The evolution of long bone microstructure and lifestyle in lissamphibians

Michel Laurin, Marc Girondot, and Marie-Madeleine Loth

Abstract.—The compactness profile of femoral cross-sections and body size of 105 specimens of 46 species of lissamphibians was studied to assess the effect of lifestyle (aquatic, amphibious, or terrestrial). Several tests that incorporate phylogenetic information (permutational multiple linear regression incorporating phylogenetic distances, logistic regression using phylogenetic weighting, concentrated-changes tests) show that the return to a fully aquatic lifestyle is associated with an increase in the compactness of the femur and an increase in body size. However, amphibious taxa cannot be distinguished from terrestrial ones solely on the basis of size or compactness. Body size and compactness profile parameters of the femur exhibit a phylogenetic signal (i.e., closely related taxa tend to be more similar to each other than to distantly related taxa).

  Mathematical equations obtained from our data by using logistic regression with phylogenetic weighting are used to infer the lifestyle of four early stegocephalians. The results are generally congruent with prevailing paleontological interpretations, which suggests that this method could be applied to infer the lifestyle of early taxa whose lifestyle is poorly understood.

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Introduction

It is notoriously difficult to infer the lifestyle (aquatic to terrestrial) of early stegocephalians. This is, in part, because their body shape is fairly conservative; unlike more recent amniotes, such as plesiosaurs or cetaceans, few Paleozoic stegocephalians had a body shape suitable only for an aquatic lifestyle (Carroll 1988). Another factor contributing to our difficulty in inferring stegocephalian lifestyles is that the closest extant taxa of many early stegocephalians are very large clades that include fully aquatic to fully terrestrial taxa. For instance, depending on which phylogeny is used, the extant clade most closely related to Embolomeri (or Anthracosauria) is either Tetrapoda (Laurin 1998) or Amniota (Ruta et al. 2003). The parsimony criterion cannot be used to infer the lifestyle of such taxa because the ancestral lifestyle of Tetrapoda is uncertain.

The difficulty in inferring the lifestyle of early stegocephalians may explain the diversity of opinions that have been expressed about the conquest of land by vertebrates during the twentieth century. Some paleontologists inferred that the appearance of the limb coincided with a shift from an aquatic to a terrestrial (or at least amphibious) lifestyle (Carroll 1988: p. 158). Others, such as Romer (1957, 1958), argued that the earliest stegocephalians (sensu Laurin 1998; this includes all limbed vertebrates, whereas Tetrapoda is restricted to the crown group of terrestrial vertebrates) were still largely aquatic, and that a truly amphibious and a terrestrial lifestyle appeared ''many millions of years'' later, in the late Carboniferous. Romer (1958) also concluded that in the Carboniferous most stegocephalians were still primarily aquatic. Romer's ideas received support from the discovery of grooved ceratobranchials that may have housed afferent branchial arteries in *Acanthostega* (a Devonian stegocephalian) and a postbranchial lamina that may indicate the presence of an internal gill chamber in a few Devonian and Early Carboniferous taxa (Coates and Clack 1991). Furthermore, as previously noted (Lehman 1955), the presence of grooves for the lateral-line organ on the skulls of many Carboniferous and Permian stegocephalians suggests

primarily aquatic lifestyles for these taxa because this organ is found only in primitively aquatic extant vertebrates and in the most aquatic lissamphibians, such as the salamander *Siren* and pipid frogs (Duellman and Trueb 1986). Thus, it seems that stegocephalians were primitively aquatic, but the primitive lifestyle of tetrapods remains uncertain.

However, even if the presence of grooves for the lateral-line organ is good evidence that an extinct taxon was aquatic, the absence of these grooves cannot be taken as evidence that an animal was terrestrial. Indeed, in extant lissamphibians, this organ never leaves a trace on the skull (Duellman and Trueb 1986, personal observation). Even the presence of grooves for the lateral-line organ is not absolute proof that an organism was aquatic because the grooves may simply indicate the presence of a functional organ in juveniles, whether or not the organ was functional in adults. The degree of ossification of the endoskeleton has long been taken as evidence of either an aquatic or a terrestrial lifestyle (Williston 1911: p. 398) because the epiphyses of long limb bones and the carpus and tarsus are largely cartilaginous in aquatic taxa, but clade-specific variation in this character, and uncertainties about the ontogenetic age of fossilized individuals, may diminish its utility. This difficulty may, in part, explain why so few attempts have been made to reconstruct the history of the conquest of land by vertebrates. Some of the most explicit and detailed scenarios for the conquest of land by vertebrates are those of Romer (1957, 1958), who argued that the earliest amniotes were primitively amphibious. Romer's (1957, 1958) scenario was based on his hypothesis that the Permo-Carboniferous diadectomorph *Limnoscelis* and the contemporaneous synapsid *Ophiacodon* were aquatic or amphibious (but see Sumida 1997: p. 392 for a divergent opinion). Because Romer (1957, 1958) thought that these taxa were ''early and primitive,'' he thought that they represented the ancestral condition for amniotes, and that their ancestors never had been terrestrial. More recently, Skulan (2000) argued that the amniotic egg was not required for reproduction on dry land and that lissamphibians cannot be assumed to retain a more primitive method of reproduction. Thus, his study raises a possibility opposed to Romer's (1957, 1958) suggestion, because it is compatible with the hypothesis that the last common ancestor of lissamphibians and amniotes (the first tetrapods) already lived and reproduced on dry land.

