

QUANTITATIVE PHYLETICS AND THE EVOLUTION OF ANURANS

ARNOLD G. KLUGE AND JAMES S. FARRIS

Abstract

In the quantitative phyletic approach to evolutionary taxonomy, quantitative methods are used for inferring evolutionary relationships. The methods are chosen both for their operationism and for their connection to evolutionary theory and the goals of evolutionary taxonomy. As an example of this approach, a detailed analysis of a set of anuran characters is presented and taxonomic conclusions based on those characters are drawn. The methods and conclusions of the quantitative phyletic analysis are compared and contrasted with the methods of previous workers in the field of anuran classification.

Classical evolutionary taxonomy has been widely criticized for the lack of precision in its methods, while the far more precise numerical phenetic taxonomy has been even more widely censured for its failure to take into account the evolutionary basis of relationships among organisms. We believe it is worth while to develop still another taxonomic methodology, incorporating the precision of numerical techniques and the power of evolutionary inference. We refer to this hybrid methodology as quantitative phyletic taxonomy.

In the present paper we combine an exposition of techniques of quantitative phyletics with some rationale for them, and with examples of their application in the form of a study of the relationships between families of anuran amphibians. We have chosen anurans both because of their intrinsic interest and because of the long history of controversy surrounding frog classification. Since the relationships of frogs have been much debated, several discussions of taxonomic principles are included in the literature. These discussions provide a convenient set of reference points through which we can readily discuss the philosophy underlying the techniques of quantitative phyletics.

To achieve a convenient framework for discussing principles, we have sacrificed in this paper some detail on the frogs themselves. We have used only those relatively few characters that have been commonly

referred to in the literature on anurans. We have deleted some families of frogs from the study in order to produce evolutionary trees directly comparable to those given by authors who have studied only a few families. As a least common denominator of sets of families to be included, we chose those treated by Inger (1967) in his most recent work on anuran phylogeny. We realize that the use of a restricted number of family names may cause some confusion as to just what our assertions on frog affinities are. To alleviate this confusion partially, we have included in Appendix I a list, according to family, of those genera that we have referred to in the text.

CHARACTER WEIGHTING

Quantitative phyletic analysis differs from other taxonomic methods in that it attempts to employ biological information in selecting optimal coding of characters and in weighting of characters; its objective is to discover the evolutionary relationships of organisms, rather than simply the phenetic relationships. The weighting of characters is done in the interest of efficiency in discovering the relationships; if convergence is a real phenomenon, then not all characters are equally correlated with the evolutionary history of organisms. If valid means of character weighting can be found, they will tend to improve our chances of inferring the correct phylogeny. In order to achieve an accurate estimate of

the real relationships of organisms, however, we must carefully select our procedures in such a way that personal bias has little chance of influencing the outcome of the analysis and that the methods are not implicitly circular in the logic of inferring the evolutionary pathways.

Several methods for prior selection of "good" taxonomic characters have been suggested, but not all are equally well suited to quantitative analysis. Among the criteria that are the most subjective are those that depend upon weighting characters according to the individual taxonomist's opinions (presumably formed before any taxonomic analysis has been performed) concerning the functional importance of characters, the significance of the biological roles of characters, the implications of functional relationships between characters, and the "most logical" direction of evolution for characters. We regard evidence of this type as too conjectural to be of any importance in taxonomic procedure. That such methods depend on an individual's understanding of a phenomenon immediately opens the possibility of endless argument between different taxonomists with different understandings of the same situation. Where such evidence for weighting has been used in a qualitative manner, it cannot be regarded as well founded. Serious taxonomic errors may be produced directly by the taxonomist's speculation on biological phenomena. An example of the perils of using subjective weighting is the work of Ghiselin (1966), who produced quite plausible-sounding "biological" arguments to the effect that torsion must have preceded coiling in the evolution of gastropods, since otherwise some intermediate form would pass through an adaptively impossible stage. Batten, Rollins and Gould (1967), however, noted that the subclass Cyclomya consists entirely of proto-gastropods with coiling but no torsion. We believe that "biological" and "functional" evidence for inferring importance and direction of evolution of characters should be excluded from

objective taxonomic studies, at least until such evidence can be interpreted in a more rigorous way than is generally possible.

A form of subjective taxonomic judgment, which we believe represents a type of weighting, is the interpretation of some characters being more "fundamental" than others. Usually, the worker who uses this type of reasoning decides which characters are most fundamental, and then he creates the major branches of a phylogenetic tree in such a way that the principal groups are each characterized by a single state of one of the fundamental characters. Finer branches of the tree are created later on the basis of other evidence.

Often this type of weighting leads to phylogenetic conclusions that seem indefensible from any other standpoint. Hecht (1963), for example, concluded that Orton's (1953) tadpole types were the most reliable indicators of anuran relationships, and then he went on to conclude that the aberrant tadpoles of the Microhylidae implied an ancient origin for that group. Hence, the Microhylidae occupied alone one of the branches of one of the earliest furcations of Hecht's tree. We cannot condone such a conclusion. That the Microhylidae differ in some set of features from other anurans could be taken as evidence for an ancient origin for that group only if it were supposed that change in those features were more probable in the distant past than in the more recent past. We know of no way in which evidence on the truth of that supposition can be obtained from available data.

Griffiths (1963) also seemed to rely on fundamental characters. He argued that the number of presacral vertebrae is a highly significant character and concluded that the Anura are diphyletic, one phyletic line characterized by nine presacrals, the other by eight or less. Quite aside from the issue of whether number of presacrals is a "good" character, it is clear that Griffiths overemphasized it. We could conclude from the distribution of presacral

counts in frogs that the Anura are diphyletic only if we assumed that it is evolutionarily impossible for a frog with nine (or eight) presacral vertebrae to be the ancestor of one with eight (or nine). We do not see how such an assumption could be defended. Griffiths never attempted to do so.

Inger (1967) introduced a new form of character weighting in his modification of Wilson's (1965) concept of "uniqueness." A character state is unique if the set of organisms that possess it form a monophyletic group. That is, it has originated just once during phylogeny. According to Wilson, a state is *unique and unreversed* if it appears just once in phylogeny, and is never lost in any of the lines possessing it. Inger used "unique" to mean "unique and unreversed." Using the term in Inger's sense, there is a complete correspondence between the set of organisms that have a unique character state and the set of members of a monophyletic group. Inger pointed out quite correctly that unique character states, if they can be identified, can serve as excellent indicators of phyletic relationships. The difficulty lies in the identification.

Inger (1967:381) offered four criteria by which unique character states can be recognized: (1) there is no obvious selective difference between the states of the character; (2) the state occurs in many taxa of the group being studied; (3) the character has low variability within taxa; (4) the unique state has an unusual developmental pattern. We do not believe the first and last of these to be realistic criteria. To establish that there is no selective difference between states of a character, it would be necessary to understand completely the selective forces shaping the species under study. Such knowledge is not available at the present level of development of evolutionary theory, and to try to fill the gaps in knowledge with speculation would only lead to the same kind of difficulties discussed above in connection with "understanding." Use of "un-

usual developmental pattern" is equally unsatisfactory for want of a stable criterion of "unusual"-ness. Criteria 2 and 3 are much more useful. They are both functions of variation within OTUs (Operational Taxonomic Units, see Sokal and Sneath, 1963) of some rank, and therefore capable of being objectively measured. Note that if criteria of uniqueness are restricted to measures of within-OTU variability, then uniqueness becomes operationally equivalent to conservatism as estimated through the within-OTU variability. This is not a surprising connection. We would expect that a highly conservative character would be more likely to have a state characteristic of a monophyletic group than would a less conservative character. Further, since conservative characters by definition evolve slowly, large reversals would be less likely than in less conservative characters, so that the states of a highly conservative character would have a higher probability of being unreversed than would states of a less conservative character. While the concept of uniqueness is an important one in evolutionary taxonomy, uniqueness is simply another facet of conservatism, when viewed from the standpoint of prior weighting of characters.

Inger (1967) has found reason to dismiss certain sets of characters altogether before the taxonomic analysis is initiated. For example, he generalized (p. 370) that "fossils cannot contribute much to our understanding of the phylogeny within the Order Salientia." And, as evidence for this generalization he stated (p. 369) that "The simplification of the skeleton [of anurans] . . . makes parallelism within the order or convergence between families likely. . . ." As an example of the simplicity of the skeletal system, he considered the shape of the vertebral centrum, and from that discussion he concluded that the character does not provide "a sound clue to phylogeny" (p. 370). His argument for this thesis is of two kinds, only one of which appears to be valid. Inger referred to a "relatively

simple change in developmental pattern involving only one structure, one kind of tissue, and one process" (p. 370) as the cause of the interfamilial and intrafamilial variability in the shape of the centrum. On the contrary, we would argue that the phenotypic simplicity, and even genotypic simplicity if it were known, of the skeletal system of anurans need not imply evolutionary simplicity. We are not aware of any work that has demonstrated the genetic basis of the formation of the anuran centrum or that the genetic basis is any simpler than that, for example, underlying cytochrome *c*. Cytochrome *c* is both genotypically and phenotypically "simple" and, yet, it appears to be of considerable value in reconstructing the phylogeny of life (Fitch and Margoliash, 1967). In summary, we contend that there need not be a relationship (1) between "simplicity" of genotype and "simplicity" of phenotype, (2) between simplicity of genotype and/or phenotype and taxonomic variability, and (3) between simplicity of genotype and/or phenotype and their use in the reconstruction of phylogeny.

One method of weighting characters that does seem to be objective is the use of variation within OTUs as an index to the relative evolutionary rates of characters. Farris (1966) pointed out that the variability of a character within a species (or a higher taxonomic category) would be expected to be inversely related to the conservatism of the character. He suggested weighting characters by dividing each unit character difference by the standard deviation of the character within biological populations. The relationship between intra-OTU variability, evolutionary rate, and character weighting is easily expressed. The rate at which a character can change in evolution is necessarily limited by the variability of the character within populations. Selection, no matter how intense, cannot change the average character state of a population in some unit time by a greater amount than the range of values

available in the population in that unit time. If a character has high variability within OTUs, then a large difference between two OTUs does not imply lack of close relationship, since the variable character could have changed rapidly. If on the other hand, a character has low variability within OTUs, then a large difference between OTUs is probably indicative of lack of close relationship, since the highly stable character probably could not evolve rapidly. Hence, in drawing taxonomic conclusions, we place greater weight on characters with low variability within OTUs.

If the rate of evolution of characters is directly related to the amount of their within-population variation, multiplying each character by the reciprocal of the within-population standard deviation would be expected to transform the characters onto new scales in which the rates of evolution would be approximately equal over characters. If the characters in a study are so scaled that they have approximately equal average rates of evolution, we can use another type of weighting; for if the rates of evolution are approximately equal, the characters should all display about the same range of variation *between* OTUs, unless some characters are markedly more prone to convergence than are others. Characters prone to convergence would tend to show less variation between OTUs than other characters. Thus, the between-OTU variation (on the *transformed* character scales) can be used directly to weight characters. If we want to use weighting of this type without first transforming the characters, we would do so by multiplying each of them by the ratio of the between-OTU variation divided by the within-OTU variation.

In this paper OTUs are families and the characters have all been given a binary coding. We have weighted characters inversely according to their variation within OTUs. Our rationale for this kind of weighting is simply that a character known to vary within a group of closely related

species is apparently capable of changing quite rapidly in evolution, hence seeming to be less conservative than a character known to vary only between distantly related forms. For a character that in reality has only a few discrete states, the conservatism corresponds inversely to the probability of a change from one state to another in a unit time. Most of the characters that we have coded as discrete binary variables, on the other hand, probably pass through a number of morphologically transitional stages in the process of changing from one of the coded states to another. In such characters, the conservatism corresponds inversely to the rate at which such a transition can take place. It is realistic to speak of the "rate" of even a binary character precisely, because the actual evolution is more continuous than the discrete coding would suggest. Since the rate at which a transition between two morphological states might be accomplished no doubt depends on the complexity of the genetic control of the character, indeed, on a wide variety of biological parameters, our weighting procedure is to some extent equivalent to weighting based more directly on detailed biological information. However, we do not assume this connection between ours and other conceivable weighting methods. Our position is simply that the rate of evolution itself is an accurate guide to the reliability of characters for phylogenetic inference. The outstanding advantage of using the variability of a character within OTUs—an indirect index to the rate of evolution—to carry out character weighting is that the weighting is objectively fixed by the data itself; it can be performed even when the detailed biological information necessary for valid application of other weighting schemes is not available.

ESTIMATION OF PRIMITIVE STATES

We use the term "primitive state of a character" to refer to that state of a character which we infer to have been present

in the common ancestor of the set of OTUs in the study. We do not intend by "primitive" any of the other attributes of characters often associated with that term. In particular, we do not consider a primitive state to be unique or unreversed necessarily, nor do we imply that a character is conservative or irreversible merely by asserting that it has a primitive state.

Subjective taxonomic studies often make use of the idea that a character has a particular direction of evolution, which cannot be reversed. Ideas of this type are used to infer the primitive condition of a character, as well as to infer membership of monophyletic groups through the assumption that character states are both unique and unreversed. This is a different sort of reasoning from that involved in asserting that a particular character state is likely to be unreversed because the character is highly conservative; irreversibility is often assumed even for characters that do not appear to be very conservative. While we have no doubt that many characters have indeed evolved primarily in one direction, we doubt that any character is completely irreversible. Further, we are aware of no objective criteria by which the relative reversibilities of characters might be established prior to a taxonomic analysis. In this study we exclude prior speculation on irreversibility and assume that all characters are at least potentially capable of undergoing reversal. To infer the primitive states of characters, we rely on available fossil material and on the criteria for primitiveness established by Wagner (see Wagner, 1961 and references therein, and Kluge, 1967). In order of reliability these criteria are:

- (1) The primitive state of a character for a particular group is likely to be present in many of the representatives of closely related groups.
- (2) A primitive state is more likely to be widespread within a group than is any one advanced state.
- (3) The primitive state is likely to be associated with states of other characters known from other evidence to be primitive.

In applying our criteria 1 and 2, we note that "closely related groups" can be selected through estimates of overall similarity that make no assumptions about primitive conditions. In evolutionary studies, the overall similarity would be computed through a weighted average unit character difference, where the weighting is done on the basis of conservatism as estimated by intra-OTU variability. In deciding on the "wide-spread" of criterion 2, we do not simply count numbers of taxa showing a particular state. A character state is widespread if it occurs in several taxa that otherwise have little in common. Thus, if a particular phyletic line were much more successful than others and produced many more species, genera, or families, we would not erroneously consider the state of a character in that line to be primitive merely because many taxa showed that condition.

