STUDIES IN COMPARATIVE BIOCHEMISTRY AND MORPHOLOGY OF THE SALAMANDER GENUS DESMOGNATHUS (AMPHIBIA: CAUDATA)

by

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This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Introduction

One of the prime purposes of this study was to test the usefullness of biochemical characters such as lactate dehydrogenase. The variation of electrophoretic patterns and morphology was investigated. It was then possible to determine the correlations of these characters to each other, and to the adaptive trends and systematics of the genus Desmognathus. Based on the techniques outlined below, I have determined the clearest limits possible using this approach and written a diagnosis which sets off the group defined by the limits.

The genus Desmognathus, restricted to the eastern half of North America, (see Fig. 1), exhibits the range of adaptive zones for the entire lungless family Plethodontidae. The range is from aquatic to terrestrial, with no paedomorphic species. Martof (1962) discusses a possible case of incipient neoteny in Leurognathus marmoratus. The subfamily Desmognathinae includes, besides Desmognathus, the monotypic genera Leurognathus and Phaeognathus. These have been shown to be distinctive from the twenty genera of the lungless subfamily Plethodontinae (Wake, 1966). The desmognathines, (referring to the subfamily), are an old group which has remained primarily within the ancestral adaptive zone of semi-aquatic, montane, warm temperate niches. The family's center of distribution is North America, with the greatest concentration and diversity of species in the southern Appalachians. This region of eastern North America is postulated to be the center of origin for the Plethodontidae (Dunn, 1926). Of the Desmognathinae, only D. fuscus,

perhaps D. ochrophaeus, and Phaeognathus hubrichti have successfully invaded the coastal plain. In the most recent treatment of the family, Wake (1966) recognized eight species of Desmognathus. While only six of these are considered here, the two questionable forms, D. ocoee and D. auriculatus are considered in the systematic summary.

Hairston (1949) clearly demonstrated an adaptive trend within the genus. His study, and those of Organ (1961a and b) and Harrison (1967) have placed each species within a progressive series from aquatic to terrestrial; quadramaculatus-fuscus-monticola-ochrophaeus-aeneus-wrighti. Leurognathus can be placed at the beginning of this series. Phaeognathus is tentatively located at the end with D. wrighti since its life history is not completely known.

If evolution occurs at different levels of organization (e.g. biochemical, tissue, absolute size) within the individual, then evidence for it, whether concordant or discordant, should be found in biochemical as well as morphological characters. The study of lactate dehydrogenase is of interest because the genetics of most morphological characters in salamanders is not understood. Laboratory crosses have not been possible as in anurans (Moore, 1944; Volpe, 1954). Therefore, genetic similarity and gene flow within a species can at best be implied. Lactate dehydrogenase appears to be controlled by two genes in those forms studied by Kaplan (1964) and Markert and Massaro (1968). Electrophoresis indicated similar control of lactate dehydrogenase in Desmognathus. This appraach is not enough to make phylogenetic conclusions. The fact that Desmognathus and the subfamily Desmognathinae are a natural group of species more closely

related to each other than any other plethodontid salamander made them an ideal group to test the usefullness of biochemical characters. In a closely related group, similar patterns imply close relationship and convergence can less easily confuse the results. The correlation of biochemical and morphological approaches offers a powerful tool for our perception of species relationships.

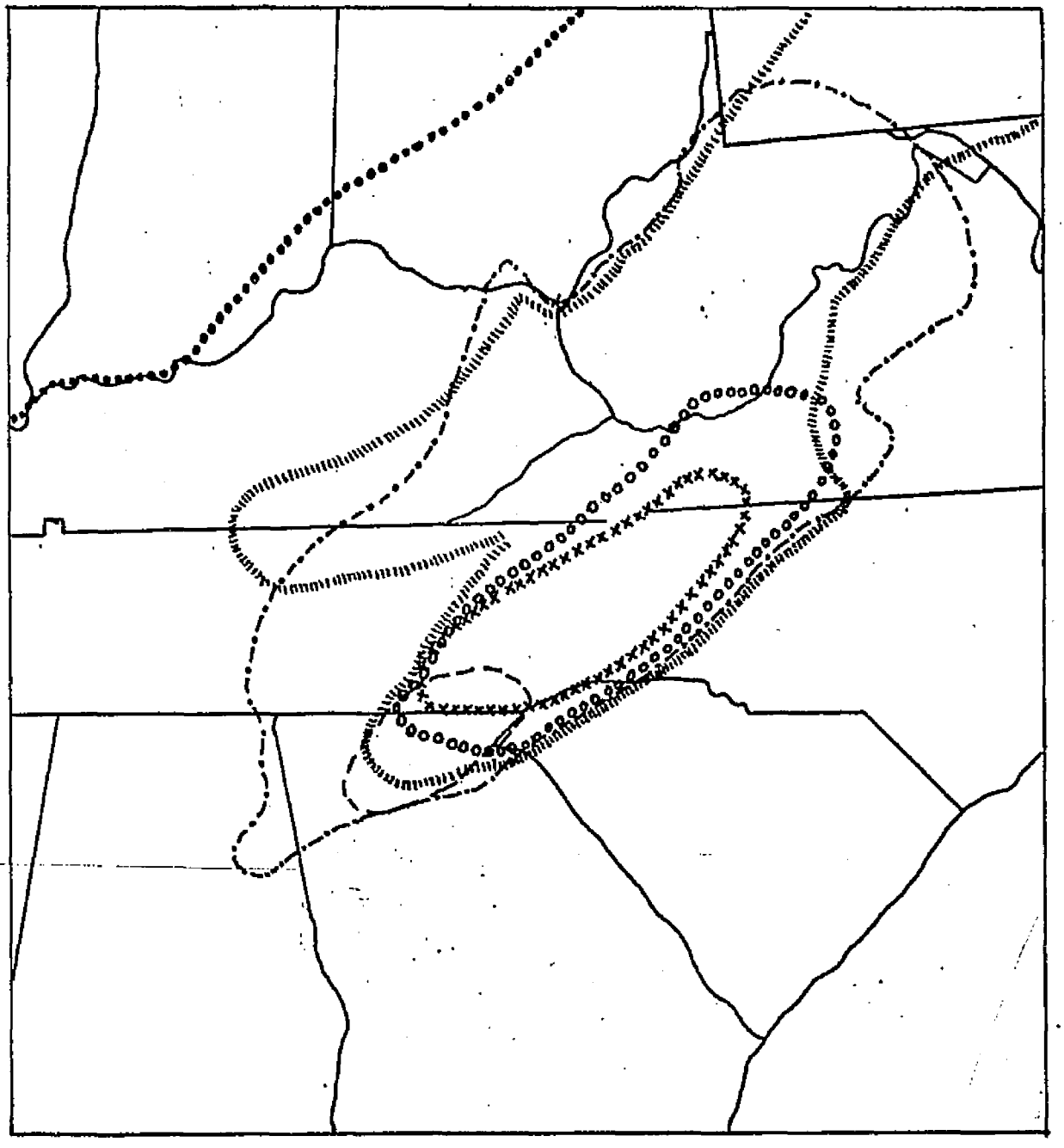


Fig. 1. Distribution of Desmognathus species in southern Appalachians.

Materials and Methods

Field work was conducted during the summer of 1968 to acquire fresh specimens for the study of lactate dehydrogenase. The proteins of preserved specimens are denatured and unavailable for the type of analysis used. Each sample was quick frozen in liquid nitrogen as described below. The majority of specimens used in this study were from the author's personal collection made during the summers of 1966 and 1968. Where material was inadequate or lacking for morphological comparison, as in the case of Leurognathus, specimens were borrowed from the American Museum of Natural History.

Desmognathus is easy to find and collect in most streams of the southern Appalachians. The following states were sampled, (see Figs. 1 and 6); Virginia, West Virginia, North Carolina, South Carolina, Tennessee, Kentucky and Arkansas.

In the course of collecting, a number of the localities listed by Martof and Rose (1963) were sampled for comparison with their data on D. ochrophaeus. An attempt was made to collect from ten to twenty specimens per locality per species, although this was not always possible. At the time of collection, field numbers were assigned and the species identified. Air and water temperatures were taken with a Schultheis "Quick Recording Thermometer". Elevation was measured by an altimeter (Airguide Instrument Co., Chicago, Illinois) which was found to be accurate within ± 100 ft. Time of day was recorded along with habitat notes.

Electrophoresis of Lactate Dehydrogenase (LDH)

Specimens were individually thawed and dissected ventrally. After sexing and recording the condition of the gonads, tissue for electrophoresis was removed for study. In males, teste lobes were counted and their length measured. In females, ova number and size were recorded as well as the relative amount of yolk. Stomachs, the principle tissue used, were removed, emptied and washed with Amphibian Ringer's solution. Each animal was tagged and refrozen until electrophoresis was completed, at which time it was preserved for additional study. Tissue was ground in 0.25 M sucrose solution (0.1 - 1.0 ml. depending on the quantity of tissue). Crude extracts were stored frozen until used. In most cases it was not necessary to centrifuge stomach extracts as it was for the liver extracts used by Shontz (1968). Since all electrophoresis was carried out with individual samples, (see Fig. 6), extracts were never pooled.

The following equipment was used for the study of LDH: 1.) Hiller "V" set for vertical starch gel electrophoresis, 8 samples/gel, (Otto Hiller, Madison, Wis.); 2.) Heath Variable Power Supply - 0 to 400 volts, with choice of voltage or current regulation; 3.) Zeiss spectrophotometer modified for temperature control. With current regulation, voltage drops of over 100 volts occurred. For electrophoresis, the best results were obtained with the power supply set for voltage regulation. After 10 hours, voltage changes were less than 5 volts and current changes less than 3 milliamps. The gel was run (power supply on) in a

refrigerator at about 4° C.

Chemicals and solutions used were as follows:

1) Hiller Electro Starch (lot 98)

2) Tris-borate buffer (Shontz, 1968, after Nance, et. al., 1963) pH 8.6

stock solution 0.02 M EDTA
0.50 M boric acid
0.90 M tris (Sigma Trizma Base)

stock dilutions gel 1:20
cathode 1:8
anode 1:6

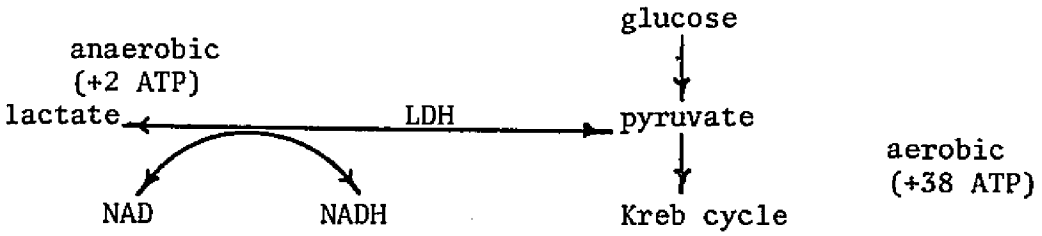
3) stain for LDH
2.25 ml. 1.8 M sodium lactate (50 ml. Sigma syrup and 29.5 ml. distilled water)
0.90 ml. NAD (30 mg./ml.)
1.20 ml. nitro blue tetrazolium (1 g./100 ml.)
0.18 ml. phenazine methosulfate (5 g./ml.)
34.92 ml. 0.1 M Tris (prepared fresh from 3.49 ml. 1.0 M Tris and 30.67 ml. distilled water)

Eleven percent starch gels (55 g. starch/500 ml. buffer) were found easy to handle in slicing and staining. All electrophoretic runs lasted 10 hours at 330 volts, 8 milliamps.

Migration of bands (reduced Nitro Blue Tetrazolium) was measured with a millimeter ruler to the nearest 0.5 mm. As a control, maximum migration was recorded relative to the migration of band 3 (M_2H_2) of purified rabbit muscle LDH (Sigma) which was run on every gel. Enough extract was prepared for the entire series of experiments. The control allowed for differences in migration between gels due to variation in time, voltage, gel consistancy, buffer pH, etc. While differences were small, comparison between gels even of the same extract might have been questionable without

this control. Relative migration made possible inter-gel comparison with some degree of confidence. Markert and Faulhaber (1963) used relative migration to compare thirty species of fish. This was tested by comparing runs of 10 and 12 hours and finding the same values for relative migration. The rate of reaction (conversion of NADH to NAD) for studies of substrate inhibition was measured in the Zeiss spectrophotometer at 25° C.

Fig. 2 Pyruvate-lactate Pathway



Lactate Dehydrogenase

When pyruvate is produced in excess, as in rapidly exercising muscle, oxygen (the final acceptor of the hydrogens stripped from glucose) is rapidly used up. As a result, (see Fig. 2) pyruvate is converted to lactate, which is stored temporarily. In the process NAD is regenerated. Cells then continue to net 2 ATP from glycolysis.

Two major types of LDH have been found (Kaplan, 1964; Markert, 1963) which fit into the above explanation. The first, found in muscle usually subjected to stress and an oxygen debt (e.g. skeletal muscle), is non-substrate inhibited. With increasing concentrations of pyruvate the reaction to lactate increases in rate to a maximum and then reaches a plateau. This type

is designated muscle-type LDH or MLDH. The second, found primarily in heart muscle or well oxygenated tissue is substrate inhibited. With increasing concentrations of pyruvate the reaction rate will increase to a maximum and then decrease. This type is designated heart-type LDH or HLDH. In reality, both types are substrate inhibited, but MLDH to a lower degree, (see Fig. 7). Cells grown in culture under aerobic conditions will produce HLDH primarily. If conditions are made anaerobic the same culture begins to produce more MLDH. This phenomenon has been observed to a similar extent in a great number of amphibians. Salthe (1965) found that forms living in what he characterized as aerobic environments (cold montane streams, terrestrial lunged amphibians) had a predominance of HLDH. The lungless plethodontids, while predominantly terrestrial and found in montane habitats, showed substrate inhibition characteristic of anaerobic forms. This was true for Desmognathus. My results for Desmognathus agree with Salthe's (1965), and the substrate inhibition curves of Phaeognathus hubricthi and Leurognathus marmoratus showed a similar pattern.

Enzyme Structure

The active LDH enzyme is a tetramer resulting from the random combination of two subunits (Kaplan, 1964; Fondy and Kaplan, 1965). These subunits appear to be controlled by two different loci. If designated M and H, the subunits combine to form five different forms or isozymes; M_4, M_3H, M_2H_2, MH_3 and H_4. The subunits are designated as such because MLDH has relatively more of the isozymes

M_3H and M_4, while HLDH has relatively more H_4 and MH_3. The subunits differ primarily in amino acid content and net charge (resulting from amino acids with different charges). If the charges are considered to be additive when the subunits are combined in a tetramer, (see fig. 3), then an M with a net charge of -1 will combine to form an M_4 with a net charge of -4. If the H has a value of -2 then the H_4 will be -8, the H_3M will be -7, etc. In cells heterozygous for the gene producing H subunits a total of fifteen isozymes could be formed (Costello and Kaplan, 1963; Salthe, 1969). Subbanding, as shown in fig. 3, would be indicative of heterozygotes. Its absence, however, does not necessarily mean that there are no heterozygotes. The negative charge will determine how far the isozyme migrates towards the anode when the enzyme mixture is placed in an electrical field across a supporting medium such as starch gel. Actually, the charges are not strictly additive since there is a certain degree of interaction between the subuints in the tetramer which may be characteristic for a species (see discussion below). Staining for LDH (Fine and Costello, 1963) consists of soaking the gel in the stain listed above (see p 17). Wherever there is a concentration of an LDH isozyme, lactate will be converted to pyruvate with hydrogens being transferred to PMS (phenazine methosulfate) via NAD. The reduced PMS then combines with the colorless NBT (nitro blue tetrazolium) to form an insoluble, dark blue product. The bands seen on a stained gel are the reduced dye, showing how far each isozyme has migrated. In this paper, the bands are shown in outline as only their position and not staining intensity is considered.

Fig. 3 Result of charge differences in two H subunits on the position (migration) of LDH isozymes following vertical starch gel electrophoresis.

Net charge is additive. A hypothetical example.

Subunit	Net Charge	Homozygotes		Heterozygote	Total Migration
M	0.0				
H	+1.0	O^{-}_{+}— M_4 ——	M_4 ——	M_4 ——	0.0
H'	+1.2				
		M_3H		M_3H	1.0
			M_3H	M_3H	1.2
		M_2H_2		M_2H_2	2.0
				M_2HH'	2.2
			$M_2H'_2$	$M_2H'_2$	2.4
		MH_3		MH_3	3.0
				MH_2H'	3.2
				MHH'_2	3.4
			MH'_3	MH'_3	3.6
		H_4		H_4	4.0
				H_3H'	4.2
				$H_2H'_2$	4.4
				HH'_3	4.6
			H'_4	H'_4	4.8

Morphology

Several of the samples analyzed electrophoretically were also studied morphologically (see Appendix). Drawings were made with a Wild binocular microscope fitted with a camera lucida. Specimens were measured and dissected under an AO binocular microscope with an ocular micrometer. Certain larger measurements were by necessity taken with a Helios replaceable point dial caliper. Readings were taken to the nearest 0.1 mm. A description of the measurements follows. Numbers refer to figs. 4 and 5. These measurements were chosen because they help define head and body shape, allowing comparison between species.

1. standard length - (snout-vent) distance ventrally from tip of snout to anterior point of vent.
2. trunk length - distance between points of forelimb and hindlimb insetions, mid-ventrally.
3. head width - measured dorsally at widest dimension, usually just posterior to angle of jaw, and including the bulk of the m. interhyoideus posterior.
4. tail depth - measured at posterior point of vent.
5. tail width - measured at posterior point of vent.
6. right hindlimb length - antero-ventral point of insertion to bend at tarsus, not including pes.
7. head length - snout to gular fold, measured ventrally.
8. tail length - from posterior point of vent to tip of tail. Broken or obviously regenerating tails were measured but were not included in the analysis.
9. snout length - snout to eye measured dorsally from tip of snout the shortest distance to the eye.
10. nares-eye distance - measured dorsally from posterior edge of nares to eye.
11. eye diameter - measured dorsally along the antero-posterior axis of the eye.

12. distance between eyes - the shortest distance.

13. distance between external nares - the shortest distance.

14. snout-forelimb insertion - measured ventrally from tip of snout to antero-ventral point of insertion.

15. right forelimb length - antero-ventral point of insertion to bend at carpus, not including manus.

16. number of trunk vertebrae - equal to costal grooves plus one (Highton, 1957); counted directly on cleared and stained specimens.

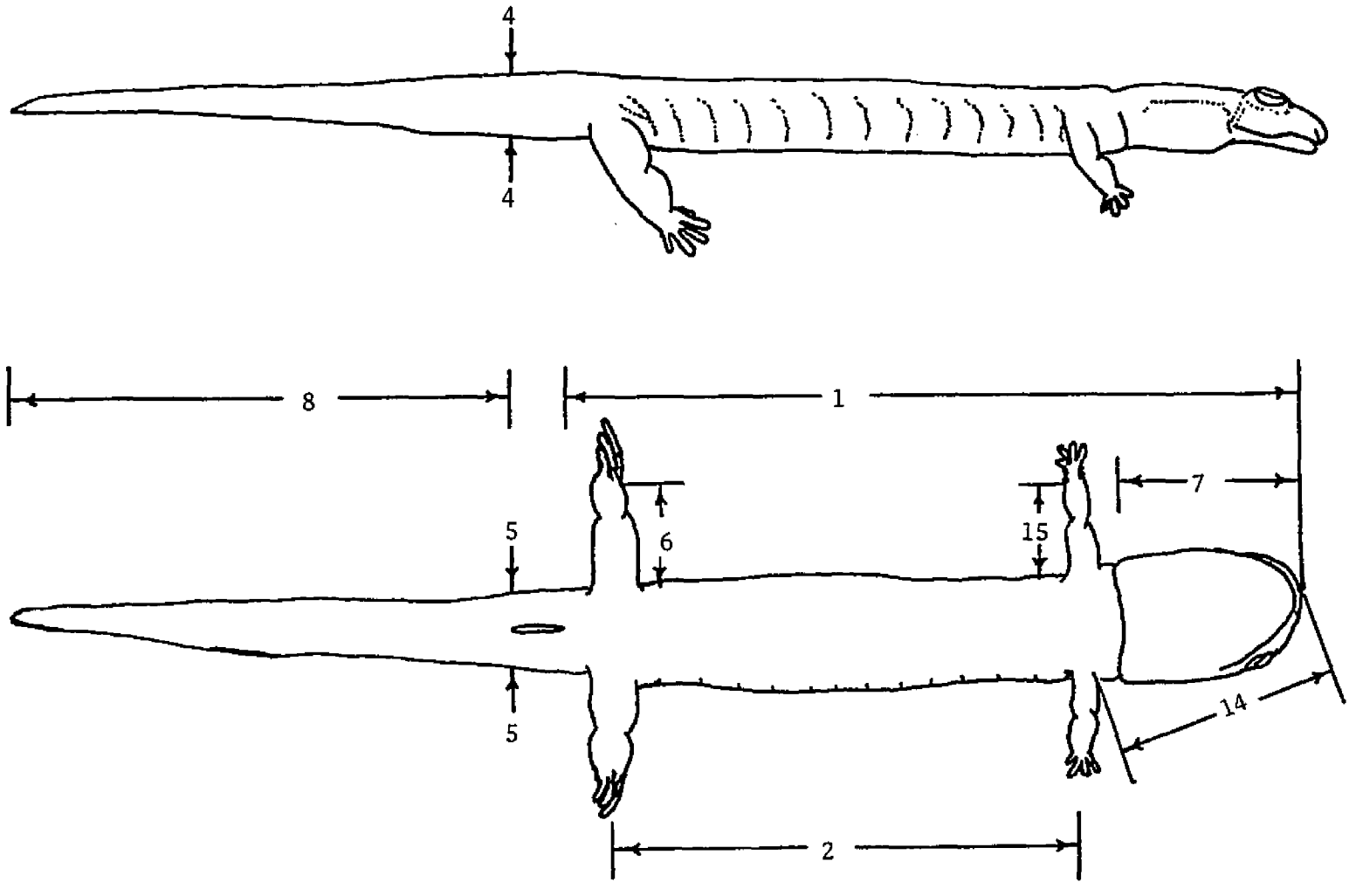


Fig. 4 Body Measurements - lateral and ventral view. (See text for explanation.)

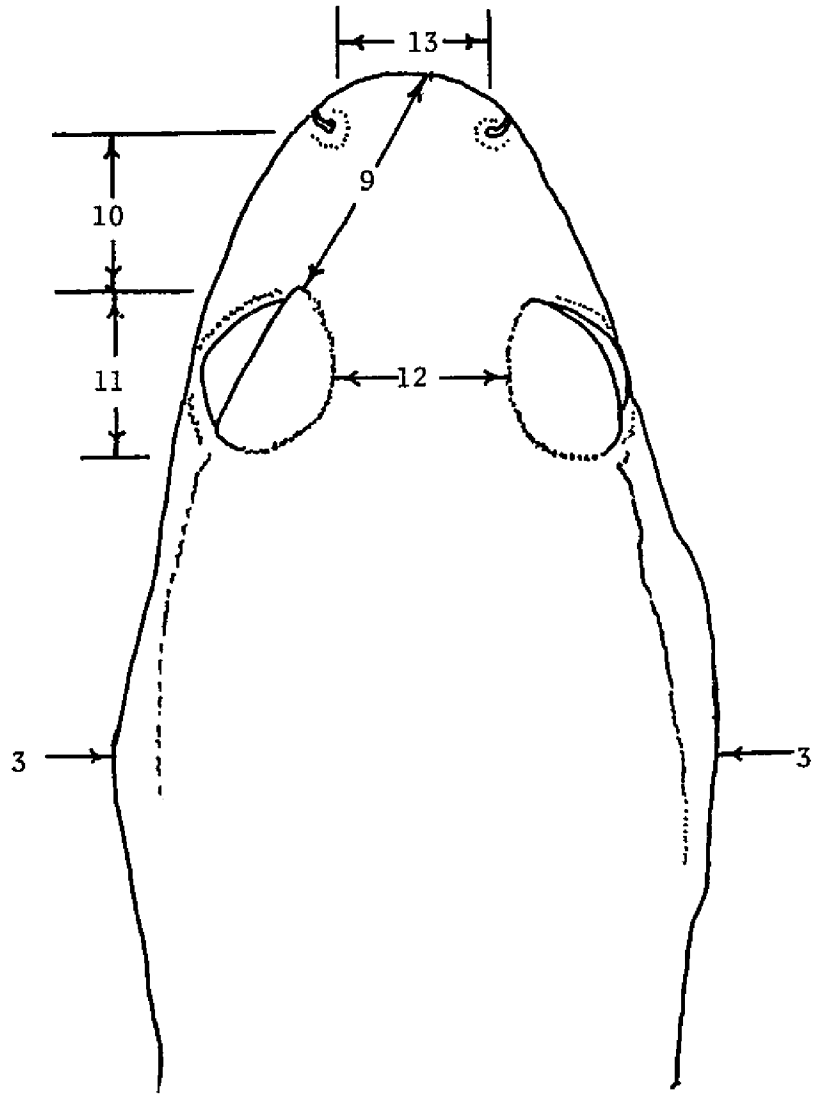


Fig. 5 Dorsal Head Measurements (See text for explanation.)