Bone microstructure and histology have been used to study the lifestyles of several extinct vertebrates, such as early stegocephalians (Ricqlès 1981), early sauropsids (Ricqlès 1974a), early synapsids (Ricqlès 1974b), champsosaurs (Buffrénil et al. 1990), ichthyosaurs (Buffrénil et al. 1987; Buffrénil and Mazin 1990, 1993), placodonts (Buffrénil and Mazin 1992), plesiosaurs (Wiffen et al. 1995), crocodilians (Hua and Buffrénil 1996), and early cetaceans (Buffrénil et al. 1990; Madar 1998). Throughout this paper, the term ''early'' is a strictly temporal (not topological) concept that denotes relative geological age. These studies included detailed qualitative analyses of bone microstructure and histology of extinct taxa, and some comparisons with extant taxa, but they did not include detailed, quantitative analyses of extant taxa.

A few other studies used quantitative methods to investigate the relationship between bone microstructure and lifestyle in extant vertebrates. Fish and Stein (1991) sampled six species of mustelids and concluded that bone is denser in aquatic than in terrestrial species. Leclair et al. (1993) concluded, on the basis of a survey of seven anuran species, that the proportion of the body mass represented by the skeleton of anurans is lower in aquatic than in terrestrial taxa. Castanet and Caetano (1995) reached similar conclusions based on a study of four species of *Rana*.

Few studies have combined a quantitative study of skeletal microstructure of extant and of extinct taxa to assess the lifestyle of early vertebrates. Buffrénil and Rage (1993) compared global compactness of vertebrae of nine species of extant snakes of various lifestyles with that of the Cenomanian (Upper Cretaceous) squamate *Simoliophis*, and concluded that the latter was aquatic. This is the closest approximation to the method that we use in this study. Wall (1983) showed that several extant aquatic placental mammals have higher

bone density than terrestrial mammals. In his study, Wall recorded the dry weight of bones and estimated their wet volume by water displacement; the bones were soaked in water before their volume was measured, presumably to avoid artificially low values caused by water seepage into the marrow, spongy tissues and vascular spaces of compact bone. Using long bone sections, Wall also showed that the increase in compactness among several aquatic mammals results from increased amounts of compact bone. He concluded that similar sections obtained from fossilized mammalian bones could be used to infer the lifestyle of extinct mammals. Neither Wall (1983) nor Buffrénil and Rage (1993) addressed the problem of the conquest of land by vertebrates, but they provided examples of how this problem could be investigated by using quantitative, comparative methods.

Bone compactness and microstructure has the potential to yield much paleoecological information because the relationship between these aspects of the skeleton and the lifestyle has been studied extensively (e.g., Ricqlès 1977; Fish and Stein 1991; Leclair et al. 1993). There is widespread consensus that an organism's lifestyle is often reflected in the skeleton at various levels ranging from gross morphology to histological structure. Some of the most frequent gross morphological adaptations to an aquatic lifestyle include the modifications of limbs into paddles and the elongation and lateral flattening of the tail (Carroll 1985). At the microanatomical level, the presence of spongy tissue in the center of the medullary region of long limb bones, as in cetaceans, or of massive pachyostosis, as in mesosaurs (Ricqlès 1974a), placodonts (Buffrénil and Mazin 1992), and sirenians (Fawcett 1942; Buffrénil and Schoevaert 1989), is thought to indicate an aquatic lifestyle (Ricqlès 1977). At the histological level, the presence of much calcified cartilage often is associated with an aquatic lifestyle, although it may also reflect neoteny; indeed, much calcified cartilage often remains in the endoskeleton of some amphibians that are both aquatic and neotenic (Ricqlès 1975a, 1989).

Thus, the skeleton can be studied at several levels of integration to assess the lifestyle of early stegocephalians. We have chosen to work primarily at the microanatomical level for two reasons: First, because the body shape of most early stegocephalians is fairly generalized, it is compatible with aquatic, amphibious, and terrestrial lifestyles. Using gross morphological features to assess the lifestyle of such taxa is more difficult than for mammals. Second, the interpretation of histological characters is equivocal. For example, the presence of calcified cartilage in an early taxon might indicate that the organism was aquatic, neotenic, or both.

Before bone microanatomical data can be used to infer the aquatic or terrestrial lifestyle of early stegocephalians, we must understand how bones change in response to shifts between aquatic and terrestrial lifestyles (and vice versa). This is best done by studying extant tetrapods because we know their lifestyle; in the case of extinct vertebrates, we can only infer it with a variable degree of uncertainty. The main purpose of this study is to report our findings on how the compactness of the femur of lissamphibians evolves in response to returns to an aquatic lifestyle, as well as to show how such data can be used to infer the lifestyle of early stegocephalians.

Lissamphibians are especially relevant to studies on the relationship between bone microstructure and habitat (aquatic to terrestrial) because of their great diversity of lifestyles (Duellman and Trueb 1986). From a presumably primitively amphibious lifestyle (suggested by the optimization of lifestyle on the phylogeny), several taxa have independently become terrestrial or aquatic (Appendix 2, Fig. 1). Furthermore, their morphology, physiology, and development are often thought to resemble those of early stegocephalians (Romer 1966: p. 78; Duellman and Trueb 1986: p. 435). However, few systematic studies of skeletal modifications that reflect lifestyle have been performed (Leclair et al. 1993; Castanet and Caetano 1995). Thus, the paucity of currently available information on the skeletal adaptations of lissamphibians to an aquatic or a terrestrial lifestyle prompted us to do this research.