CONSTRUCTION OF TREES

In constructing a phyletic tree, we employ the method of Wagner (1961), which is a simplified procedure for producing a most parsimonious tree in the sense of Camin and Sokal (1965). We choose this algorithm for its ease of calculation and because it makes no assumptions about reversibility of characters. Further, it is capable of being applied to continuously coded data and, hence, to data in which the characters are rescaled in order to weight for conservatism. If c is the relative conservatism of a character with numerical states x , y , and z , the weighting is accomplished simply by multiplying each state to obtain new values cx , cy , cz . When weighting of this type is used, the most parsimonious tree based on the weighted data is influenced more by the most conservative characters. In these capabilities the method of Wagner differs from the procedure of Camin and Sokal, and is, we believe, superior to that procedure.

We provide here a short description of the Wagner method for constructing trees. For further discussion and derivations of

results, the reader is referred to Farris (in press). We use the following conventions: $X(A,i)$ denotes the state of character i for OTU A , and the *difference*, $D(A,B)$, between OTU A and OTU B is defined to be

$$D(A,B) = \sum_i |X(A,i) - X(B,i)|. \quad (1)$$

The objective of the Wagner method is to form a network, or tree, by connecting all original OTUs and realize in the process a minimum number of changes ("steps" in the sense of Camin and Sokal, 1965) on the tree. This is a network of minimum *length* in the space in which "length" is defined a certain way. To define the length of a tree, we note first that on a tree, each OTU is connected directly to one of the branching points on the tree (ie., the most recent depicted ancestor of that OTU). For example, in Fig. 1B, Y is the most recent ancestor of A . In that figure, $ANC(Y)$ is an ancestor of A , but it is not the most recent ancestor of A . The connection between OTU A and its most recent ancestor we shall call *interval A*, using the OTU name to index the interval. The difference, as defined in equation (1), between OTU A and its most recent ancestor, will be called the *length of interval A*. The *length of the tree* is the sum of the lengths of all intervals of the tree. The tree of minimum length is defined to be *most parsimonious*. We assume throughout that the $X(A,i)$ and the D 's computed from them are weighted values. It should be clear that the choice of weighting coefficients can usually affect which tree is most parsimonious.

A most parsimonious tree usually has incorporated into it one or more hypothetical intermediates. These are artificial OTUs used as branching points on the tree. Their purpose is to minimize the length of the tree, and their character states are chosen to achieve that end.

The Wagner method itself proceeds as follows:

- (1) Choose an ancestor OTU. Go to 2.

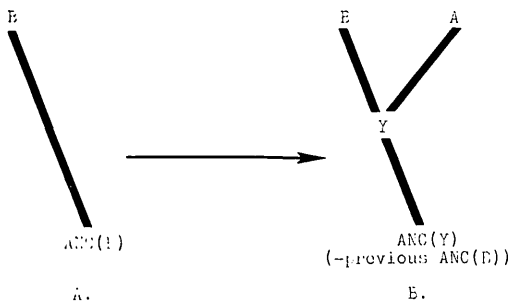


FIG. 1.—Inserting a new OTU and intermediate onto a tree. **A.** Before insertion. **B.** After insertion. In **A** there is one interval, **B**. In **B** there are three intervals, **B**, **A**, and **Y**.

- (2) Find the OTU that has the smallest difference, as defined in equation (1). Connect it to the ancestor to form an interval. Go to 3.
- (3) Find the unplaced OTU, **A**, that differs least from the ancestor. Go to 4.
- (4) Find the interval from which the OTU identified in 3 differs least. The difference, $D(A,INT(B))$, between OTU **A** and interval **B**, is computed as follows: $INT(B)$ is a connection between OTU **B** and OTU **B**'s most recent ancestor on the existing tree. Let this most recent ancestor be denoted $ANC(B)$. Then $D(A,INT(B)) = (D(A,B) + D(A,ANC(B)) - D(B,ANC(B)))/2$. Go to 5.
- (5) Attach OTU **A** to the interval found in 4, denoted **B**. To do this construct an intermediate, **Y**, and insert it into the tree. The insertion is shown in Fig. 1. For each character, i , $X(Y,i)$ is computed as the median of $X(A,i)$, $X(B,i)$ and $X(ANC(B),i)$. Go to 6.
- (6) If any OTUs remain unplaced, go to 3. Otherwise, stop.

Readers who so desire may obtain a FORTRAN IV program to perform the above algorithm from the junior author.

THE PARSIMONY CRITERION

The use of most parsimonious trees has been attacked by Inger (1967) and by Rogers, Fleming, and Estabrook (1967). Inger stated (p. 369) that “[Parsimony] does not adequately take into account the numerous parallelisms that may occur within a taxon. Neither does it allow for alteration in the conditions of selection that may lead a population to head first in one

genetic direction and then in another. . . .” and (p. 381) “I can adduce no biological reasons for using this criterion as a basis for choosing [among²] many alternatives.” We believe that these criticisms lack force. The parsimony criterion, like any other criterion used in evolutionary study is intended ultimately to detect parallelism. Parsimony does this by erecting an evolutionary pattern that is most consistent with available data. Parallelisms can then be detected once the evolutionary pattern is established. Certainly one could not objectively detect parallelism by assuming that it existed prior to the analysis! In using parsimony, we take the possibility of reversals into account by assuming no irreversibility of characters and by using the Wagner method. Parsimony operates by finding a pattern of relationships that is most consistent with the data. This may not be a “biological” reason for choosing between alternative trees; but the principle of tailoring theories to fit known facts is an irreplaceable part of science in general.

Rogers et al. (1967) contend that the use of the parsimony criterion assumes that evolution itself is parsimonious, and that while the most parsimonious tree may indeed be the tree most likely to be true, it still may have very low probability of being correct—so low, in fact, as to invalidate the principle of choosing the most parsimonious tree. That the first claim is untrue is shown by the fact that a most parsimonious tree may show a large number of convergent and parallel changes, demonstrating that evolution is not parsimonious. The second criticism is implicitly a criticism of the use of any kind of maximum likelihood estimation procedure. Considering the general and successful use of maximum likelihood estimators in statistics, this argument cannot be accorded much weight.

A MEASURE OF CONSISTENCY

As we have indicated above, one of our objectives is to produce a tree that is most consistent with the original data. In com-

paring trees, we may wish to measure the degree to which they are consistent with data. Camin and Sokal (1965) use "number of extra steps" to measure the deviation of the tree from a perfect fit to data. We will use a somewhat more general index of consistency for this purpose.

We now define the *index of consistency*, c . The *range*, r , of character i , $r(i)$, is defined as the difference between the numerically largest and numerically smallest states of the character. The *size*, R , of the data is defined as

$$R = \sum_i r(i).$$

Letting L stand for the length of the tree, we define the index of consistency of a tree to a set of data as $c = \frac{R}{L}$, where R , L , and

the tree have been computed on the set of data for which c is specified. The value of c lies between 0 and 1. It is 1 if there is no convergence on the tree, and tends to 0 as the amount of convergence on the tree increases. Since c is monotone decreasing on L , c is maximal over trees for a set of data on the most parsimonious tree. The index c is influenced by weighting coefficients, it being assumed that L and the $r(i)$ are computed on weighted data.

We use c instead of "number of extra steps," because it varies between fixed limits, and can be used on weighted and continuously coded data and to compare the fits of trees to different data sets. We

note that $c = \frac{R}{R + S}$, where S is "extra length," is some weighted continuous analog of "number of extra steps."

PRELIMINARY WAGNER TREES

One of the first steps in our taxonomic analysis was the computation of Wagner trees using an initial suite of characters taken from the literature. We followed Inger's (1967) coding of these characters.

We present the results of this analysis here, both as an example of the capabilities of most parsimonious trees to summarize data and because the preliminary trees bear on our later conclusions.

We formed a Wagner tree for the data presented in Inger's Table 1. The cladogram is depicted in our Fig. 2. We have made one modification in Inger's coding, that of arciferal versus firmisternal pectoral girdle condition. Inger's state g , "transitional," is a description of the variation in the character in a family and does not correspond to any condition of an organism. Accordingly, we have eliminated that state from the analysis by breaking each family having that state into two OTUs, one coded for arciferal, G , and one for firmisternal, g . How well our tree fits the data depends partly on exactly how one numerically encodes two of Inger's characters. The tadpole spiracle and the type of centrum each have three states, and for each Inger gave one state as being primitive and the other two as derived. He did not stipulate, however, whether one derived state is supposed to be a precursor to the other, or whether both derived states are supposed to arise directly from the primitive state. If we make no assumptions about obligatory sequential relations between character states, we are effectively using a *trivial metric* on that character (viz., a unit character difference between two OTUs is zero if the two OTUs have the same state in the multistate character, and is one otherwise). With such a metric, the number of evolutionary changes necessary to fit the data is 12, and the tree has 20 steps, so that there are eight extra steps. Some of the loss of parsimony in the tree of Fig. 2 is owing to Inger's opinions as to primitive states. If the state in the Ascaphidae is taken to be primitive for each character, a tree with 19 steps can be obtained with the same cladistic topology (Fig. 3).

While we do not prefer the trivial metric, its use is necessary in order to compare the fit of our Fig. 2 to the data of Inger's three

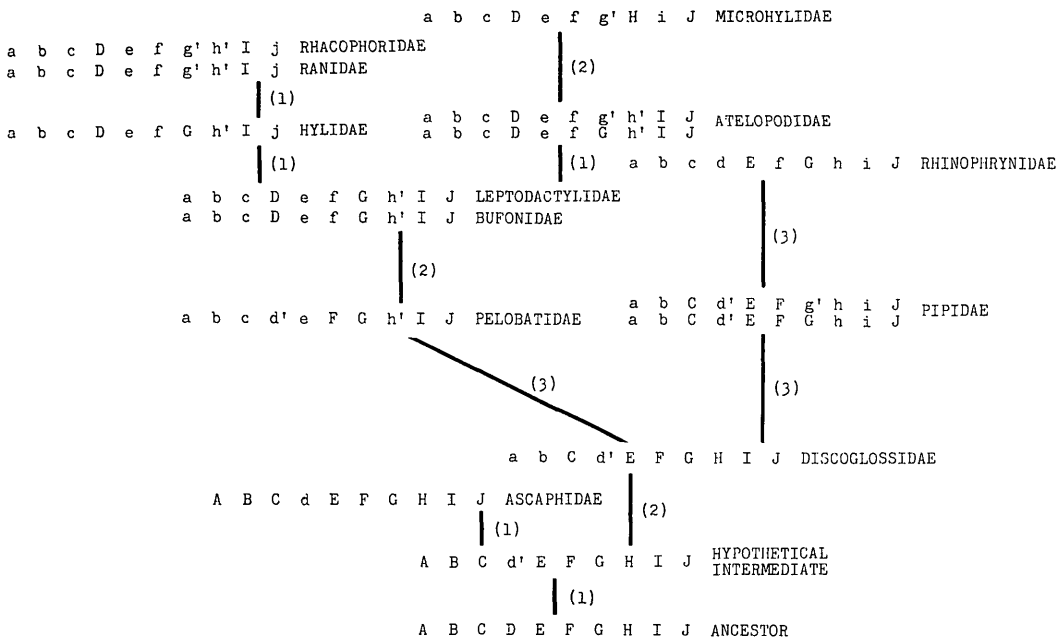


FIG. 2.—A maximum parsimony phylogeny of anuran families constructed according to the procedure of Wagner (1961). The 10 characters are those used by Inger (1967); see page 8 for further discussion. The phylogeny has a total length of 20, and it includes four cases of simple homoplasies, c, d, f and i, one example of multiple homoplasies, g', and two evolutionary reversals, D and H (reversals are only another form of homoplasies).

trees as summarized in his Table 2. We are forced to compare fits through the table because Inger did not list the character states of the intermediates on his trees. (We presume throughout that Inger's listings, "Fig. a," "Fig. b," and "Fig. c" in his Table 2 refer to his Figs. 4, 5, and 6, respectively). His Table 2 gives the number of "convergent changes" for his three trees. These values can be converted into the number of extra steps, the usual measure of degree of parsimony of a cladogram (Camin and Sokal, 1965), only if it is assumed that the metric on individual characters is trivial. Under that assumption, the number of extra steps for a tree is equal to the sum over characters of extra steps for each character. The number of extra steps for a character is the sum over character states of a function of number of convergent changes (nc) for the state. The function is

$$f(nc) = 0, \text{ if } nc = 0$$

$$f(nc) = nc - 1, \text{ if } nc \geq 1.$$

The necessary computation on Inger's Table 2 reveals that his Figs. 4, 5, and 6 have 15, 11, and 10 extra steps, respectively.

Thus, for the most consistent assumptions about primitive states, the Wagner tree for Inger's data has $c = .63$, while the trees that Inger proposed have c between .445 and .545; these c values were all computed with all characters assigned the same weight. This illustrates how the most parsimonious tree is the most consistent one for a set of data.

CHARACTER ANALYSIS AND RECORDING

That the highest c value, .63, for Inger's data is substantially less than unity indicates disagreement between the characters, as coded, on the relationships of frog families. It is possible that all this

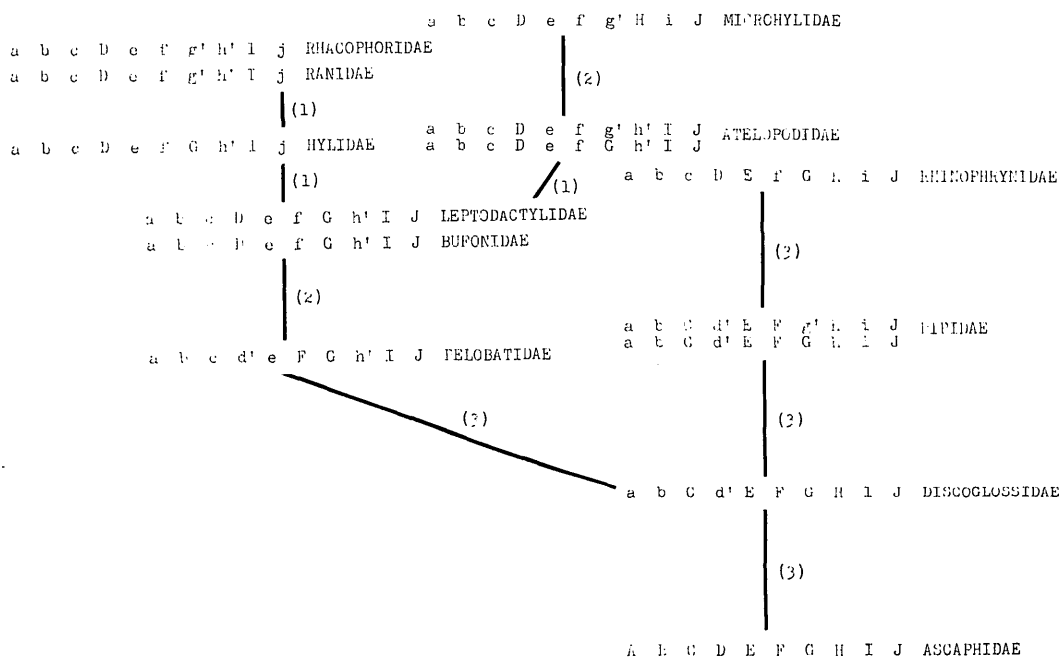


FIG. 3.—A maximum parsimony phylogeny of anuran families constructed according to the procedure of Wagner (1961). The 10 characters are those used by Inger (1967), except that all character states exhibited by the Ascaphidae are treated as primitive; see page 8 for further description of data modification. Compared to Fig. 1, this phylogeny is shorter by one evolutionary step, 19, and it includes one less homoplasy, d, and one extra reversal, D.

disagreement is owing to true convergence within frogs, (*viz.*, that some structure in line A is identical to a structure in line B, though they have evolved independently). Such convergence can be detected only after one succeeds in forming a fairly accurate tree of frog relationships.

