones are in contact over the nasal processes of the premaxilla in recently metamorphosed *Pseudotriton* and that the nasals separate in old adults. Martof and Rose (1962) disagreed with Grobman and stated that the nasals of *Pseudotriton* develop long before metamorphosis and move together, not apart, as development proceeds. During this investigation, two recently metamorphosed *Pseudotriton* specimens showed the nasals already in contact and three old adults showed the nasals apart (Figs. 28, 30, 32), agreeing with Grobman (1959). Apparently this character is variable. The skull of *Pseudotriton* is more stubby and robust (1.20 to 1.53 times longer than width at ends of maxillae) than the skull of most *G. palleucus* specimens, which were artificially metamorphosed.

The genus *Eurycea* has a single premaxilla and there are usually separate (Fig. 34) or sometimes fused nasal processes of this bone (Rose and Bush, 1963). Adult *Eurycea* always have a distinct gap between the prevomerine and parasphenoid teeth (Fig. 35) instead of a continuous row of teeth from one bone to the other. In addition, the snout anterior to the eyes is much shorter in *Eurycea* than in any *Gyrinophilus* salamanders (Fig. 8).

Although the species *G. palleucus* now consists of three subspecies (Lazell and Brandon, 1962 and Brandon, 1965), little would be accomplished by describing a new genus to accommodate it. Its body proportions and therefore external appearances are certainly much more like other species of *Gyrinophilus* than like those of *Pseudotriton* and *Eurycea*. Its skull, although differing from others in the genus, differs to a greater degree from those of *Pseudotriton* and *Eurycea*. Finally, the

genus *Eurycea* contains species which differ among themselves to a greater degree (Rose and Bush 1963, Mitchell and Reddell 1965) than do *Gyrinophilus* species including *G. palleucus*.

Figures 1, 2, 3, and 4 illustrate changes associated with metamorphosis and old age in the same individual *G. palleucus* which lived 9 years and 7 months after capture and start of thyroid treatment. After metamorphosis, it was examined by R. A. Brandon, J. N. Dent, J. S. Kirby-Smith, J. D. Lazell and Dr. E. McCrady. Brandon noted, as did the author, the external resemblance in old age (Fig. 4) to metamorphosed *Typhlotriton spelaeus* Stejneger (See Fig. 103 (a) in Bishop 1943).

Neoteny may result from poor blood circulation in the thyroid so that thyroxine cannot escape in sufficient amounts into the blood stream. In such a case, the removed thyroid can be macerated and injected into the donor and produce metamorphosis. It may result from deficient blood circulation to the skin and lungs (when present) so that any metamorphosing salamanders die during gill reduction and only those retaining gills throughout life survive. It may result from iodine insufficiency or from inability of the body to react to thyroxine or to a very sluggish or a slow-iodine-absorbing thyroid. According to Dent and Kirby-Smith (1963), injected radioactive sodium iodide was taken up very slowly by the thyroid in *G. palleucus*. Enough iodine is present in Sinking Cove Cave water to enable metamorphosis in *Desmognathus fuscus* and *Eurycea bislineata* salamanders living in water flowing from the cave entrance.

Due to the absence of green plants (producers) in caves, and to the direct and indirect dependency of cave organisms on organic material washed in or brought in from the outside, food is frequently the most important limiting factor in the survival and reproduction of cave organisms. Apparently much energy is needed by salamanders to accomplish metamorphosis. In food-scarce caves, natural selection might select for survival those individuals which through random mutations fail to undergo metamorphosis, but become sexually mature. The other individuals may starve during metamorphosis and not survive to reproduce. This may account for the frequency of neoteny in cave environments.

If proven neoteny is not acceptable as a character for separating species of animals, then artificially metamorphosed (if possible) specimens must be used for comparisons with known species. In the case of artificially metamorphosed specimens of *Gyrinophilus palleucus*, this species has proven to be distinct from any other species of the genus. This experiment of artificial metamorphosis is a sort of induced evolution in reverse, for thyroxine treatment has returned the derived form to its ancestral morphology.

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the use of specimens of *G. palleucus* collected prior to 1950, Mr. J. C. Nicholls, Jr. for specimens of *G. danielsi dunnii*, and Mr. J. D. Lazell for the specimen of *G. p. duryi*.