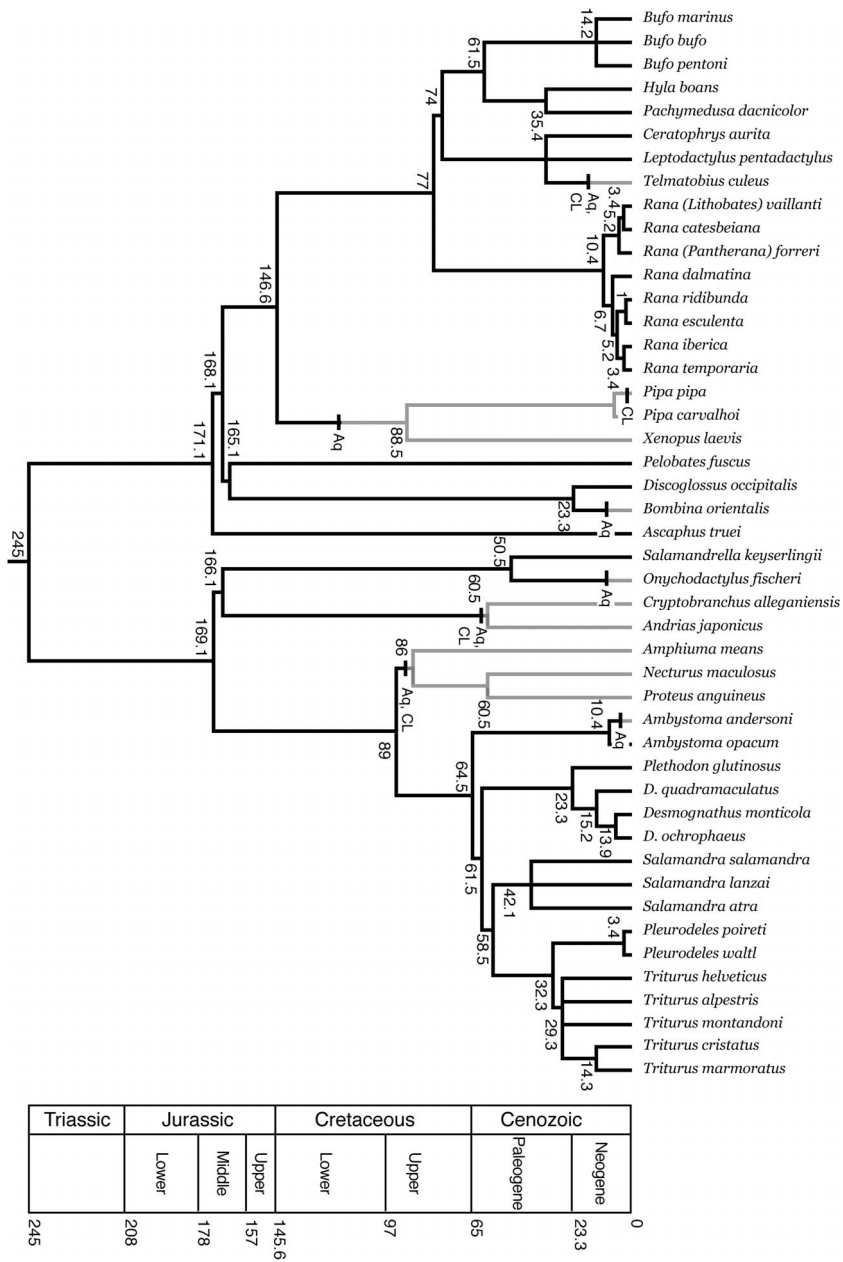We selected the femur for this study because several earlier works have investigated its

FIGURE 1.   Phylogeny used in this study (see Appendix 1 for the list of studies from which this was compiled). The large numbers appearing next to the nodes (at the base of the branches) represent the geological age of each node. The evolution of lifestyle and of an index representing the product of axial length and global compactness are traced onto the tree to show their coevolution. Branches leading to aquatic taxa are in grey. The geological time is expressed in Myr BP. Legend: Aq, aquatic lifestyle (the primitive condition is a terrestrial or an amphibious lifestyle); CL, increase in the product of femoral compactness and the logarithm (ln) of axial length (cm) to a value greater than 1.8. To display all the information, the divergence between both species of *Pipa* has been drawn lower than it should actually be.

compactness (e.g., Buffrénil et al. 1987, 1990; Buffrénil and Mazin 1993; Hua and Buffrénil 1996), allowing comparison between our results and those of others. Furthermore, we possessed thin-sections of this bone for more taxa (extant and extinct) than of any other long bone. Finally, Buffrénil and Schoevaert (1989) noted that in *Dugong dugon* there is a decreasing proximo-distal gradient of compactness in the pectoral limb; *D. dugon* has lost the pelvic limb. They suggested that for mechanical and energetic reasons the extra bone, which acts as a ballast, should be located close to the center of gravity of the animal. Thus, the proximal limb elements (femur and humerus) might undergo more noticeable changes in compactness as the lifestyle of taxa changes (from terrestrial to aquatic or vice versa) than distal limb bones.

In this study, we develop mathematical models that can be used to infer the lifestyle of stegocephalians, and we apply one of them to four early stegocephalians to assess the plausibility of our inferences. We present these models and paleobiological interpretations as examples of what can be accomplished by using bone microanatomical data and the mathematical methods that we develop, but our study does not attempt to assess when vertebrates became terrestrial. This important question will be studied after similar comparative, evolutionary analyses of bone microanatomy in amniotes have been performed, and after inferences on the lifestyle of numerous other early stegocephalians have been obtained. These lifestyle inferences will then be entered on a time-calibrated phylogeny of stegocephalians and the lifestyle will be optimized by using parsimony or likelihood to reconstruct the history and timing of the conquest of land and early returns to the aquatic environment.