Some "convergence" however, may be owing to the miscoding of data. That is, two structures that actually differ and are nonhomologous may have been coded erroneously as the same state of some character. This pseudoconvergence would also be detectable on an accurate tree. Unlike true convergence, however, pseudoconvergence is capable of being detected simply by scrutinizing the organisms in the study, without reference to any tree-forming procedure.

We have undertaken a morphological re-analysis of the characters in the study.

The aims of this analysis were to establish the relative variation of the characters within OTUs, to rectify as many cases of miscoding as possible, and to achieve a numerical coding of the data that most accurately represents our state of knowledge of the data. Detailed discussion of individual characters has been relegated to Appendix II. We will summarize here the main features of our procedures.

Since we expect the reliability of characters for evolutionary inference to be inversely related to within-OTU variability, we have sought to define character states so as to minimize their within-OTU variation. For example, in defining the states "arciferal" and "firmisternal" of the character "pectoral girdle type" we have used the definitions of those terms proposed by Griffiths (1963), rather than the classical definitions as used, for example, by Inger

TABLE 1. NUMBER OF PRESACRAL VERTEBRAE.

Ascaphidae	9 (8 ^a)
Discoglossidae	9 or 8 (7 ^a)
Pipidae	8 or 7 (6 and 5 ^a)
Rhinophrynidae	8
Pelobatidae	9 or 8 (7 ^a)
Bufo	8 or 7
Atelopodidae	8, 7, 6 or 5 ^b
Leptodactylidae	8 or 7
Hylidae	8 or 7
Ranidae	8 or 7
Rhacophoridae	8 or 7
Microhylidae	8 or 7

^a Apparently owing to a synostosis of two or more vertebrae; not coded for in Table 2.

^b The presence of five vertebrae in *Oreophrynella* corresponds more closely to the extreme reduction in atelopodids (*sensu stricto*) than bufonids. This suggests that *Oreophrynella* is an atelopodid and not a bufonid.

(1967), because the newer definitions lead to lower within-OTU variation for this character.

Some characters have been deleted from the character set because of extremely high within-OTU variability. This is done because highly variable characters are difficult to code meaningfully for OTUs of family rank. Further, for some such characters we suspect much of the variation to be purely phenotypic and so not readily subject to evolutionary interpretation.

Characters which contained more than one piece of information have been subdivided into a series of characters. For example, "tadpole type" has been resolved into "mouth armed or not," "spiracle median or sinistral," "spiracle anterior or posterior" and "operculum origin." Degree of subdivision of characters is a function of redundancy of information content.

After deletions, we retained 6 of the 10 "characters" used by Inger (1967). Following subdivision, these 6 "characters" are represented as 11 unit characters. Each unit character has been resolved into two states, so that each is conveniently numerically coded as a binary variable (*viz.*, each character takes on numerical values 0 and 1). Steps of the analysis and coding procedure are summarized in Fig. 6 and Tables 1-4.

Most of the 11 binary characters are assigned weight 1. Characters IIIa and IIIb (centrum type), IV (pectoral girdle type), and Vb (median versus sinistral spiracle) were assigned lower weight because of within-OTU variation. The weights are given in Table 3.

CLADISTIC CONCLUSIONS

A most parsimonious tree for the recoded, weighted data is presented in Figs. 4 and 5. We emphasize that we do not regard these diagrams as the "Ultimate Truth" on frog relationships. We do feel, however, that they are somewhat more reliable than the conclusions of, say, Hecht (1963), Griffiths (1963), or Inger (1967), by virtue of the attention we have paid to the way in which our trees were constructed and the character analysis. Fig. 4 is a ground-plan diagram in the sense of Wagner (1961). Fig. 5 is a more conventional phyletic tree diagram with the same cladistic relationships of OTUs shown in Fig. 4.

Although in constructing our tree we have attempted merely to find the phylogeny most consistent with the 11 characters coded, our results are very similar to the classical notions of anuran phylogeny held by many workers in the past (see the numerous dendrograms reproduced by Hecht, 1963, and Inger, 1967). It seems that the opinions of those workers were based in part on data other than what we coded (the actual data used is not explicitly stated by many). If this is true, it would appear that our tree has considerable predictive value. By a similar argument, we would expect that other trees would have less predictive value, to the extent that they differ from ours. Our phylogeny is relatively robust in the sense that major changes in topology can be achieved only by adding many discordant characters or by extreme weighting of characters. The detailed topology of the Wagner diagram (Fig. 4) would probably be somewhat labile under the addition of new characters. For example, a new intermediate form con-

TABLE 2. THE VARIABILITY (A) AND TAXONOMIC DISTRIBUTION (B) OF SIX CHARACTERS.^a

TABLE 2A

Characters	States and Polarity	
	(primitive)	(derived)
I Tail muscle (<i>M. caudalipuboischiotibialis</i>) (present or absent)	present (A)	absent (a)
II Ribs (free, fused or lost)	free in both subadults and adults (B)	free in subadults, fused in adults (b) → lost in both subadults and adults (b*)
III Vertebral ossification (modes of)	ectochordal (C)	stegochordal (c') holochordal (c)
IV Pectoral girdle (epicoracoidal cartilages free—arciferal, or fused—firmisternal)	arciferal (D)	firmisternal (d)
V Spiracle	coded according to Fig. 6	
VI Scapula and clavical (overlap or juxtapose)	overlap (F)	juxtapose (f)

“fused” in both subadults, and adults (b')

^a Table 2A above corresponds to Table 1 of Inger with the following exceptions: the number of presacral vertebrae, *M. adductor longus*, *M. sartorius*, cornified beaks and denticles, and vent characters are not included (see text for reasons). The scapula-clavicle character is not the same as that used by Inger (see Appendix II).

TABLE 2B

Families	Characters	Character states					
		I	II	III	IV	V	VI
Ascaphidae	A	B	C	D	E, e, e*	F	
Discoglossidae	a	B	c'	D	e	F	
Pipidae	a	b	c'	D, d	E*	F	
Rhinophrynidae	a	b*	C	D	E*	F	
Pelobatidae	a	b'	c'	D	e'	f	
Bufo	a	b'	c	D	e'	f	
Atelopodidae	a	b'	c	D	e'	f	
Leptodactylidae	a	b'	c	D	e', e*, e**, e***	f	
Hylidae	a	b'	c	D	e', e**, e***, e-	f	
Ranidae	a	b'	c	d	e', e**	f	
Rhacophoridae	a	b'	c	d	e'	f	
Microhylidae	a	b'	c	d	e", e***	f	

necting the Pipidae and the Rhinophrynidae might replace the current direct connection between those two taxa. Such altera-

tions of the ground-plan diagram would not, however, affect the topology of the phyletic tree (Fig. 5). It is in this sense

TABLE 3. CHARACTERS OF TABLE 2 CODED IN A BINARY FORM.^a

Families	Binary form of characters										
	I	II			III		IV	V			VI
		a	b	c	a	b		a	b	c	
Ascaphidae	0	0	0	0	0	0	0	0	0	0	0
Discoglossidae	1	0	0	0	0	1	0	0	0	0	0
Pipidae	1	0	1	0	0	1	0	1	-	-	0
Rhinophrynidae	1	-	-	1	0	0	0	1	-	-	0
Pelobatidae	1	1	1	0	0	1	0	0	1	1	1
Bufo	1	1	1	0	1	0	0	0	1	1	1
Atelopodidae	1	1	1	0	1	0	0	0	1	1	1
Leptodactylidae	1	1	1	0	1	0	0	0	1	1	1
Hylidae	1	1	1	0	1	0	0	0	1	1	1
Ranidae	1	1	1	0	1	0	1	0	1	1	1
Rhacophoridae	1	1	1	0	1	0	1	0	1	1	1
Microhylidae	1	1	1	0	1	0	1	0	0	1	1
Weight of character	1	1	1	1	¼	¼	½	1	½	1	1

^a See Appendix II for further explanation.

that we consider our conclusions to be robust. For example, to disengage the Microhylidae from the line leading to the Ranidae and Rhacophoridae, and to shift its origin to the Ascaphidae-Rhinophrynidae-Pelobatidae lines as Inger (1967) proposed, would require that the pseudo-operculum (character Va) be considered primitive or that spiracle position (Vb) be weighted about five times more than any other character.

The greatest single point of difference between Inger's conclusions and ours is the placement of the Microhylidae. His opin-

ions on that point seem to be predicated on the variability of the "firmisternal" condition (p. 369) and the uniqueness of the sinistral spiracle (p. 378). We have removed the variability of the "firmisternal" condition by a more judicious coding of the character. As we pointed out above, the irreversibility of sinistral spiracle does not follow from its uniqueness. Further, in the Hylidae we have pointed out that *Phyllomedusa* has a nearly median spiracle, *Nototheca fissilis* a median spiracle, and that *Hyla goeldii* is polymorphic (including the extremes of median and sinistral open-

TABLE 4. OCCURRENCE OF THE CHARACTER STATES OF BEAKS AND DENTICLES IN ANURAN FAMILIES.^a

	Beak		Denticles (rows)			
	present	absent	triple	double	single	absent
Ascaphidae	×	×	×	×	×	×
Discoglossidae	×		×	×	×	
Pipidae		×			?	×
Rhinophrynidae		×				×
Pelobatidae	×	×			×	×
Bufo	×	×			×	×
Atelopodidae	×				×	
Leptodactylidae	×	×		?	×	×
Hylidae	×	×		?	×	×
Ranidae	×	×		?	×	×
Rhacophoridae	×	×			×	×
Microhylidae		×				×

^a The questionable occurrence of states (?) are discussed in Appendix II.

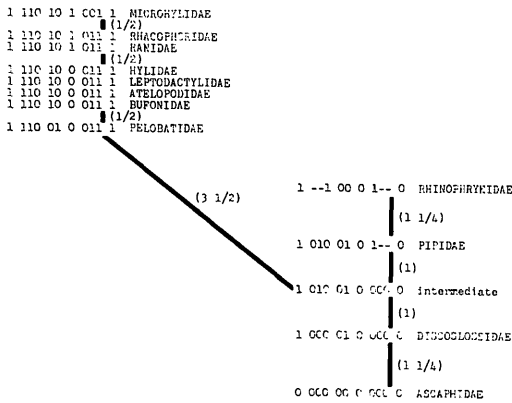


FIG. 4.—A phylogeny of anuran families constructed according to the maximum parsimony procedure of Wagner (1961). The six sets of binary coded characters are discussed on pages 19–32; see Tables 2 and 3 for summary. The phylogeny has a total length of 9½. Character IIIb is both convergent and reversed, and Vb is reversed.

ings). The most obvious interpretation of these facts is that there has been at least one case of reversal from sinistral to a median condition in the hylids. If the reversal has occurred in the hylids, why could it not have also occurred in the microhylids? The median spiracle of almost all microhylids is not only median, but considerably distant from the branchial chamber; in ascaphids and discoglossids the spiracle is on the edge of the chamber. Of our 11 characters, the microhylids differ from the ranid-rhacophorid group in only one. Therefore, we believe that available evidence quite strongly supports our placement of the microhylids with the ranid-rhacophorid line.

Our Fig. 4 is so drawn as to imply that some of the modern families of frogs are derived from other modern families, and this requires clarification. The Wagner diagram is not intended to imply, for example, that the common ancestor of ranids and microhylids was one of the known ranids—or even any ranid. The ground-plan diagram merely implies that the common ancestor of ranids and microhylids was like modern ranids with respect to the 11

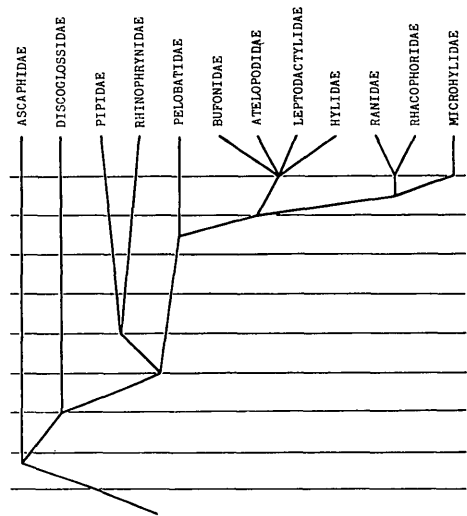


FIG. 5.—The maximum parsimony phylogeny of anuran families (of Fig. 4) translated into the more classically formed dendrogram. The horizontal parallel lines represent equal degrees of evolutionary divergence.

characters coded on the diagram. On the other hand, we cannot concur with Inger's criticism (p. 372) of postulating the derivation of one modern taxon from another. The "modern" families of frogs certainly had fossil members. We see no reason why, for instance, all species currently classified in the Microhylidae could not have a common ancestor that would fit within the Ranidae, as that family is usually conceived. Our position would be that while modern frog families may not be derived from each other, there is no biological law that would prevent this from being the case. To take a well-known example, we believe it is quite reasonable to state, "Mammals are derived from reptiles," implying merely that all mammals had a common ancestor that was a reptile, quite independently of the fact that Mammalia and Reptilia are both "modern" classes.