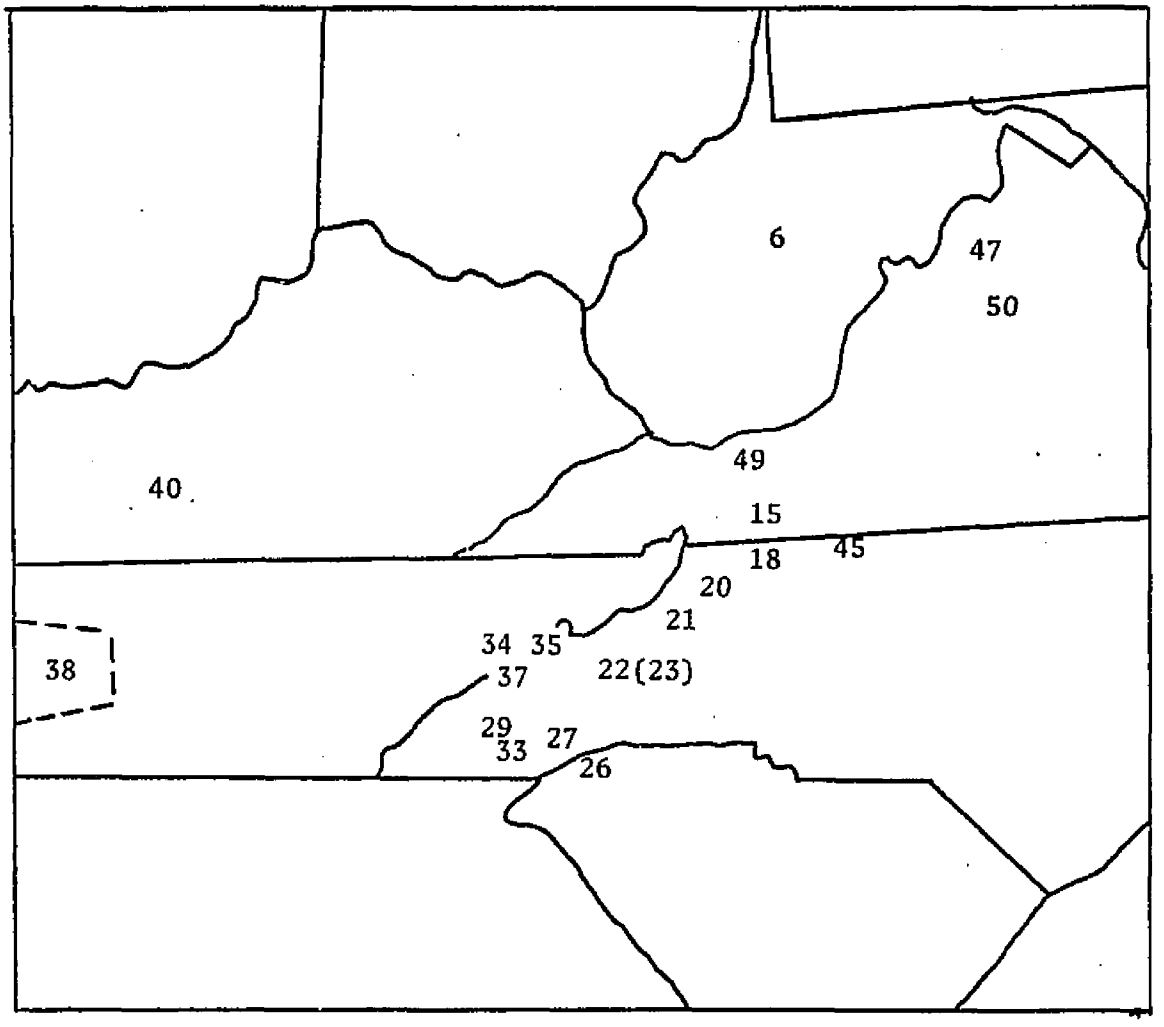


Fig. 6. Field numbers and localities of Desmognathus samples.

Field No.	Locality	County	State
6	Holly River State Park	Webster	West Virginia
15	Rock Castle Gorge	Floyd	Virginia
18	Doughton Park	Alleghany	North Carolina
20	Grandfather Mt.	Avery	" "
21	Linville River	Burke	" "
22(23)	Mt. Mitchell State Park	Yancy	" "
26	Route 178 (U.S.)	Pickens	South Carolina
27	U.S. Route 64	Transylvania	North Carolina
29	Deep Gap	Macon	" "
33	Bridal Veil Falls	Macon	" "
34	Clingman's Dome	Sevier	Tennessee
35	Fish Camp Prong Little River-Smokies	"	"
37	Little Pigeon River-Smoky Mountains	"	"
38(inset)	Ouachita National Forest	Montgomery	Arkansas
40	Mammoth Cave National Park	Edmonson	Kentucky
47	U.S. Route 33	Greene	Virginia
49	Cascades-Jefferson Nat'l Forest	Giles	"
50	Sugar Hollow- Va. Rt. 614	Albemarle	"

Comparative Studies of Lactate Dehydrogenase

Results

Approximately 240 individuals of Desmognathus have been analyzed electrophoreticall and have yielded recognizable zymograms. A series of electrophoretic and catalytic controls were done to determine:

1) which of the electrophoretic bands, cathodal or andodal, corresponded to MLDH or HLDH; i.e. which band corresponded to M_4, M_3H, M_2H_2, MH_3, and H_4 respectively.

2) which tissue extract gave the best representitive zymograms, i.e. clearly showed all separable isozymes.

The zymograms of tail muscle and belly muscle always showed very heavy staing of the cathodal bands with the anodal bands either faint or not staining at all. This suggested that the most cathodal band was the M_4 tetramer based on the evidence that MLDH predominates in skeletal muscle subject to occasional periods of relatively anaerobic activity (Cahn, et. al. 1962; Kaplan, 1964; Salthe, 1965).

Catalytic evidence that the cathodal bands corresponded to MLDH and the anodal bands HLDH was obtained from a consideration of zymograms and substrate inhibition curves for stomach and tail muscle of D. quadramaculatus (specimen 37-3, see Fig. 7). MLDH is more substrate inhibited than HLDH. A measure of substrate inhibition is the L/H ratio, i.e. the ratio of the activity at an arbitrary low pyruvate concentration ($L=3.3X10^{-4}M$; see Fig. 7), to the activity at an arbitrary high pyruvate concentration ($H=10^{-2}M$). Tail muscle showed considerable activity at the cathod and virtually none for the anodal bands. Stomach muscle exhibited the same cathodal activity but more anodal activity. The difference between the two was that stomach muscle had relatively more of the anodal isozymes than tail msucle. The former was more substrate inhibited; L/H=1.5, to 1.3 for the

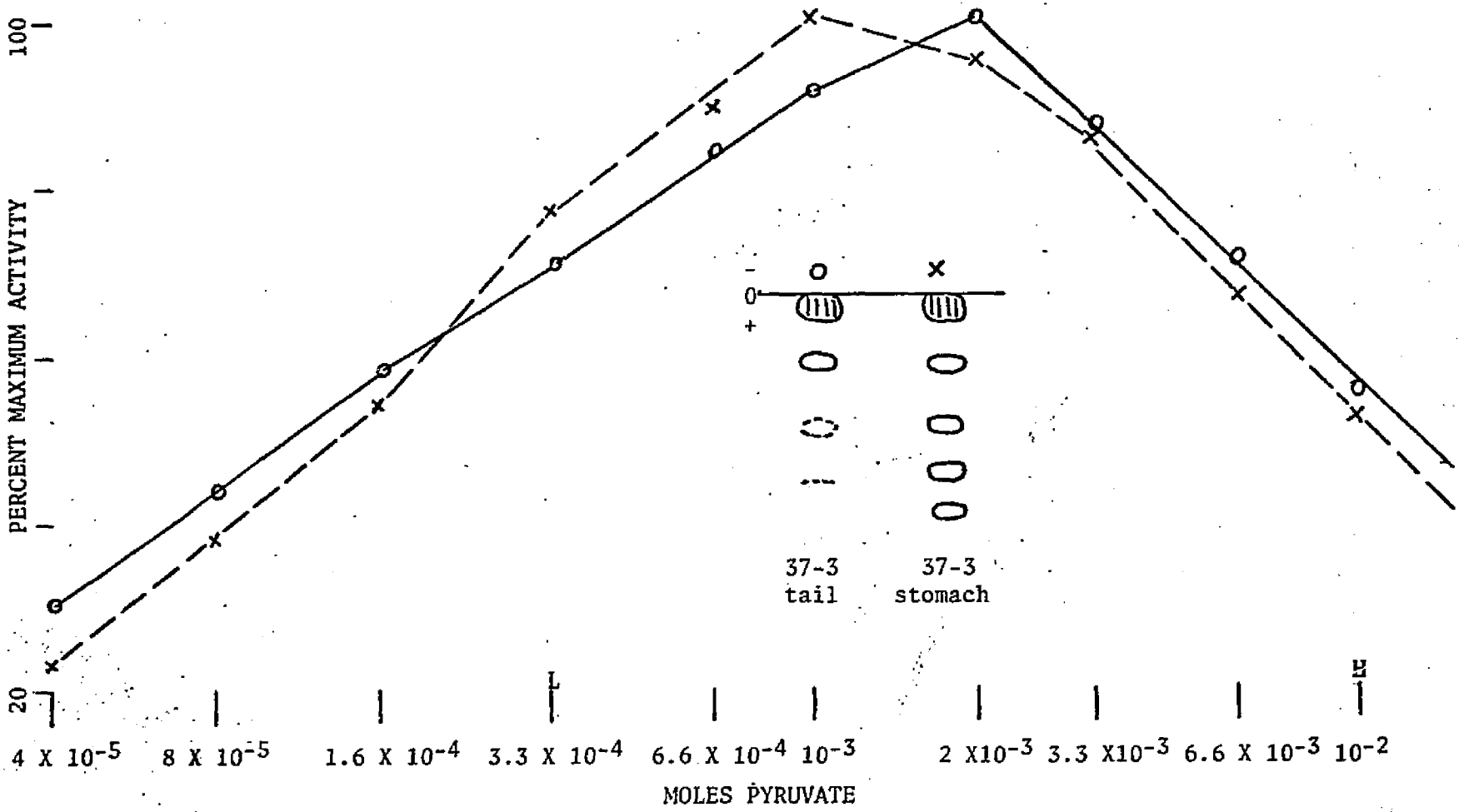
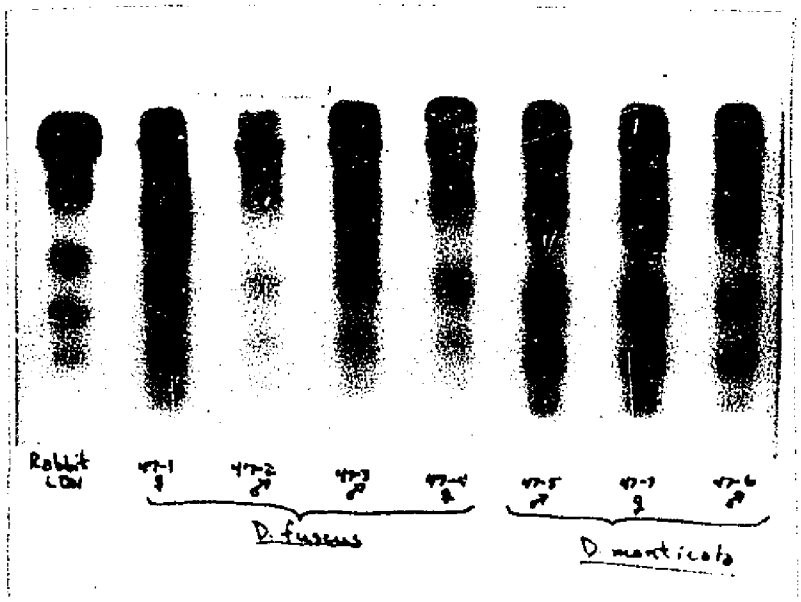


Fig. 7. Comparison of zymograms and substrate inhibition curves of stomach muscle and tail muscle LDH. 37-3= Desmognathus quadramaculatus, Great Smoky Mountains National Park. Vertical lines within bands indicate heavy staining, dashed lines indicate bands with faint staining. L and H are arbitrary low and high pyruvate concentrations for computing the L/H ratio (Salthe, 1965).

latter. This is evidence that the anodal isozymes are more like HLDH than the cathodal isozymes, which are more like MLDH. Salthe (1965) considered L/H ratios of less than 2.0 as being characteristic of MLDH. The most cathodal band is then the M_4 tetramer, and progressing towards the anode, with increased mobility are the M_3H, M_2H_2, MH_3, and H_4 bands.

Figure 8 is a photograph and diagram of a starch gel to indicate how gels are represented in the diagrams. Figure 9 shows zymograms of crude liver, heart, testes, belly wall muscle and stomach extracts from D. ochrophaeus. The ideal zymogram would be a mixture of these tissues or an extract of the entire animal. This was impractical, especially with larger animals. Stomach tissue was used for all individual Desmognathus studied. In most specimens it was large enough, and centrifugation of the extract was not required as it was for the liver homogenates used by Shontz (1968). Heart muscle gave the best zymograms, but due to the heart's small size, enough material with enough LDH activity was present only in large D. monticola, D. quadramaculatus, Leurognathus marmoratus, and Phaeognathus hubrichti.

Accompanying the following discussion are zymograms for all the specimens considered. In some instances specimens, larvae for example, were too small and the enzyme yield from individual specimens were too low. These specimens had to be ground whole



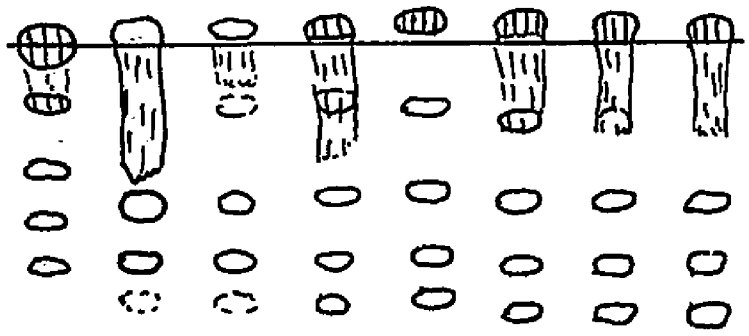


Fig. 8. Comparison of Photograph and Diagram (made from photograph) of Zymograms of Lactate Dehydrogenase. Note Rabbit muscle LDH standard, and representation of streaking (vertical dashes) and dark staining (vertical lines).

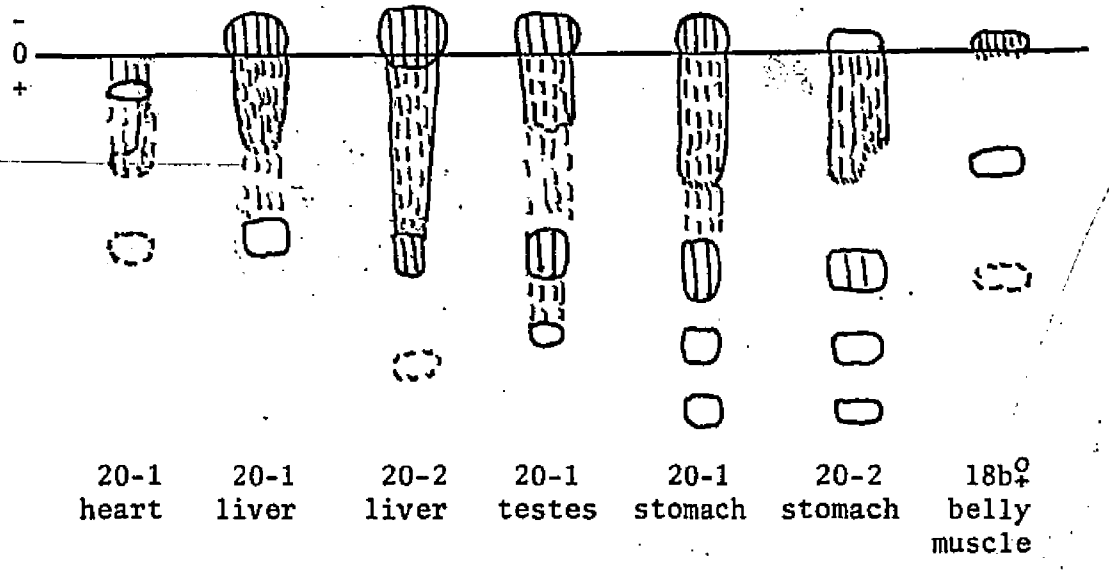


Fig. 9. Control zymograms of crude extract of Desmognathus ochrophaeus LDH which determined that stomach muscle was an appropriate tissue for resolving all isozymes. See also fig. 17 for zymograms of tail muscle LDH, and fig. 16 for zymograms of crude extract of entire specimens. 20-1, 20-2= Grandfather Mt., North Carolina. 18b♀= Doughton Park, North Carolina. Vertical dashed lines indicate streaking.

which rendered them unavailable for morphological study. Therefore, electrophoretic analysis of egg, larva, juvenile and adults was done only for D. ochrophaeus. This was the only species for which the entire life cycle was available. The characteristic banding pattern is found throughout the life cycle, differences were found only in relative amounts of each isozyme. Also, included in the figures are summary zymograms of each species and species group, (see figs. 9, 10, and 11). Specimens from the various samples were run on the same gel as a control in addition to the use of relative migration values (see p 17). This permits the use of summary figures.

Subbanding, which may be indicative of heterozygotes, (see fig. 3) was not encountered in this study. This also appears true for Shontz' (1968) samples of Desmognathus.

The relative migration for the fastest isozyme, H_4, was determined. The values for each species and their distribution, (see figs. 12, 13, and 14), show the range of variation for each species and species group.

Discussion

The results of the electrophoresis of lactate dehydrogenase indicated that the general patterns of isozyme mobility were species specific. Visual comparison suggested that several species had similar patterns within the range of variation exhibited by each. Similarity in pattern was determined by the spacing of bands (see p 37) and the absolute migration of each band. These species have been placed in species groups. This implied close relationship and means that within each group similar mobility results from the same subunit, and between groups similar mobility results from convergence, i.e. the tetramers have the same net charge but different subunits.

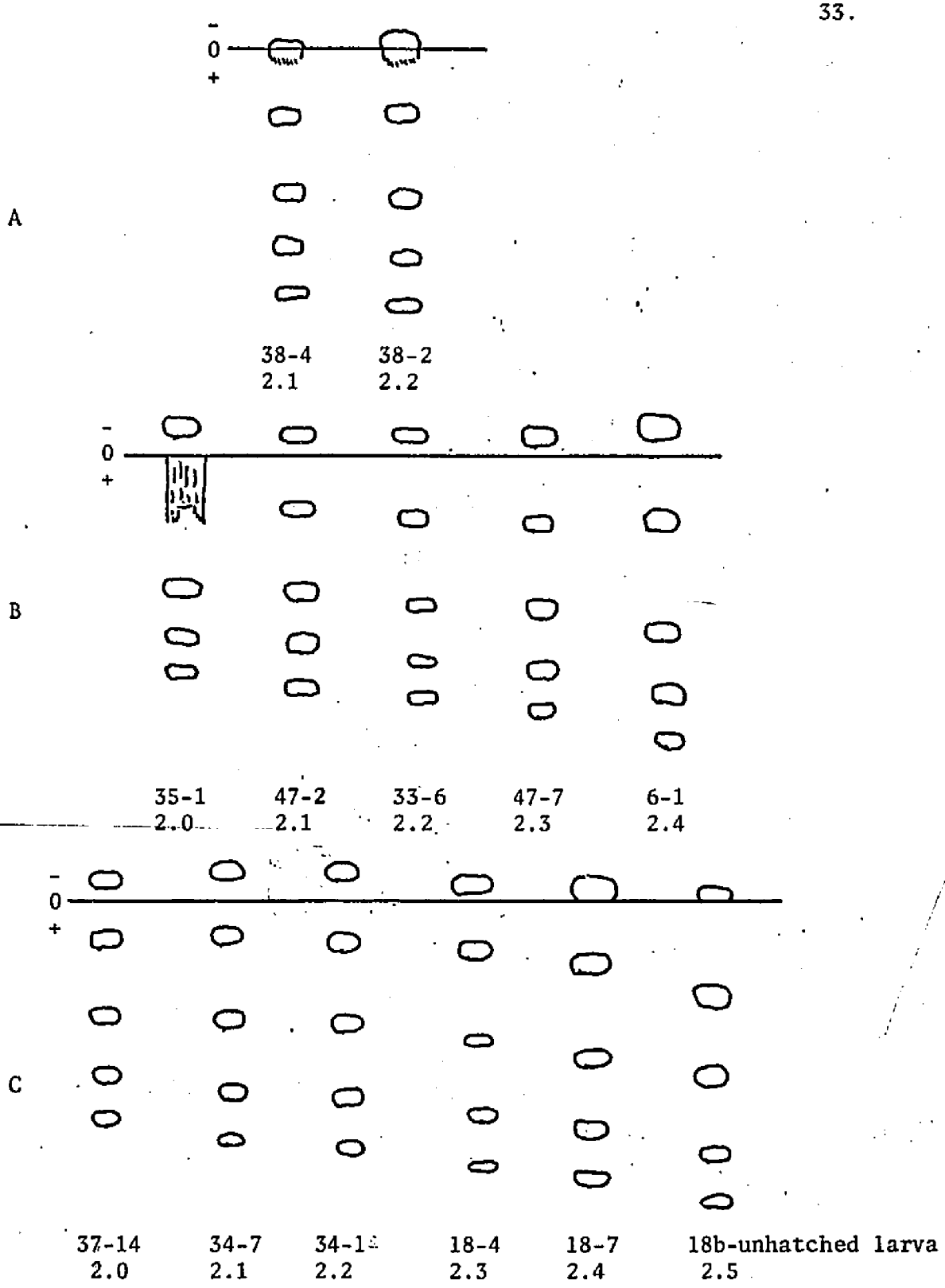


Fig.10. Comparison of H_4 relative migration for the species of Group I. A= D. fuscus; B= D. monticola; C= D. ochrophaeus. Distances adjusted for comparison. Vertical dashed lines indicate streaking. Band size is a result of the amount of isozyme.

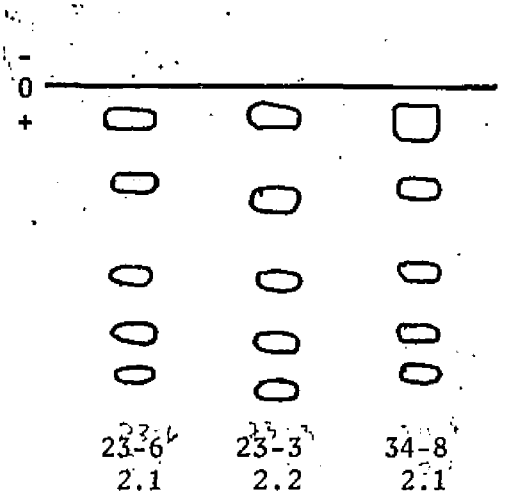


Figure 11. Comparison of H_4 Relative Migration for Desmognathus wrighti. 23= Mt. Mitchell, North Carolina. 34= Clingman's Dome, Great Smoky Mountain National Park, Tennessee. Distances adjusted for comparison.

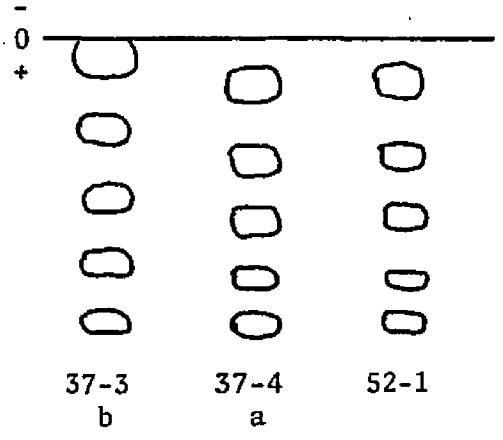


Fig. 12. Comparison of zymograms of type a and type b Desmognathus quadramaculatus, Great Smoky Mountains National Park, and the zymogram of Leurognathus marmoratus, Jefferson National Forest, Virginia.

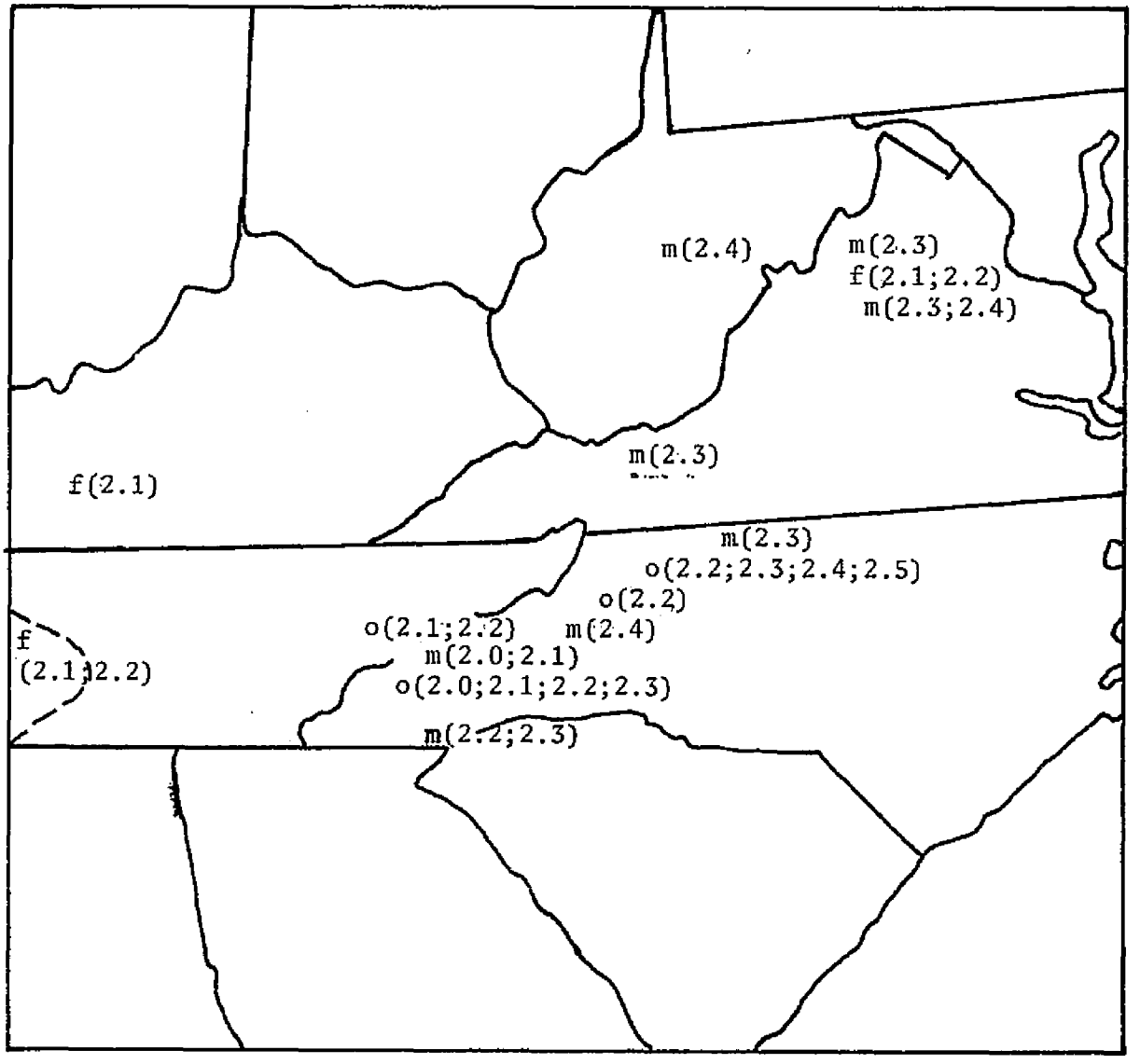


Fig. 13 . Distribution of Group I Desmognathus samples. f= D. fuscus, m= D. monticola, o= D. ochrophaeus. Numbers in parenthesis are the H_4 relative migration values for each sample (see text for explanation).