Materials and Methods

Building the Reference Phylogeny

For most of our analyses, as well as for a coherent evolutionary interpretation of the microstructural data, a phylogeny that incorporates branch length (here, based on divergence time) had to be produced (Fig. 1). The phylogeny was compiled from various sources (Appendix 1), and the most recent likely divergence times were determined from the literature, usually based on the fossil record. In some cases, these divergence dates are approximate because the affinities of many extinct species are uncertain and the fossil record is incomplete. A good example is provided by the divergence date between *Pipa* and *Xenopus*, within Pipidae. Pipids are abundantly represented in the fossil record, but the oldest pipoids, from the Lower Cretaceous, may be stem-pipids that predate the divergence among the extant genera (Trueb 1999; Báez et al. 2000). The Coniacian or Santonian (Upper Cretaceous) pipid *Pachybatrachus* is thought to be more closely related to *Pipa* than to *Xenopus* (Báez and Rage 1998), and the Upper Cretaceous pipid *Saltenia* is thought to be more closely related to *Xenopus* than to *Pipa* (Báez and Pugener 2003). Furthermore, the fact that *Pipa* is found in South America, and *Xenopus* in Africa, suggests that the divergence between these two genera is no later than the completion of the separation between Africa and South America. Therefore, we have dated the divergence between *Pipa* and *Xenopus* from the Coniacian (Upper Cretaceous), although it could have occurred much earlier.

In cases of conflict among various phylogenetic studies, those based on the densest taxonomic sampling were preferred. Also, studies that incorporated both morphological and molecular data were preferred over those that incorporated only molecular data. For instance, the phylogeny of Caudata by Larson and Dimmick (1993) conflicts with parts of the phylogeny of lissamphibians proposed by Hay et al. (1995). We followed Larson and Dimmick's (1993) paper because it included morphological and molecular data, and 20 OTUs (operational taxonomic units) were sampled, whereas Hay et al. (1995) included only molecular data, and only nine OTUs were included. Because some of our statistical tests required fully resolved phylogenies, we favored studies with the best topological resolution. For instance, Hay et al. (1995) provided a nearly fully resolved phylogeny that had a great influence on our chosen topology, whereas the phylogeny of Ford and Cannatel-

la (1993) is less informative because it includes large polytomies. Our phylogeny incorporates data from all these studies, and the conflicts were resolved by using the criteria mentioned above (Appendix 1).

Bone Compactness Profile Data

This study is based on detailed anatomical drawings (incorporating information about vascularization, bone trabeculae, and resorption spaces in the cortical compacta) of femoral mid-diaphyseal cross-sections of 105 specimens representing 46 species and 26 genera of extant lissamphibians (Fig. 1). Most large clades of Anura and Caudata are represented in our sample, as well as some of the most highly aquatic (e.g., cryptobranchids, *Amphiuma*, pipids) and most terrestrial (e.g., *Bufo*, *Plethodon*, *Hyla*) lissamphibians. Our sample also includes closely related species of neotenic and transforming Caudata (*Ambystoma andersoni* and *A. opacum*). Most size classes are represented in the sample, from taxa of minute body size (Plethodondidae, Hynobiidae) to some of the largest extant lissamphibians (*Andrias japonicus*, *Amphiuma means*, *Bufo marinus*). In addition, bone sections of four Paleozoic stegocephalians (*Doleserpeton*, *Chroniosaurus*, *Cardiocephalus*, and *Phaanthosaurus*) from four major clades (*Temnospondyli*, Embolomeri, Amphibia, and Amniota) are used to infer the lifestyle of these early taxa (Amphibia is here defined as the largest clade that includes *Ascaphus truei* but not *Homo sapiens*). All these taxa are relevant to the history of the conquest of land by vertebrates because they are outside the large clades of extant tetrapods (Apoda, Caudata, Anura, Mammalia, Testudines, Sauria). All bones represent adults, as far as we know; for *Doleserpeton*, the ontogenetic stage is not certain, although it is certainly a postmetamorphic individual.

It is inappropriate to study bone density in early stegocephalians because the volume of bone tissue is difficult to estimate in extinct taxa, as Wall (1983: p. 206) recognized. Typically, permineralization has filled most or all of the cavities of the bone including, in many cases, the marrow cavity. Thus, methods that evaluate global compactness of a bone are difficult to apply to fossil material. We have circumvented this problem by studying compactness of cross-sections made at the mid-diaphyseal level, a method more similar to that of Buffrénil and Rage (1993). By mid-diaphyseal, we mean the region of the diaphysis that is the narrowest, and where the cortical compacta is the thickest; this is not necessarily the midpoint between proximal and distal ends of the bone. Radiographs were taken to localize this region, when the mid-diaphysis was poorly defined. Simple image analysis techniques can yield accurate data on the compactness of a section. A further advantage of this method is that information about the distribution of the bone tissue can also be gathered by applying a mathematical model that we developed, and by using computer software that greatly simplifies data collection and analysis (Girondot and Laurin 2003). Our model shows the distribution of the bony tissue in the element along the radius of the bone, as seen in cross-section. To our knowledge, only Buffrénil and Schoevaert (1988: Fig. 6) have produced graphs showing the distribution of the bone tissue, but these graphs were not fit to a mathematical model, which would have hampered statistical comparisons. Our method could (at least in theory) reveal differences in the distribution of the bony tissue between aquatic and terrestrial taxa, even if the global compactness is equal in both groups. We believe that this advantage is important because the long limb bones of some aquatic taxa, such as extant whales, are not especially compact, as Wall (1983) recognized. However, the distribution of bone tissue (as seen in cross-section) in cetaceans differs from that in terrestrial taxa; in cetaceans, there is porous bone in the cortical and medullary areas, whereas most terrestrial vertebrates have a compact cortex and a large medullary cavity.