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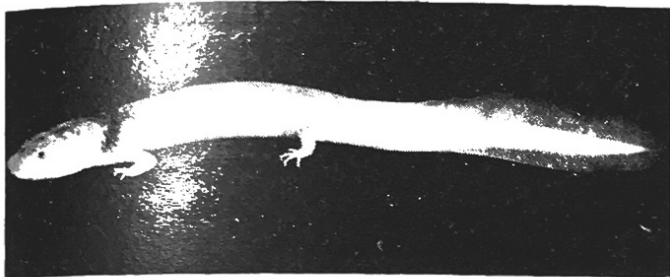


Fig. 1. Neotenic, live *Gyrinophilus palleucus* 138 mm. in length. Note large gills, large caudal fin, and small eyes.

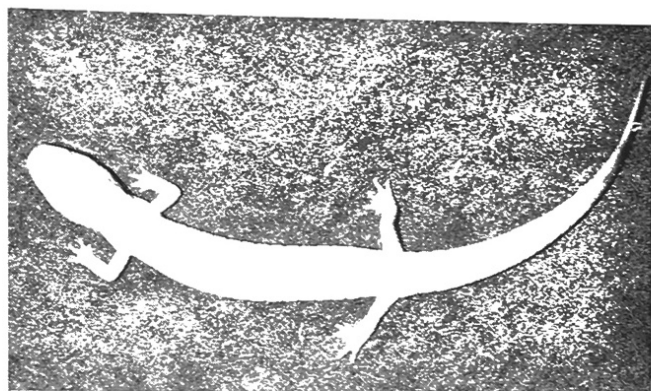
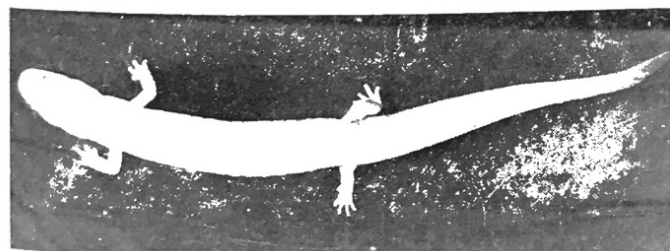
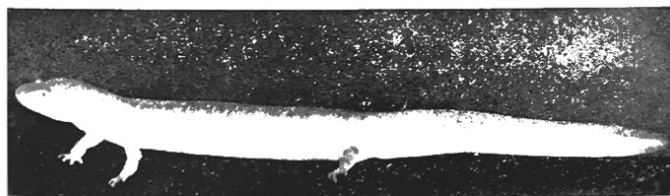


Fig. 4. Dorsal view of live *Gyrinophilus palleucus*, 9 years and 7 months from start of metamorphosis with thyroxine. Note pigment pattern still faint and eyes inconspicuous, snout elongated, and absence of light line from eye to nostril.



Figs. 2 and 3. Dorsal and lateral views of live *Gyrinophilus palleucus*, metamorphosed 9 months with thyroxine. Note faint pigment pattern, much reduced caudal fin, absence of gills, but eyes still inconspicuous, and snout elongated.



Fig. 5. Dorsal view of alizarin red preparation of skeleton of *Gyrinophilus palleucus* metamorphosed 3 years with thyroxine, lower jaw removed. Note narrow snout and 18 ribs.

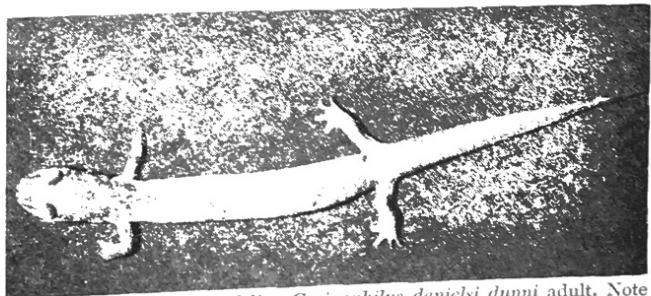


Fig. 6. Dorsal view of live *Gyrinophilus danielsi dunnii* adult. Note prominent eyes and conspicuous light line from eye to nostril.



Fig. 7. Dorsal view of aliarin red preparation of skeleton of *Grinophilus danieli durni* adult, lower jaw removed. Note rounded snout and 19 ribs.