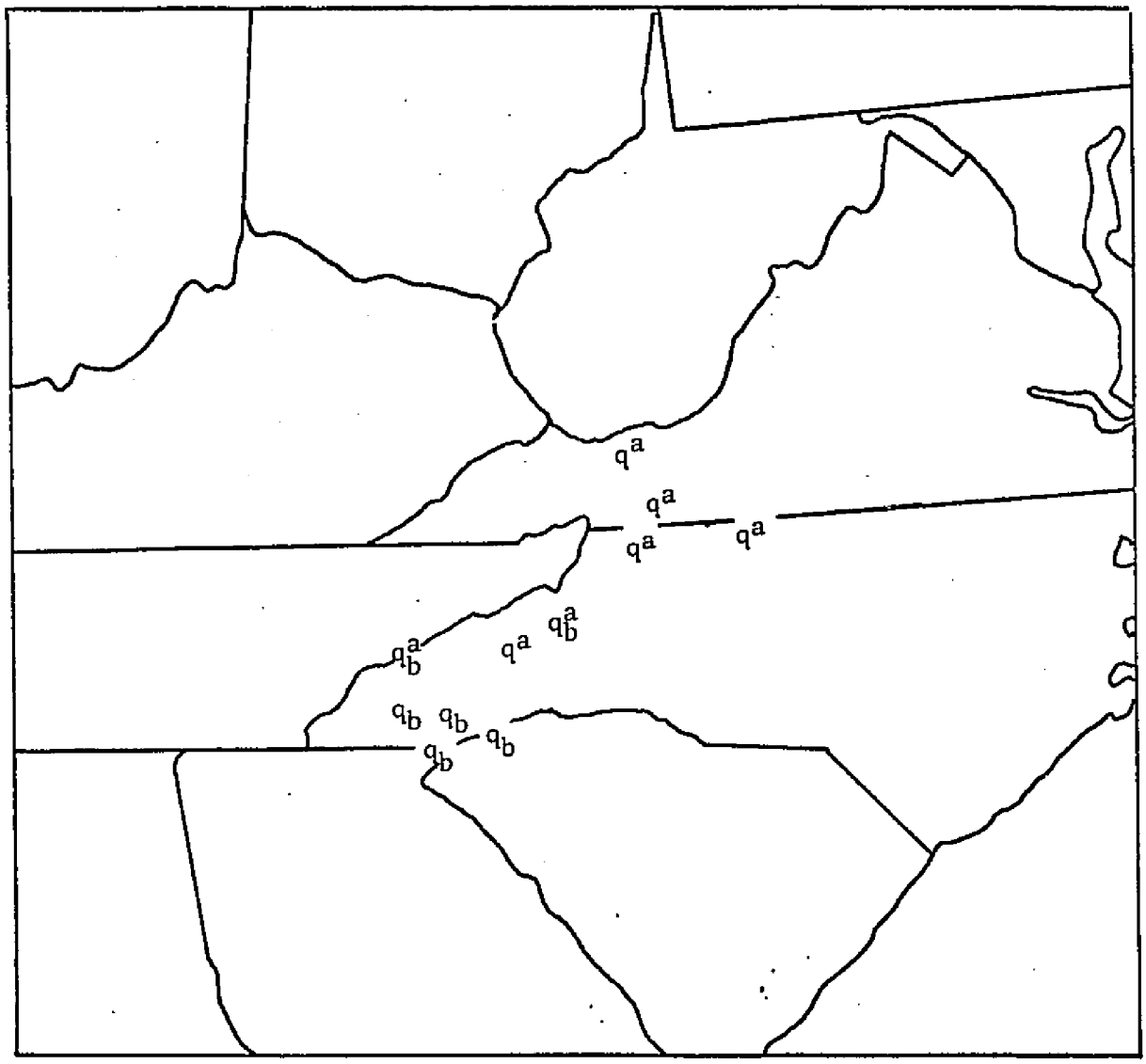


Fig. 14. Distribution of Desmognathus quadramaculatus samples (q). a and b refer to type a and b M subunits, lactate dehydrogenase (see text for explanation).

Patterns of Interaction, or the Spacing of Bands

As discussed above, the subunits have a net charge dependent upon the amino acid composition. Theoretically each LDH tetramer should have a mobility which is the total of its subunits' charges. The example in Fig. 3 uses this assumption. However, subunit interaction in the quaternary structure of LDH reduces or increases the expected mobility. This can be expressed by analyzing zymograms in reverse using the same assumptions as in the theoretical example of Fig. 3. For example, specimen 6-3, a male D. monticola from Holly River State Park, West Virginia gave the following mobilities after a 10 hour run, (see Fig. 15):

Subunit	Mobility (mm. from origin)	M	H
M_4	-4.0	-1.0	
M_3H	+11.5	-1.0	+14.5
M_2H_2	+28.0	-1.0	+15.0
MH_3	+39.0	-1.0	+13.3
H_4	+46.5		+11.6

The net mobility of the M subunit was assumed to be one-fourth of the M_4. The M subunit was given the same value for the other isozymes since it varied the least intraspecifically throughout this study. The H values were computed on the basis of the M value. The different observed values of H apparently reflect the interaction between M and H. Graphs of these values for this method of analysis, (see Fig. 15), have a characteristic shape for each species group. While the graph patterns are a function of subunit interaction, they also reflect a characteristic band spacing. This criterion led me to consider the LDH patterns of species within a group as identical, especially those of group I.

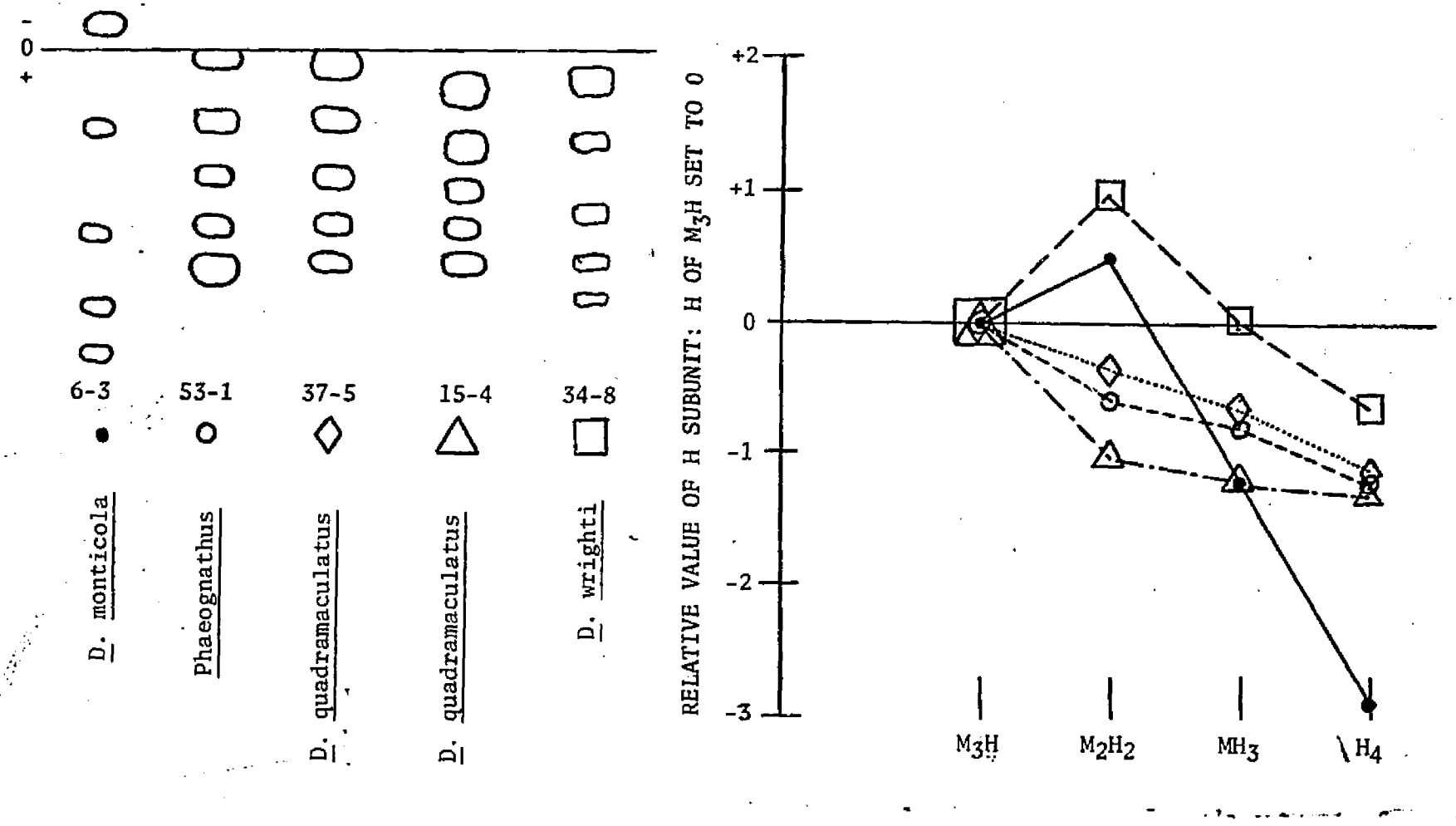


Fig. 15. Patterns of subunit interaction and spacing of isozymes in zymograms of crude extract of stomach muscle LDH. Zymograms show specimens for which relative H values are plotted. Symbols below specimen number correspond to symbols of graph. 6-3 D. monticola, Holly River State Park, West Virginia; 53-1 Phaeognathus hubrichti, Alabama; 37-5 D. quadramaculatus, Great Smoky Mountain National Park; 15-4 D. quadramaculatus, Rock Castle Gorge, Virginia; 34-8 D. wrighti, Clingman's Dome, Great Smoky Mountain National Park.

The lack of electrophoretic evidence for heterozygotes, i.e. subbanding, is not explainable at this time. Zymograms of the clutch of a D. ochrophaeus female, (18b♀, see Fig. 16), demonstrated that two H subunits were expressed. An unhatched specimen (ground whole) from the clutch was identical to the female. The recently hatched young, also ground whole, showed a slightly faster H_4. This is evidence that one or both of the parents was a heterozygote or that the female courted more than once with different males, and one of the offspring was heterozygous. Organ (personal communication) has noted that multiple courtship may occur under laboratory conditions. It is doubted that this is the maternal effect noted by Wright and Moyer (1966) for frogs of the genus Rana. In their study maternal subunits were present during early development and were gradually replaced by the individual's subunits. Heterozygotes probably occur and the absence of subbanding may be explained by one or both of the following, 1) inadequate separation and resolution of subbands, 2) repression of one subunit, either on the gene, messenger RNA or protein level. The possibility exists that there is severe selection against heterozygotes. The absence of data from many individuals of a single population precludes consideration of the explanations.

Group I: fuscus-monticola-ochrophaeus

The results indicated that only three species, D. fuscus, D. monticola and D. ochrophaeus had a negatively migrating band. These results agree with those of Shontz (1968), yet she considered the fuscus pattern different. Shontz (1968) made use of liver homogenates

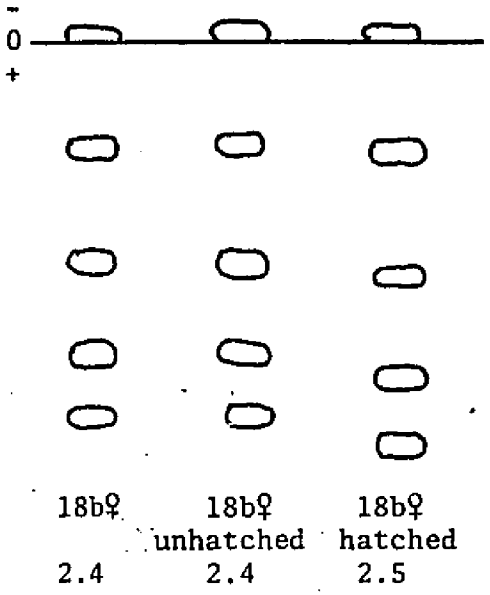


Fig. 16. Comparison of zymograms and H_4 relative migration values of a female Desmognathus ochrophaeus and two individuals from her clutch of eggs, Doughton Park, North Carolina. The latter two were ground whole.

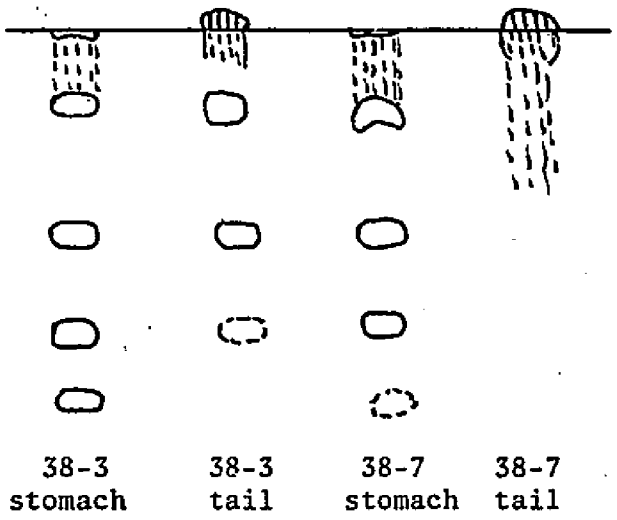


Fig. 17. Comparison of zymograms of crude extracts of stomach and tail muscle from the same specimens. Note negative aspects of M_4 present in tail muscle LDH but absent in stomach muscle LDH. Compare with fig. 18. Desmognathus fuscus brimleyorum, Ouachita National Forest, Arkansas.

which showed monticola and ochrophaeus to have a clearly negative band. Her fuscus demonstrated two patterns, with mountain specimens essentially similar to monticola and ochrophaeus. The other pattern was of lowland fuscus which did not have a negatively migrating band. Possibly these were the subspecies (or species of Wake, 1966) auriculatus. I found the latter pattern in fuscus from Arkansas. In stomach extracts a negative band is lacking. However, the zymograms of tail muscle extract from the same individuals clearly showed a negative aspect identical to numerous ochrophaeus, several montane fuscus, and monticola, (see Figs. 17 and 18). This demonstrated a closer relationship of low altitude fuscus with high altitude fuscus. The former are primarily allopatric, the latter sympatric with D. monticola and D. ochrophaeus. Huheey (1966) suggested that D. fuscus may not be present to any great extent (if at all) in the Great Smoky Mountains in particular and the southern Appalachians in general, and that competitive exclusion by D. monticola and D. quadramaculatus was responsible for this. Organ (1961a) found fuscus at Whitetop Mountain, Virginia from several altitudinal transects primarily at the higher altitudes with monticola replacing it at lower altitudes in large streams. In small streams the two species coexisted. It is not clear why competition with other Desmognathus species is responsible for the absence of D. fuscus in the southern Appalachians as Huheey (1966) suggests, since it is successful at higher altitudes in Virginia (Organ, 1961a).

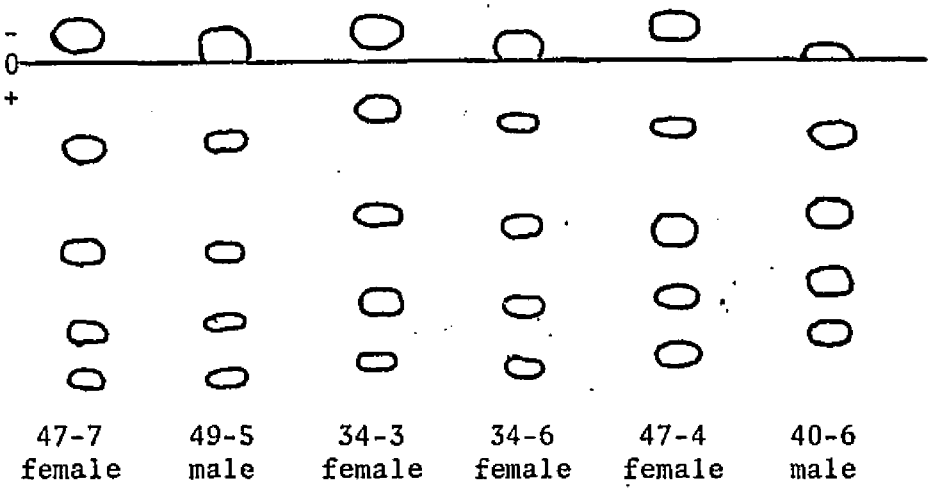


Fig. 18. Zymograms of crude extract of stomach muscle which demonstrate the two types of M_4 found in Desmognathus Group I species. Compare with fig. 17. 34-3, 34-6= D. ochrophaeus, Great Smoky Mountains National Park, Tennessee; 40-6= D. fuscus, Mammoth Cave National Park, Kentucky; 47-4= D. fuscus; 47-7= D. monticola, Route 33, Greene Co., Virginia; 49-5= D. monticola, Jefferson National Forest, Virginia.

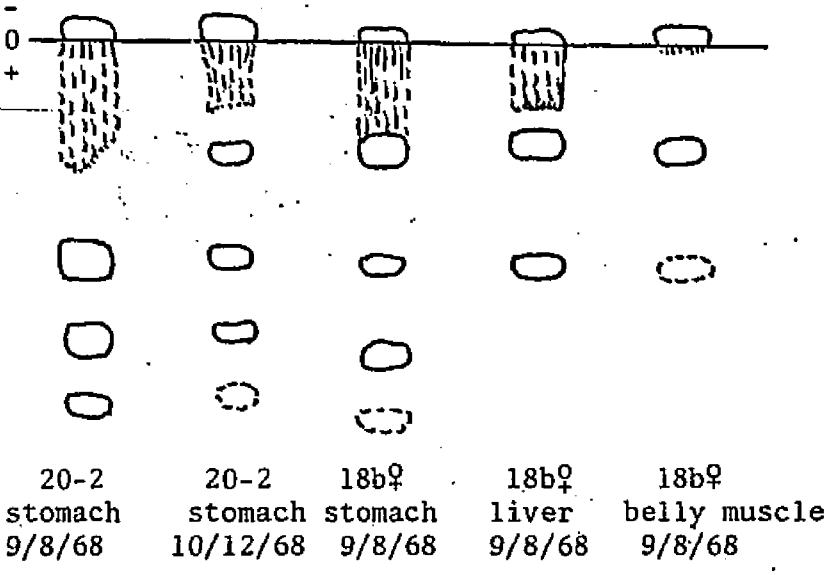


Fig. 19. Zymograms comparing the degree of streaking between two Desmognathus ochrophaeus: after one month of frozen storage, and between tissues of the same specimen. 20-2= Grandfather Mt., North Carolina; 18b♀= Doughton Park, North Carolina.

The distribution of bands in fuscus LDH zymograms clearly relate it closely to monticola and ochrophaeus, (see Fig. 18). Each species has individuals showing one of two types of negative bands; one in contact with the origin and one separate from it. Lowland fuscus only show the former pattern. The absence of a negative band in stomach and liver (Shontz, 1968) extracts indicates a departure in pattern. Variability of the fuscus pattern is in keeping with its wide distribution. Polymorphism in enzyme systems is greater when the requirements (i.e. selective parameters) are less restricted (Gillespie and Kojima, 1968). Fuscus in invading the coastal plain and as far west as Arkansas and western Oklahoma has experienced a vast range of selective forces including higher mean temperatures and lower annual rainfall.

An additional characteristic of zymograms for this group was a streaking in some individuals extending from the origin towards the anode and often covering the M_3H band. Control stains without lactic acid did not reveal this streaking which suggested it is a result of LDH. It did not form distinct bands and was unstable. With time, the extent of the streak diminished, revealing the previously obscured band. In the same individual the streak distance varied between tissues, (see Fig. 19).

The distribution of H_4 relative migration values, (see Fig.10), for the dusky group allows the following conclusions. The ranges of the individual species values overlap. D. ochrophaeus has the widest range of H_4 mobility compared with the variation of D. monticola and D. fuscus. D. ochrophaeus occurs over the greatest

altitudinal range of any desmognathine (Huheey, 1966; Organ, 1961a). The samples of D. fuscus exhibit low H_4 mobility with relative migrations of 2.1 to 2.2. D. monticola showed relative migrations from 2.0 to 2.4. The relative migrations for D. ochrophaeus range from 2.0 to 2.5. The overlap between species and localities does not permit the drawing of isophenes, (Martof and Rose, 1963). The unique variability of the M_4 (discussed above) in D. fuscus still allows consideration of it as having the most variable LDH pattern even though the H_4 variability is low.

Group II D. quadramaculatus-Leurognathus. Relationships of Phaeognathus.

D. quadramaculatus exhibited LDH patterns distinct from the other members of the genus. In all the specimens tested the H_4 relative migrations were the same, equal to 1.9. Two variations of the M subunit were found. One, (a) migrating approximately 7 mm. towards the anode, and (b) resulting in an M_4 touching the origin on the anode side. No intermediates were found among the samples studied which is in keeping with the absence of expressed heterozygosity. Two of eleven samples contained both types, (see Fig. 14). However, samples were small and the emphasis is placed here on the presence rather than the absence of types.

Shontz (1966) reported only one variant, the one designated (b) in this study, with specimens from Macon Co., North Carolina in the extreme western part of the state. (I sampled this area in the summer of 1968.) Shontz missed the other variant by sampling a small part of the distribution. This led to the erroneous conclusion that the D. quadramaculatus LDH pattern did not vary, which

was correlated to the relatively low morphological variation (Shontz, 1968). This possibility is discussed below under morphology.

A single specimen of Leurognathus marmoratus from Virginia was tested electrophoretically. The pattern is identical to D. quadramaculatus type a, as shown in Fig. 12. This warrented tentative inclusion of these species in group II. The question of the generic status of L. marmoratus that Martof (1962) considered, will be reconsidered below.

The migration and spacing of bands of types a and b are graphed in Fig. 15. Both curves follow the same trend but are distinctly different. The correspondence of type b D. quadramaculatus with that of Phaeognathus is of interest but is viewed by this author with caution. The question of the relationship of Phaeognathus to the rest of the desmognathines has been raised by Highton (1961). Wake (1966) considers it a very early branch of the subfamily which previously had a wider distribution. The LDH data offer a clue for a relationship between Phaeognathus and type b D. quadramaculatus. Morphological correlation other than general dark coloration is unlikely due to the divergent adaptations.

Group III D. wrighti and D. aeneus.

D. wrighti, located primarily above 5,000 ft., (Blair, 1958; Conant, 1958; Organ, 1961B), apparently consists of numerous disjunct populations. Zymograms of samples from two populations, (see Fig. 14), showed that the M subunits were similar. Relative migration values for Mt. Mitchell, North Carolina, were 2.1 to 2.2,

and for Clingman's Dome, Great Smoky Mountains National Park, Tennessee the value was 2.1, [illegible]. The spacing of bands resulted in the same pattern of interaction curve, (see Fig. 15). D. aeneus from Deep Gap, Macon Co., North Carolina, suggested similarities with D. wrighti, although they did not yield clear zymograms.

Apparent molecular divergence between two isolated D. wrighti populations is small. This agrees with the degree of specialization, Organ, 1961b), and very likely with their more recent evolution. This is discussed further following the discussion of morphology below.

Studies in Comparative Morphology

The studies of lactate dehydrogenase demonstrated three species groups within the genus Desmognathus. The studies of comparative morphology were undertaken for several reasons. One reason was to test whether morphological characters would divide the species into groups similar to the LDH study. Numerous characters have been considered characteristic of the genus and of the subfamily Desmognathinae. A comparison of size and body proportions (relative size) was necessary to clarify descriptive phrases frequently found in the literature such as "Desmognathus monticola - size moderately large, to about 75 mm snout-vent length" (Huheey, 1966). Generalized descriptions of the heads of the various species were found in the literature, in field guides, and in personal communications, e.g. narrower snout, longer snout, wider or longer head. These characteristics have never been analyzed, but are based primarily on field experience and what has been termed gestalt taxonomy by various desmognathine workers. Only two species have been adequately investigated, Leurognathus marmoratus (Martof, 1962) and D. ochrophaeus (Martof and Rose, 1963). Therefore, several characters have been quantified and analyzed.

Diagrams of dorsal head views, head musculature, and skulls are included. These are essential to a proper understanding of the differences and similarities between the species and species

groups. The figures were the result of the study of the desmognathine jaw mechanism, (see discussion below).

Results

The data in Tables 1 - 23 has been subjected to the Kruskal-Wallis one way analysis of variance by ranks (Siegal, 1956). This test is useful for determining if the species fall into the same species groups of the lactate dehydrogenase study. The computed H values listed below each table indicate the degree of confidence. The Null hypothesis that the samples are random was rejected with probabilities below .05. Probabilities are included in the following discussion.

The data from several localities (see Appendix) have been included in the range for each character. In some cases samples within each species did not fully overlap. The lactate dehydrogenase species groups are based on the total species variation for the localities sampled. Therefore, the total variation of morphological characters and its relation to the species as a whole was considered.

Although sexual dimorphism occurs in Desmognathus (Martof and Rose, 1963), the data from males and females was lumped. Few females were collected and consideration of the sexes separately or alone does not change the conclusions.

The values for Phaeognathus were not included in the computation of the value of H, (see Tables 1 - 23.).

Table 1 Body Size. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	35.54	5.0952	23.8-48.2
D. m.	14	47.30	9.7648	33.1-60.3
D. f.	18	46.43	10.0291	24.4-60.0
D. q.	14	55.66	16.4496	34.7-87.8
L. m.	3	59.30	8.6007	50.8-68.0
P. h.	1	110.40	--	110.4
D. w.	13	23.86	2.0000	20.0-26.1
D. a.	3	21.60	0.8326	21.0-22.6

H = 5.3571

Table 2 Tail Length.

Species	N	$\bar{X}$	s	Range
D. o.	21	38.26	7.9752	23.9-51.4
D. m.	11	53.09	14.6381	32.6-73.6
D. f.	8	43.43	10.4244	33.6-65.4
D. q.	6	47.75	14.1942	33.0-71.6
L. m.	3	43.27	6.6425	38.8-50.9
P. h.	1	114.10	--	114.1
D. w.	10	17.78	1.8861	14.7-20.9
D. a.	2	20.10	0.7070	19.6-20.6

H = 3.74999

Table 3 Relative Tail Length. (tail length/snout-vent length). (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	21	1.078	0.1299	0.83-1.27
D. m.	11	1.085	0.1030	0.88-1.23
D. f.	8	0.973	0.1007	0.85-1.09
D. q.	6	0.860	0.0522	0.81-0.95
L. m.	3	0.730	0.0436	0.68-0.76
P. h.	1	1.030	--	1.03
D. w.	10	0.743	0.0411	0.68-0.81
D. a.	2	0.950	0.0424	0.92-0.98

H = 4.71428

Table 4 Trunk Length.