Our model is described in detail elsewhere (Girondot and Laurin 2003), but a brief summary may be useful. Bone compactness C as a function of the distance to the center d can be described by a sigmoidal function:

$$C(d) = \frac{1}{1 + e^{(1/S)(P-d)}}(\text{Max} - \text{Min}) + \text{Min},$$

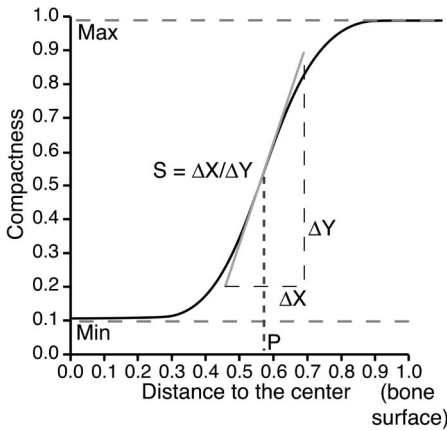where P is the relative distance from the cen-

FIGURE 2. Mathematical model that shows the distribution of mineralized tissues as a function of distance to the bone center. In the chosen example, the positive value of *Min* indicates that there is a medullary spongiosa (this is not the case in most lissamphibian femoral mid-diaphyseal sections). See text for an explanation of *S*, *P*, *Min* and *Max*.

ter to the point of inflection, where the most abrupt change in compactness is observed (Fig. 2). This relative distance is the ratio between the distance to the center and the radius. This generally corresponds to the position of the transition zone between the cortical compacta and the medullary cavity or spongiosa. *P* generally reflects (is directly proportional to) the size of the medullary cavity (or spongiosa). *P* has a simple relationship with the corticodiaphyseal index, or CDI (Castanet et al. 2000); it corresponds to $1 - CDI$.

S is the reciprocal of the slope of the compactness change at point *P*. It usually reflects the width of the transition zone between the cortical compacta and the medullary cavity (or the medullary spongiosa), because a low slope indicates a broad region where the compactness is higher than 0 but lower than 1. More precisely, the width of the region that encompasses a compactness from min +5% to max −5% is simply $2 \cdot S \cdot \ln(0.95/0.05)$. *Max* is the upper asymptote. It generally reflects the compactness in the outermost cortex. *Min* is the lower asymptote. It generally reflects the compactness in the center of the medulla.

In the original implementation, our software (Bone Profiler) calculated bone compactness in concentric zones equal to 2% of the bone radius, except the most superficial part

of the bone, where two zones of 1% of the radius were sampled. This was done to better model the bones with a very thin cortical compacta. Thus, 51 compactness values were incorporated into a single global compactness profile of the bone. This method has the drawback of generating high S values when the outline of the medullary cavity is irregular or does not match closely the outline of the bone, as well as when a transition zone of spongy bone is present between both zones. To discriminate between these two situations (which correspond to distinct phenotypes), we modified the Bone Profiler software to take measurements of 51 concentric zones (z) in 60 sectors (s) measuring 6° each (Fig. 3). On each 6° sector, the 51 compactness values are used to generate a compactness profile. The average value and standard deviation of the 60 resulting compactness profiles are calculated, and the outliers (defined as the profiles for which at least one of the four parameters was more than four standard deviations away from the mean value) are eliminated. This screening procedure is repeated as many times as necessary to eliminate all outliers. Bone Profiler then reports the final statistics (global compactness profile parameter values, plus the average and standard deviation of the parameters calculated from the 60 6° sectors). This new method should extract more information from a bone section because the mean value and standard deviations of the compactness profile parameters can be studied, and these can reflect heterogeneity in the section. The advantages of this new method of analysis will be greater when studying bones of larger organisms (e.g., amniotes, temnospondyls), which are more complex. However, even among lissamphibians, some femora show a complexity that could benefit from the new model (Fig. 4). In this study, the reported compactness values used for all calculations include all mineralized tissues (calcified cartilage and bone).