DISCUSSION

If the weighting criterion that we have used is a biologically realistic one, we would expect that characters with high variability within-OTUs would be more prone to

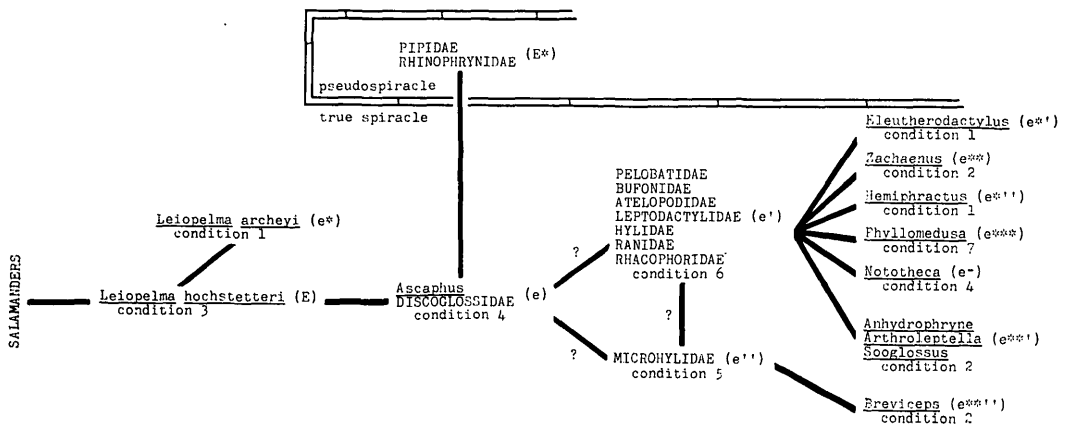


FIG. 6.—The probable course of evolution of the major operculum and spiracle conditions found in anurans. Each condition is defined and other taxonomic examples are presented on pages 26–30.

convergence than would other characters. Inspection of Fig. 4 seems to indicate that this is indeed the case. That this agreement of our tree with our expectations is not an artifact of our weighting procedure is indicated by two points. As we noted above, the Wagner tree for these data is robust and could be changed only by extremely different weighting coefficients from those used. In fact, *the* most parsimonious tree for these data would have the same form as our Fig. 4, if all the characters were weighted equally, or if the weighting coefficients were varied in any manner from equal weighting within factors of five! Certainly, the Wagner tree for our data does not depend strongly on the choice of weighting factors. The second consideration is that much the same picture of amounts of convergence in different characters is obtained from the Wagner tree for Inger's (1967) data (our Fig. 2). These data were not selected or weighted by us in any way. When analyzed by a most parsimonious tree, the data on frogs, both before and after our recoding, seem to show a stable set of relationships. It is not trivial that the concordance between single characters and the pattern of the data as a whole are largely predictable from the within-OTU variabilities of char-

acters. We believe that this predictability provides support for the practice of weighting by variation within OTUs.

The selection, weighting, and recoding of characters that we performed was done entirely on the basis of morphological data that had not been taken fully into account by earlier workers. It is interesting that the character reevaluation resulted in a data set with a high consistency index ($c = .84$). Related to this point is the fact that the characters showing the most convergence on the preliminary Wagner tree (Fig. 2) turned out to be the ones which seemed morphologically to be the most in need of recoding. That this is the case is again not trivial; it seems to suggest that most parsimonious trees have some power to detect miscoded characters. Trees produced by other means, for example, Fig. 4 of Inger (1967), seem to have less power in this respect. We would expect that a reasonably reliable method for producing evolutionary trees would indeed have some power of this kind. We would predict, in fact, that detection of miscodings in "rough" sets of data would be one of the most valuable applications of most parsimonious trees. Since Wagner trees seem to have some ability to predict which characters are miscoded or over-weighted, even when

other sources of weighting and coding information are unavailable, it should be possible to construct algorithms to find a series of trees, all but the first, based in part on weighting information supplied by the previous trees. Such algorithms would provide weighting coefficients generated entirely by a computer. We are currently investigating that type of program.

SUMMARY

A new taxonomic methodology, termed quantitative phyletic taxonomy, is proposed. It is founded on the precision of numerical techniques and the power of evolutionary inference. Quantitative phyletic analysis employs biological information in selecting optimal coding and weighting of characters. The objective of quantitative phyletics is to discover the evolutionary relationships of organisms.

Characters are weighted according to the concept of conservatism as estimated through within- and between-OTU variability. Multiplying each character by the reciprocal of the within-population standard deviation transforms characters on to scales in which rate of evolution would be approximately equal over characters. With equal rate of evolution, the range of variation between OTUs should be approximately equal; characters prone to convergence would tend to exhibit less between-OTU variability and they are weighted accordingly. The advantage of weighting characters by their OTU variability is that the weighting is fixed objectively by the data itself.

The primitive state of a character, that which occurred in the common ancestor of a set of OTUs, is inferred from evidence derived from fossils and on the degree and kind of distribution of the character states over the OTUs. Preliminary estimates of overall similarity, that make no assumptions about primitiveness, can be used to infer the character state distribution. We assume that all characters are, at least potentially capable of undergoing reversal.

The Wagner method for producing most parsimonious trees is described and an algorithm presented. The method makes no assumptions about reversibility of characters, and it can be applied to continuous variables and to those that are weighted. OTU linkage is determined by a difference equation, and the interval length between pairs of OTUs is equal to the sum of their character state differences. The length of the tree is the sum of the lengths of all intervals of the tree, and the tree of minimum length is the *most* parsimonious. Hypothetical OTUs are formed usually at branching points to minimize the length of the tree. The parsimony criterion is likened to a kind of maximum likelihood estimation procedure, and it is intended ultimately to detect homoplasy. A general index of consistency is defined; it measures the deviation of a tree from a perfect fit to data.

The Wagner method for producing most parsimonious trees was applied to a suite of characters from anuran families which were taken directly from the literature. These data had been used previously to form trees by different numerical taxonomic methods. The most parsimonious tree gave the best fit to the data. That the highest consistency value is substantially less than unity indicates considerable disagreement between characters, and the discordance is owing to either true or pseudoconvergence, or both. Pseudoconvergence is produced by miscoding data and it is capable of being detected simply by scrutinizing the organisms without reference to any tree forming procedure. In our reanalysis and recoding of the original suite of characters, we defined character states so as to minimize their within-OTU variation. This is in accord with our thesis that the most reliable characters for evolutionary inference are the most conservative. Some characters were deleted because of their extremely high within-OTU variability, while others were subdivided into a set of independent variables. After

reanalysis and recoding, the remaining characters were each resolved as a binary variable and weighted according to their within-family variability. The final most parsimonious tree, using these data, appears to have considerable predictive power and to be relatively robust. The close relationship of the Microhylidae to the Ranidae is a major difference between our tree and the conclusions of other recent investigators.

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- sternum*, *Gigantorana*, *Hyperolius*, *Oxyglossus*, *Ptychadena*, *Rana*, *Sooglossus*, *Stauroids*, *Trichobatrachus*.
- Rhacophoridae: *Afraxalus*, *Chiromantis*, *Kassina*, *Rhacophorus*.
- Microhylidae: *Breviceps*, *Chaperina*, *Elachistocleis*, *Hoplophryne*, *Kalophrynus*.

APPENDIX II

Tail muscle.—Inger (1967:371) noted that the "tail muscles," presumably referring to both the *M. pyriformis* and the *M. caudalipuboischiotibialis* (see Griffiths, 1963:262), are primitive "according to everyone's opinions," and he coded them together as two character states, namely present in the Ascaphidae and absent in all other modern families (p. 375, Table 1). He reasoned (p. 370) that "As frogs are one of the very few groups of vertebrates that have lost their tails and the only amphibians known to have done so, taillessness is almost certainly one of the derived conditions of the order." We agree with the kind of reasoning employed by Inger. In this example, however, it must be pointed out that, as recently as 1955, Ritland (1955a; also see 1955b:259, 272–9) discussed the likelihood that the *M. caudalipuboischiotibialis* is merely a coccygeal head of the *M. semimembranosus*, not homologous with one of the three tail wagging muscles of salamanders and, therefore, not a primitive feature of the tailed pro-Salientia. In addition, it must be further noted that the *M. pyriformis* appears to be uniformly present in all families of anurans with the exception of *Pelobates*, *Pipa* and *Hymenochirus* (Noble, 1922:33; Dunlap, 1960:17). In the absence of other critical investigations into this problem, we have chosen tentatively to accept the classical thesis that the *M. caudalipuboischiotibialis* of anurans is homologous with one of the tail wagging muscles in salamanders. We have coded the character in two states in anurans, present or absent (Tables 2 and 3). Based on our criterion 1, we have coded the former state as primitive and the latter as derived. In Table 3 the primitive state is coded as 0, the derived as 1. The *M. pyriformis* has not been encoded because of the kind and degree of variability that it exhibits.

Presacral vertebrae.—Inger (p. 372) referred to the number of presacral vertebrae as two character states, 9 and < 9 (p. 375, Table 1). He accepted the presacral number of nine as diagnostic of only the ascaphids among modern anurans, thereby not considering the variability previously described by Tihen (1960b; also see Kluge, 1966:7, 8) for pelobatids, which have eight or nine, and discoglossids, which also have eight or nine (Boulenger, 1897:39, reported on specimens with as many as 11 vertebrae, but this probably included the sacrum and the urostyle). The references to eight vertebrae in *Leiopelma* and *Ascaphus*

APPENDIX I

The following is a list of the extant anuran genera that we cite in the text. They have been arranged according to those families recognized by Inger (1967). In that the families were not defined, by either characters or taxa-included, we have been forced to make our placements by inference (both from Inger, 1967, and Griffiths, 1959, 1960, and 1963).

- Ascaphidae: *Ascaphus*, *Leiopelma*.
- Discoglossidae: *Alytes*, *Barbourula*, *Bombina*, *Discoglossus*.
- Pipidae: *Hymenochirus*, *Pipa*, *Pseudhymenochirus*, *Xenopus*.
- Rhinophrynidae: *Rhinophrynus*.
- Pelobatidae: *Aelurophryne*, *Leptobrachium*, *Megophrys*, *Pelobates*, *Pelodytes*, *Scaphiopus*, *Scutigera*.
- Bufoinae: *Bufo*, *Cacophryne*, *Nectophrynoides*.
- Atelopodidae: *Atelopus*, *Brachycephalus*, *Melanophryniscus*, *Oreophrynella*.
- Leptodactylidae: *Ceratophrys*, *Eleutherodactylus*, *Engystomops*, *Heleioporus*, *Heleophryne*, *Lepidobatrachus*, *Leptodactylus*, *Limnodynastes*, *Mixophyes*, *Notaden*, *Odontophrynus*, *Physaelasmus*, *Pleurodema*, *Rhinoderma*, *Sminthillus*, *Telmatobius*, *Zachaeus*.
- Hylidae: *Centrolene*, *Cochranella*, *Hemiphractus*, *Hyla*, *Nototheca*, *Phrynohyas*, *Phyllomedusa*, *Pseudis*, *Ptychohyla*, *Teratohyla*.
- Ranidae: *Anhydrophryne*, *Arthroleptella*, *Caco-*

(Ritland, 1955a:142; Stephenson, 1960:480), seven in pelobatids and discoglossids, and six and five in pipids all appear to involve a synostosis of two or more of the normally free nine, eight or seven presacral vertebrae in their respective families. It seems reasonable to us that only this specific category of variability may be logically ignored (see Table 1) in the discussion of most of anuran phylogeny, since it probably represents a developmental anomaly (*sensu stricto*).¹ On the contrary, however, it seems that the presence of nine vertebrae cannot be ignored in pelobatids and discoglossids on this basis. In these two families the individual nine free presacral vertebrae very often all appear to be normal. The larger number seems to be a reflection of the more posterior position of the sacral vertebra, relative to those species which have eight presacral vertebrae, and not some subdivision of a trunk segment. The number and position of the spinal nerves appear to be the best indication of the normalcy of the vertebral column (Ritland, 1955a).

As shown in Table 1 we have attempted to recognize the "normal" variability in vertebral number in anuran families, and it seems reasonable to code the states according to the progressive trend of decrease in number of vertebrae (9 → 8 → 7 → 6 → 5). Our interpretation of primitive and derived states rests on the assumption that pronurans, e.g., *Triadobatrachus* (*Protobatrachus*), salamanders and caecilians, and all other vertebrates for that matter, have larger numbers than modern anurans (*vide* fossils and criterion 1).

It appears likely that Inger's reason for considering all vertebrae variability of less than nine as only a single character state is best illustrated by his statement that (p. 372) "the lower number eight or less could very well have developed several times in independent lines and could have been derived from a stock (ascaphoid?) which had nine." In this example he has lumped character states on the basis of their variability and potential independent loss, while in the same example he did not consider the condition of nine vertebrae in pelobatids and discoglossids.

We argue that when character states are so similar as to appear to be homologues (as at least a first approximation, see Key, 1967), then convergence can only be discerned after a phylogeny has been constructed using more than one character. This procedure is particularly critical for homonomous series of structures, such as vertebrae, where some total meristic of the individual parts is the character.

We have not used the number of presacral vertebrae in our phyletic reconstruction because of the lack of critical study of normal variability, particularly among the more primitive frogs.

¹Exceptional in *Pelodytes* where the actual fusion of the first and second vertebrae has been fixed evolutionarily.

Ribs.—Inger (1967:371, 375, Table 1) treated ribs as two character states, either present in some stage of their life history, or absent. He did not specify the absence as owing to actual loss of the center of ossification, or that the center had fused to some part of the vertebra. In our opinion, to more accurately describe the known variation in anurans, this character must be coded in at least the following three states: (1) ribs present in both subadults (unfortunately precise aging, in the form of normal tables, is available for only a few species) and adults, (2) ribs present in subadults, but absent in adults, or (3) ribs absent in both subadults and adults. Noble (1931:233) pointed out that ribs are present in pipid larvae and that they fuse to the vertebral diapophyses in adults. As a more precise example, in the pipid *Xenopus laevis* ribs appear relatively late, stage 52 of its normal table, and only become fused to the diapophyses in postmetamorphic individuals, following stage 66 (Nieuwkoop and Faber, 1956:107). It is this specific process of rib fusion during ontogeny that most strongly supports our contention that character state (2), as described above, must be encoded.