Species	N	$\bar{X}$	s	Range
D. o.	29	22.62	3.6353	14.7-32.3
D. m.	14	30.00	6.4461	20.7-39.0
D. f.	18	30.02	6.4879	15.0-39.8
D. q.	14	35.21	10.5539	21.0-54.4
L. m.	3	38.33	5.8011	32.6-44.2
P. h.	1	76.90	--	76.9
D. w.	13	14.80	1.3565	12.1-16.6
D. a.	3	13.70	0.5568	13.2-14.3

H = 5.35714

Table 5 Relative Trunk Length. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.636	0.0235	0.59-0.68
D. m.	14	0.634	0.0191	0.60-0.67
D. f.	18	0.646	0.0259	0.58-0.69
D. q.	14	0.632	0.0233	0.60-0.67
L. m.	3	0.647	0.0058	0.64-0.65
P. h.	1	0.690	--	0.69
D. w.	13	0.618	0.0264	0.58-0.66
D. a.	3	0.630	0.0058	0.63-0.64

H = 3.74999

Table 6 Index of Tail Flattening. (depth/width).

Species	N	$\bar{X}$	s	Range
D. o.	29	0.838	0.1929	0.59-1.08
D. m.	14	0.927	0.1350	0.76-1.20
D. f.	17	0.975	0.1000	0.82-1.15
D. q.	14	0.915	0.0989	0.75-1.06
L. m.	3	1.080	0.0971	1.00-1.19
P. h.	1	0.890	--	0.89
D. w.	13	0.915	0.1324	0.75-1.18
D. a.	3	0.913	0.1858	0.70-1.04

H = 0.85401

Table 7 Head Length. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	8.69	1.4070	5.7-12.3
D. m.	14	11.71	2.4601	8.3-15.6
D. f.	18	11.12	2.3579	6.8-15.2
D. q.	14	14.11	4.3436	8.0-23.6
L. m.	3	14.93	2.1825	13.0-17.3
P. h.	1	23.2	--	23.2
D. w.	13	5.72	0.7496	4.5-6.7
D. a.	3	4.97	0.2309	4.7-5.1

H = 5.35714

Table 8 Relative Head Length. (head length/snout-vent length.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.244	0.0194	0.21-0.30
D. m.	14	0.247	0.0123	0.21-0.26
D. f.	18	0.240	0.0152	0.22-0.28
D. q.	14	0.253	0.0155	0.23-0.27
L. m.	3	0.251	0.0056	0.25-0.26
P. h.	1	0.210	--	0.21
D. w.	13	0.239	0.0241	0.20-0.28
D. a.	3	0.229	0.0123	0.22-0.24

H = 5.35714

Table 9 Head Width. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	6.21	1.0686	4.3-8.6
D. m.	14	8.74	1.7252	5.7-11.1
D. f.	18	8.21	2.0682	4.2-12.8
D. q.	14	9.82	2.7389	6.3-15.2
L. m.	3	9.40	1.0149	8.3-10.3
P. h.	1	11.70	--	11.7
D. w.	13	4.12	0.3059	3.7-4.9
D. a.	3	3.57	0.3512	3.2-3.9

H = 5.35714

Table 10 Relative Head Width. (head width/snout-vent length).

Species	N	$\bar{X}$	s	Range
D. o.	29	0.169	0.0358	0.15-0.20
D. m.	14	0.185	0.0137	0.16-0.21
D. f.	18	0.176	0.0148	0.15-0.21
D. q.	14	0.177	0.0139	0.16-0.20
L. m.	3	0.158	0.0067	0.15-0.16
P. h.	1	0.106	--	0.106
D. w.	13	0.174	0.0201	0.15-0.22
D. a.	3	0.165	0.0221	0.14-0.19

H = 1.17856

Table 11 Relative Eye Diameter. (eye diameter/head length). (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.241	0.0308	0.15-0.30
D. m.	14	0.257	0.0297	0.21-0.29
D. f.	18	0.245	0.0244	0.20-0.28
D. q.	14	0.246	0.0322	0.16-0.28
L. m.	3	0.239	0.0385	0.20-0.28
P. h.	1	0.168	--	0.168
D. w.	13	0.268	0.0631	0.19-0.38
D. a.	3	0.248	0.0115	0.24-0.26

H = 2.7499

Table 12 Snout Length.

Species	N	$\bar{X}$	s	Range
D. o.	29	2.79	0.5108	1.7-4.1
D. m.	14	3.69	0.9429	2.6-5.4
D. f.	18	3.21	1.2144	2.0-5.2
D. q.	14	4.49	1.7217	2.6-7.8
L. m.	3	4.37	0.9074	3.7-5.4
P. h.	1	5.50	--	5.5
D. w.	13	1.78	0.1819	1.6-2.1
D. a.	3	1.47	0.0577	1.4-1.5

H = 5.35714

Table 13 Relative Snout Length. (snout length/head length). (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.321	0.0278	0.27-0.37
D. m.	14	0.312	0.0275	0.27-0.35
D. f.	18	0.308	0.0389	0.26-0.41
D. q.	14	0.313	0.0232	0.26-0.39
L. m.	3	0.291	0.0187	0.28-0.31
P. h.	1	0.237	--	0.237
D. w.	13	0.314	0.0289	0.27-0.36
D. a.	3	0.295	0.0023	0.29-0.30

H = 0.71117

Table 14 Pre-nares Snout Length. (snout length minus external nares to eye distance.)

Species	N	$\bar{X}$	s	Range
D. o.	29	1.02	0.3219	0.3-1.8
D. m.	14	1.27	0.3791	0.8-2.2
D. f.	18	1.32	0.3650	0.8-2.1
D. q.	14	1.56	0.6134	1.0-3.0
L. m.	3	1.07	0.3512	0.7-1.4
P. h.	1	1.70	--	1.7
D. w.	13	0.68	0.1214	0.5-0.8
D. a.	3	0.70	0.1000	0.6-0.7

H = 3.9102

Table 15 Relative Pre-nares Snout Length. (snout length/head length). (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.1150	0.0276	0.038-0.157
D. m.	14	0.1072	0.0141	0.082-0.141
D. f.	18	0.1184	0.0206	0.080-0.164
D. q.	14	0.1102	0.0184	0.089-0.148
L. m.	3	0.0703	0.0144	0.054-0.081
P. h.	1	0.0730	--	0.073
D. w.	13	0.1203	0.0201	0.081-0.156
D. a.	3	0.1413	0.0201	0.118-0.157

H = 4.46069

Table 16 Distance Between the Eyes.

Species	N	$\bar{X}$	s	Range
D. o.	29	2.16	0.3591	1.5-2.9
D. m.	14	2.57	0.8260	1.7-4.3
D. f.	18	2.44	0.5670	1.6-3.4
D. q.	14	2.91	0.9742	1.8-5.0
L. m.	3	2.43	0.4041	2.2-2.9
P. h.	1	4.40	--	4.4
D. w.	13	1.23	0.4273	1.1-1.8
D. a.	3	1.30	0.1000	1.2-1.4

H = 3.9102

Table 17 Distance Between External Nares. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	1.74	0.3933	1.1-2.7
D. m.	14	2.61	0.6120	1.9-3.7
D. f.	18	2.21	0.5472	1.0-2.8
D. q.	14	3.12	1.2040	2.0-5.8
L. m.	3	2.93	0.3512	2.6-3.3
P. h.	1	4.30	--	4.3
D. w.	13	1.07	0.0947	0.9-1.2
D. a.	3	0.87	0.0577	0.8-0.9

H = 5.35714

Table 18 Ratio of Distance between Eyes/Distance between Nares.

Species	N	$\bar{X}$	s	Range
D. o.	29	1.267	0.2040	1.00-1.77
D. m.	14	0.976	0.1607	0.66-1.21
D. f.	18	1.124	0.1439	0.96-1.60
D. q.	14	0.952	0.1143	0.69-1.10
L. m.	3	0.840	0.1652	0.69-1.00
P. h.	1	1.020	--	1.02
D. w.	13	1.2454	0.2327	0.92-1.80
D. a.	3	1.500	0.1570	1.33-1.63

H = 4.46428

Table 19 Snout-Forelimb Insertion. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	10.79	1.5440	7.0-14.1
D. m.	14	14.07	2.5381	10.5-17.9
D. f.	18	13.74	2.9839	8.3-18.6
D. q.	14	17.12	5.0937	10.9-28.4
L. m.	3	17.50	2.0000	15.6-19.6
P. h.	1	26.7	--	26.7
D. w.	13	7.15	0.5517	5.8- 8.0
D. a.	3	6.30	0.3606	5.9- 6.6

H = 5.35714

Table 20 Relative Snout-forelimb Insertion. (snout-forelimb insertion/snout-vent length).

Species	N	$\bar{X}$	s	Range
D. o.	29	0.3037	0.0290	0.231-0.343
D. m.	14	0.2994	0.0181	0.275-0.332
D. f.	18	0.2964	0.0167	0.261-0.340
D. q.	14	0.3080	0.0137	0.286-0.329
L. m.	3	0.2970	0.0095	0.288-0.307
P. h.	1	0.2420	--	0.242
D. w.	13	0.3035	0.0180	0.282-0.333
D. a.	3	0.2900	0.0150	0.281-0.308

H = 0.85714

Table 21 Hindlimb Length. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	6.20	1.1163	3.8-8.5
D. m.	14	8.09	1.6986	5.4-10.4
D. f.	18	7.57	1.5873	3.8-10.1
D. q.	14	9.46	2.7723	5.3-15.0
L. m.	3	9.50	1.0500	8.7-10.7
P. h.	1	8.30	--	8.3
D. w.	13	4.15	0.7067	3.0-5.8
D. a.	3	3.57	0.3215	3.2-3.8

H = 5.35714

Table 22 Forelimb Length.

Species	N	$\bar{X}$	s	Range
D. o.	29	4.74	0.9927	2.3-7.0
D. m.	14	6.39	1.6513	3.9-9.7
D. f.	18	5.52	1.3149	2.7-6.9
D. q.	14	7.56	2.4719	4.7-13.0
L. m.	3	7.10	0.9644	6.4-8.2
P. h.	1	6.80	--	6.8
D. w.	13	3.48	0.7672	2.5-5.4
D. a.	3	2.8[illegible]	0.1528	2.7-3.0

H = 5.35714

Table 23 Ratio of Hindlimb/Forelimb. (Species and genera are abbreviated by the first letter.)

Species	N	$\bar{X}$	s	Range
D. o.	29	0.769	0.1146	0.49-0.94
D. m.	14	0.786	0.0737	0.63-0.94
D. f.	18	0.731	0.0794	0.57-0.89
D. q.	14	0.7986	0.0698	0.67-0.91
L. m.	3	0.7500	0.0173	0.74-0.77
P. h.	1	0.820	--	0.82
D. w.	13	0.836	0.950	0.65-1.00
D. a.	3	0.803	0.0321	0.78-0.84

$H = 3.92142$

Discussion:

The results showed musch overlap in the characters at the species level. Figure 20 illustrates the snout-vent length range of males for each testis lobe class, for each species. The number of lobes per testis was determined by Humphrey (1922) to be correlated with age. This was reconfirmed in Desmognathus by Organ (1961a). The figure illustrates the overlap, intra- and interspecifically, as well as the progressive increase in size from D. aeneus and D. wrighti to Phaeognathus. Phaeognathus hubrichti was the only specis which showed significant differences for most characters and was therefore not included in the analysis of variance of the species groups. The study of lactate dehydrogenase had cautiously placed it with group II - D. quadramaculatus and L. marmoratus. Despite the overlap, the Kruskal-Wallis one way analysis of variance yielded significant H values (probability less than .05) for eleven of the twenty-three characters. Nine of these were absolute size measurements rather than ratios. This indicates that in the evolution of the desmognathines body proportions have remained the same and the differences between the species are of absolute size. This indicates a lack of allometry and suggests that the adaptive radiation of Desmognathus is primarily associated with changes in size and surface to volume ratios. This further suggests that the correlations of enzyme and morphology may not be related directly to ecology but to the size of the species.

The apparent trend in decreased size with increased terrestrialism is not accompanied by changes in relative size (e.g. to snout-vent length of head, trunk, or tail), (see Fig. 40).

Within the range of size for Desmognathus and Leurognathus the trunk vertebral number has remained constant at fifteen. Phaeognathus has evolved in a different manner. It is a highly terrestrial salamander, yet according to Valentine (1963), it is possibly the largest terrestrial salamander known. Elongation has been accomplished by an increase in the number of trunk vertebrae (21 to 23; Brandon, 1965) and probably an increase in the number of tail vertebrae. There has not been a corresponding increase in the head length. The head measurements relative to snout-vent length are both significantly lower than the other Desmognathinae (see Tables 8 and 10).

The three absolute size characters which did not give significant differences were tail length, (Table 2),pre-nares snout length (Table 14), and the distance between the eyes (Table 6). The latter two may be errors produced by preservation. The high variation of tail length probably resulted from incompletely regenerated tails which appeared complete. The H value for relative tail length (Table 3) indicated it was significant at the .05 level. This was the only character for which the analysis of variance indicated non-random grouping but did not detect that a Group II and III species had reversed expected values. D. wrighti was more like Leurognathus than D. aeneus was more like D. quadramaculatus

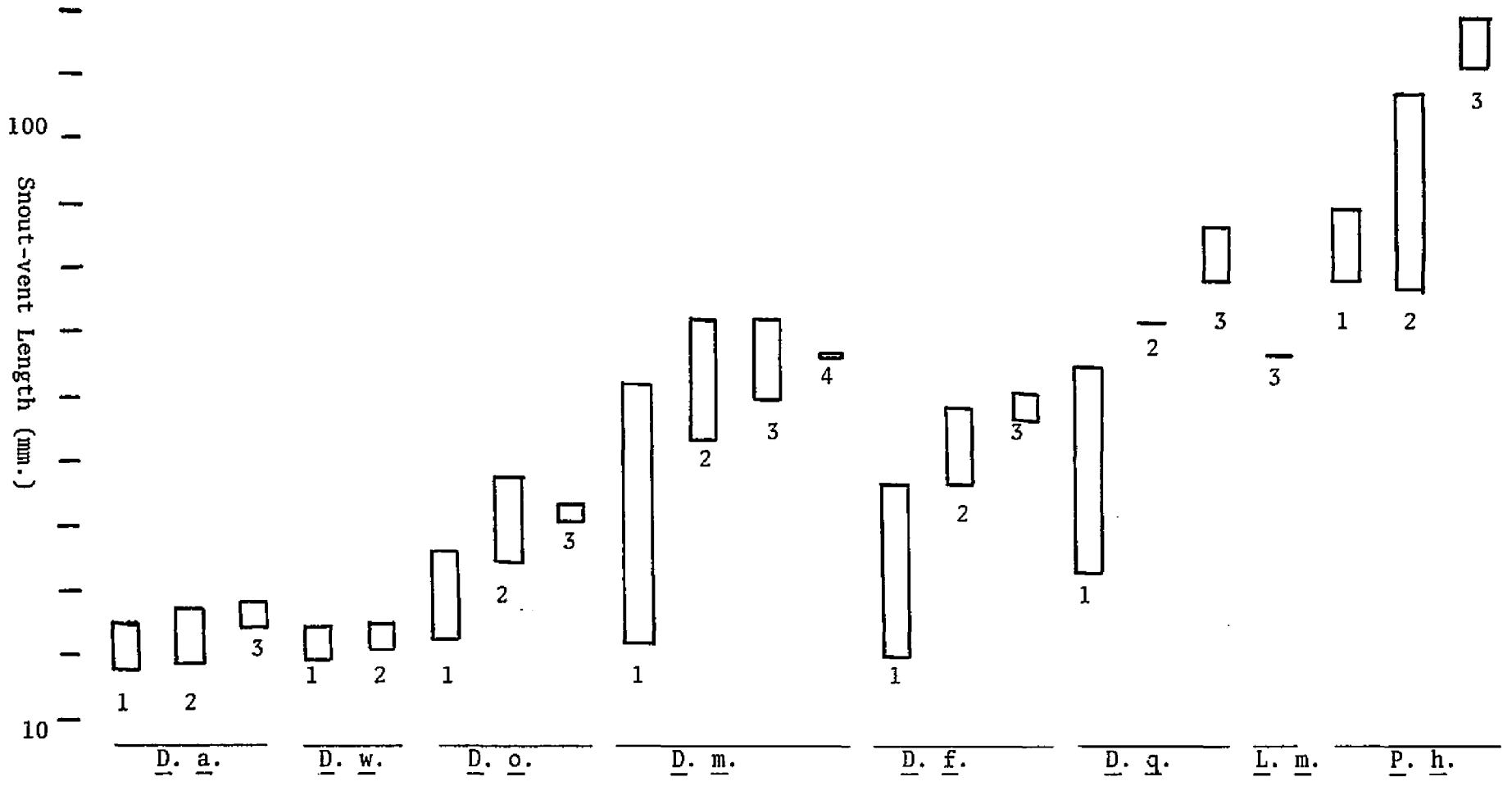


Fig. 20 Range of snout-vent length for the number of lobes per testis in the Desmognathinae. (Species and genera are abbreviated by the first letter. Data from the following references are included: D. monticola - Organ (1961a), D. fuscus - Spight (1967), P. hubrichti - Brandon (1965), D. aeneus - Harrison (1967).

than D. wrighti. D. ochrophaeus, D. fuscus, and D. monticola grouped as usual in Group I.

Desmognathine Head Musculature and Jaw Apparatus

The desmognathine jaw apparatus is a unique adapatation of the subfamily whereby the skull is raised to open the mouth. This is accomplished by partial immobilization of the lower jaw by the atlas-mandibular ligament and by various modifications of the skull as described below. The nature of the mechanism and the variation of this character was studied in all the species of the subfamily. The complex of characters associated with mouth opening could be considered primitive because it is found in all the species. It was of interest to determine whether there were any modifications correlated with the adaptive trend away from the primitive aquatic to the terrestrial adaptive zone. All the elements of the mechanism were studied to determine if there were differences associated with size, and if there were differences, to see if they corresponded to the species groups as elucidated by the studies of lactate dehydrogenase. This included dissections of those muscles which participate in mouth opening and investigations of the skull of each species (see figs. 22 - 36). Fig. 21 shows, for comparison, dorsal head views of the species, drawn twice natural size.

Head Musculature

No obvious differences in the musculature and associated structures were observed other than those associated with absolute size. It has been determined (reconfirmed in most cases) which muscles participate, their origin(s), insetion(s), and action(s).

The muscles of the adult head associated with mouth opening follow. The descriptions and abbreviations apply to Figs. 28 - 36, and are taken from Francis (1934) unless otherwise noted.

m. intermandibularis posterior (m.i.m.p.) - ventrally the most anterior muscle in metamorphosed individuals. The m. intermandibularis anterior disappears at metamorphosis in the Hynobiidae, Ambystomidae, Salamandridae, and Plethodontidae (Piatt, 1940). Origin: mesial surface of mandible. Insertion: median raphe. Action: to raise the floor of the mouth.

m. interhyoideus (m.i.) - fans out at the level of the previous muscle, with the anterior fibers lying dorsal to it. Origin: by a short thin tendon from the postero-mesial edge of the quadrate. Insertion: median raphe. Action: constricts hyobranchial skeleton and posterior part of mouth.

m. interhyoideus posterior (m.i.p.) - Origin: by tendon from the ventro-lateral surface of the quadrate and squamosal. In Desmognathus and Leurognathus the origin is also from the ventrolateral surface of the mandible, just anterior to its articulation with the quadrate (see fig. 34). The same is probably true for Phaeognathus. Insertion: primarily on the skin of the gular fold. Action: constricts pharynx, depresses head. As Wake (1966) noted this is an important aspect of the jaw mechanism. Based on the insertion of the muscle on the mandible it also serves to hold the prearticular firmly against the quadrate

when the mouth is being snapped shut.

This last muscle is the bulge so obvious on Desmognathus, Leurognathus, Phaeognathus and other salamanders. Its condition is considered unique in the Plethodontidae and many authors call it the m. quadrato-pectoralis of Druner (1901). As reviewed by Piatt (1940) this muscle and the m. interhyoideus arise from the same mass of mesoderm. They remain unaltered after metamorphosis in those species in which metamorphosis is normal in the Ambystomidae, and in addition the Cryptobranchidae and Amphiumidae. At metamorphosis in the Plethodontidae a new muscle arises from the m. interhyoideus posterior. Smith (1920) called this the m. gularis (m.g.) and since then there has been disagreement about the terminology. Piatt (1935; 1940) argued that since the m interhyoideus posterior gives rise to a new muscle at metamorphosis, it (the m. interhyoideus posterior) is not identical to the larval muscle and should therefore be known as the m. quadrato-pectoralis. In Desmognathinae and Aneides the m. interhyoideus posterior is the dominant muscle. The latter is different in that the m. gularis lies internal to it rather than external. In all other plethodontid genera the m. interhyoideus posterior disappears almost completely and the m. gularis takes its place. Eaton (1937) proposed calling both muscles, in larvae and adults, the m. gularis because of their common ontogenetic origin.

The nomenclature can be clarifed as follows. The homology of the m. quadrato-pectoralis and the m. interhyoideus posterior is obvious; they are the same muscle and the latter name is used here. The additional slip which becomes the dominant muscle in plethodontids other than the Desmognathinae and Aneides is still termed the m. gularis.

m. depressor mandibulae (m.d.m.)- Origin: posterior edge of squamosal, lateral ridge of occipito-otic, and the anterior portion of the fascia cephalodorsalis. Insertion: in Desmognathus, Leurognathus, and Phaeognathus on the ventral surface of the prearticular by a short tendon. It does not appear to insert on the dentary besides, as Baird (1951) found in Pseudoeurycea. Action: depressor of the lower jaw, (see discussion of jaw mechanism below).

m. levator mandibulae anterior (superficial portion) (m.l.m.a.)- Origin: in desmognathines, on raised postero-lateral ridge of atlas. The atlas is highly modified. Insertion: on well-developed coronoid process of prearticular. Action: this muscle encloses the atlas-mandibular ligament (l.a-m.) that passes from the atlas to the lower jaw. Correlated with this is a well-defined parietal-otic trough through which the muscle and ligament pass. Soler (1950) called this the m. temporalis, while Wake (1966) referred to this and the other levators as the adductor mandibulae musculature. The ligament is unique to the Desmognathinae and was one of the major reasons Soler (1950) wanted to separate the group as the family Desmognathidae (see Table 24). The action of the muscle in other salamanders is to raise the lower jaw. In the desmognathines the atlas-mandibular ligaments limit movement of the lower jaw independently of the skull (see Figs. 37 and 38). Upon dissecting out the muscle and ligaments, if the latter are lifted out of the parietal-otic troughs the lower jaw is free to swing open wide.

m. levator mandibulae anterior (deep portion) (m.l.m.a.d.)- Origin: lateral borders of frontal and parietal bones. Insertion:

coronoid process. Action: with the previous muscle.

m. levator mandibulae posterior (m.l.m.p)- Origin: anterior edge of squamosal and quadrate. Insertion: prearticular. Action: with the two previous muscles.

m. dorsalis trunci (m.d.t.)- This major muscle mass is differentiated at the head. The parts responsible for raising the skull in desmognathines have their origin on the first, second, and third vertebrae. The lateral muscles insert over the postero-lateral aspect of the occipito-otic immediately behind the dorsal end of the squamosal. The medial portion of the m. dorsalis trunci is mainly responsible for raising the skull and is quite well developed. It has an origin on the atlas and an insertion over the otic crests behind the troughs and along a ridge on the parietal, anterior to the parietal-otic troughs.

Jaw Apparatus

The atlas-mandibular ligament runs through the m. levator mandibulae anterior (superficial portion) and limits the lower jaw from being depressed. However, it should be noted that the path of the muscle is similar in other salamanders (Baird, 1951; personal observations) and may serve to limit lower jaw depression in general. The maximum mouth opening would then be limited by the maximum stretch of the m. levator mandibulae anterior (superficial portion). The ligament strengthens the position of the lower jaw whether open or closed. The mouth is then opened by the action of the dorsal spinal muscles which insert along a parietal ridge. Anterior to the insertion of these muscles the frontals, nasals, premaxillae, and maxillae bear no musculature. Relative to other plethodontids the entire skull is strengthened by

the development of the parietal-otic troughs resulting in longitudinal movement and lateral restriction of the muscle and ligament, modification of the atlas, and the specialized skull and atlas articulation (Wake, 1966).

As a result of the thickened bone and relatively firm sutures, the skull is raised as a unit. This results in the nerve cord being bent sharply. In non-desmognathines with sessile occipital condyles the nerve cord would be pinched by the resulting degree of flexure. The stalked condyles found in this group (see Figs. 22-28, 37, 38) leave a space between the atlas add skull (0.5mm in a D. ochrophaeus male, 29-4, with a snout-gular fold length of 10.3mm), for swinging the skull up (see Figs. 31 and 32). As Fig. 38 shows, the broad atlantal-condylar facets of the atlas allow the occipital condyles to shift down and accentuate the swing of the skull.

The regular mode of opening the mouth in non-desmognathines appears to be by the action of the mandibular depressor swinging the lower jaw down while the dorsal spinal muscles hold the skull in the same plane (see Fig. 37). The fulcrum is located at the articulation of the quadrated and prearticular. In desmognathines the opposite occurs. Because of the inelastic nature of the ligament, compared to the muscle, the lower jaw cannot be swung down from the horizontal plane. The depressor mandibulae muscles hold the lower jaw in the same plane while the skull is swung up by the action of the dorsal spinal muscles (see Fig. 37). This requires that the atlas-mandibular ligament and associated musculature move in the parietal-otic trough. The ligament serves several functions, one of which was originally selected

for. As the skull is raised the squamosal and quadrate (both firmly attached to the skull) are rotated forward, thrusting the lower jaw forward (see Figs. 37 and 38). To close the mouth, only the relaxation of the dorsal spinal muscles is required. The tension on the atlas-mandibular ligament would serve to pull the skull down. With the levator mandibulae muscles and the m. interhyoideus posterior (pulling the quadrate posteriorly) the mouth can be rapidly and firmly snapped shut. The lower jaw is linked to the skull at two points, the insertion of the ligament and the quadrate-prearticular articulation. Because of a pivot point anterior to the regular jaw articulation, the gape of the mouth in desmognathines is less than other salamanders (adult mouth structure, Regal, 1966). The inelastic ligament and the m. depressor mandibulae serve to hold the lower jaw firmly in place against the quadrate with the mouth open.

The lower jaw in Desmognathus, Leurognathus, and Phaeognathus is specialized (Wake, 1966:28). The dentaries are large, massive bones, broad ventrally (see Figs. 24, 27, 31, 34, 35, and 38). The primary selective force suggested for the evolution of the jaw mechanism was correlated to the behavior of desmognathines burrowing beneath rocks and into crevices, and perhaps feeding (Dunn, 1926). As described, the jaw apparatus as a functional unit appears to be well adapted for stream bottom feeding. The size and solid nature of the dentary would aid as an anchor for a salamander feeding in a current. The heavier the lower jaw, the less effort required to be stationary. As the muscles were already present to raise the skull, selection would be for greater freedom of movement such as the stalked occipital condyles allow. The whole system was made more efficient and the possibility of

damage at the point of flexure was reduced by the appearance of a ligament in the m. levator mandibulae anterior (superficial portion). This muscle is apparently subject to tension when non-desmognathine salamanders open the mouth. Overall strengthening (and therefore increased weight of the head) would not be of advantage to a salamander which had to raise the head in order to drop the lower jaw. It would be in a form resting its head on the substrate. The adaptations for holding the lower jaw firmly against the quadrate function best (i.e. more tension) when the mouth is open. The result in the Desmognathinae was a mechanism whereby a salamander could rest on the stream bottom with a minimum of movement and without lifting itself off the bottom, to drop the lower jaw. This hypothesis stressis feeding as the primary selective force.