Body Size

We incorporated body size into the analysis because an initial examination of many sections revealed that the bones of small animals usually have a much simpler structure than
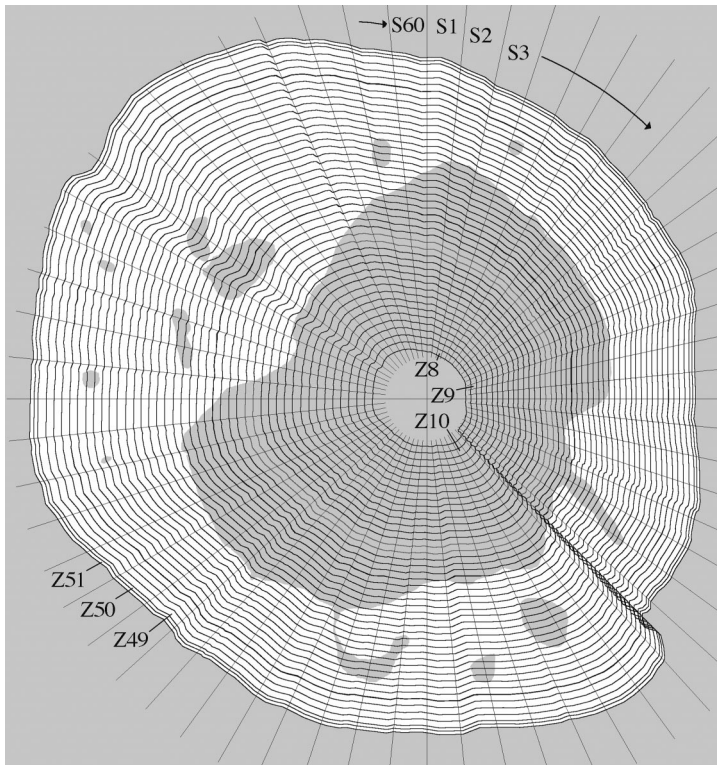
FIGURE 3.   Sampling method for establishing the compactness profile in Bone Profiler. The bone section is divided into 60 sectors (S 1–60; only a few are identified), each of which measures 6° in width. Each sector is further divided into 51 zones (zones 1–7, near the center, were not drawn for want of space). The compactness values of the 51 zones of each sector are used to generate compactness profiles (60 profiles, for complete sections, but fewer, for fragmentary sections). The section is from the femur of the aquatic, neotenic *Ambystoma andersoni*.

bones of mid-sized or large animals. For instance, the bones of most organisms with small body size lack a spongiosa (Ricqlès 1976: p. 15). Therefore, we could expect that the difference between aquatic and terrestrial taxa might be reflected by the relative cortical thickness for small animals. However, for larger organisms, this same ecological difference could result in changes in the development of the spongiosa, in addition to (or instead of) potential changes in the relative cortical thickness. Furthermore, body size itself might yield paleoecological information, because body size has already been suggested to differ between aquatic taxa and their terrestrial relatives (Titus and Larson 1996; Bininda-Emonds et al. 2001). In all cases (Caudata, various extinct stegocephalians), axial length refers to the length from the atlas to the sacrum, except for anurans, in which it was measured

to the posterior end of the urostyle (Appendix 1).

The way in which body size can be incorporated into an analysis depends on the analytical method used, and this is explained separately for each test (see below).
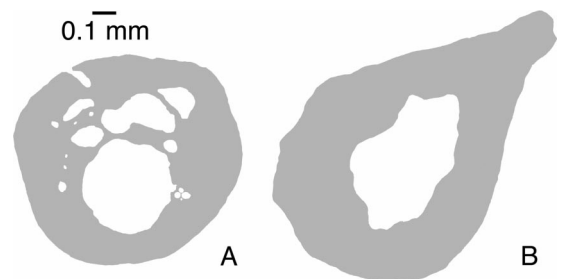

FIGURE 4.   Cross-sections of femora with a broad (A) and a narrow (B) transition zone between the cortical compacta and the medullary cavity. A, *Bombina orientalis*. B, *Pleurodeles waltl*.

Detecting a Phylogenetic Signal in Compactness Profile Data

To detect a phylogenetic signal in microstructural and size characters, we performed both phylogenetic permutational linear regression analysis (a modified Mantel test) using Permute (Legendre et al. 1994) and measures of number of character steps (squared length) on random trees using a random tree generation algorithm from the TreeFarm package (Maddison et al. 2002) of Mesquite (Maddison and Maddison 2002).

For the regression analysis, the phylogenetic distance between two taxa (independent variable; here, in Myr) is regressed against the phenotypic distance (the difference between two taxa for a certain character). This test has the advantage of quantifying the proportion of the variance of the dependent character explained by the independent character, but it assumes a linear relationship between both variables. Of course, transformations can be performed to linearize the relationship. The significance of the regression coefficients is then tested with a permutation procedure that amounts to a random allocation of OTUs on the branches of the phylogeny.

The random tree generation algorithm used (for the measure of numbers of steps on random trees) was implemented in Mesquite for this analysis. It reshuffles the terminal taxa on the tree while keeping the topology and branch length fixed. This algorithm is more appropriate for our purpose because the other tree generation algorithms implemented in Mesquite would have yielded trees of a different height than the original tree. This would have biased the results because the squared length of a character on a tree depends on the tree height (Maddison 1991). The number of steps (squared length, for continuous characters) was scored for each random tree and compared with the number of steps on the reference tree. If fewer than 5% of the randomly generated trees yield numbers of steps (squared length) equal, or inferior, to the reference tree, we conclude that there is a phylogenetic signal in the character.

Analysis of the Ecological Signal in Compactness Profile Data

We studied the correlation between bone compactness profile data, lifestyle, size, and phylogenetic position by using methods for continuous and discrete characters.

Tests for Continuous Characters.—We performed a phylogenetic permutational multiple regression analysis (a modified Mantel test) using Permute (Legendre et al. 1994). This method, contrary to many other available statistical tests, can incorporate phylogenetic data (topology and branch length) into this analysis. This is advisable because standard statistical methods assume that species are statistically independent entities. However, this is known to be false because closely related species can be expected to be more similar to each other than more distantly related species. To build the files representing the phylogeny and branch lengths for CAIC, we used TreeEdit 1.0a10 (Rambaut and Charleston 2001). The permutational regression analyses using Permute are performed on distance matrices (Legendre et al. 1994). Thus, the sign of the regression coefficients is always positive; other methods must be used to determine whether the relationships between the original variables are positive or negative. We do not present an independent contrasts analysis using CAIC (Purvis and Rambaut 1995) because this method is less powerful when one of the characters is discrete and because it provided no additional information.