The cladogram derived from Inger's data (Fig. 1) indicates that ribs are absent (c) in the Rhinophrynidae and in the Pelobatidae and all other derived families. Superficially then, the absence of ribs in these two phyletic lines must be considered an example of independent loss. The following data seem to suggest, however, that independent evolution is not involved. The cleared and stained tadpoles of *Rhinophrynus dorsalis* that we prepared, at stages approximately equivalent to 47 to 58 of *Xenopus laevis* (Nieuwkoop and Faber, 1956), do not appear to possess ribs, either as separate centers of ossification, or as presumptive synostotic areas at the ends of the vertebral diapophyses. In addition, the similar proportions of diapophysis length to vertebra width, including intra- and intersegmental comparisons of vertebrae two through four, in tadpoles of these same stages with adults, further suggests that ribs have not developed after stage 58 and then fused to the ends of the diapophyses. Lastly, the ends of the diapophyses of postmetamorphic *Rhinophrynus* do not indicate, even vaguely, the presence of fused ribs. In contrast to these findings, our examination of numerous cleared and stained tadpoles and recently transformed individuals of the pelobatid genus *Scaphiopus* (*bombifrons*, *couchii*, *hammondii*, *intermontanus*) seems to suggest that in this phyletic line the absence of ribs is owing to rib-diapophysis fusion, similar to that so readily seen in pipids. For example, in a recently transformed *Scaphiopus holbrookii* (20 mm snout to vent length), one can clearly see a large separate center of ossification at the distal end of both diapophyses of the fourth, postcranially located, vertebra. If this con-

dition can be confirmed in additional material of *S. holbrookii*, and demonstrated for other vertebrae, as well as in other pelobatids and for other families derived from that stock, then a case of independent evolution in the cladogram can be explained away. Ridewood (1897) indicated the likelihood of rib-diapophysis fusion in *Pelobates*, and in one series of *Microhyla* van der Steen (1930) found what appeared to be a separate anlagen, which later fused with short processes on the third pair of arches. And, rib-diapophysis fusion appears to be an established fact in at least two other families, Bufonidae and Ranidae, since Mookerjee (1931:191) described in detail a condition similar to *S. holbrookii* in *Bufo* and *Rana*. Mookerjee's (p. 191) point that "the mesenchymatous rib is from the beginning formed close to the distal end of the rib-bearer [= diapophysis]", particularly in the case of *Rana* and *Bufo*, might form some basis for the generally accepted thesis that ribs are absent ("lost") in the more advanced frogs. The data on *Scaphiopus*, *Pelobates*, *Microhyla*, *Rana* and *Bufo*, although admittedly very meager, tentatively suggest that the character of ribs should be coded as (1) free ribs present in both subadults and adults, (2) free ribs present in subadults, fused to diapophyses in adults, (3) ribs "fused" to diapophyses in subadults and adults, and (4) ribs lost in both subadults and adults (see Table 2). This form of coding removes the case of independent loss of ribs in anurans if state 4 is derived from 2, independent of 3. The primitive state (1) is inferred from fossil evidence and criterion 1.

In Table 3 the rib character (II) has been coded as (a) rib free in subadult = 0, rib fused in subadult = 1, (b) rib free in adult = 0, rib fused in adult = 1, and (c) rib present = 0, rib absent = 1. This form of binary coding seems to contain all of the relevant information conveyed in Table 2.

Vertebral ossification.—Inger (1967:372–3) used the three developmental modes of ossification of the centrum of vertebrae described in detail by Griffiths (1963:256–61)—ectochordy, holochordy and stegochordy—as another major character in his analysis of anuran phylogeny. He discussed Griffiths' contention that the adult husk-like ectochordal vertebra is primitive, and the partial husk-type stegochordal and the solid holochordal are derived, and concluded that there is another interpretation possible, which he followed (see p. 375, Table 1). His reasoning for this action is as follows (p. 373): "As most extinct lepospondylous amphibians had holochordal vertebrae . . . , holochordy is probably the primitive state and ectochordy and stegochordy represent derived conditions." He concluded that (p. 373) "the only evidence for the primitive nature of ectochordy is its appearance as an early ontogenetic state in many frogs."

Our discussion of this character is presented in two parts. In the first section, we will accept without qualification, as did Inger, Griffiths' concept of the kinds of vertebral ossification and their taxonomic distribution. In that context we will focus on the following points: (1) that the Lepospondyli are ancestral to the Lissamphibia is questionable, (2) and even if the Lepospondyli are ancestral to modern amphibians, the predominance of an ectochordal-like vertebra, not holochordal, in adults of that extinct group suggests that the former condition is more likely to be primitive, and (3) there are other, and more relevant, data than the time of appearance in ontogeny which suggest that ectochordy is primitive. It should be emphasized that the strict use of this character, as defined by Griffiths (p. 258), involves some knowledge of the "morphogenetic [ontogenetic] pattern," which of course is available for few, if any, fossil forms. The second part of our discussion is devoted to a reinterpretation of the kinds of vertebral ossification and their taxonomic distribution in anurans. We believe the reinterpretations to be correct over those of Griffiths'. However, we have purposely ignored ours in the final reconstruction of anuran phylogeny (Tables 2 and 3; Figs. 4 and 5). The reason for this procedure is given in the section on character analysis and recoding (page 9–11).

In a relatively recent paper, Parsons and Williams (1963) reexamined the relationships of the three modern amphibian orders, as the monophyletic Lissamphibia, and concluded that it is impossible to put forward even a tentative theory as to which Paleozoic amphibians are ancestral to the living group(s) (p. 48). Further, Baird (1965) and Estes (1965) recently presented evidence to support the derivation of the Lissamphibia from the Labyrinthodontia rather than from the Lepospondyli. To avoid confusion we note that the meaning in Estes' statement (p. 33), that it "is as possible to derive the vertebrae of modern amphibians from a primitive rhachitomous type as it is from a lepospondylous type," almost certainly follows Williams' (1959) use of vertebral terminology and not that of Griffiths (see Peters, 1964:187, 304) and it can therefore be disregarded in the immediate discussion.

It appears that the lepospondyls (aistopods, nectrideans and microsaurids) are characterized by vertebral centra which ossify as cylinders around the notochord, and it was by virtue of these "husk vertebrae" that they were named Lepospondyli (see Baird, 1965:287). In addition, apparently most of the other groups of primitive amphibians, the Labyrinthodontia, have lepospondylous-like vertebrae, either completely or incompletely encircling the notochord (terminology sensu Baird; see Romer, 1966:94, Fig. 128, and Piveteau, 1955). Watson's (1940:224) statement that "The vertebrae of *Miobatrachus* [now

considered a synonym of the rhachitome labyrinthodont *Amphibamus*] so much resemble those which must have existed in the ancestors of the Anura [*Protobatrachus*]” emphasizes the great degree of similarity between these major taxonomic assemblages (also see Hecht, 1962:41). We can see no major gross morphological difference between the lepospondylous kind of vertebrae and the ectochordal type found in adults of the anuran families Ascaphidae and Rhinophrynidae; we are, however, making this comparison without knowledge of the pattern of development in the Lepospondyli and other primitive amphibians.

Evidence that ectochordy is primitive and that stegochordy and holochordy are derived obtains from the following points: (1) that the other members of the Lissamphibia, the salamanders and the caecilians, have ectochordal-like vertebrae, (2) the earliest (Triassic) and most primitive frog-like form known *Triadobatrachus* [= *Protobatrachus*] (Piveteau, 1955; Romer, 1966:100; Watson, 1940), which may be represented by a larva or late metamorphic individual (Griffiths, 1956; also see Hecht, 1962:43), has ectochordal-like vertebrae, and (3) the predominance of ectochordal-like vertebrae in both Labyrinthodontia and Lepospondyli (*vide* fossil evidence and criterion 1).

To assume that ectochordy is a derived condition almost certainly creates numerous cases of convergence within the Anura and in the other major groups of the Amphibia, particularly the Labyrinthodontia; a more parsimonious interpretation would be to consider that state primitive as we have done (Table 2). In recoding the states of this character we have denoted both stegochordy and holochordy as derived. It appears that the change from ectochordy to either of these states involves at least a subtle increase in the deposition of calcium salts and a change in the site of calcium deposition relative to the notochord. The actual existence of the notochord in the more advanced families of frogs depends (basically) on whether one looks at the gross or microscopic anatomy and from which level in the ontogenetic continuum the study material is taken. In consideration of this point, it is not surprising that the cladogram given in Fig. 4 indicates states of homoplasy. We have used this character, even in its recoded form, primarily because of its historical popularity among anuran taxonomists.

The fact that heterochrony is more than just a possibility in some frogs (Stephenson, 1960), and that the development of the centrum is likely to be relatively strongly influenced by such a phenomenon, also should be taken into consideration when using this character in more detailed phyletic studies. Inger considered the possibility of heterochrony (p. 373), but he used it as the only evidence in support of ectochordy as the

primitive state (see our statement number 3, p. 21).

In Table 3 it has been necessary to code this character (III) in the binary forms (a) notochord retained in adult = 0, notochord not retained in adult = 1, and (b) centrum ossification ventral in position = 0, ossification not ventral in position = 1. This manner of coding does not appear to sacrifice any information conveyed in Table 2.

Inger seemingly followed Griffiths (1960, 1963), who in turn almost certainly obtained his basic interpretation of the different developmental patterns of the anuran centrum and intervertebral body from Mookerjee (1931). Our brief review of Mookerjee's major works on this subject (1931, 1936, and Mookerjee and Das, 1939), coupled with our own observations on whole and thick sectioned and stained vertebrae of a few anuran taxa, has led us to conclude that reinterpretation is in order.

Mookerjee recognized two different modes of vertebra development, perichordal and epichordal, neither of which necessarily involves any reference to the Gadovian concept of arcualia (see Mookerjee, 1936:317). According to Mookerjee's interpretation, the perichordal type of centrum formation relates to the chondrification and eventual ossification of sclerotomic cells around the notochordal sheaths, while the epichordal type involves the chondrification and ossification only of the dorsal, or the dorsal and lateral, parts of the perichordal tube of sclerotomic cells. In the latter mode, some ventral portion of the tube remains membranous and ultimately degenerates along with the notochord. The credit for the actual discovery of the two modes should probably go to Dugès (1834). Griffiths (1963) referred to the former type as the ectochordal developmental pattern, and to the latter as stegochordal; he coined the new terms only because of the historical association of perichordal and epichordal with Gadov's concept of vertebrae formation from arcualia. To these two developmental modes, Griffiths added his third category (that of holochordy) and, furthermore, he recognized two kinds of stegochordy on the basis of their supposedly different developmental pathways.

According to Griffiths (1963:260), holochordal vertebrae are those where “the notochord is completely replaced by osteoid tissue.” By this definition, holochordy is only a developmental continuation of the ectochordal (perichordal) mode. Griffiths' distinction between the two kinds of stegochordy (his Fig. 5; b3 and d3) is based on whether the ventral and lateral walls of the perichordal tube become cartilaginous before they degenerate. We see no major reason for recognizing Griffiths' terms ectochordal and stegochordal over perichordal and epichordal, respectively, *sensu* Mookerjee; in the remainder of this part of the discussion we will use the latter terms.

We have retained Griffiths' "holochordal," but not as a state of the perichordal-epichordal character. This new usage obtains from the fact that it denotes how much of the area, once occupied by the notochord, is replaced by bone; it does not denote where in the perichordal tube the centrum develops. Lastly, our observations, and apparently those of Mookerjee, do not support the contention that there are two stegochordal modes of development, at least in those families to which Griffiths applied the term.

Mookerjee and Das (1939) discussed a number of taxonomically important morphologic features in the pattern of centrum development that appear to have been overlooked by Griffiths and Inger. Mookerjee and Das recognized two kinds of perichordal and epichordal modes. These are based on whether or not the ventral hyaline cartilage (*sensu* Mookerjee and Das) develops in the sclerotomic tube of cells sometime during ontogeny. The ventral cartilage does not take part in the formation of the epichordal centrum because it is that ventral part of the perichordal tube which always degenerates. The cartilage seems to degenerate in all of the perichordal centra as well. They pointed out that in some frogs the hypochord in the region of the urostyle fuses to the epichordal portion of the perichordal tube following the degeneration of the notochord and the ventral hyaline cartilage. The presence or absence of the ventral hyaline cartilage, and the loss or fusion of the hypochord to the epichordal centrum, seem to show considerable intrataxonomic and regional (in the same vertebral column) variability, which suggests that they may not be particularly useful taxonomic characters. Thirdly, and of obvious importance to any further study of modes of vertebral development is their observation that all of the frogs that they examined had a perichordal atlas (except *Rhacophorus maculatus*). Finally, and of considerable importance, is their discovery of a form of epichordy in *R. maculatus*, which in some characteristics is intermediate between the epichordal and perichordal extremes described by Griffiths. In general, the intermediate characteristics in the mid-trunk centra in *R. maculatus* are (1) only a relatively small part of the perichordal tube degenerates, and therefore the centrum is formed of a large part of the perichordal tube (in the adult, the centrum is intermediate in depth), and (2) the notochord does not appear to completely degenerate and therefore it seems to give rise to the large spaces in the adult centrum (see Mookerjee and Das, 1939, Pl. 2, Fig. 30). Our observations (as discussed below) add support to the idea that there is a continuum of subtle change between the two developmental extremes. The numerous levels of intermediacy suggest that the two categories cannot continue to be recognized unless one adopts the dichotomous con-

ditional of "perichordal tube continuous, or discontinuous." We believe this conditional to be unrealistic, since it seems that almost all epichordal frogs have a perichordal atlas.

The following summary of the taxonomic distribution of perichordal and epichordal vertebrae is based on the observations of Mookerjee (1931) and Mookerjee and Das (1939), and our own: Ascaphidae, Rhinophrynidae, Bufonidae, Ranidae, and Microhylidae—perichordal; Discoglossidae, Pelobatidae, Atelopodidae, Hylidae, Leptodactylidae, and Rhacophoridae—both perichordal and epichordal; Pipidae—epichordal.

Our study of histological preparations of nearly continuous developmental series (*sensu* neurula to post-metamorphosis) of *Ascaphus truei*, *Rhinophrynus dorsalis*, *Bufo marinus*, *Rana sylvatica*, *Cacosternum capense*, and *Breviceps mossambicus* indicates that they are perichordal and that *Xenopus gilli* is epichordal. Our series of *Discoglossus pictus* is also nearly continuous, and the species is perichordal (*sensu stricto*), unlike the epichordal condition reported for the only other discoglossid studied, *Bombina bombina* (Mookerjee, 1931). Our interpretation of perichordy in *Discoglossus* is based on the fact that the ventral part of the perichordal tube does not disintegrate, although it becomes fibrous and shrinks, thereby restricting the notochordal cavity to a very small oval space.