Wake (1966:59) notes that Phaeognathus does not appear to have as well developed a skull raising mechanism as Desmognathus and Leurognathus. Based on my observations and analysis, Phaeognathus has all the attributes for raising the skull, and the jaw mechanism would be expected to function in a similar manner to the other desmognathines.

It can be concluded that the desmognathine head musculature and jaw apparatus show little variation the subfamily despite the adaptive trend within the group. The only differences the species show in this character complex are those associated with absolute size. The primitive function is postulated as being primarily for feeding and was possibly a preadaptive step to burrowing.

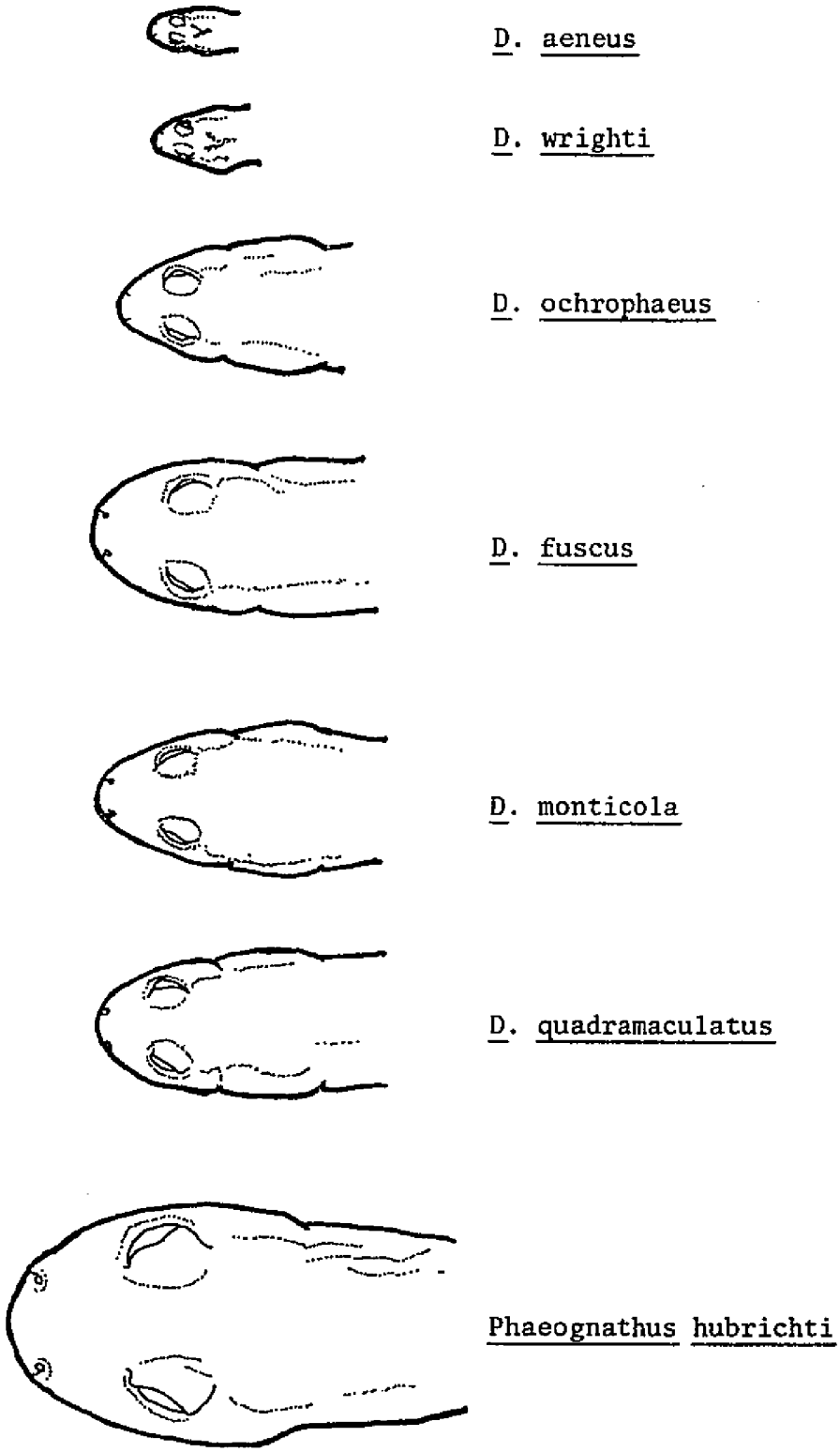


Fig. 21. Diagrams of Dorsal Head View, Desmognathus and Phaeognathus. (All drawings 2X).

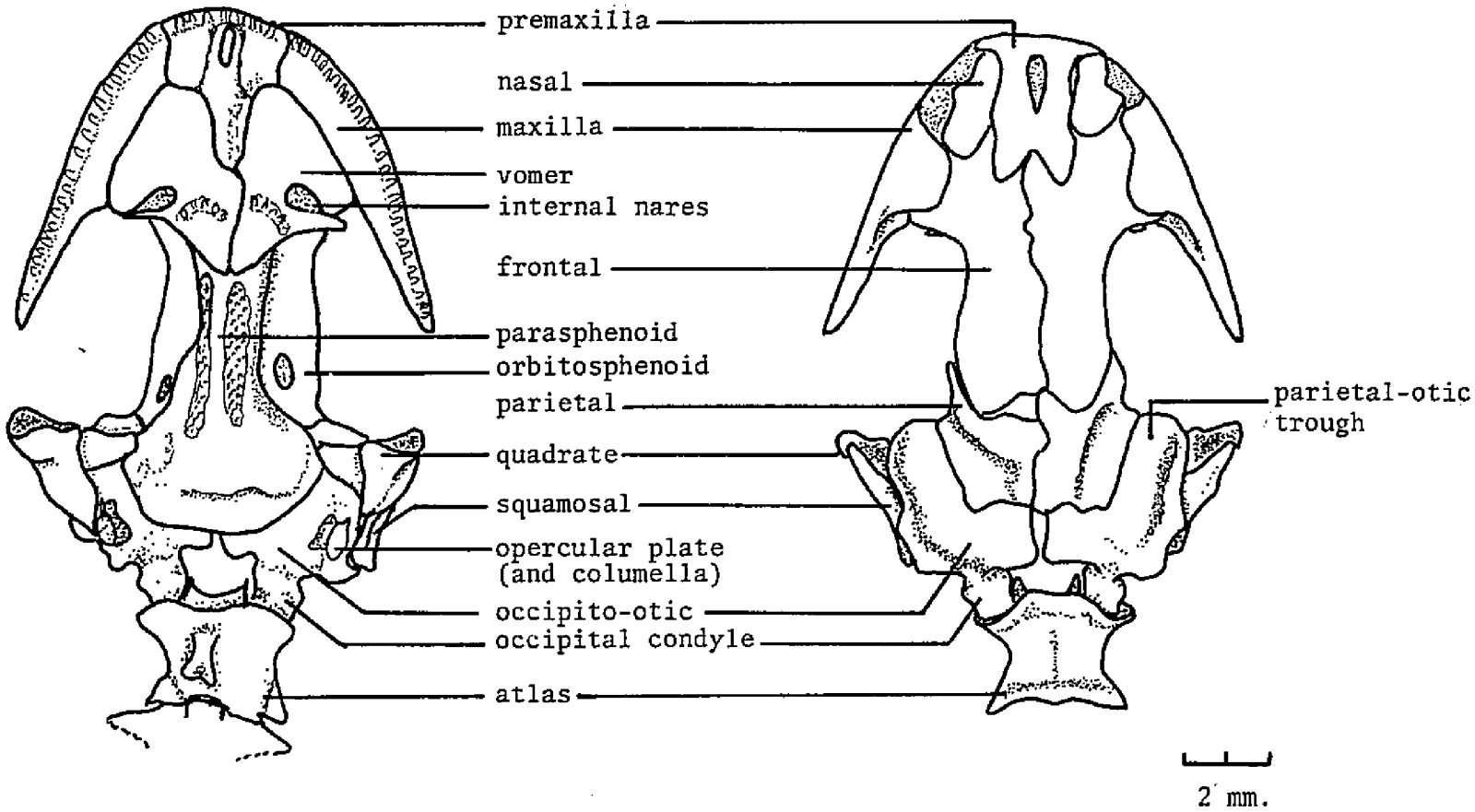


Fig. 22 Dorsal and Ventral View of D. monticola Skull. (male #47-6; Rt. 33, Greene Co., Virginia.)

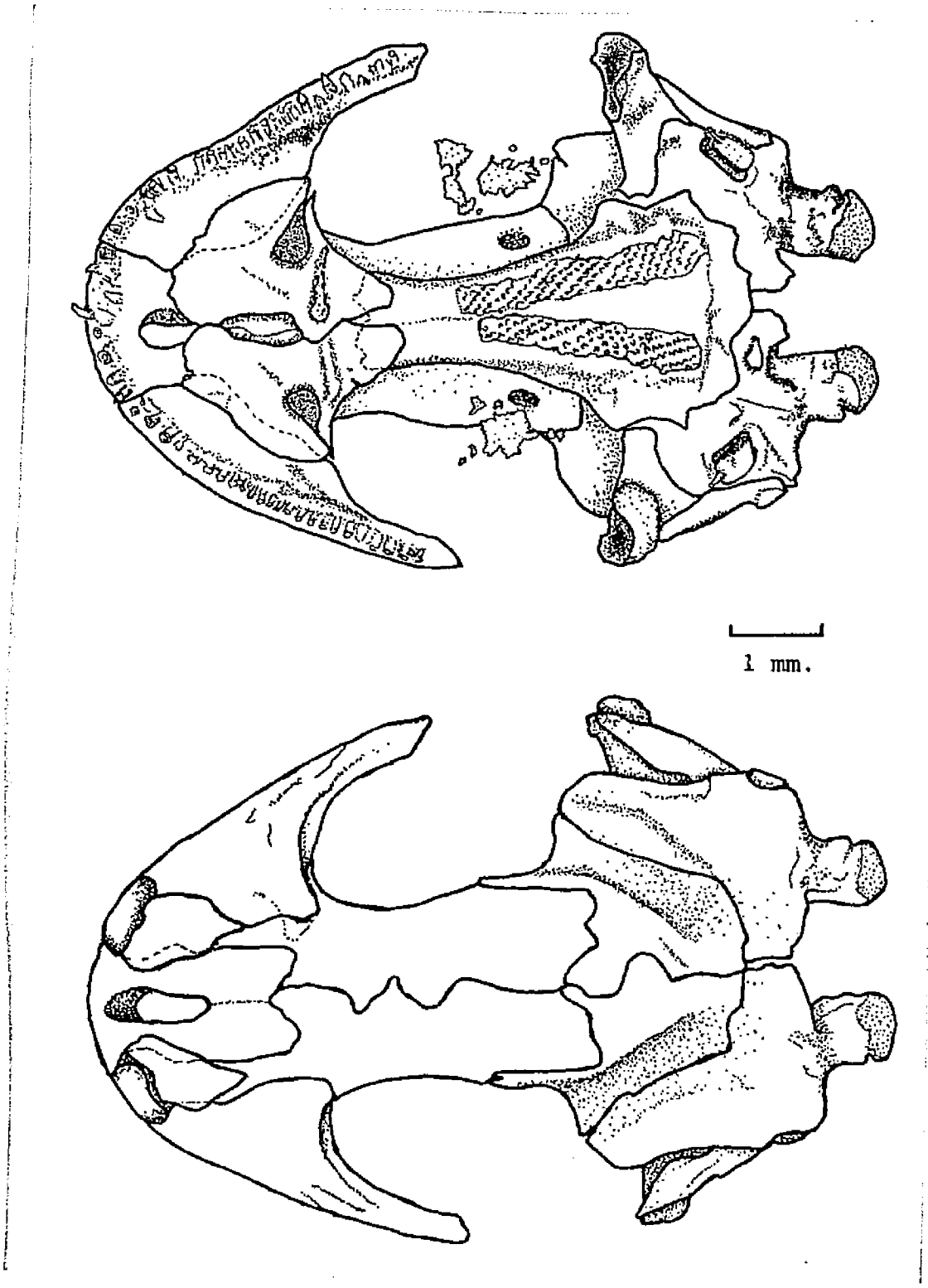


Fig. 23 Dorsal and Ventral View of D. ochrophaeus Skull. (male #20-1, Grandfather Mt., Avery Co., North Carolina)

(Note "crushing" plates" (Noble, 1931). See Fig. 27 for names of bones.)

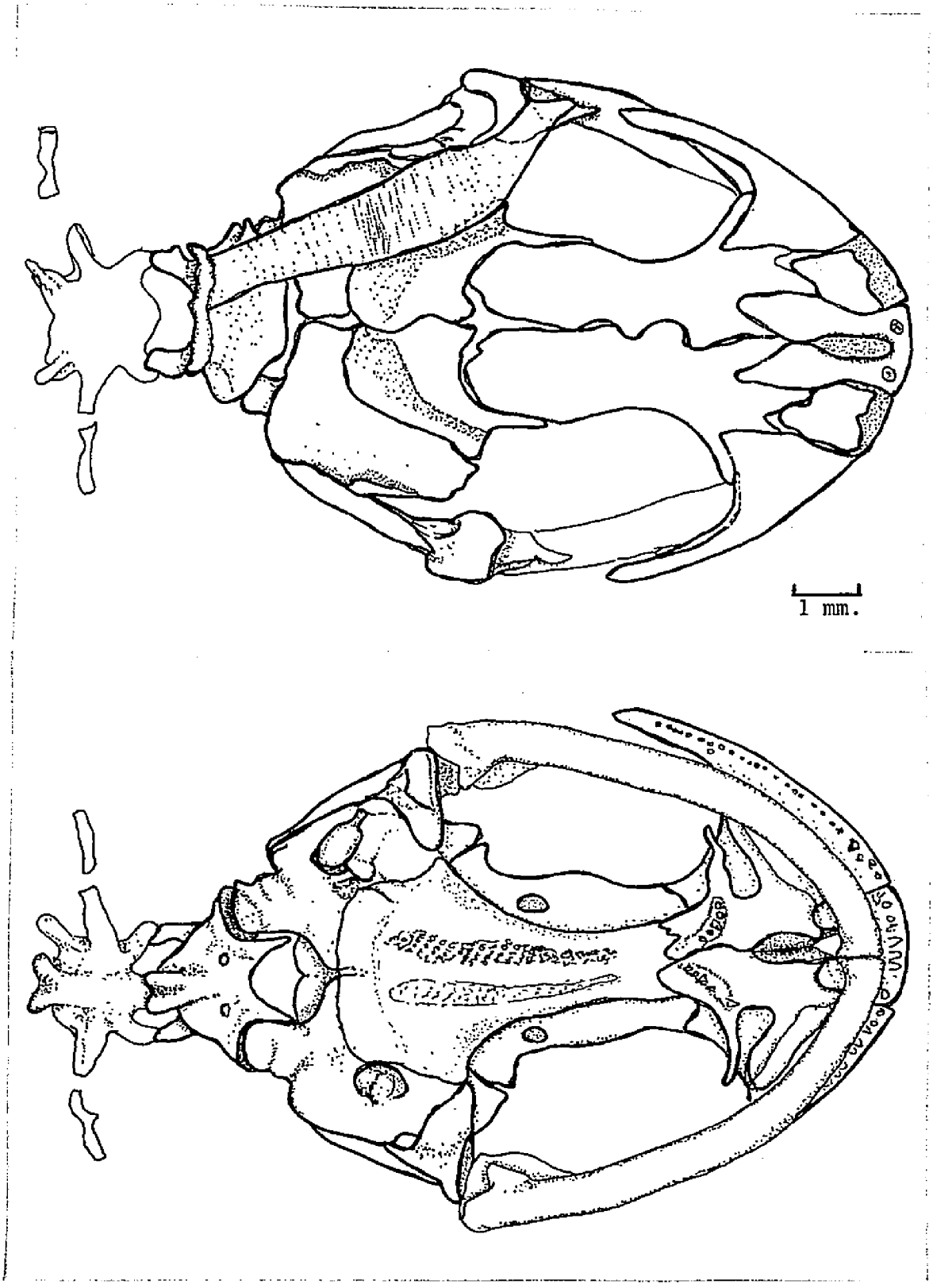


Fig. 24 Dorsal and Ventral View of D. fuscus Skull. (female; # 47-1; Rt. 33, Greene Co., Virginia)
(See Fig. 27 for labels of bones. Note atlas-mandibular ligament in dorsal view.)

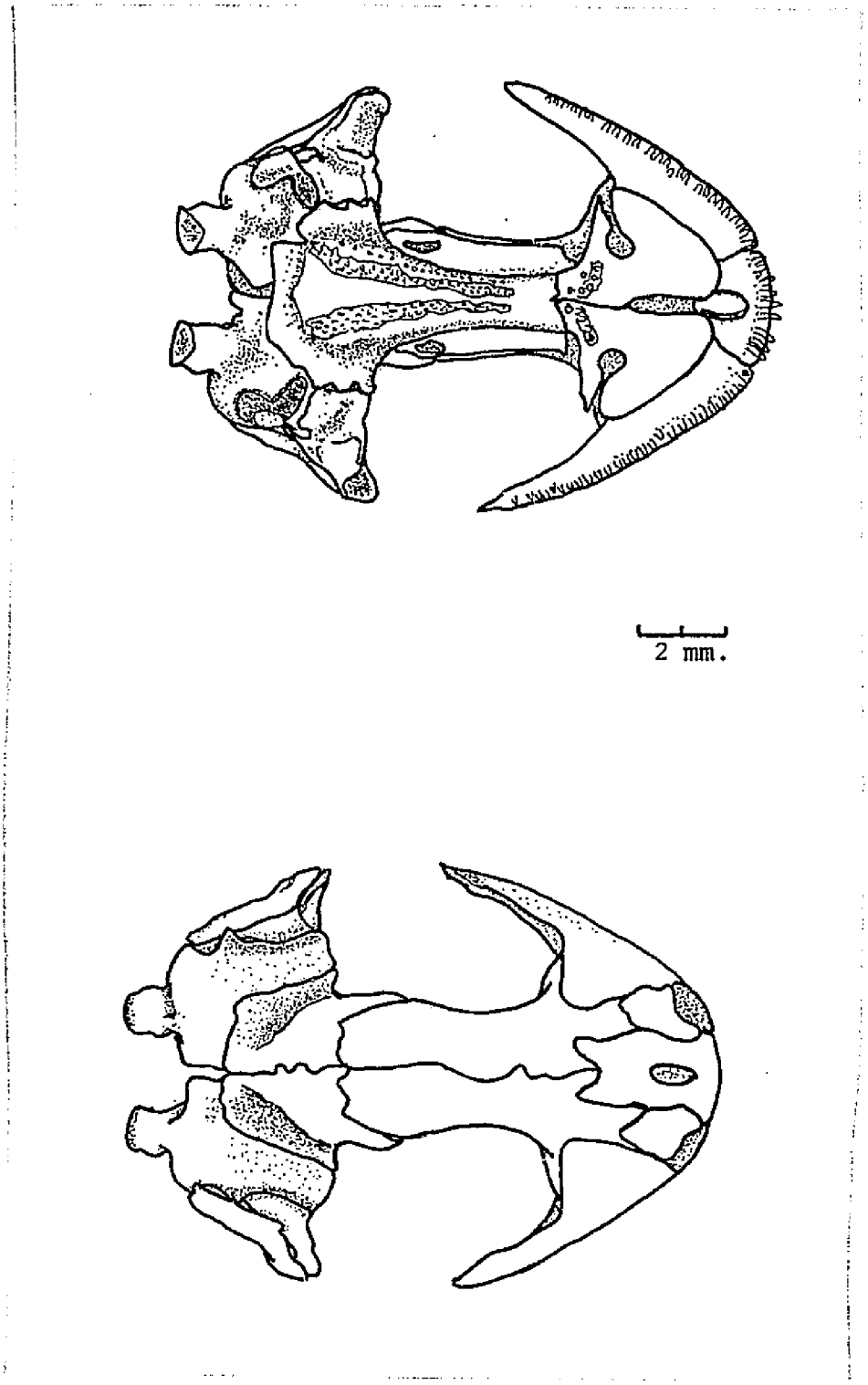


Fig. 25 Dorsal and Ventral View of D. quadramaculatus Skull.
(female # 22-1; Mt. Mitchell, Yancy Co., North Carolina)
(See Fig. 27 for labels of bones.)

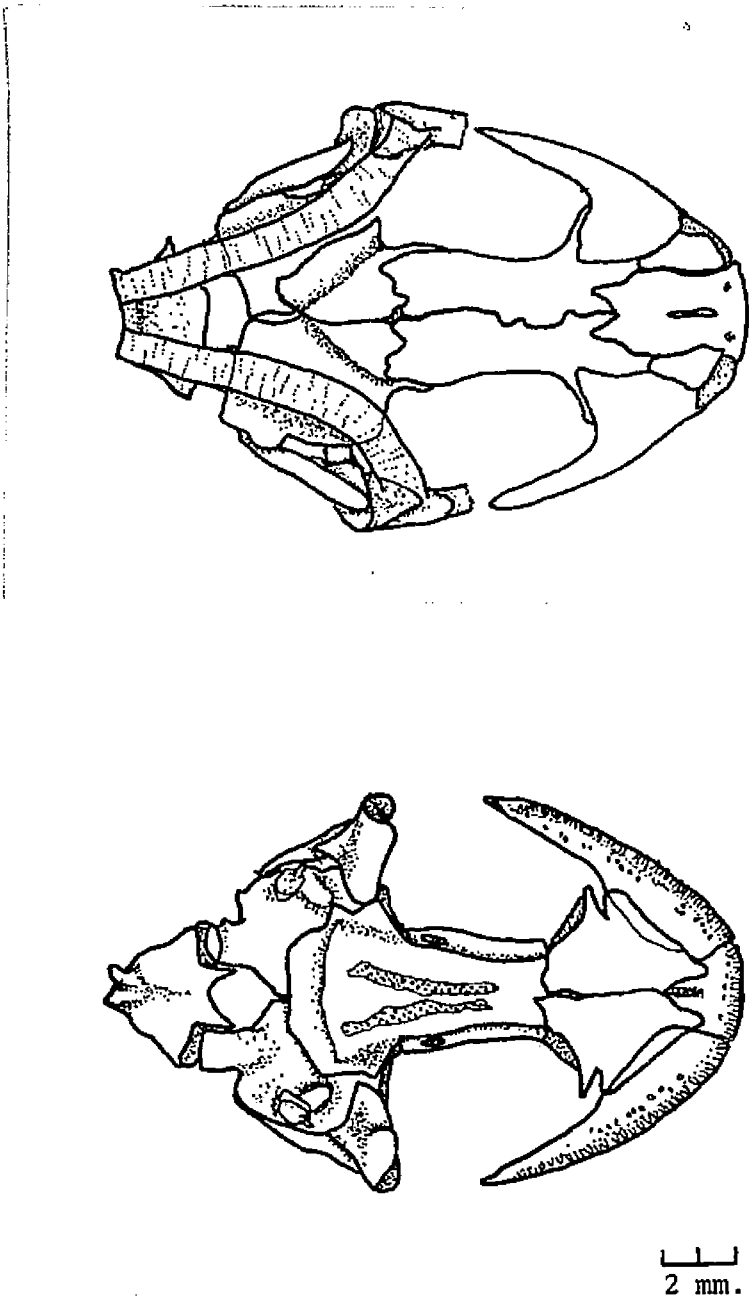


Fig. 26 Dorsal and Ventral View of Leurognathus. (female # 54-2; Greenbrier Cove, Great Smoky Mountains National Park, Sevier Co., Tennessee)
Note that the atlas-mandibular ligaments are in place.
See Fig. 27 for labels of bones.

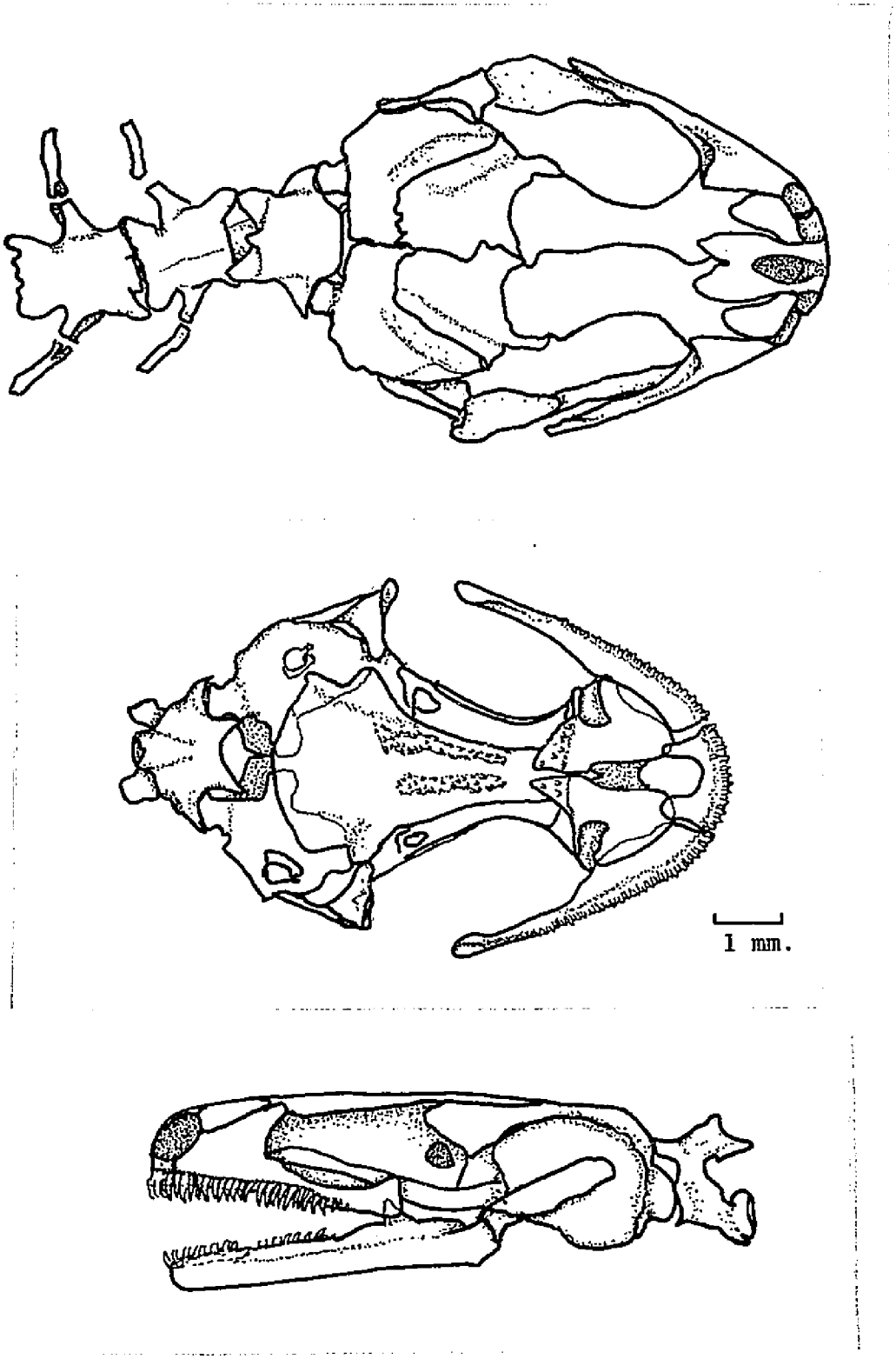


Fig. 27 Dorsal, Ventral and Lateral View of D. wrighti Skull. (female #23-6; Mt. Mitchell, Yancy Co., North Carolina) (See Fig. 27 for labels of bones.)