Concentrated-Changes Test for Discrete Data.— We also used the concentrated-changes test (Maddison 1990); this test for discrete, binary characters is intrinsically phylogenetic. For this purpose, the quantitative data must be recoded into binary characters. This is a disadvantage because this method discards part of the information, and the limit between the states must be fixed more or less arbitrarily. However, given that we have only qualitative data on the lifestyle of extant taxa, and that few statistical methods incorporating phylogenetic data adequately deal with correlations between discrete and continuous characters, we conclude that a concentrated-changes test complements the other analyses that we per-

formed. To determine the boundary between the two states, a histogram of distribution may be useful, but we tested several thresholds to determine the impact of various coding schemes.

Logistic Regression and Inference of Lifestyle in Extinct Stegocephalians

We also performed logistic regressions, in which a phylogenetic weight was incorporated. This analysis was aimed at providing a mathematical equation that enables inference of the lifestyle of an extinct taxon from size and compactness profile data. In this analysis, we optimized up to ten constants to maximize the fit between observed and inferred lifestyle of the extant taxa. Let bc_i be a parameter describing bone characteristics (maximal section diameter, compactness profile parameters S, P, Min, and Max for $i = 1$ to 5, respectively). The logarithm of axial length is used as a covariable for each of these bone characteristics. Then the logistic regression model is

$$ h = \frac{1}{1 + \exp\left[C_0 + \sum_{i=1}^{5} \left(C_{i1} bc_i^{C_{i2}\ln \text{presacral length}}\right)\right]}. $$

The 11 constants C_{ij} were optimized by using the weighted least-square sum (SSQ) over all individuals between observed habitat for individual k (H_k) and inferred habitat (h_k) based on these bone characteristics:

$$ SSQ = \sum_{i=1}^{\text{Nb individuals}} [W_k(h_k - H_k)^2]. $$

W_k is the phylogenetic weight and is the reciprocal of the product of the number of lineages issued from each hypothetical ancestor of a given terminal taxon, from the root to each terminal taxon. In a fully resolved tree, this number equals two. Thus, when several individuals in a clade are known, and the clade's sister group is represented by a single individual, the weight of both clades is equal. More generally, two sister clades always have equal weights. However, this weighting scheme does not incorporate branch length.

The general model used up to ten estimated parameters, but it can be reduced to gain degrees of freedom by setting $C_{x1} = 0$ with $x =$ 1 to 5. In this case, the bone characteristic parameter, bc_x, is removed from the analysis and two degrees of freedom are gained; C_{x2} is also unused. The parameter C_0 can also be set to 0 to gain one degree of freedom. We used a backward elimination procedure and decided to remove parameters if this resulted in a loss of correct fit of fewer than two individuals at each step. Note that classical procedures for model selection—i.e., Akaike Information Contents for likelihood (Akaike 1974) or least squares (Yamaoka et al. 1978)—cannot be used for a phylogenetic weighted sum of squares.

We applied the results of the logistic regression analysis to infer the lifestyle of a few Paleozoic stegocephalians. For this analysis, we needed the axial length of the individuals from which the sections were taken. This was obtained by indirect means because the bones that we sectioned were isolated. The dimensions (length, diameter) of these bones were measured and compared with published skeletal reconstructions or specimen drawings of the same species or, if unavailable, of a closely related species. The axial lengths of the individuals sectioned were estimated, assuming isometric growth. To assess how much uncertainty this procedure introduced, minimal and maximal plausible values were estimated (by considering the plausible range in values of presacral length of each organism), as well as our best guesses for each individual (based on the number of presacral vertebrae that can be inferred by using parsimony to have been present in each taxon). We then used all these dimensions to perform lifestyle inference. When all three sets of length gave the same results, we concluded that the uncertainty about the size of the individual sectioned is not problematic.

Results

The distribution of compactness profile parameters indicates that there is an ecological signal in compactness profile parameter P (Fig. 5): aquatic taxa tend to have a lower value of parameter P (a relatively smaller medullary cavity). The other compactness profile parameters (S, Min, and Max) do not appear to exhibit habitat-dependent variations. Thus, their
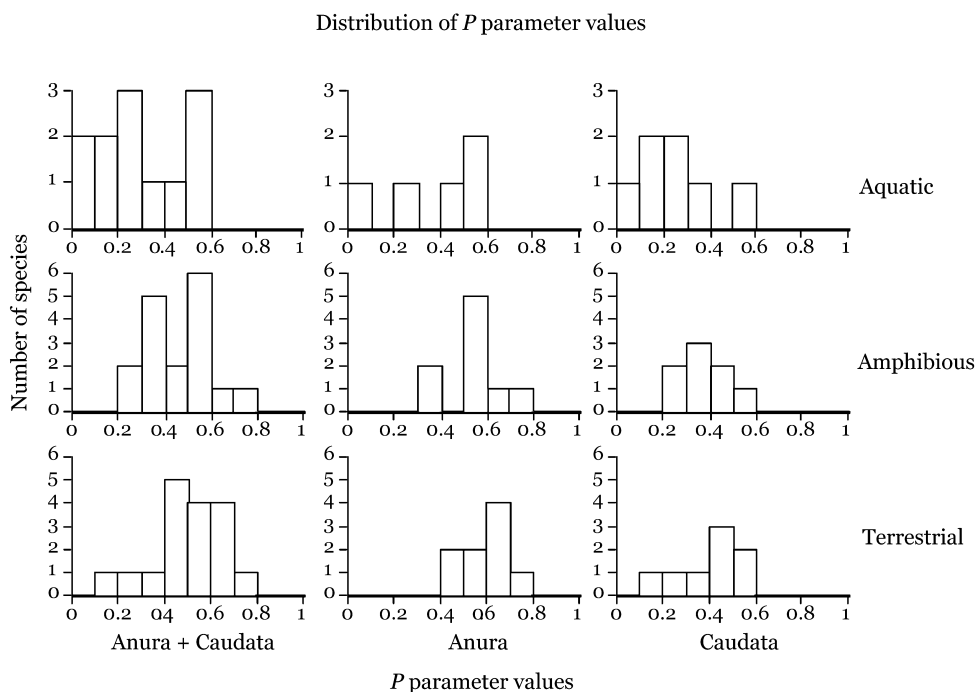
Distribution of *P* parameter values