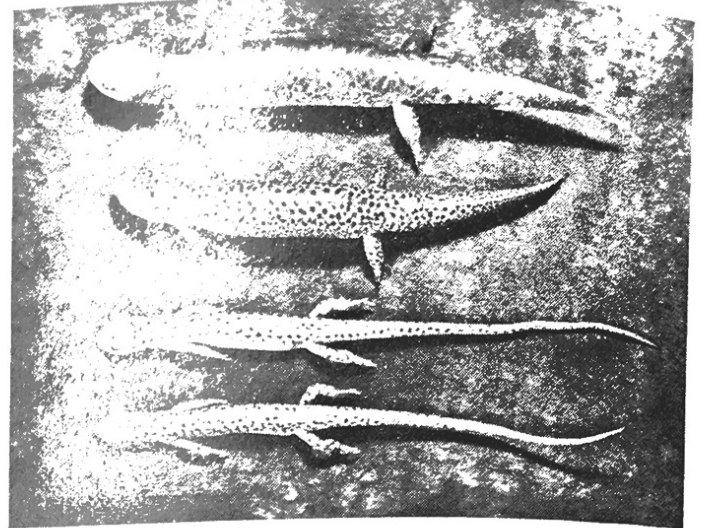
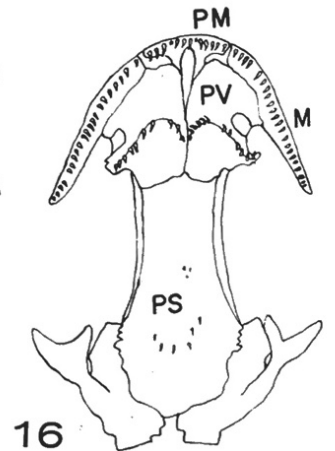
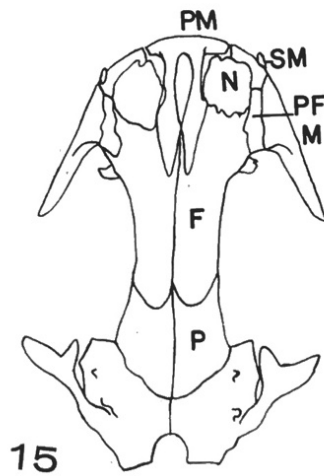
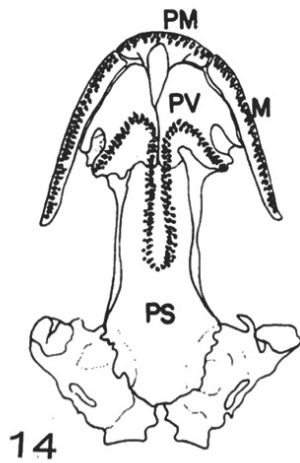
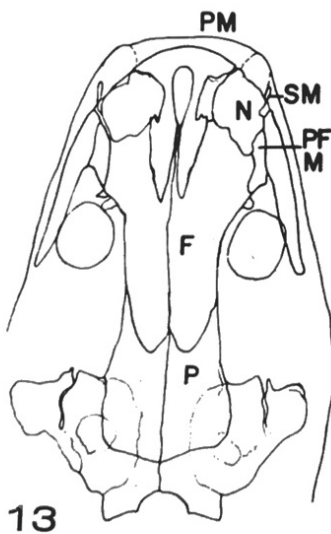
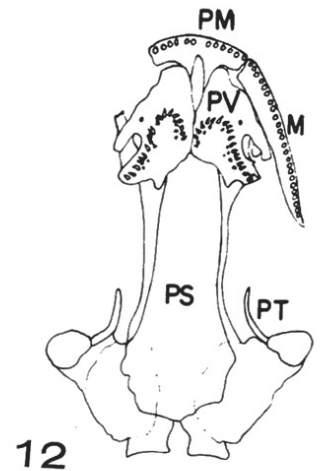
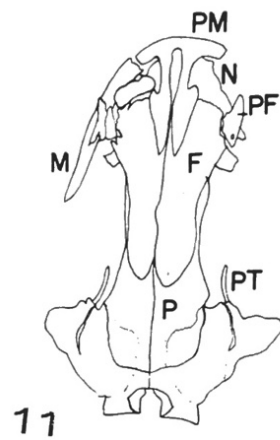
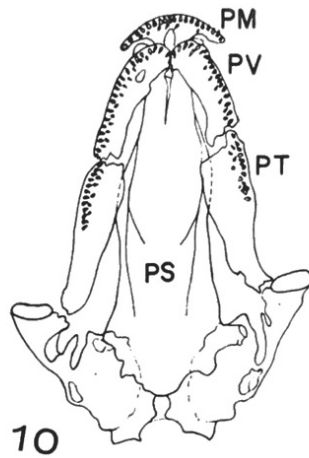
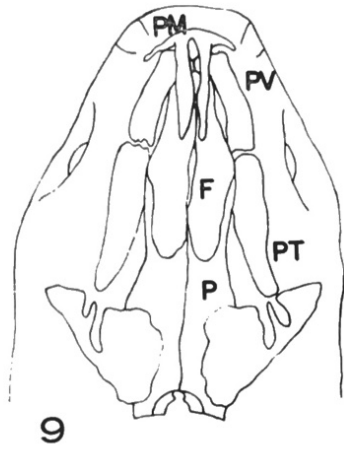