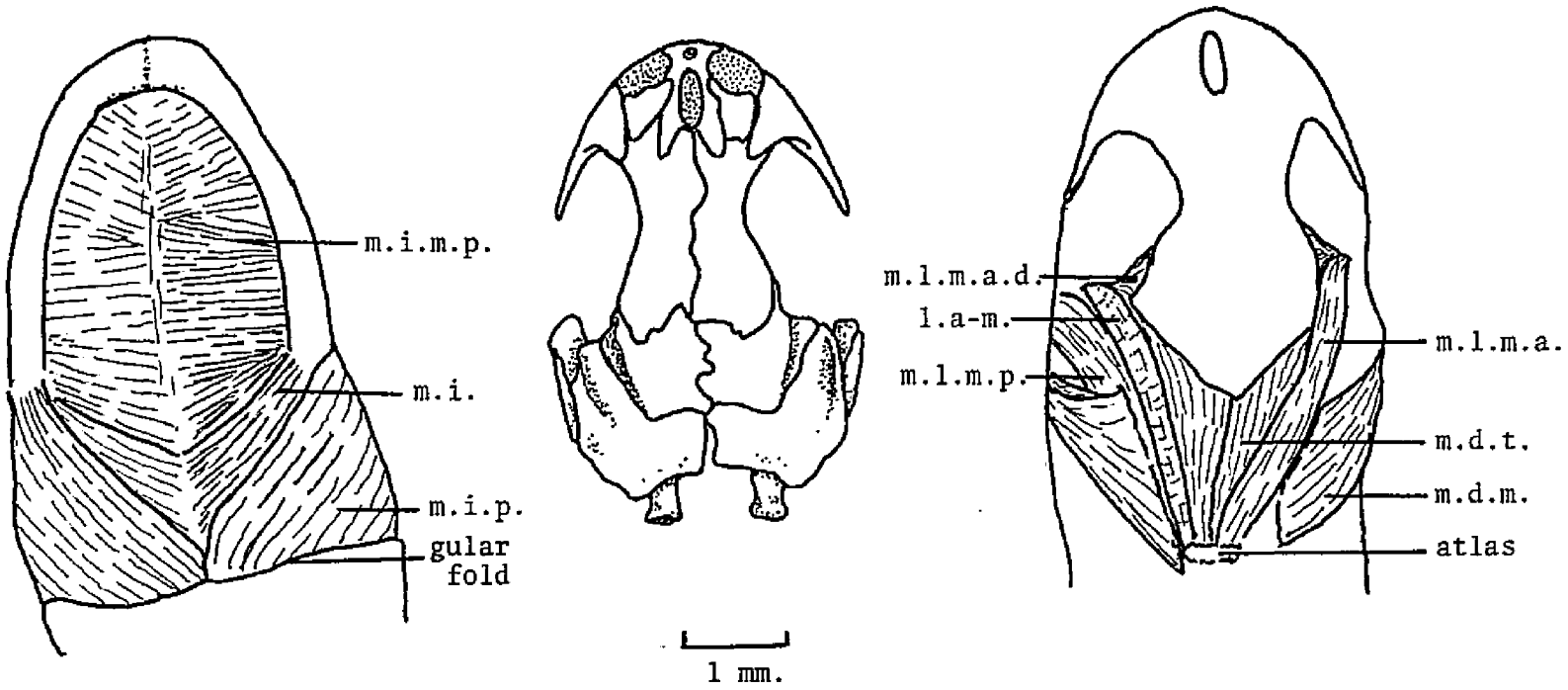


Fig. 28 Dorsal View of D. aeneus Skull and Dorsal and Ventral View of Head Musculature. (female # 29-1; Deep Gap, Macon Co., North Carolina) (See test for abbreviations. See Fig. 27 for labels of bones.)

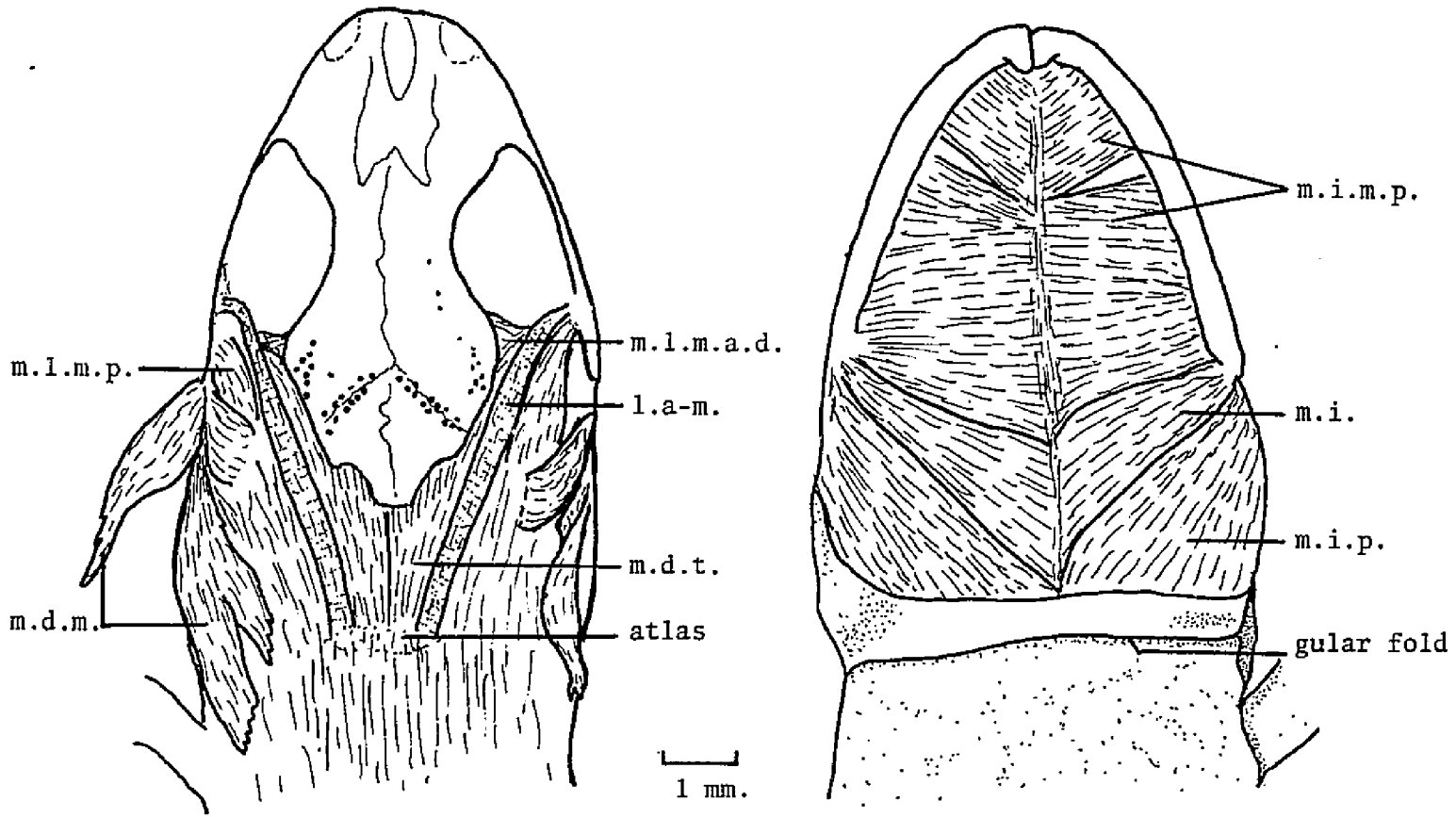


Fig. 29 Dorsal and Ventral View of D. wrighti Head Musculature. (Female # 23-6; Mt. Mitchell, Yancy Co., North Carolina) (See text for abbreviations.)

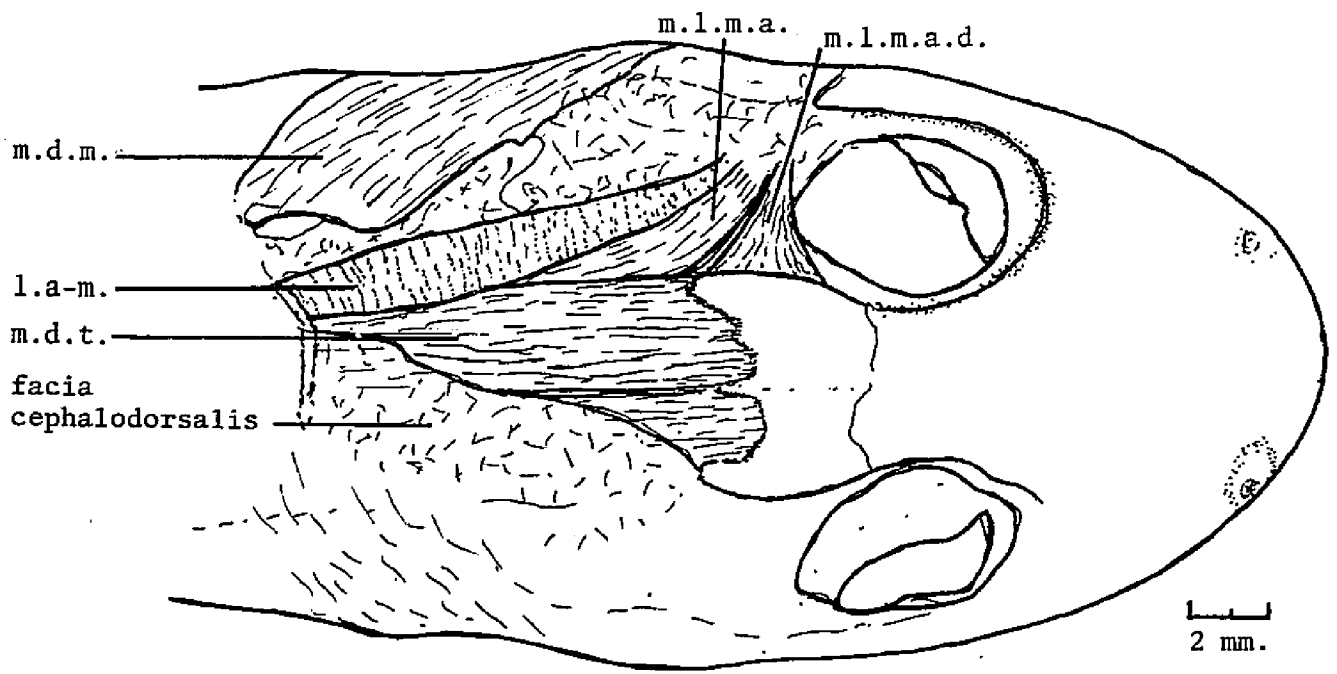


Fig. 39 Dorsal View of Phaeognathus hubrichti Head Musculature. Fascia cephalodorsalis partially removed. (female # 53-1, Butler Co., Alabama) (See text for abbreviations.)

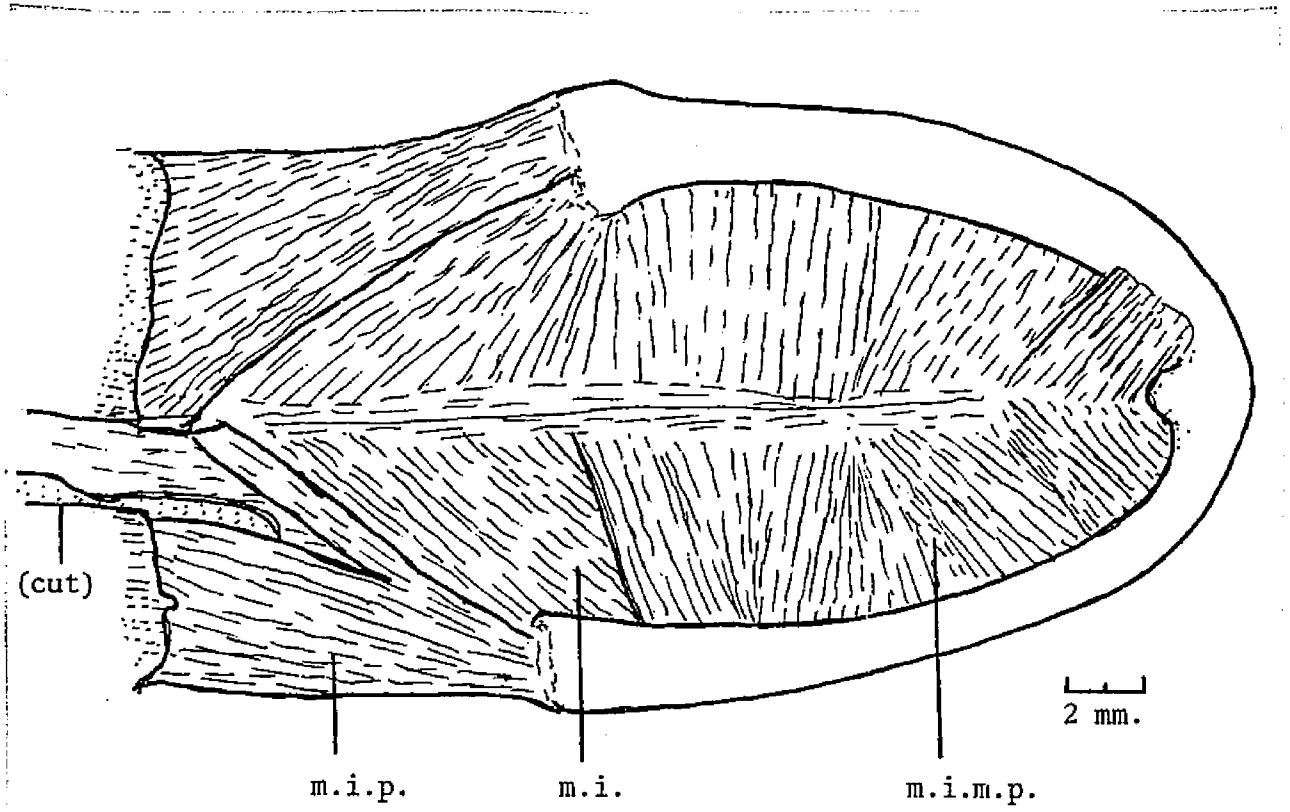


Fig. 35: Ventral View of Phaeognathus hubrichti Head Musculature. (female # 53-1, Butler Co., Alabama) (See text for abbreviations.)

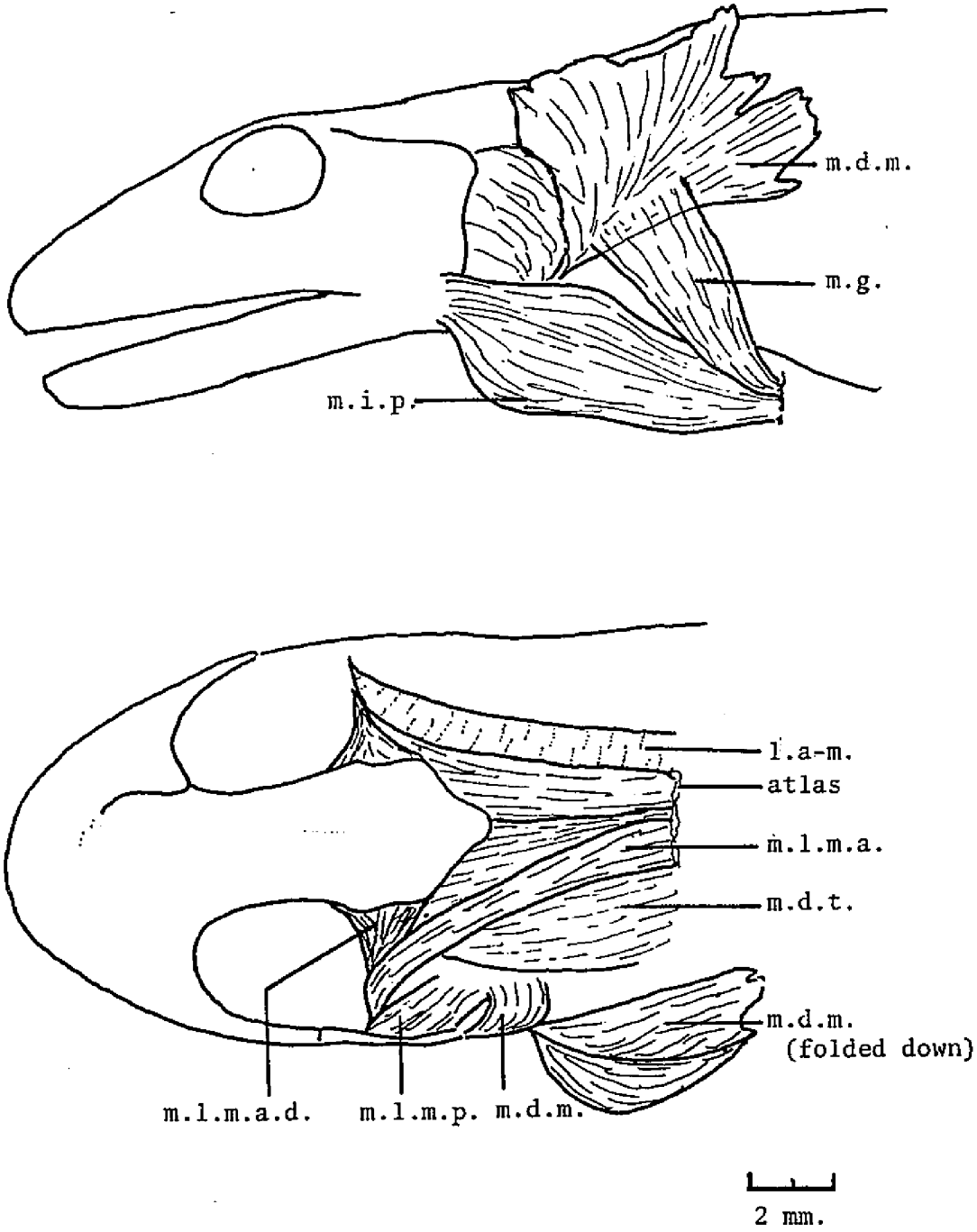
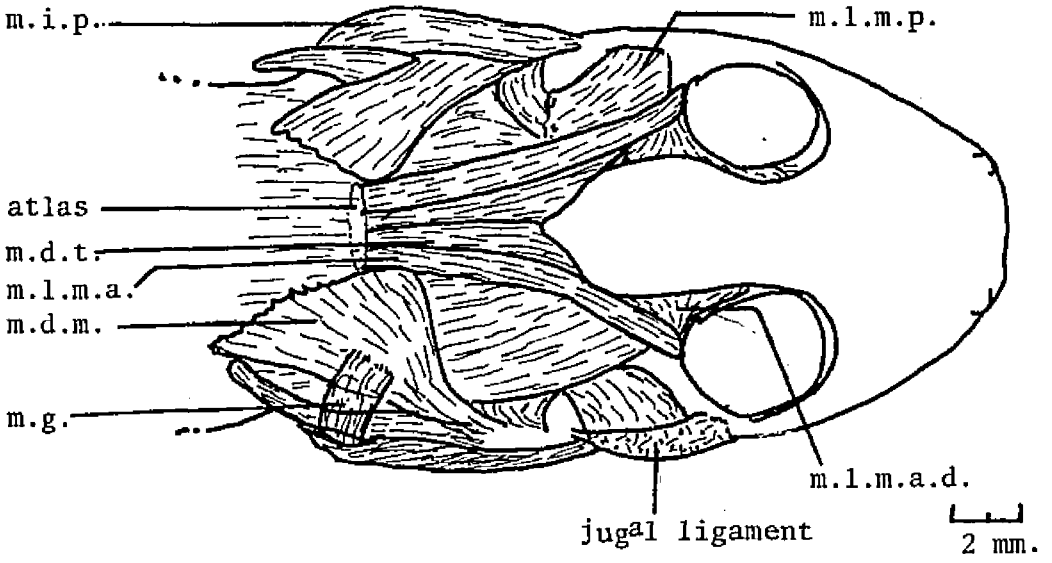


Fig. 32 Lateral and Dorsal View of D. quadramaculatus Head Musculature. (female #22-1; Mt. Mitchell, Yancy Co., North Carolina) (See text for abbreviations.)



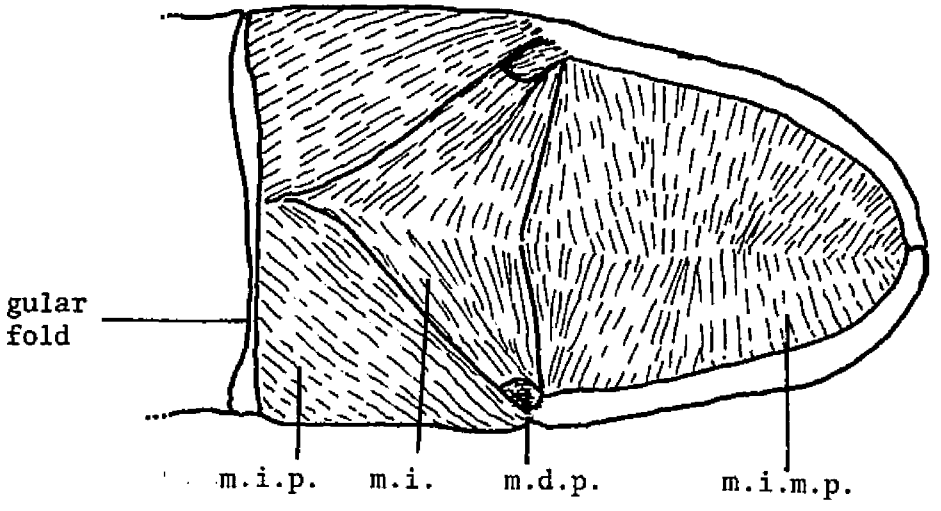


Fig. 33 Dorsal and Ventral View of Leurognathus marmoratus Head Musculature. (female # 54-2, Greenbrier Cove, Great Smoky Mountains National Park, Sevier Co., Tennessee) (See text for abbreviations.)

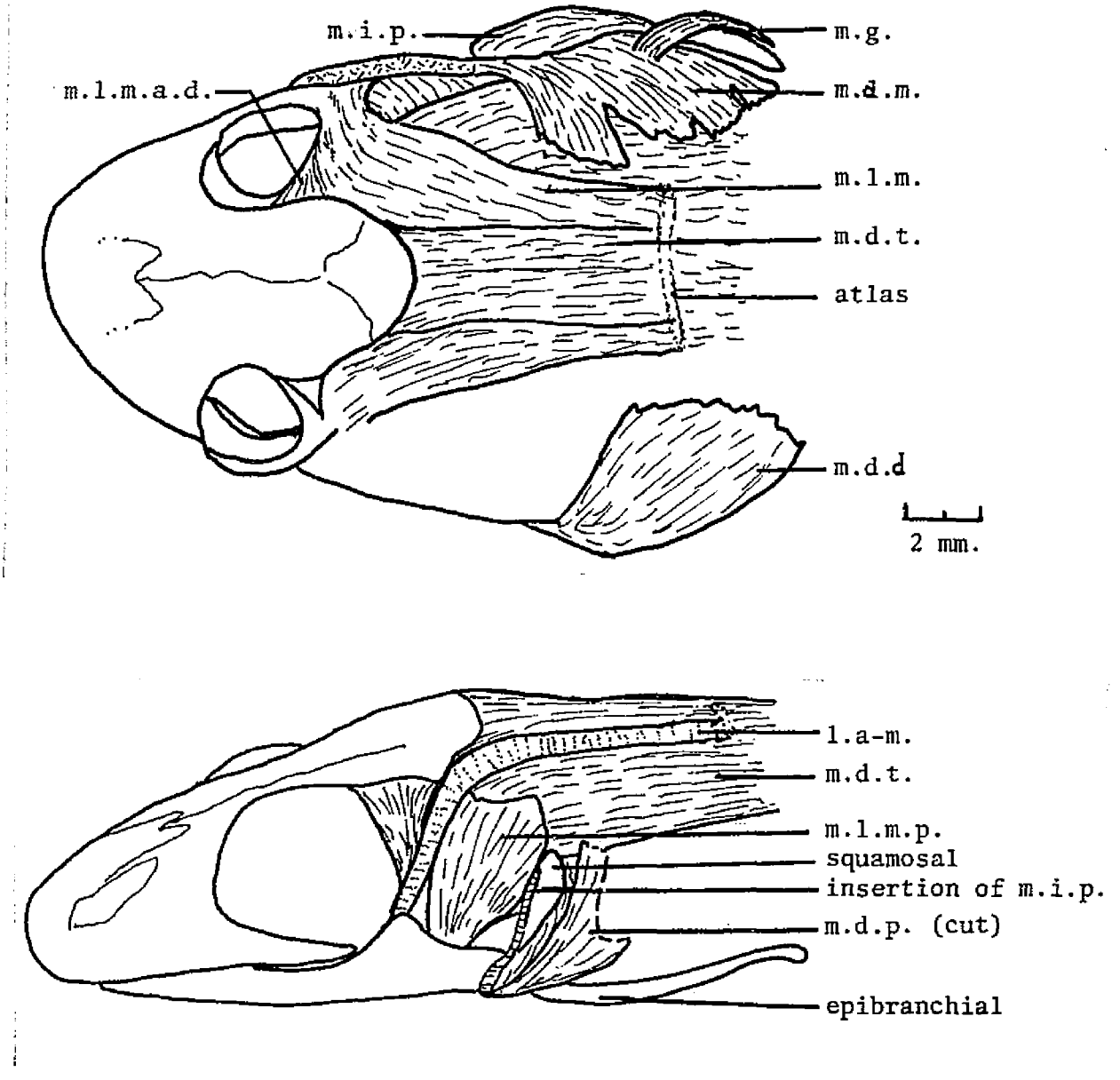


Fig. 34 Dorsal and Lateral View of D. monticola Head Musculature. (male #49-8, Cascades, Jefferson National Forest, Virginia) (See text for abbreviations.)