Of the pelobatids that we have examined, *Scaphiopus bombifrons* and *S. intermontanus* are perichordal. Although our developmental series of *S. couchii*, *S. h. holbrookii* and *S. holbrookii hurteri* are not continuous we believe the former two taxa are perichordal. In contrast to *S. bombifrons* and *S. intermontanus*, the ventral part of the perichordal tube of *S. couchii* and *S. h. hurteri* remains fibrous until well after metamorphosis, at which time it finally ossifies. The mid-trunk centra of *S. h. holbrookii* appear to be epichordal, like that condition reported for *Pelobates fuscus* (Emelianoff, 1925). Our examples of mego-phryine pelobatids, *Leptobrachium hasseltii* and *Megophrys monticola*, are perichordal.

In the Atelopodidae and the Rhacophoridae we have not been able to study continuous developmental series. Our observations indicate that in the former family, *Atelopus minutus* is very likely epichordal, while *A. varius cruciger* and *Brachycephalus ephippium* are almost certainly perichordal. In the latter family, *Afraxalus weidholzi* and *Kassina senegalensis* are perichordal, in contrast to Mookerjee and Das' conclusion that *Rhacophorus maculatus* is epichordal.

Our histological preparations of the Hylidae and Leptodactylidae, in particular, support our contention that there are conditions between the extremes of perichordal and epichordal which cannot be placed without serious question in either category. In the Hylidae, *Hyla arborea japonica*, *H. cadaverina*, *H. cinerea*, *H. eximia*, *H.*

septentrionalis and *Pseudis limellum* are classified as epichordal; the epichordal condition in *Pseudis* has been reported previously by Mookerjee (1931). In all of the *Hyla*, the ventral part of the perichordal tube and the notochord appear to degenerate. The mid-trunk centra of adult *H. septentrionalis* are vacuolated like those of the epichordal *Rhacophorus maculatus* (Mookerjee and Das, 1939: Pl. 2, Fig. 30). These spaces may be equated to that small part of the notochordal canal that is left within the epichordal arc. In *Ptychohyla* there are two distinct degrees of perichordy, one of which is very close to the epichordal type found in *Hyla*. In *P. schmidtorum* the centrum is very shallow like that of *Hyla*. Unlike those species, however, the ventral part of the perichordal tube becomes fibrous and shrinks (it does not degenerate) and forms a horizontal band across the bottom of the shallow epichordal arc of cartilage, wherein a very small part of the notochord remains. In contrast to *P. schmidtorum*, *P. spinipollex* is typically perichordal; the centrum is relatively deep and its ossification resembles that of *Rana* as described by Mookerjee and Das.

In the Leptodactylidae we have found typical perichordal centra in *Ceratophrys ornata*, *Heleophryne purcelli*, *Leptodactylus ocellatus*, *Notaden nicholsi*, *Pleurodema diplolistris* and *Telmatobius marmoratus*. Epichordal centra are present in *Eleutherodactylus biporcatus*, *E. rugulosus*, and *Leptodactylus podicipinus*. Our developmental series of the following leptodactylids lack some critical stages and our conclusions are, therefore, tentative: *Eleutherodactylus rhodopis*, *Leptodactylus labialis*, *L. pentadactylus*, *L. fuscus*—perichordal, and *Physalaemus cuvieri*—epichordal. The latter group of species have centrum depths intermediate between the perichordal and epichordal size extremes of Griffiths.

Although the number and kinds of taxa that have been studied by us are not extensive (further work is in progress), we believe that they are sufficient to indicate that considerable intra-familial variability exists among anurans, and that there is a continuum of change between the states perichordal and epichordal. In the future studies that are obviously required to better document this variability and to assess the states of the continuum, we believe the critical developmental stages to be studied most intensively are those during and shortly after metamorphosis. It is during these stages that the notochord and the ventral and lateral walls of the perichordal tube disintegrate. Because of the obvious cases of regional and ontogenetic variability and the numerous states of intermediacy between perichordal and epichordal, we believe that all future studies should uniformly focus on a specific vertebra, or set of vertebrae, such as the third, fourth or fifth. It is only in this way that the homologous equivalence throughout all studies will be maximized and

the taxonomic value of the character more accurately evaluated. We have noted during our study that all adult frogs with epichordal centra seem to exhibit relatively very shallow centra, with the shallowness being directly proportional to the degree of epichordy. In addition to these characteristics, most of the kinds of epichordal centra stained with Alizarin Red-S exhibit a granular appearance in metamorphosing and post-metamorphic individuals, unlike those with the perichordal pattern. These kinds of evaluations may provide a reasonably accurate assessment of the type of developmental pattern in the absence of continuous series.

Griffiths and Inger appear to have greatly over-generalized the taxonomic distribution of holochordy. By definition, it cannot be present in the extreme epichordal Hylidae, Atelopodidae, Leptodactylidae, and Rhacophoridae. In addition to these exceptions, we have found numerous examples of species with perichordal vertebrae in adult ranid and rhacophorid genera alone (e.g., *Rana*, *Gigantorana*, *Hyperolius*, and *Kassina* and *Afraxalus*). It must be emphasized that this kind of perichordal centrum differs from that in ascaphids, because (1) the notochordal canal does not completely pass through the entire length of the centrum, since the ossified intervertebral body is usually fused to one end of it, and (2) the persistent notochord may be relatively hard, but nevertheless it is fatty in texture and it does not contain any detectable amount of calcium salts. The variability that we have found in this character also indicates that it too possesses relatively little information on interfamilial relationships.

The terms procoelous and opisthocelous are commonly used to describe to which end of the centrum the intervertebral body attaches. When the intervertebral body does not attach to the centrum, the term amphicoelous is often applied. Unlike most interpretations, however, we believe the most judicious coding of the characters to be as follows (1) intervertebral body free or fused to the centrum, and (2) intervertebral body fused to the anterior end of the centrum (opisthocelous) or to the posterior end (procoelous). The other frequently used term, diplasiocoelous, has been interpreted in at least two very different ways by anuran systematists. It has been defined as the biconcave eighth (or last) postcranial vertebra, and as that vertebral column wherein the eighth vertebra is biconcave and the remainder are procoelous (Noble, 1931; Goin and Goin, 1962: 218). If one chooses the first definition, then diplasiocoelous seems to be equivalent to saying "the eighth presacral vertebra is amphicoelous." If the second definition is followed, which is the usual case, then diplasiocoelous obviously is not comparable to the character states procoelous and opisthocelous. If one restricts his studies to what he believes to be homologous vertebrae, say the

third, fourth or fifth, then the condition of the homonomous series of elements as a whole (diplasiocoely) is not relevant.

We believe that the concept of vertebral articulation in anurans must be changed based on the general similarity of centrum and intervertebral body formation in any one body segment, and the numerous levels of intermediacy between perichordy and epichordy. It seems only reasonable that for every state of intervertebral body development there should correspond a state of procoely, or opisthocoely. To use the two extremes of perichordy and epichordy as an example, it would follow that the procoelous or opisthocoelous perichordal vertebrae are not equivalent to the procoelous, or opisthocoelous, epichordal vertebrae. The degree to which they are homologous depends on how much of the perichordal tube is included in the intervertebral body.

M. adductor longus and *M. sartorius*.—Inger (1967:371, 375, Table 1) used the condition of the *M. adductor longus* and the *M. sartorius* as 2 of his 10 characters (see his footnote, p. 380). And, he referred to the overall evolutionary trend of progressive fragmentation and specialization of musculature—from fishes to amphibians to reptiles to mammals—as his evidence for determining primitive and derived states of these specific muscles. He coded the *M. adductor longus* as absent (primitive) or present (derived) and the *M. sartorius* as combined with the *M. semitendinosus* (primitive) or split off from it (derived). He explicitly ruled out the likelihood that the absence of these muscles in primitive anurans could be attributed to a secondary loss by reason of the general increase in locomotor efficiency resulting from division and specialization of the thigh musculature. The relationship between locomotor efficiency and muscle complexity has not yet been documented, and, in any event, it would not appear to apply in view of the high degree of similarity between primitive salamanders and frogs such as *Ascaphus* (Noble, 1922:45–46, 55–57). The following data indicate that there is considerably greater variation within the presently recognized families than Inger recorded and that there is more direct evidence available for inferring primitive and derived states.

Inger (1967:375, Table 1) recorded the *M. adductor longus* as absent in the Ascaphidae, Discoglossidae, Pipidae and Rhinophrynidae, and as present in the Pelobatidae, Bufonidae, Atelopodidae, Leptodactylidae, Hylidae, Ranidae, Rhacophoridae, and Microhylidae. No literature is cited for the source of these data and those on the *M. sartorius*, and no intrafamilial variation was noted. However, according to Noble's (1922: 23–57) classic study of anuran thigh musculature and more recently Dunlap's (1960:1–76) detailed work, there are the following exceptions. The

M. adductor longus is absent in only two discoglossid genera, *Alytes* and *Bombina*; it is present in the genus *Discoglossus*. In the family Pelobatidae the muscle is present in *Pelobates* and absent in *Scaphiopus* and both interspecifically and intraspecifically variable in *Megophrys*. Moreover, in the Leptodactylidae the muscle is absent in *Mixophyes* and one of two specimens of *Eleutherodactylus tubolus* [?], the atelopodid genera *Atelopus* and *Oreophrynella*, and the two rhacophorid genera, *Chiromantis* and *Rhacophorus*, that have been examined. In addition, Tihen's (1960a:227, Table 1, 232) study of the Bufonidae indicated that of the 10 genera recognized, the *M. adductor longus* is present only in *Bufo* and that it has been lost independently in the family at least three times (see Tihen's Fig. 1, p. 229).

Inger (1967:375, Table 1) recorded the *M. sartorius* as combined with the *M. semitendinosus* (which is equivalent to saying "adductor longus absent" in that it too is combined with another muscle, the *M. pectineus*) in the Ascaphidae, Discoglossidae, Pipidae and Pelobatidae, and as separate (which is equivalent to saying "adductor longus present" in that it has separated from the *M. pectineus*) in the Rhinophrynidae, Bufonidae, Atelopodidae, Leptodactylidae, Hylidae, Ranidae, Rhacophoridae, and Microhylidae. Again, no intrafamilial variation was noted, but according to Noble (1922:29–31) and Dunlap (1960:6–9) there appear to be the following exceptions. The *M. sartorius* is only partially separated from the *M. semitendinosus* in the pipid genus *Xenopus* (possibly interspecifically variable—compare Noble to Dunlap), in the discoglossid genus *Discoglossus*, and in *Rhinophrynus* and *Limnodynastes* of the Rhinophrynidae and Leptodactylidae, respectively. The variability in these morphologically intermediate genera, in terms of the degree of separation of the *M. sartorius* from the *M. semitendinosus* and the considerably varied origins and insertions of the heads of the two muscles (Dunlap, 1960:8, 1966), suggests that homoplasy may be involved in all cases.

The two-headed nature of the *M. sartorius*–*M. semitendinosus* in most of the more primitive frogs (Dunlap, 1960:8), the only partial separation of the *M. sartorius* from the *M. semitendinosus* in *Xenopus* (e.g., *X. mulleri*, Noble, 1922:30–31, Pl. X, Fig. 2), and the intraspecific variability in *Megophrys montana* and the other morphologically intermediate genera (Dunlap, 1960) points to more direct evidence for the form in which Inger has coded the character states.

It would appear that the intraspecific and interspecific variability described for various anuran species, genera, and families, and in particular that morphogenetic sequence described in numerous *Megophrys* species (Noble, 1922:26), and the physical relationship of the *M. adductor longus* to the *M. pectineus* (Dunlap, 1960:9–11)

provides better evidence for ascertaining the primitive and derived states of that character than does the very general evolutionary trend cited by Inger. That the *M. adductor longus* may have evolved and been lost independently in the order Salientia alone is almost certain. Arguments similar to these can also be made for the *M. sartorius*. We cannot justify the use of these muscle characters. They appear to contain little information on relationships at higher taxonomic levels in anurans and nothing is gained by considering them further.

Pectoral girdle.—Like many others before him, Inger (1967:369, 375, Table 1) used the condition of the epicoracoid cartilages of the pectoral girdle as a taxonomic character. In general he appears to have followed the more classical definition of character states (*sensu* Cope-Boulenger) delimited by Noble (1931). These states are (1) epicoracoid cartilages overlapping and free—arciferal, (2) epicoracoid cartilages fused on the midline—firmisternal, and (3) a “transitional” condition. The actual source of Inger’s taxonomic data are difficult to ascertain for the following reasons: (1) he defines “transitional” as “In families that are sufficiently variable to be considered intermediate”; this does not correspond to Noble’s (1922) intermediate “arcifero-firmisternal” condition defined on the basis of morphology, and (2) Inger did not mention the variation in both the arciferal and firmisternal categories (*sensu* Noble) which were explicitly noted by Noble (1922, 1931) and Griffiths (1959, 1960, 1963).

Inger (p. 369) cited the exceptional intermediate conditions found in the leptodactylid *Sminthillus* (first discussed in detail by Noble, 1922) and the bufonid *Cacophryne* (*sensu* Griffiths, 1959:480) as evidence that incomplete firmisterny evolved independently. He then used this example to “raise the possibility” that the major firmisternal families, Microhylidae and Ranidae, share this resemblance as the result of independent evolution. From Inger’s criticism of Griffiths’ thesis that the ranids and microhylids are closely related (p. 373), we must assume that he was aware of the fact that Griffiths had redefined the epicoracoid character of the pectoral girdle. As Griffiths summarized (1963:264, 1959:472), criteria based only on degree of fusion, freedom and overlapping of the epicoracoid cartilages are “incapable of exact taxonomic application,” and for this reason he redefined the character in terms of “whether or not they possess posteriorly-directed epicoracoid horns.” Griffiths contended (p. 265) that the two character states defined on this basis “agree broadly with, respectively, the Arcifera and Firmisternia (*sensu* Boulenger) except that such forms as *Rana rugulosa*, *R. tigrina* and *Atelopus*, etc. are correctly designated.” He also pointed out that this scheme avoids Noble’s (1922) enigmatic “arcifero-firmisternal” condition.

If we accept Griffiths’ criteria for defining the states of the epicoracoid character, then the following taxonomic distribution obtains: All anuran families considered herein are uniformly arciferal except the Ranidae, Rhacophoridae and Microhylidae (Griffiths, p. 273), which are uniformly firmisternal, and except the Pipidae which according to Griffiths’ study (p. 271) has two arciferal genera (*Xenopus* and *Pipa*) and two firmisternal genera (*Hymenochirus* and *Pseudhymenochirus*). We have recorded the states of this character according to Griffiths’ definition and his taxonomic survey in the absence of data to the contrary.