Fig. 8. Dorsal view of the very slender cave salamander, *Eurycea lucifuga* (lower) and the robust red salamander, *Pseudotriton ruber* (upper), for comparison with photos of the moderately robust species of *Grinophilus*.



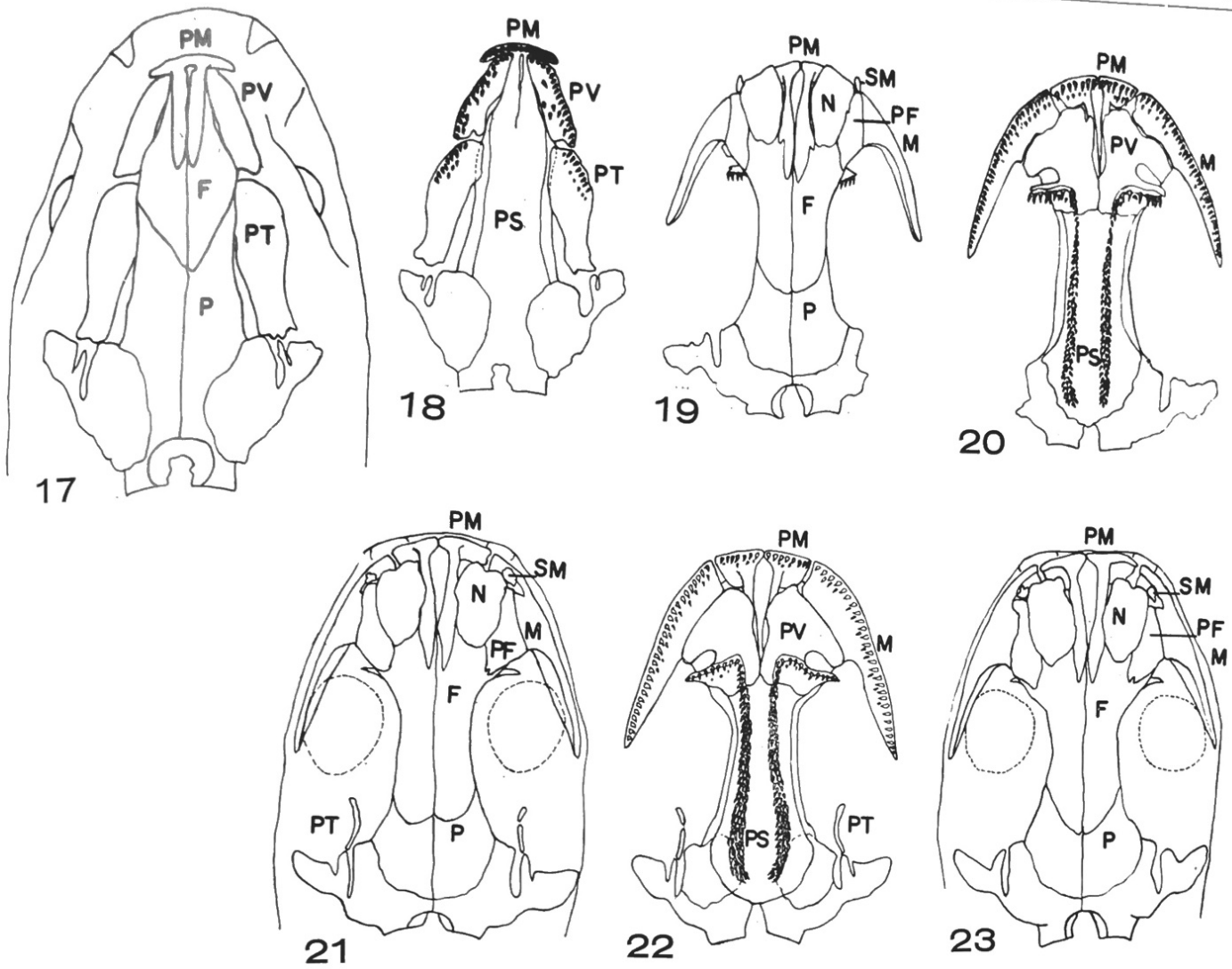
For all skull figures PM = premaxilla, M = maxilla, SM = septomaxilla, N = nasal, PV = prevomer, PT = pterygoid, F = frontal, PF = prefrontal, P = parietal, and PS = parasphenoid.