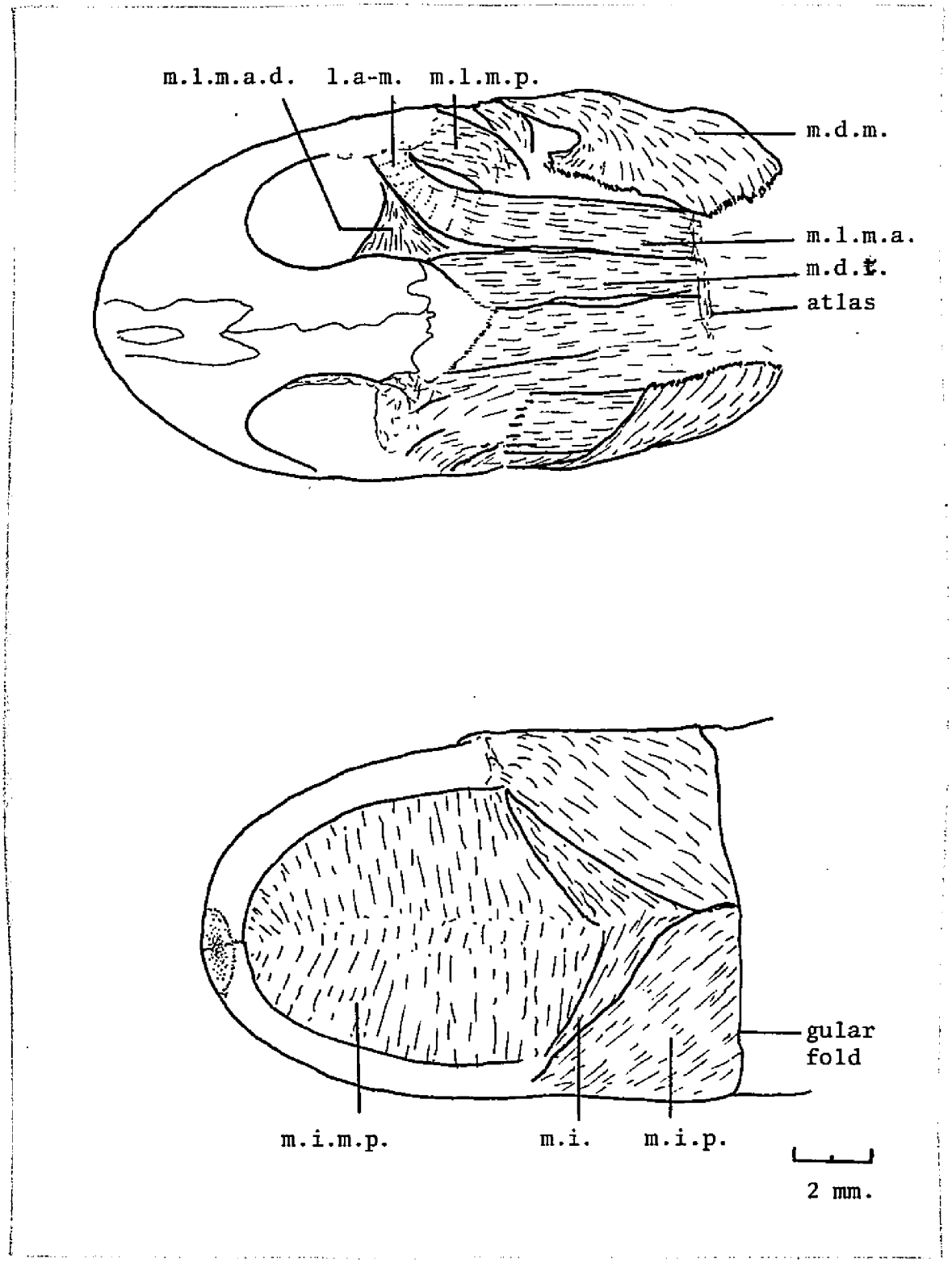


Fig. 35 Dorsal and Ventral View of D. fuscus Head Musculature. (male #38-2; Hickory Nut Mountain, Ouachita National Forest, Arkansas) (See text for abbreviations.)

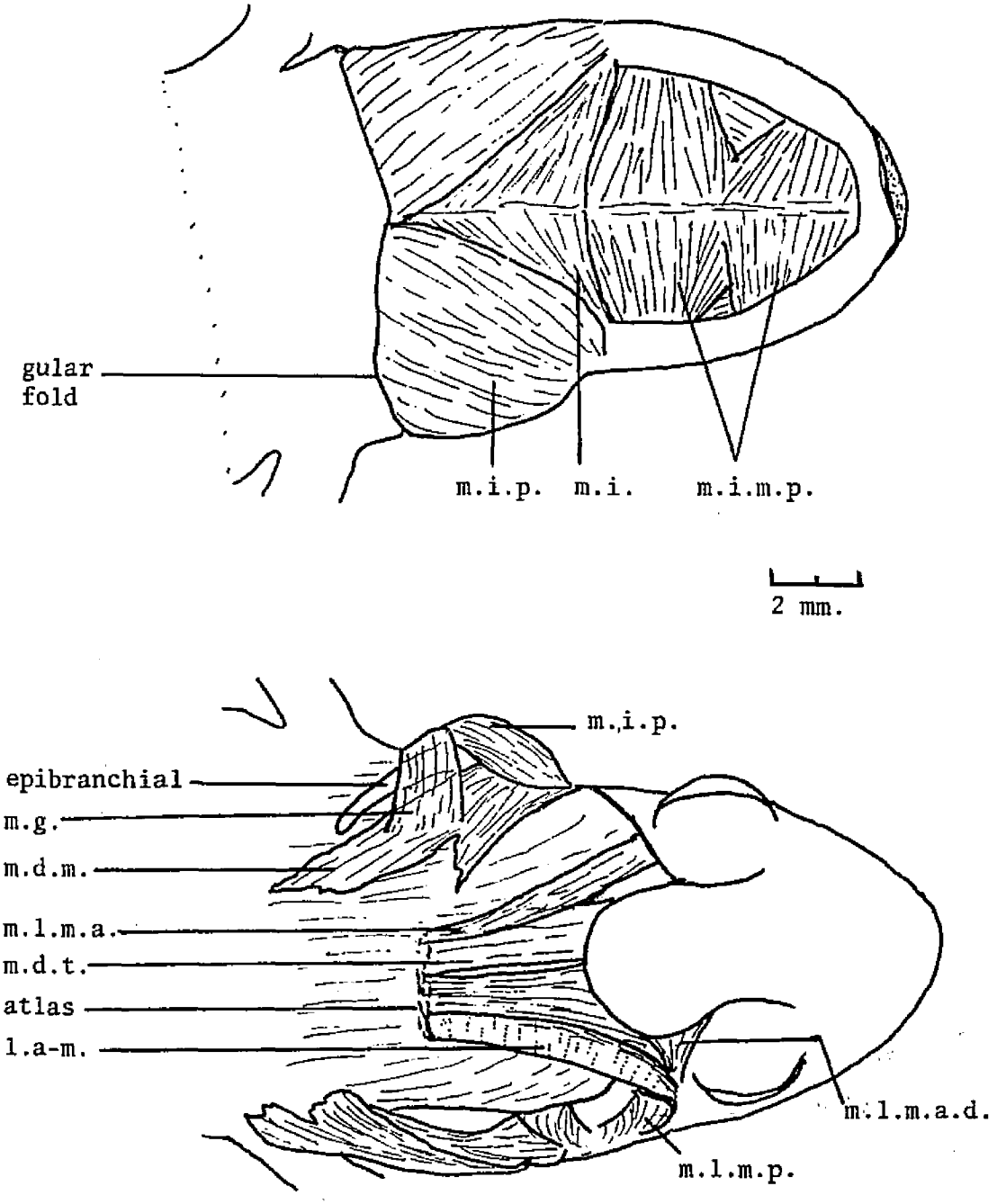


Fig. 36 Dorsal and Ventral View of D. ochrophaeus Head Musculature. (male #34-7; Clingman's Dome, Sevier Co., Tennessee) (See text for abbreviations.)

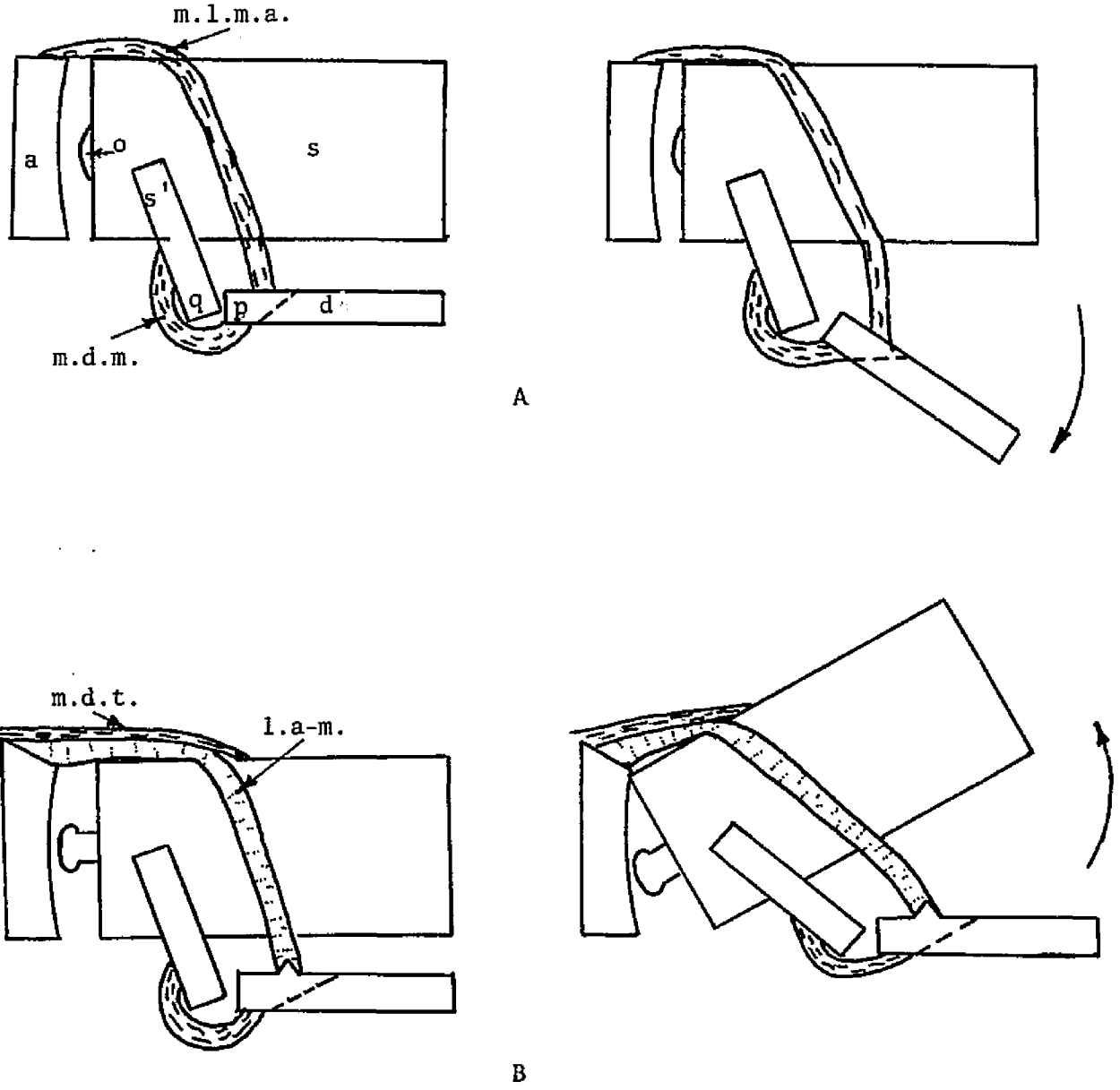


Fig. 37. Diagramatic models of heads, comparing methods of mouth opening in non-desmognathine-(A), and desmognathine-(B) salamanders. a=atlas, d=dentary, o=occipital condyle, p=prearticular, q=quadrate, s=skull, s'=squamosal, m.d.m.=m. depressor mandibulae, m.d.t.= m. dorsalis trunci, m.l.m.a.=m. levator mandibulae anterior (superficial portion), l.a-m.=atlas-mandibular ligament.

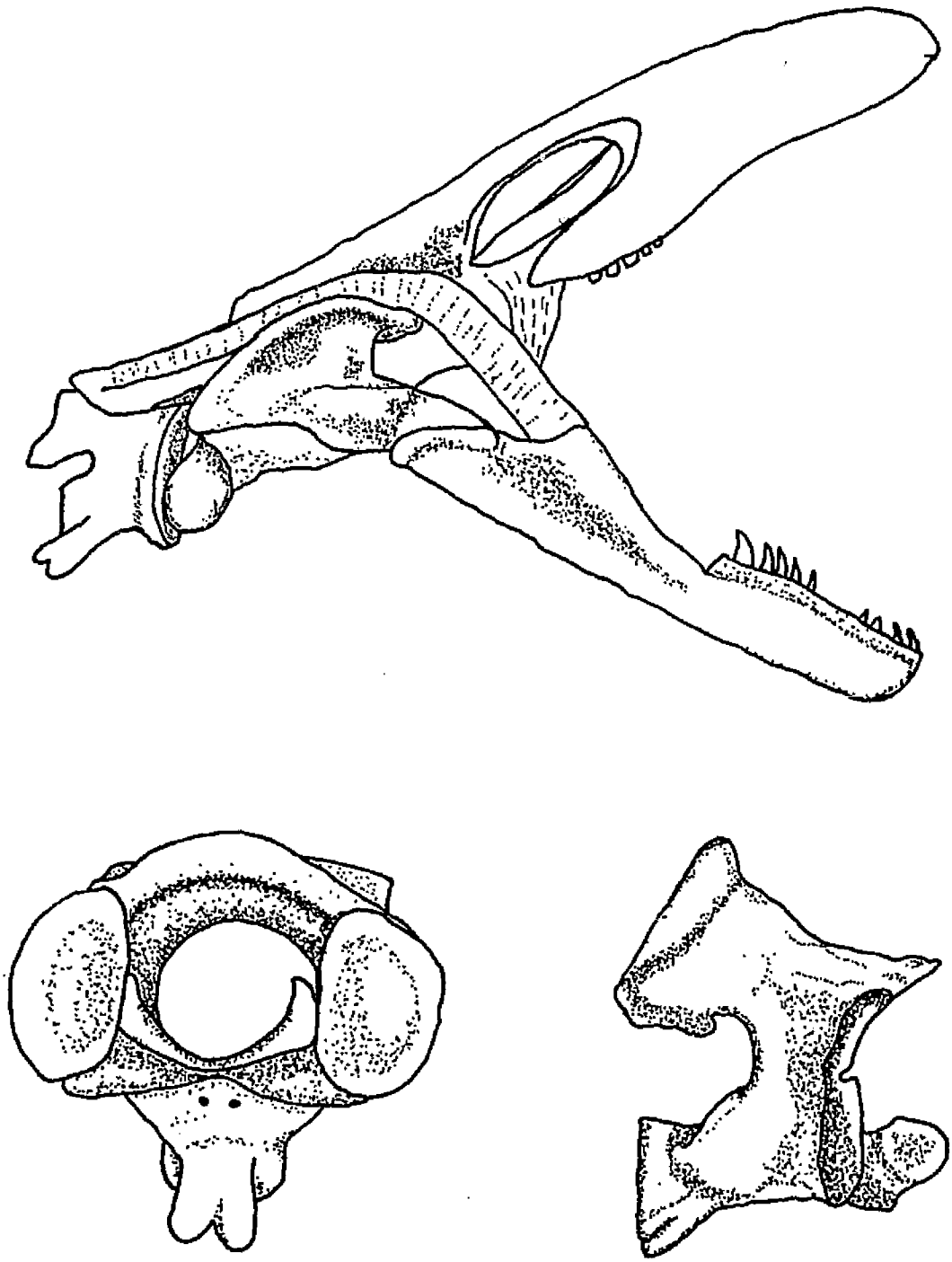


Fig. 38. The Desmognathine Atlas. Function of the Broad Atlantal-Condylar Facets in Mouth Opening (top); Anterior and Lateral View (bottom). (Dissected Male, D. ochrophaeus #20-1; Disarticulated Atlas, Female, D. fuscus #47-4.)

Systematic Summary

Subfamily Desmognathinae

Since Cope (1866) first proposed the family Desmognathidae the taxonomic status of the desmognathine higher category has fluctuated. Table 24 presents the opinions of various authors who have considered its position. There are three basic views: 1)that Desmognathus, Aeurognathus and the recently described Phaeognathus (Highton, 1961) are separated from the Plethodontidae as the Desmognathidae, 2)they are included as the subfamily Desmognathinae in the Plethodontidae, and 3)they are not separated and are included in the Plethodontidae.

In defense of the first view, Soler (1950) listed the characteristics defining the Desmognathidae:

1. true opistocoelous vertebrae.
2. hemicoelous centra.
3. naso-labial groove.
4. lungs absent.
5. tongue attached in front and by the middle.
6. larvae with four rather than three epibranchials.
7. highly modified atlas and stalked occipital condyles.
8. carpus and tarsus not ossified.
9. strong tendon (ligament) in the m. temporalis (=m. levator mandibulae anterior) which immobilizes the mandible.
10. highly developed m. quadrato-pectoralis (=m. interhyoideus posterior).

The majority of these characters are not diagnostic and the list can be reduced. Items 1 and 2 refer to the condition of the vertebrae and are therefore not separate. Naso-labial grooves and lunglessness are characteristics of all plethodontid salamanders. Items 5 and 8 are found in other genera (Regal, 1966; Wake, 1966). The m. interhyoideus posterior (see discussion of musculature above) exhibits hypertrophy primarily in males. Finally, items 7, 9, and 10 are associated with the modified jaw apparatus. I am in agreement with

Table 24. Historical Status of the Subfamily Desmognathinae. (Notes refer to subfamily or family (if no family))

Author(s)	Date	Subfamily	Family	Notes
Baird, S.F.	1850			proposed Desmognathus
Gray, J.E.	1850		Plethodontidae	included Desmognathus
Cope, E.D.	1859	Spelerpinae	Salamandridae	included Desmognathus
Cope, E.D.	1866		Desmognathidae	Desmognathus only
Cope, E.D.	1869		Desmognathidae	same; Thorius in Thoriidae
Cope, E.D.	1875		Desmognathidae	same as above
Boulenger, G. A.	1882	Desmognathinae	Salamandridae	Desmognathus and Thorius.
Cope, E.D.	1889		Desmognathidae	same as above
Stejneger, L.H.	1892		Desmognathidae	same; Typhlotriton included
Cope, E.D.	1893	Desmognathinae	Desmognathidae	Desmognathus only; Haptoglossa (Costa Rica), Thorius and Typhlotriton in Thoriinae
Moore, J.P.	1899		Desmognathidae	Leurognathus and Desmognathus
Moore, J.P.	1900	Desmognathinae	Plethodontidae	same; separated on basis of articulation of vertebrae
Fowler, H.W.	1907		Desmognathidae	same
Brown, A.E.	1908		Desmognathidae	same; D. fuscus fuscus type species of family
Brimley, C.S.	1912		Plethodontidae	no subfamily

Table 24 (continued)

Author(s)	Date	Subfamily	Family	Notes
Wilder, I.L.W.	1913		Desmognathidae	Desmognathus and Leurognathus only
Stejneger, L.H. and Barbour, T	1917		Plethodontidae	no subfamily
Fowler, H.W. and E.R. Dunn	1917		Plethodontidae	same
Wilder, I.L.W. and E.R. Dunn	1920		Desmognathidae	Desmognathus and Leurognathus only
Dunn, E.R.	1926		Plethodontidae	no subfamily
Noble, G.K.	1927		Plethodontidae	same
Noble, G.K.	1931		Plethodontidae	same
Bishop, S.C.	1943		Plethodontidae	same
Smith, H.M. and E.H. Taylor	1948		Desmognathidae	Desmognathus and Leurognathus only
Soler, E.I.	1950		Desmognathidae	same (see discussion p 96)
von Wahlert, G.	1957		Desmognathidae	same
Blair, A.P.	1958	Desmognathinae	Plethodontidae	Desmognathus and Leurognathus only
Highton, R.	1961	Desmognathinae	Plethodontidae	same; Phaeognathus included
Wake, D.B.	1966	Desmognathinae	Plethodontidae	same
Brame, A.H. Jr.	1967	Desmognathinae	Plethodontidae	same

Wake (1966:58) and others, that while the desmognathines are distinct from other plethodontids, the many observable changes are correlated with adaptation to a particular habitat. Desmognathus, Leurognathus, and Phaeognathus are a natural grouping, the Desmognathinae.

Fig. 39 is a diagramatic representation of the species relationships within the subfamily Desmognathinae. On the generic level it is similar to Wake's (1966:59) phylogeny. The species are arranged in the species groups as demonstrated by studies of lactate dehydogenase and as supported by analysis of absolute size differences.

Desmognathus Baird, 1850

Diagnosis (after Wake, 1966):

Differs from Leurognathus (see Fig. 26) in having internal nares which open medially; vomerine preorbital processes well separated from vomers and not extending beyond vomer bodies; an internasal fontanel in adults; vomers and premaxillae joined by septum-like processis which form fontanel border; skull not strongly depressed (see Figs. 22-28). Also differs in having a less prominent nasal eminence (Martof 1962:13).

Differs from Phaeognathus in having a lower number of trunk vertebrae, fifteen versus 21-23; small maxillary jugal processes; vomerine preorbital processis not extending laterally beyond vomer bodies; neural crests present only on first two or three trunk vertebrae; parapophysis and diapophysis spparated for most of lengths; ribs more than one-half the distance across parapophysis; stocky (non-elongated) appearance; longer limbs and digits.

A light line from the eye to the angle of the jaw can be diagnostic (see Fig. 4). However, this is often obscured in highly

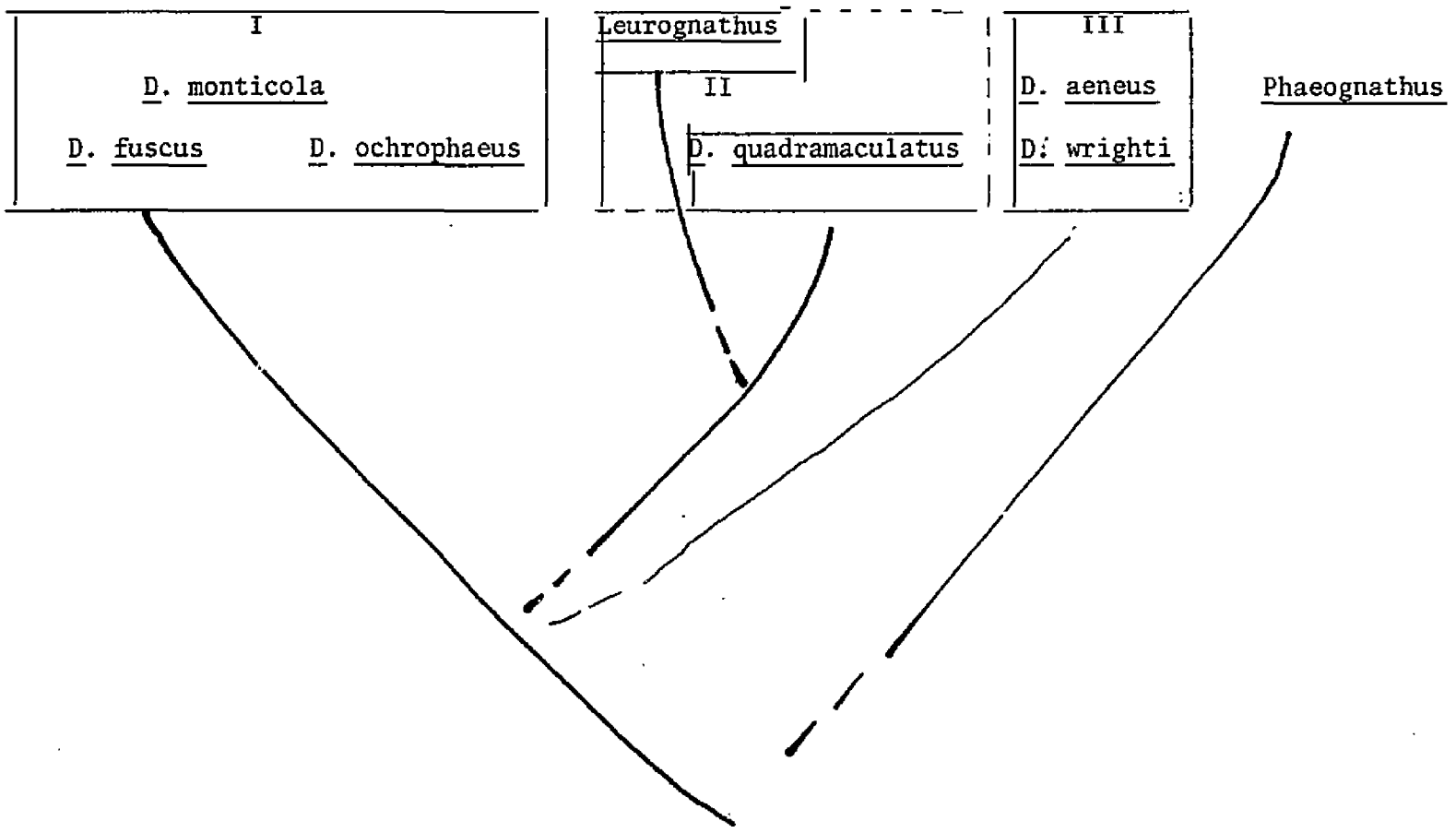


Fig. 39. Relationships within the subfamily Desmognathine. Groups based on studies of lactate dehydrogenase.

melanic specimens and in most preserved specimens. Martof (1962:13) notes that a faint line may occur in Leurognathus.

Description: There is considerable morphological overlap of the Desmognathus species as shown by comparative osteology (Wake, 1966), and the ranges of absolute and relative size measurements (see above, p 61). No apparent species differences were observe in the head musculature or the jaw apparatus. Species differences (with overlap) have been demonstrated for size-fecundity relationships (Tilley, 1968) and differential mortality (Organ, 1961a).