We must come to some decision as to which of the two states, arcifery or firmisterny, is primitive. It is clear that one cannot rely on the fossil record for an interpretation, since epicoracoid cartilages have yet to be found preserved. Nor does a comparison, in the sense of Griffiths’ definition, with salamanders appear to be logical. Therefore, we are forced to rely on the estimate that arcifery is primitive (our criterion 2); this results solely from the fact that the greater proportion of anuran taxa exhibit that condition (Table 2). In Table 3, the character (IV) is translated into a binary form (arciferal = 0, firmisternal = 1).

Spiracle.—Inger discussed at length (1967: 373–383) the usefulness of “two” morphological characters of larvae proposed by Orton (1953, and later expanded upon by her in 1957) and the taxonomic distribution of the states of those characters. The two characters are (1) spiracle paired or single, emerging from the ventral midline or the left side of the body, and (2) cornified beaks and denticles present or absent (denticles in one or two rows per ridge, or absent, were also conditions described by Orton, 1953). In attempting to substantiate the taxonomic usefulness of these characters, Inger criticized Griffiths (1963), who had concluded that the intrataxonomic variability (= “polymorphism” of Griffiths) of the characters was too great for them to be of any real value in delimiting major groups of anuran taxa (also see Griffiths and Carvalho, 1965). The data of most of the other workers (Boulenger, 1891; Walker, 1938; Turner, 1952) that Griffiths cited in support of his conclusion, do not appear to document his thesis (see below), nor do his references to the “medio-ventral” condition of the spiracle in the hylid *Phyllomedusa trinitatus* and the presence of denticles in *Pseudhymenochirus*. Our discussion of the three larval characters attempts to give a better estimate of the degree of variability than that presented by Griffiths.

Inger’s statement (p. 375) that “Each of . . . the four types of tadpoles . . . is characteristic of one or several families of adults, and each family of adults has only one of these major types of free swimming tadpole” might be attributed to Orton’s knowledge of variability in 1953. This relationship results from her statement (1957:84)

that "The apparent integrity of the four larval types [I-IV] and their apparent reliability as indicators of basic evolutionary lines are inferred from their restriction to, and constancy within, the particular taxonomic group which each larval type characterizes." Such character constancy appears to have been responsible for her postulate that the tadpoles of the Pipidae and Rhinophrynidae (Type I) and the Microhylidae (Type II) are the most primitive. Her awareness of the importance of understanding variability is clearly recognized, however, when she noted that future discoveries would probably necessitate changing her conclusions (p. 85).

The fact that the "operculum" and, therefore, the spiracle of pipids is not homologous to the operculum of fish, salamanders and most other amphibians is such a discovery that radically changes Orton's thesis. Nieuwkoop and Faber (1956:141) described the development of the "operculum," and thereby the "spiracle," in the pipid *Xenopus laevis* as follows: "At stage 40 the third visceral arch has formed an ectodermal fold, the operculum, which grows caudalwards. At stage 44 it overarches the sinus cervicalis, leading to the formation of the cavity of the filter apparatus or the gill chamber. After the external gills have been completely covered over, only a narrow opening is left at stage 46. This opening forms a ventral oblique slit at stage 48, while the operculum has formed a thin caudal border. At stage 50 a fold has grown out from the hyoid arch, forming the basal portion of the operculum while the original outgrowth of the third arch forms its apical portion." We have found a similar developmental pattern in our whole material of *X. laevis*, and in *X. gilli* and *Rhinophrynus dorsalis*. These observations suggest that the entire pipid-rhinophrynid phyletic line has evolved a unique operculum and spiracle. It therefore follows that these data must be coded separately from the usual condition, namely (1) true operculum and spiracle formed mostly of an ectodermal outgrowth of visceral arch II (hyoid), and (2) pseudo-operculum and spiracle formed mostly of an ectodermal outgrowth of visceral arch III; an outgrowth from arch II occurs late and forms only the base of the pseudo-operculum. The relatively late ontogenetic appearance of the ectodermal fold from arch II in pipids and rhinophrynids, and the fact that the operculum is a composite (not in the sense of Orton, 1949:263-264) formed of folds from arches II and III, suggests that this character state is unique among fishes, salamanders and anurans. It follows that the pipid-rhinophrynid state is almost certainly derived. The apparent restriction of the composite pseudo-operculum and pseudo-spiracle to the phyletic line appears to correspond with the evolution of a very different type of gill chamber. In addition to this correspondence, the Pipidae exhibit a further unique

modification of the gill chamber, namely the foreleg develops in a separate enclosed space whose walls appear to be derived from the presumptive opercular epithelium just before the opercular folds begin to form (Newth, 1949; Nieuwkoop and Faber, 1956). Among anurans, this chamber appears to be restricted to pipids, and it would therefore be judged the derived state.

It is convenient for us to note at this time that we have been able to confirm Griffiths' statement (1963:254) that the leptodactylid *Lepidobatrachus* has paired spiracles (Cei, 1968; Cei, pers. comm.) and that these paired openings are not the result of the eruption of the forelegs (UMMZ 128836). On the other hand, Griffiths (1963; Griffiths and Carvalho, 1965) has repeatedly cited Turner's (1952) work on *Scaphiopus* as evidence of polymorphism in the oral characters of tadpoles. And, that his work can be cited as evidence is predicated on Turner's contention that *S. bombifrons* and *S. intermontanus* are conspecific with *S. hammondii*, which does not appear to be the case (Kluge, 1966). Owing to the lack of critical early developmental stages of *Lepidobatrachus*, we have not been able to determine whether its operculum is derived from visceral arch II or III. Therefore, we have not attempted in this paper to encode the paired spiracle condition in the Leptodactylidae.

If we can temporarily ignore the distinction between the categories "free-living larvae" and "direct development" in frogs with a type "1" operculum (see above), and consider all embryos at a stage close to the onset of metamorphosis (*sensu* tail reabsorption), then at least seven major known modifications of the spiracle (*sensu* Orton, and Inger) can be easily discerned. These modifications, as characterized and exemplified taxonomically below, are recognized on the basis of the degree of development of the true operculum and the dermal fold (Orton, 1949:263) and the extent and place of their fusion (*viz.*, in the formation of the spiracle). Life history terminology follows Jameson (1957:76, Table 1).

Condition 1. Spiracle absent (e.g., direct developing Leptodactylidae, such as *Eleutherodactylus*, and Ascaphidae, *Leiopelma archeyi*, Stephenson, 1955:787, and Archey, 1922; and the hylid genus *Hemiphractus* where the eggs and developing young are carried on the back of the parent, Orton, 1949:264). The development of the operculum appears to be completely suppressed and the dermal fold is only poorly developed, thereby leaving a wide gap through which the branchial region and the foreleg buds are exposed.

Condition 2. Spiracle absent (e.g., in such non-aquatic embryos as the rapid genera *Anhydrophryne*, Hewitt, 1919; *Arthroleptella*, Power and Rose, 1929, and de Villiers, 1929b; and *Sooglossus*, Brauer, 1898; the microhylid genus *Breviceps*, de Villiers, 1929a; and tentatively suggested for the terrestrial nest building leptodactylid genus

Zachaeus, Lutz, 1944). In contrast to condition 1, where the operculum is absent, and conditions 4–7, where the operculum is present and partially fused, the operculum-dermal fold fusion is complete, and therefore the branchial region and the foreleg buds are entirely covered.

Condition 3. Spiracle absent (e.g., the ascaphid *Leiopelma hochstetteri*, Stephenson, 1955:787–788). Like condition 2, with the exception that the operculum does not fuse with the dermal fold, the forelegs are usually covered by the posterior edge of the well-developed operculum.

Condition 4. Spiracle single and restricted to the ventral mid-line very near the posterior rim of the branchial chamber (e.g., the ascaphid genus *Ascaphus*, the Discoglossidae, and the hylids *Hyla goeldii* [intraspecifically variable] and *Nototheca fissilis*; Griffiths and Carvalho, 1965). The position of the single spiracle and its slit-like external opening are owing to the absence of operculum-dermal fold fusion on the midline very close to the rim of the branchial chamber.

Condition 5. Spiracle single and restricted to the ventral midline, with the external aperture, in contrast to condition 4, usually located immediately below the vent (e.g., most Microhylidae). The arboreal east African *Hoplophryne rogersi* (Noble, 1929:301, Fig. 6) exemplifies the variant where the external aperture is not located immediately below the vent, but is closer to the middle of the coiled gut. The South American *Elachistocleis ovalis* exemplifies an additional variant where the spiracular opening is sinistral and directed upwards along the caudal fin (Griffiths and Carvalho, 1965). The usual position of the spiracle and its relatively complex tube-like canal and external aperture are owing to the absence of operculum-dermal fold fusion on the midline. Early in development the external aperture of the spiracle is slit-like and is located very close to the rim of the branchial chamber and only later grows over the coiled gut on the ventral midline towards the vent in the form of a long tube. This ontogenetic pattern is clearly shown by Orton (1946:243, Figs. 1a–d). There appears to be a weak positive relationship in conditions 4 to 7 between the length of the free tubular portion of the spiracular canal and the shape of its external aperture, slit-like or round, relative to the distance the external aperture is from the rim of the branchial chamber (*viz.*, the longer the free part of the spiracular tube and the more oval the form of the external aperture, the longer the spiracular canal).

Condition 6. Spiracle single and restricted to the left side of the body, usually much above the ventrolateral margin of the trunk (e.g., the majority of the species in the Pelobatidae, Ranidae, Rhacophoridae, Bufonidae, Atelopodidae, Leptodactylidae, and Hylidae, *vide* Inger, 1967). The sinistral position of the spiracle and its relatively

complex tube-like free extension and usually round external aperture are owing to the absence of operculum-dermal fold fusion near the left posterolateral corner of the branchial chamber. Early in development the external aperture of the spiracle is usually slit-like and located very close to the rim of the branchial chamber, and it only later grows around the left side of the body in the form of a tube. This state is not restricted to larvae with aquatic development (e.g., it occurs in the larvae of the bufonid *Nectophrynoides tornieri* and the leptodactylid *Rhinoderma* which have direct development and where the embryo is carried by the parent until birth). Defined only by the position of the spiracular opening, without reference to the body, the microhylid *Elachistocleis ovalis* would be referred to condition 6.

Condition 7. Spiracle single and restricted to slightly left of the ventral midline (e.g., the arboreal nest building hylid genus *Phyllomedusa*, Starrett, 1960; and *Hyla goeldii* [intraspecifically variable], Griffiths and Carvalho, 1965). The nearly midline position of the sinistrally located spiracle and its slit-like external aperture are owing to the absence of operculum-dermal fold fusion slightly to the left of the midline near the posterior rim of the branchial chamber. There is variation in the position of the external aperture of the spiracle in both sinistral conditions 6 and 7, particularly in the latter, but of the two conditions, *Phyllomedusa* and *Hyla goeldii* appear to be exceptional in the degree to which the spiracle is located close to the midline. We cannot agree with Griffiths' (1963) statement that the spiracle in *Phyllomedusa trinitatis* is located exactly on the midline and, therefore, in some sense equivalent to condition 4 (see Griffiths and Carvalho, 1965: 115).

To evaluate the polarity of all seven character states, it is most convenient to consider them in the form of a binary set of comparisons. First, we can ask, is the absence (condition 1) or the presence of a true operculum (all other conditions) primitive? The great similarity of anlage, developmental pattern, and final form of the operculum of fishes, salamanders and frogs, excepting that condition found in pipids and rhinophrynids as described above, clearly suggests that it is primitive (criterion 1). We have acknowledged this dichotomy by recognizing the character as true operculum present (primitive) or absent (derived); see Table 2 and Fig. 6. Next we can evaluate the relative primitiveness of the operculum in terms of complete fusion with the dermal fold (condition 2) versus only partial fusion (conditions 4 to 7) or no fusion at all (condition 3). Again, similar to the previous argument, our conclusion that condition 2 is probably derived rests on the fact that the anuran taxa that exhibit that modification appear to represent relatively minor exceptions (criteria 2 and 3)

compared to the majority operculum-spiracle condition in the major taxonomic categories to which they are usually assigned on the basis of other characteristics. For example, the genera *Anhydrophryne*, *Arthroleptella*, and *Sooglossus*, and *Breviceps*, and *Zachaeus* are almost certainly related to three natural groups of genera, namely the Ranidae, Microhylidae and Leptodactylidae, respectively; the vast majority of these related taxa have aquatic larvae with a single sinistral spiracle (operculum-dermal fold fusion incomplete). Our argument is the most parsimonious one, for to assume that all of the examples of condition 2 are homologues, and the taxa possessing this modification are primitive, dictates that the spiracle has evolved independently many times and is not homologous (see Fig. 6).

The explanation of condition 3 in *Leiopelma hochstetteri* must be considered differently from the above, because a simple parsimonious taxonomic approach cannot be applied. This obtains if we accept the Ascaphidae as a natural group of only two genera, *Leiopelma* and *Ascaphus*. *Leiopelma* has direct development (operculum absent or present and completely free), while *Ascaphus* has a typical aquatic larva with a spiracle (the operculum and dermal fold are fused in part). The fact that the operculum of *L. hochstetteri* is similar to the probable ancestral form, namely that seen in most salamander larvae where the spiracle has not formed owing to the absence of operculum-dermal fold fusion, suggests that its state is primitive (criterion 1). Additional support for this relationship obtains from Stephenson's (1955) point of view (which counters Noble's, 1927:63, conclusion) that the mode of development of *Leiopelma* is more like urodeles than frogs. In the absence of relevant data to the contrary, we tentatively recognize the condition 3 "spiracle" (found in *L. hochstetteri*) as primitive. Accepting this thesis requires that the condition of *L. archeyi* be derived from condition 3 of *L. hochstetteri* and that conditions 4 to 7 are also derived from 3. If data are found to contradict our contention that one of the states is primitive, condition 3 will still be considered primitive relative to 1, but in itself derived from some anuran where the spiracle (*sensu stricto*) is present (e.g., like conditions 4 to 7).