Figs. 9 and 10. Dorsal and ventral views of unmetamorphosed larval *Grinophilus pallescens* skull.

Figs. 11 and 12. Dorsal and ventral views of *G. pallescens* skull (damaged), 9 months from start of metamorphosis. Note parasphenoid teeth have not been developed and pterygoids have not completely disappeared.

Figs. 13 and 14. Dorsal and ventral views of *G. pallescens* skull, 3 years from start of metamorphosis.

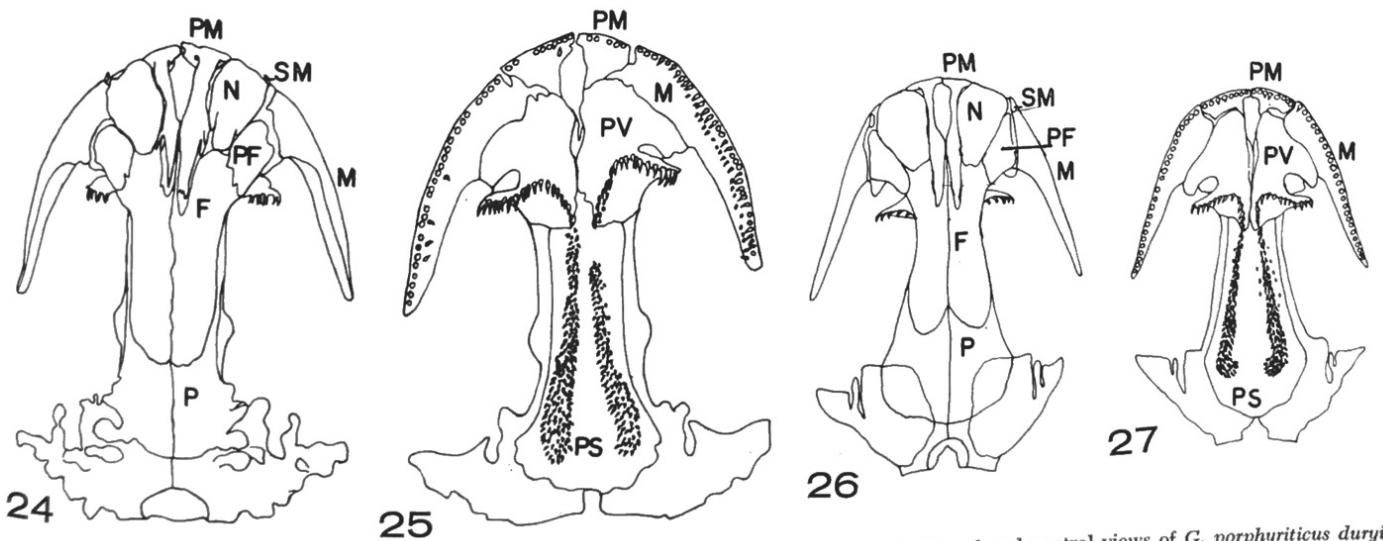
Figs. 15 and 16. Dorsal and ventral views of *G. pallescens* skull, 9 years and 7 months from start of metamorphosis. Parasphenoid teeth almost entirely lost and premaxilla still undivided, but with separate nasal processes.



Figs. 17 and 18. Dorsal and ventral views of larval *G. danielsi* skull.