Using two approaches to taxonomy, comparative biochemistry and morphology, the clearest group limits with Desmognathus are not at the species level but at the species group level. This raises a question, not answerable here, as to the status of these species. They are obviously closely related and differences of a less obvious nature, such as reproductive ecology aid in defining species of Desmognathus. The high incidence of sympatry throughout the southern Appalachians suggests that these are real species. This study has defined three distinct groups.

Group I: Desmognathus ochrophaeus Cope, 1859

Desmognathus monticola Dunn, 1916

Desmognathus fuscus (Rafinesque, 1820)

Diagnosis: differs from other desmognathines in having a negatively migrating isozyme of lactate dehydrogenase (M_4) following electrophoresis in Tris-borate buffer, pH=8.6, for 10 hours (see Fig. 9). The pattern of subunit interaction for the enzyme is characteristic (see Fig. 15).

Differs from Group III, D. wrighti and D. aeneus in having a larger body size (and other measurements, see Tables 1-23), with the

ranges just overlapping.

Description: All three species have a highly variable color pattern. Martof and Rose (1963) note that too much taxonomic emphasis has been put on color pattern. They reduced D. ocoee Nicholls, 1949 to synonymy with D. ochrophaeus. They also question the usefullness of the subspecies D. o. ochrophaeus and D. o. carolinensis which are separated mainly by color pattern. Wake (1966) lists the darker (dorsal and ventral) subspecies D. fuscus auriculatus as a full species while Brame (1967) retains it as a subspecies of D. fuscus.

Dorsal views of the species (see Figs. 21 and 22) illustrate the typical impression of each species. Martof and Rose (1963:422) note that D. fuscus has a short-blunt snout and D. ochrophaeus a long pointed snout. D. monticola also has a long snout. The size relationships as noted previously were similar.

Group II. Desmognathus quadramaculatus Holbrook (1840)

Leurognathus marmoratus is included in this group. This suggests a closer relationship with D. quadramaculatus then previously admitted. Other than a different adaptive zone, the totally aquatic habitat, its generic status might be removed. However, the clear differences found in the skull (internal nares) make it distinct enough to retain the genus. Phaeognathus. pn the basis of its subunit interaction curve (see Fig. 15) only, is simply noted here. No other indications of relationship were noted for Phaeognathus.

D. quadramaculatus differs from group I and III in having two distinct M subunits while only one type of H subunit was detected for lactate dehydrogenase. Electrophoresis was done in Tris-borate buffer, pH=8.6, for 10 hours (see Fig. 11). This gives rise to two subunit

interaction curves

interaction curves (see Fig. 15). Type a is similar to the single Leurognathus specimen analyzed and type b is similar to the single Phaeognathus specimen.

Differs from group III, D. wrighti and D. aeneus, in having a larger body size (see Tables 1 - 23). The ranges do not overlap.

Differs from groups I and III in having a more highly keeled tail, and in adults in having a dark ventrum throughout its range. The latter character may not hold if D. fuscus auriculatus is raised to full species status.

Description:

The dorsal view of D. quadramaculatus suggests the head shape of D. fuscus (see Fig. 21). The snout is short and blunt, but in general has a wider post-orbital width. While these may be typical shapes, quantitatively they are similar (see Tables 10, 13, and 15).

Group III Desmognathus wrighti King, 1936

Desmognathus aeneus Brown and Bishop, 1947

Diagnosis:

Differs from groups I and II in its subunit interaction curve (see Fig. 15).

Differs from group I in having a positively migrating M_4 isozyme of lactate dehydrogenase following electrophoresis in Tris-borate buffer, pH=8.6, for 10 hours (see Fig. 10).

Differs from groups I and II in having a smaller body size, (see Tables 1 - 23). The ranges barely overlap group I species, and do not overlap group II.

Differs from group II in not having a keeled tail.

Description:

The dorsal views of D. wrighti and D. aeneus (see Fig. 21) are different, even though the outline in Fig. 21 of the latter is from a female specimen. The color patterns are similar but D. aeneus normally lacks the chevrons located mid-dorsally on D. wrighti. Pigmentation around the border of the lower jaw in ventral view is characteristic of D. wrighti but is not found in D. aeneus. D. wrighti often has a brassy (irridescent) aspect to the ventrum.

Summary

This study demonstrates that within the genus Desmognathus there is a correlation between biochemical characters and absolute size characters, but that these did not differentiate the species studied. The enzyme lactate dehydrogenase was chosen because ecological correlations had been demonstrated in the Amphibia (Salthe, 1965). This study suggests that the primary correlation in Desmognathines is with absolute size. The characters used did define limits for three species groups as indicated by electrophoresis of lactate dehydrogenase and supported by morphological studies. The three groups are:

I. D. fuscus, D. monticola, D. ochrophaeus

II. D. quadramaculatus

III. D. wrighti, D. aeneus

Leurognathus marmoratus was biochemically and morphologically in Group II. This indicates the close relationship with D. quadramaculatus. However, it does not reflect the high degree of adaptive convergence. Leurognathus is clearly a derived form which has entered a new adaptive zone within the desmognathine aquatic zone (Wake, 1966). Its generic status is not therefore questioned.

Furthermore, it is concluded that the jaw mechanism, probably evolved as an improved feeding mechanism, may have been one of the preadaptive steps to burrowing and increased terrestrialism as found in the subfamily Desmognathinae.

Appendix- Absolute Size Measurements of Specimens by Species and Locality (see p22 for descriptions).
(measurements in mm)

Desmognathus ochrophaeus

Specimen	Sex M	Sex F	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
#22 Doughton Park, Blue Ridge Parkway, Allegany Co., North Carolina, 7/14/68																	
18b		x	33.4	21.3	6.0	1.9	3.2	5.0	7.5	--	2.4	1.5	1.1	1.5	1.5	10.5	3.8
18-1	x		35.2	22.0	6.9	3.2	4.2	6.3	8.6	42.1	2.8	2.0	2.4	2.0	2.0	11.8	5.0
18-2	x		23.8	14.7	4.3	2.2	2.6	3.9	5.7	23.9	1.7	1.7	1.5	1.5	1.1	7.0	3.2
18-3	x		39.0	25.9	7.6	3.9	5.0	6.5	8.3	32.9	2.6	2.0	2.0	2.2	1.9	9.9	5.7
18-4		x	32.7	19.2	5.9	2.6	3.2	5.9	7.8	--	2.2	1.9	1.9	1.9	1.3	8.2	4.7
18-6		x	31.8	21.0	5.4	2.9	3.3	5.9	7.0	--	2.5	1.7	1.5	1.7	1.2	10.0	4.0
18-7	x		32.1	21.6	6.4	2.8	3.9	6.1	7.0	26.8	2.6	1.6	1.6	1.8	1.5	11.0	5.0
18-8	x		37.6	24.2	7.5	4.0	4.9	7.3	8.5	47.3	2.8	1.6	2.4	2.3	1.3	11.3	6.5
#20 Grandfather Mt., Avery Co., North Carolina, 7/15/68																	
20-1	x		41.5	27.8	7.0	3.2	3.5	8.5	9.8	39.2	3.5	2.0	2.4	2.4	2.4	11.1	5.3
20-2		x	39.4	23.4	6.2	2.9	3.4	6.7	9.6	40.3	2.6	1.6	1.8	2.0	1.9	11.7	3.3
20-3	x		36.8	22.7	6.4	3.5	3.7	6.0	9.3	45.9	2.9	1.8	1.9	2.2	2.0	11.6	4.9
20-4	x		32.6	21.4	5.2	2.8	2.8	6.6	8.8	38.0	2.5	1.5	2.2	2.3	1.7	10.8	4.1
20-5		x	33.8	21.0	5.1	2.3	2.8	7.0	8.2	37.5	2.4	1.7	1.8	2.0	1.6	8.5	4.5
20-6	x		37.0	22.5	6.0	3.0	3.0	6.8	8.3	--	2.9	2.0	2.2	2.4	1.9	10.1	5.5
20-8	x		38.4	24.3	5.7	2.9	3.1	6.5	8.3	33.6	3.1	1.8	1.8	2.6	1.7	12.5	5.0
20-9	x		36.3	22.5	6.0	2.9	3.4	6.1	9.1	36.9	2.8	1.7	2.3	2.4	1.5	11.4	5.1
20-10	x		40.0	26.5	7.2	3.0	3.6	7.6	9.9	46.2	3.2	2.1	2.4	2.6	2.1	11.1	4.0
20-11		x	36.9	23.9	6.2	2.3	3.2	7.4	9.0	--	2.7	1.7	2.1	2.3	1.8	10.4	5.4
20-12	x		40.5	25.4	6.7	3.3	3.9	7.4	9.8	51.4	3.1	1.9	2.7	2.4	1.9	12.5	5.2
20-13		x	33.3	21.3	5.4	2.9	2.9	5.3	8.3	35.5	2.6	1.7	2.0	2.1	1.4	10.7	4.5
20-14	x		30.7	18.6	5.1	2.6	2.9	5.6	7.8	--	2.8	1.7	2.3	2.3	1.4	10.3	4.7
20-15		x	30.2	19.2	5.1	2.7	2.5	4.9	8.0	36.8	2.7	1.5	1.8	2.0	1.5	9.5	3.7
#29 Deep Gap, Macon Co., North Carolina 7/20/68																	
29-4	x		39.9	25.6	8.0	4.3	4.2	6.5	10.3	40.9	3.6	2.1	2.3	2.5	2.3	13.0	5.3
29-5		x	29.2	17.9	4.4	2.6	3.1	3.8	6.6	30.3	2.1	1.2	1.9	1.6	1.4	9.4	2.3
#34 Clingman's Dome, Great Smoky Mountains National Park, Sevier Co., Tennessee, 7/22/68																	
34-1	x		31.1	19.3	6.0	2.6	2.8	5.1	8.6	32.0	2.8	1.8	2.1	1.9	1.7	10.3	4.7
34-2	x		44.9	28.6	8.0	3.5	4.2	6.4	12.0	43.8	3.9	2.4	2.9	2.8	2.5	14.1	6.0

Appendix (continued) (measurements in mm)

Specimen	Sex M	Sex F	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
34-3		x	30.2	18.6	5.6	2.2	2.6	4.9	8.9	36.6	2.5	1.6	2.2	2.1	1.6	10.2	4.0
34-6		x	34.3	23.2	6.3	3.2	3.2	5.9	8.7	--	2.5	1.7	2.3	1.9	1.7	10.2	5.1
34-7	x		48.2	32.3	8.6	4.5	5.6	8.0	12.3	48.4	4.1	2.3	3.0	2.9	2.7	13.7	7.0
Desmognathus monticola																	
#21 Linville River Area, Burke Co., North Carolina, 7/16/68																	
21-5		x	57.6	36.8	10.3	5.6	6.4	9.5	14.2	64.7	4.1	2.7	3.6	2.0	3.0	16.9	7.5
21-6		x	37.3	22.8	7.7	3.6	4.3	7.4	9.8	--	3.1	2.0	2.8	1.8	2.1	12.4	5.5
21-7		x	41.1	26.8	8.5	4.3	4.5	7.3	10.4	42.7	2.8	1.7	2.8	1.9	2.1	12.1	6.1
21-8		x	38.3	24.2	7.8	4.1	4.1	6.3	9.7	--	3.1	1.9	2.7	1.7	2.0	12.7	5.0
21-9	x		60.4	38.4	11.1	5.9	7.5	10.0	15.6	67.7	5.4	3.2	3.7	3.2	3.4	17.9	7.8
21-10		x	40.2	25.9	7.5	3.4	4.5	6.4	10.1	40.8	2.8	1.8	2.7	1.9	2.0	12.0	4.8
#47 Rt. 33, Greene Co., Virginia, 8/6/68																	
47-5	x		51.9	32.1	10.1	6.6	5.9	8.6	13.0	50.9	4.6	3.1	2.8	3.4	2.8	16.2	5.4
47-6	x		54.5	33.4	10.0	5.9	6.0	10.4	13.2	--	4.6	3.2	2.8	3.4	2.9	15.2	7.9
47-7		x	46.6	29.3	8.3	5.9	4.9	8.1	9.8	51.6	3.3	2.5	3.0	3.0	2.5	12.9	6.3
#49 Cascades, Jefferson National Forest, Giles Co., Virginia, 8/25/68																	
49-2	x		60.3	39.0	10.9	5.2	6.1	9.7	14.9	73.6	4.9	3.2	3.5	3.2	3.4	16.6	8.5
49-5	x		45.7	30.7	7.9	4.4	4.9	7.9	10.9	52.3	3.2	2.1	3.0	2.4	2.7	13.8	6.1
49-8	x		58.7	37.9	10.3	5.3	6.4	10.3	14.8	72.0	4.4	2.9	3.2	4.3	3.7	17.2	9.7
49-12		x	36.2	22.0	5.7	2.8	3.5	6.0	9.3	31.7	2.7	1.7	2.6	1.8	2.0	10.5	5.0
49-14	x		33.1	20.7	6.2	3.6	3.3	5.4	8.3	36.0	2.6	1.8	2.3	2.0	1.9	10.6	3.9
Desmognathus fuscus																	
#38 Hickory Nut Mt., Ouachita National Forest, Montgomery Co., Arkansas, 7/26/68																	
38-1	x		47.4	30.9	7.9	3.6	4.8	8.4	10.8	--	3.3	2.1	2.8	2.3	2.3	13.8	5.6
38-2	x		55.3	31.9	9.2	6.0	5.3	8.5	13.3	--	4.2	2.7	3.1	2.8	2.8	16.8	6.5
38-3		x	47.4	29.8	7.9	4.9	4.5	8.1	11.7	44.0	3.2	2.1	2.9	2.5	2.1	14.5	4.6
38-4	x		60.0	39.7	9.7	5.8	6.0	10.1	15.2	--	4.3	2.8	3.1	3.1	2.8	18.6	7.8
38-7	x		51.1	31.3	7.8	4.8	4.8	8.2	13.2	35.7	3.9	2.4	2.9	2.6	2.2	15.7	6.7
#40 Three Springs, Mammoth Cave National Park, Edmonson Co., Kentucky, 7/29/68																	
40-1	x		40.2	25.9	7.3	4.0	4.8	6.8	9.9	36.3	3.0	1.8	2.6	2.2	2.0	11.9	5.1
40-2		x	44.3	29.8	7.9	5.6	5.3	7.7	10.0	--	2.6	1.8	2.7	2.1	1.8	13.5	5.0

Appendix (continued) (measurements in mm)

Specimen	Sex M	Sex F	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
40-3		x	43.5	28.7	7.7	5.2	5.4	7.3	10.4	37.0	3.0	1.7	2.9	2.0	1.9	12.1	5.3
40-4	x		45.4	29.8	7.8	4.7	5.5	7.5	10.7	48.5	3.1	1.8	2.7	2.0	1.8	13.4	5.4
40-5	x		55.6	35.6	10.6	5.7	6.3	9.0	12.6	--	4.2	2.6	3.1	3.0	2.7	15.7	6.9
40-6	x		59.8	39.8	12.8	8.2	8.5	8.6	14.7	65.4	4.7	2.8	3.3	3.4	2.8	16.9	6.8
#47 Rt. 33, Greene Co., Virginia, 8/16/68																	
47-1		x	35.6	23.1	6.7	3.8	4.0	6.2	9.1	36.1	2.5	1.6	2.2	2.1	1.8	11.0	4.5
47-2	x		24.4	15.0	4.2	2.2	2.5	3.8	6.8	--	2.1	1.1	1.5	1.6	1.0	8.3	2.7
47-3	x		53.5	34.9	9.2	5.5	5.5	8.6	11.9	--	4.0	2.7	2.5	2.8	2.8	15.9	6.2
47-4		x	35.2	24.3	6.3	3.2	3.9	5.8	7.7	--	2.4	1.4	2.1	1.8	1.7	9.2	3.5
47-8		x	31.0	20.4	4.8	2.9	3.0	4.6	7.7	33.6	2.0	1.2	1.7	1.6	1.6	8.8	4.1
#55 Monroe, Union Co., North Carolina, 4/26/69 (Bernard S. Martof)																	
55-1	x		51.8	33.3	10.0	7.1	6.2	8.6	11.7	--	4.5	2.8	3.2	2.7	2.8	15.3	6.4
55-2	x		54.3	36.1	10.0	6.8	6.4	8.4	12.8	46.5	5.2	3.1	3.4	3.4	2.8	15.9	6.3
Desmognathus quadramaculatus																	
#22 Mt. Mitchell, Yancy Co., North Carolina, 7/17/68																	
22-1		x	56.4	36.4	9.4	5.6	7.0	10.5	13.4	48.9	4.3	3.0	3.3	2.8	2.7	17.9	8.5
22-2	x		34.7	21.0	6.4	3.8	3.6	5.3	8.0	33.0	3.0	2.0	2.1	2.0	2.1	10.9	4.8
22-3	x		79.0	48.7	12.6	9.0	9.2	11.4	20.3	--	7.3	4.3	3.2	4.4	4.3	23.0	9.3
#37 West Prong Little Pigeon River, Great Smoky Mountains National Park, Sevier Co., Tennessee, 7/23/68																	
37-1	x		41.3	24.8	7.9	4.6	5.1	7.1	11.3	--	3.3	2.1	3.1	2.1	2.3	13.0	6.2
37-2	x		55.1	33.8	10.5	5.8	6.6	9.5	15.1	44.8	4.8	3.1	4.1	3.5	3.4	16.3	7.5
37-3	x		72.8	46.0	13.8	8.7	8.5	13.6	17.7	--	6.9	4.3	4.5	4.0	5.8	21.7	11.3
37-5	x		87.8	54.4	15.2	11.1	10.5	15.0	23.6	71.6	7.8	5.6	5.3	5.0	5.2	28.4	13.0
37-4	x		46.8	29.3	9.4	4.6	5.1	9.9	12.8	--	3.9	2.5	3.4	2.5	2.6	15.4	7.4
#49 Cascades, Jefferson National Forest, Giles Co., Virginia, 8/25/68																	
49-1	x		66.0	43.9	10.5	5.7	7.6	10.5	16.7	--	4.8	3.3	3.7	2.8	3.5	21.5	8.3
49-3	x		38.8	23.3	6.3	3.4	3.5	6.8	10.0	--	2.6	1.5	2.3	1.8	2.1	11.7	4.7
49-4	x		64.6	42.6	10.8	7.2	7.5	9.8	15.1	53.7	4.7	3.2	4.2	3.1	2.9	19.0	8.2
49-6	x		39.3	25.1	6.7	3.8	4.2	6.8	9.8	34.5	2.7	1.6	2.6	2.2	2.0	12.3	4.9
49-7	x		42.2	27.6	7.6	3.5	4.5	6.5	10.8	--	3.0	1.9	2.4	2.2	2.1	13.0	5.3
49-9	x		54.5	36.0	10.4	5.0	5.9	9.7	12.9	--	3.7	2.5	3.4	2.4	2.7	15.6	6.5

Appendix (continued) (measurements in mm)

Specimen	Sex M	Sex F	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Leurognathus marmoratus																	
#52 Whitetop Mt., Smythe Co., Virginia, Summer, 1968 (Della J. Organ)																	
52-1	x		68.0	44.2	10.3	5.6	5.6	10.7	17.3	50.9	5.4	4.0	4.8	2.9	2.9	19.6	8.2
#54 Greenbrier Cove, Great Smoky Mountains National Park, Sevier Co., Tennessee, 9/28/52 (American Museum of Natural History Herpetological Collection #62072-76, Roger Conant)																	
54-1		x	59.2	38.2	9.6	6.8	5.7	9.1	14.5	40.1	4.0	2.9	3.5	2.2	3.3	17.5	6.7
54-2		x	50.8	32.6	8.3	5.0	4.7	8.7	13.0	38.8	3.7	3.0	2.6	2.2	2.6	15.6	6.4
Phaeognathus hubrichti																	
#53 Butler Co., Alabama, Summer, 1968 (John Woods)																	
53-1	x		110.4	76.9	11.9	5.4	6.1	8.3	23.2	114.1	5.5	3.8	3.9	4.4	4.3	26.7	6.8
Desmognathus wrighti																	
#23, 24 Mt. Mitchell, Yancy Co., North Carolina, 7/17/68																	
23-1	x		25.2	15.1	4.1	1.5	2.0	4.1	6.2	--	1.7	1.2	1.6	1.6	1.1	7.5	3.5
23-2	x		24.2	15.1	3.8	1.6	2.1	3.8	6.2	--	1.8	1.1	1.5	1.5	1.1	7.4	3.0
23-3	x		25.0	15.9	4.1	1.8	2.0	4.6	6.5	17.9	2.1	1.3	1.2	1.3	1.1	7.5	3.9
23-4	x		24.4	15.3	4.2	2.0	2.1	4.6	5.8	18.5	2.1	1.3	1.3	1.4	1.0	8.0	4.2
23-6		x	25.9	15.8	4.3	2.0	2.0	4.0	6.5	20.9	1.8	1.0	1.6	1.8	1.0	7.3	4.0
24-1	x		26.1	16.3	4.1	1.8	2.2	5.8	6.7	19.2	2.0	1.3	1.3	1.1	1.0	7.7	5.4
#34 Clingman's Dome, Great Smoky Mountains National Park, Sevier Co., Tennessee, 7/22/68																	
34-8	x		22.0	14.3	4.2	1.7	2.0	3.2	6.2	16.0	1.8	1.0	1.6	1.5	1.2	7.0	2.7
34-9	x		20.7	12.1	4.2	1.6	2.1	3.0	5.2	--	1.6	0.9	1.1	1.1	1.1	6.8	2.5
34-10		x	25.5	16.6	4.9	2.2	2.4	4.0	5.6	18.0	1.8	1.0	1.8	1.2	1.2	7.2	3.5
34-11	x		22.7	13.6	3.8	1.7	1.9	3.8	4.7	15.4	1.6	1.1	1.8	1.2	0.9	6.6	3.1
34-12	x		23.1	13.6	3.9	1.6	1.5	4.6	4.5	18.7	1.6	0.9	1.6	1.2	1.0	7.0	3.0
34-13	x		20.0	13.1	4.3	2.0	1.7	4.5	4.7	14.7	1.6	1.1	1.6	1.2	1.0	5.8	3.0
34-14	x		25.4	15.6	3.7	2.2	2.1	4.0	5.6	18.5	1.7	1.1	1.5	1.1	1.2	7.2	3.4

Appendix (continued) (measurements in mm)

Specimen	Sex M	Sex F	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Desmognathus aeneus																	
#29 Deep Gap, Macon Co., North Carolina, 7/20/68																	
29-1	x		22.6	14.3	3.2	1.4	1.4	3.2	5.1	--	1.5	0.7	1.2	1.3	0.8	6.4	2.7
29-2	x		21.4	13.6	3.6	1.4	2.0	3.7	4.7	19.6	1.4	0.7	1.2	1.4	0.9	6.6	2.9
29-3	x		21.0	13.2	3.9	2.2	2.1	3.8	5.1	20.6	1.5	0.9	1.3	1.2	0.9	5.9	3.0

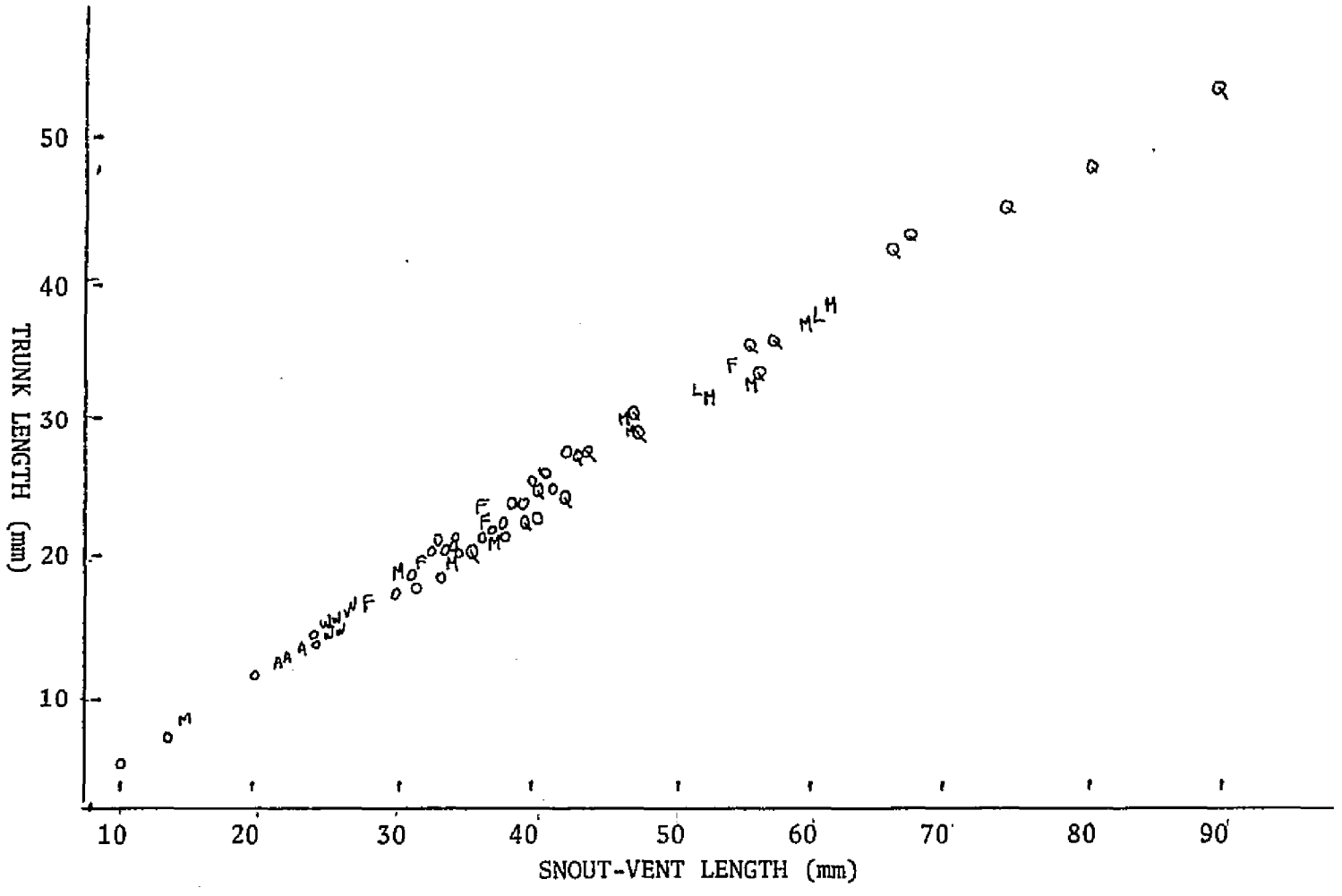


Fig. 40. Graph showing the Relationship of Trunk Length to Snout-Vent Length (Body Size). (All specimens shown for Desmognathus and Leurognathus (L);;Desmognathus species shown by first letter of species name. Phaeognathus trunk length=76.9 mm, snout-vent length=110.4 mm.)

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VITA

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