Our binary comparison of the remaining conditions 4 to 7 involves the position of the external aperture of the spiracle relative to the rim of the branchial chamber. In condition 4 the spiracle is slit-like and located on or very close to the posterior rim of the chamber. The ontogenetic pattern leading up to this final position has been investigated in some detail by Callien and Houillon (1951) in *Discoglossus pictus* (also see Boulenger, 1897:98, for data on *Bombina variegata pachypus*). In contrast to this final ontogenetic position, the external aperture of the spiracle in all remaining

conditions 5 to 7, following an extended developmental sequence, are located some relatively great distance from the rim of the chamber. It seems most likely that the primitive condition is 4 (*sensu Ascaphus* and Discoglossidae), wherein the external aperture is not carried any relatively great distance, if indeed it is moved at all, from the rim of the chamber owing to the development of only a short spiracular tube. Our argument here is based on the similarity of the earlier phases of the ontogenetic pattern of all anurans and, that to realize the final position of conditions 5 to 7, the pattern always passes through a stage like the position of condition 4 (criteria 2 and 3).

The median spiracular opening in *Nototheca fissilis* is almost certainly derived from condition 6 (typical sinistral spiracle). Here again, as we discussed in greater detail above, our argument rests on criterion 2; our stand results from our accepting *Nototheca fissilis* as a hylid and recognizing that almost all other hylids have a sinistral spiracle and that a similar sinistral spiracle is present in most other families (e.g., Pelobatidae, Ranidae, Rhacophoridae, Bufonidae, Atelopodidae, and Leptodactylidae). We fully recognize that this implies an *a priori* decision as to the limits of the higher taxonomic categories. This problem is not encountered when the study is initiated at the individual or infraspecific populational level, and moreover, closely related groups can be recognized prior to analysis of the group under study through estimates of overall similarity that make no assumptions about primitive conditions. The developmental sequence of spiracle formation in *Nototheca fissilis* should be examined closely for evidence of similarity to condition 6.

Within the remaining conditions, 7 (nearly median spiracle, as exemplified by the hylid genus *Phyllomedusa* and 80 per cent of the *Hyla goeldii* examined, Griffiths and Carvalho, 1965) is almost certain to have been derived from condition 6. Our argument for this conclusion is based on criterion 2. That *Hyla goeldii* is polymorphic, namely it exhibits conditions 4, 7 and probably 6, clearly indicates the probable pattern of evolutionary reversal (*viz.*, from condition 6 to 7 to 4). It is interesting that *Hyla goeldii* and *Nototheca fissilis* are probably very closely related (Cochran, 1955:191).

We know of no biologically realistic *a priori* way to relate the remaining conditions 5 and 6, in terms of polarity. We cannot accept Inger's (1967:377) reasoning that the sinistral spiracle is derived from one in a median position because "In organisms that are essentially bilaterally symmetrical, a bilateral developmental pattern seems more primitive than an asymmetrical one." Because of this difficulty we have coded condition 5 (e.g., Microhylidae) as derived from both condition 4, independent of 6 and from 6, and similarly we have coded condition 6 as derived

from both condition 4, independent of 5, and from 6 (see Fig. 6). There can be little doubt that the "sinistral" spiracle of *Elachistocleis ovalis* is derived from the typical median position of the Microhylidae (criterion 2).

From the preceding discussion it seems quite clear that the "spiracle character" embodies relatively little information that relates to the interpretation of phylogeny. Like the other characters, we have considered the different modifications in a binary form (Table 3, V). We believe the following three codings contain most of the phyletically useful information: (a) true operculum = 0, pseudo-operculum = 1; (b) true spiracle not strongly sinistral = 0, true spiracle strongly sinistral = 1; and (c) true spiracle located on or near margin of branchial chamber = 0, true spiracle not located on or near margin of branchial chamber = 1.

Cornified beaks and denticles.—The second larval "character" that Inger (1967:375, Table 1) used, the presence or absence of cornified beaks and denticles, deserves reinterpretation and must be reevaluated as a meaningful estimate of phyletic affinity. To code both beaks and denticles as a single character is inconsistent with the known variability that each of these two sets of structures exhibits, namely they are to a large degree independently variable (see Table 4). One can only accept them as a single character at the family level if the intrataxonomic variability is not coded for (the procedure followed by Inger). Moreover, Orton (1953:69) recognized not only the presence or absence of rows of denticles as discrete states, but also referred to double versus single rows of denticles per ridge (apparently she overlooked the "triple" rows in some discoglossids and *Ascaphus*). The denticles and the beak should be treated as separate characters and the form of the rows of denticles (triple, double or single per ridge) included as states of that character (Table 4).

As seen in Table 4, both beak and denticle characters are highly variable. It is because of this degree of variability that we believe there is little, if any, diagnostic value derived from using either of them in the phyletic analysis of the classically delimited anuran families considered herein. We acknowledge that in most of these families a beak and a single row of denticles are present in the great majority of the species. In addition, it is clear that in some species where the beak and the rows of denticles, or the denticles alone, are absent their loss is positively correlated with the occurrence of some form of direct development; however, this correlation does not hold for all examples of beak-denticle loss. Even if all of the relatively few exceptions of beak and denticle loss in each family could somehow be rationally explained on the basis of cause and effect related to direct development, the char-

acters would still not be admissible for the following reasons. There are many kinds and degrees of direct development involved (Lutz, 1948; Jameson, 1957). Direct development is known in two of the three families, Pipidae and Microhylidae, which primitively are almost certain to have had a free swimming kind of tadpole without beaks and denticles. Also, there is the purely practical problem of assessing the considerable degree of ontogenetic change (Limbaugh and Volpe, 1957). For meaningful interspecific comparisons, the times of appearance and disappearance of beaks and denticles in the premetamorphic phase of ontogeny must be determined. These necessary data are given in the form of normal tables, of which not more than 20 species of anurans have been described in sufficient detail (Nieuwkoop and Faber, 1956). An additional problem will be to include the condition where the larval mouth parts are typical, except that they are not cornified (e.g., *Rhinoderma*).

Of all of the states of the beak and denticle characters delimited in Table 4, the presence or absence of double or triple rows of denticles might be considered taxonomically useful. However, even here we do not have confidence in the condition of the character, because of the extreme difficulty in distinguishing between species with double rows per ridge (sensu *Ascaphus*) and those that have two "single" rows very close together (e.g., ranids *Trichobatrachus robustus*, *Rana rugulosa* and *R. crassipes*, hylid *Hyla claresignata*, and the leptodactylid genus *Heleophryne*). Equally important, these taxonomic examples almost certainly indicate that tooth rows have secondarily evolved *de novo* in anurans, and in more than one phyletic line. Future studies that consider denticle characteristics must attempt to homologize the individual rows and treat them as separate characters.

Griffiths' (1963:254) statement that small denticles are present in the pipid *Pseudhymenochirus* (presumably *P. merlini*) must be reinvestigated, because Lamotte (1963:946) described them as absent in his study of *P. merlini*. If Griffiths' observation can be confirmed, then another major parameter of denticle variation must be taken into consideration.

Vent (larvae).—The position of the external aperture of the vent, either median or dextral, has been noted in most of the descriptions of anuran larvae from the mid-Eighteenth Century to the present day. Noble (1927:74) pointed out that, although the position of the anal opening had been considered as both a "good" generic and familial character for many years, its variability was too great for it to be of any real significance. Apparently for similar reasons, Orton (1953, 1957) altogether omitted the position of the vent in her survey of taxonomic characters of anuran larvae.

Inger (1967:375, Table 1; also see 1958:382) did not discuss the conclusions of Noble, Orton and others, and he used the position of the anus in his analysis of anuran phylogeny. He stated (p. 379) that "The position of the vent is remarkably constant within families" and reasoned that "As the anus is median in adult frogs and most vertebrates, including other amphibians, I am treating the dextral vent of larvae as the derived state." We do not agree that the character is "remarkably constant within families."

To assess the variability of the position of the vent in aquatic larvae, we cursorily surveyed the relevant literature at hand. Our sources were Alcalá (1962), Griffiths and Carvalho (1965), van Kampen (1923), Lee (1967), Liu (1950), Noble (1927), Pyburn (1967), Starrett (1960, 1967), numerous papers by Lamotte and his colleagues, 1954 to 1965, and Inger (1954, 1966). The exceptions to Inger's familial definitions of the state of the vent are listed below in parentheses. Pelobatidae—median (*Aelurophryne brevipes*, *A. glandulata*, *Leptobrachium*, *Megophrys oshanensis*, *Scutigera pingii*, *S. popei*, *S. schmidti*); Atelopodidae—median (*Melanophryniscus moireirae*); Leptodactylidae—median (*Ceratophrys*, *Engystomops pustulosus*, *Heleioporus*, *Odontophrynus americanus*); Hylidae—dextral (*Centrolenella fleischmanni*, *C. granulosa*, *C. prosoblepon*, *C. reticulata*, *C. spinosa*, *Hyla annectans*, *H. montana*, *Phrynohyas venulosa*); Ranidae—dextral (*Oxyglossus laevis*, *O. lima*, *Ptychadena maccarthiensis*, *P. mascarensis*, *P. submascarensis*, *P. tournieri*, *Rana jerboa*, *Staurois chunganensis*, *S. richetti*); Microhylidae—median (*Chaperina fusca*, *Elachistocleis ovalis*, *Kalophrynus* sp.). Many are both intrafamilial and intrageneric exceptions, and the degree of variability within both taxonomic categories ranges from single exceptions to nearly 40 percent of those species surveyed. It should be pointed out that a polymorphic condition might be inferred if age is not taken into account in those larvae that have dextral vents. A truly polymorphic situation has been reported by Lee (1967, pers. comm.) in the leptodactylid genus *Heleioporus*. Both the median and dextral vents have been found in the same egg mass in each of the following species: *H. albopunctatus*, *H. eyrei*, *H. inornatus*, and *H. barycragus*. From these findings, we cannot accept the position of the vent as a meaningful character in delimiting anuran relationships. Our cursory survey of the literature strongly suggests that both states of that character have evolved numerous times in parallel.

Scapula and clavicle.—Inger (1967) gave some consideration to the use of the scapula to clavicle ratio as a character in his analysis of anuran phylogeny. He noted that a short ratio (greater than three, *vide* Griffiths, 1963) "may be" primitive, and as evidence for this possibility he used the argument of (p. 374) "the occurrence of short

scapulae in the families of frogs generally held to be primitive." He (p. 380) used the character in the phylogeny generated by his computer program, coding the character as ≥ 3 or < 3 with the primitive state not specified. The same character was also tabulated in his other phylogenies (Figs. 4 and 5, Table 2).

Griffiths (1963:265) almost certainly served as Inger's primary source of data on the scapula to clavicle ratio; he in turn explicitly (at least in part) obtained some information from Proctor (1921). Griffiths' contention (p. 265) that the Ascaphidae, Discoglossidae and Pipidae have a scapula to clavicle [=precoracoid of Proctor] ratio greater than three is not supported by Proctor's raw data. We recorded the following ratios from skeletal material in our possession, which add further evidence that the "primitive" families have a ratio greater than three, while the more advanced families (including the Rhinophrynidae) have a ratio of less than two.

Ascaphidae: *Ascaphus truei* (n = 4; orv = 1.4–1.9; \bar{x} = 1.58¹; also see Ritland, 1955a:146, Fig. 6). Also see Stephenson (1960:481, Fig. 4) for contradictory data on *Leiopelma*. Discoglossidae: *Alytes obstetricans* (n = 3; orv = 2.1–2.3; \bar{x} = 2.17); *Barbourula busuangensis* (n = 1; 2.8); *Bombina bombina* (n = 4; orv = 2.9–3.3; \bar{x} = 3.1); *B. orientalis* (n = 2; orv = 1.9–2.2; \bar{x} = 2.05); *B. variegatus* (n = 1; 3.1); *Discoglossus pictus* (n = 5; orv = 2.2–2.7; \bar{x} = 2.36). Pipidae: *Hymenochirus boettgeri* (n = 1; 1.4); *Pipa pipa* (n = 2; orv = 2.9–3.3; \bar{x} = 3.1); *Xenopus gilli* (n = 1; 2.8); *X. laevis* (n = 3; orv = 2.8–3.6; \bar{x} = 3.07); *X. mulleri* (n = 5; orv = 2.6–3.2; \bar{x} = 2.88).

We believe that there is a very general trend in the scapulae of frogs towards increased size and complexity (see Tihen, 1965:311), but the trend is not a simple dichotomy as Inger and Griffiths accepted; the ratio exhibits considerable variation within genera, and between families of primitive frogs as described above. Equally important, before the character can be considered again, the degree of ontogenetic change (which we believe to be significant) must be described, and a more precise method of measuring the elements must be defined and followed by all investigators. The acute angle of the clavicle of some species of anurans makes that measurement extremely difficult to take and certainly contributes to the greater variance in those species.

Griffiths (1963:265) first discussed the scapula to clavicle ratio in the context of two other characters: (1) proximal end of scapula, uncleft or cleft, and (2) scapula overlain anteriorly by the clavicle or not. We have attempted to substantiate Griffiths' contention that the Ascaphidae and the Pipidae have a cleft scapula. The degree

¹ n = number of specimens examined; orv = observed range of variation; \bar{x} = mean.

of variation that we found intrataxonometrically at all levels in the Pipidae, and the conspicuous effects of increased ossification of the glenoid cartilage with age, suggests that a much more detailed study of the character is in order before it can be considered further. The second scapula character of Griffiths shows considerably greater promise in elucidating anuran phylogeny. We have examined representatives of all genera of the Ascaphidae (except *Leiopelma*), Discoglossidae, Pipidae, and Rhinophrynidae and find that the distal end of the clavicle overlaps much of the scapula. All of the material of the other families that we have examined shows little or no anterior overlap of the clavicle (the plane of contact is nearly vertical). Of the forms examined in the latter group of families, *Pelodytes punctatus* most closely approaches the overlapped state of the former group of families.

We have used the scapula to clavicle overlap character instead of the scapula to clavicle ratio character of Griffiths. We cannot accept the scapula to clavicle ratio as a very meaningful taxonomic character, because of its extreme degree of variability as described above. We believe the scapula to clavicle overlap character is consider-

ably less variable than Griffiths' other scapula associated characters within the classically accepted families used herein, and the simple dichotomy of overlap or no overlap is certainly much easier to record. We have coded the overlapped state as primitive (0 in Table 3) on the basis of similar conditions occurring in almost all other amphibians where the clavicle is present and where there is no suggestion of loss of limbs (*vide* fossil evidence and criteria 1 and 2). Before we can consider the dichotomy of clavicle to scapula overlap with greater confidence, all species of *Leiopelma* must be studied and preadult stages of *Xenopus* species must be followed through metamorphosis. In the case of adult *Xenopus*, the scapula and clavicle are fused together and, thereby, make the overlap of the two elements difficult to interpret.

Museum of Zoology and Department of Zoology, University of Michigan, Ann Arbor, Michigan 48104. Present address of junior author is Department of Biological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11790.