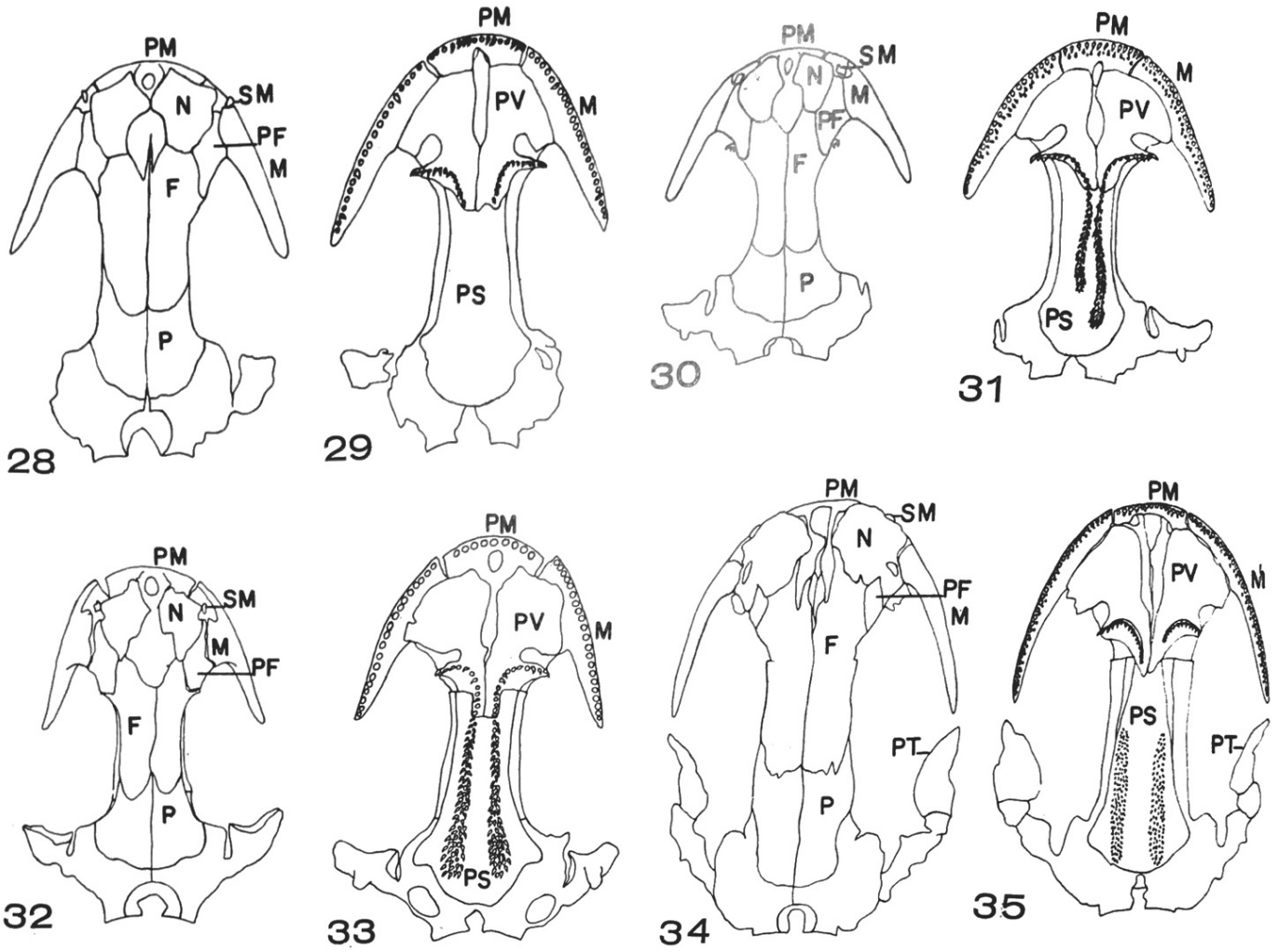
Figs. 19, 20, 21 and 22. Dorsal and ventral views of *G. d. dunni* skulls, both about 9 months from start of metamorphosis.

Fig. 23. Dorsal view of *G. d. dunni* skull; old adult.



Figs. 24 and 25. Dorsal and ventral views of *G. porphyriticus porphyriticus* skull; old adult.

Figs. 26 and 27. Dorsal and ventral views of *G. porphyriticus duryi* skull.



Figs. 28 and 29. Dorsal and ventral views of *Pseudotriton ruber ruber* skull, recently metamorphosed. Damaged, but note fusion of nasal processes of premaxilla, nasals touching in mid line, and absence of parasphenoid teeth.

Figs. 30 and 31. Dorsal and ventral views of *Pseudotriton ruber ruber* skull, damaged; old adult.

Figs. 32 and 33. Dorsal and ventral views of *Pseudotriton ruber schenki* skull; old adult.

Figs. 34 and 35. Dorsal and ventral views of *Eurycea lucifuga* skull, year old adult. Premaxilla single, nasal processes of premaxilla unfused, gap between prevomarine and parasphenoid teeth.