FIGURE 5. Histogram showing the distribution of compactness profile parameter *P* as a function of lifestyle.

values are not presented (Appendix 2). Axial length apparently reflects lifestyle (Fig. 6): aquatic taxa tend to be larger than terrestrial and amphibious ones. Bone diameter (at the mid-diaphysis) shows, at best, a weak relationship with lifestyle (Fig. 7).

Phylogenetic Signal in the Data

The random trees demonstrate that body size (axial length), femoral diameter, global compactness at the femoral mid-diaphysis, compactness profile parameter *P*, and lifestyle contain phylogenetic information (Table 1). A permutational multiple linear regression analysis confirms that the compactness profile parameter *P* contains phylogenetic information (Table 2). A similar test fails to show that axial length includes phylogenetic information (Table 3).

Ecological Signal assessed by Permutational Multiple Linear Regression Analyses

A permutational multiple linear regression analysis was performed to determine which factors influence lifestyle. For this purpose, we performed a multiple linear permutational regression analysis with the habitat as the de-pendent variable, and the axial length, bone section diameter (both log-transformed), phylogenetic distance, and compactness profile parameter *P* as independent variables (Table 4). This analysis indicates that the parameter *P* and the axial length influence the lifestyle. *P* is lower in aquatic taxa than in terrestrial ones (i.e., the medullary cavity is smaller). Aquatic taxa tend to be larger than terrestrial and amphibious taxa (Figs. 5, 6). Bone section diameter and phylogenetic position do not seem to have a significant effect, according to this analysis. Forward selection and backward elimination procedures confirm that only the axial length and compactness profile parameter *P* influence the lifestyle.

The distributions of axial length and compactness profile parameter *P* suggest that there is a difference between aquatic taxa, on the one hand, and amphibious and terrestrial taxa, on the other (Figs. 5, 6). However, there is no clear separation between amphibious and terrestrial taxa. Furthermore, even if amphibious taxa were distinct from the terrestrial ones, they might be more similar to terrestrial taxa than to aquatic ones. Thus, to obtain the best results from a permutational linear
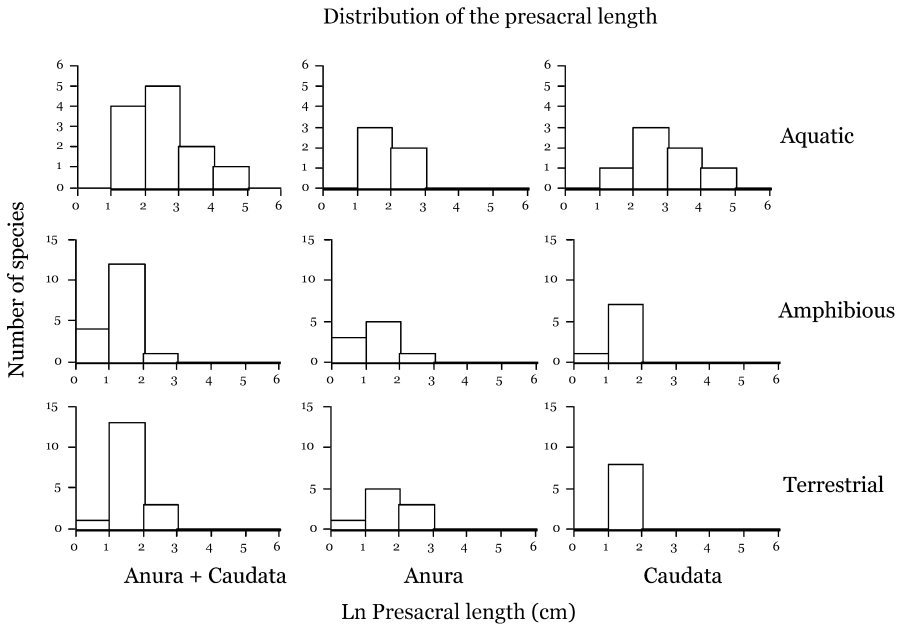
Distribution of the presacral length

FIGURE 6. Histogram showing the distribution of axial length as a function of lifestyle.

regression analysis, the simple lifestyle coding 0 (aquatic), 1 (amphibious), and 2 (terrestrial) might not be adequate. To verify whether it was better to treat the amphibious and terrestrial taxa separately, or to regroup them into a single ecological category for our analysis, and to determine the best coding for am-

phibious taxa, permutational regression analyses using several coding alternatives of amphibious taxa were performed (Table 5). The proportion of explained variance is maximal when amphibious taxa are coded between 2.0 and 2.2, with a maximum located near 2.1. If taken at face value, the maximum near 2.1

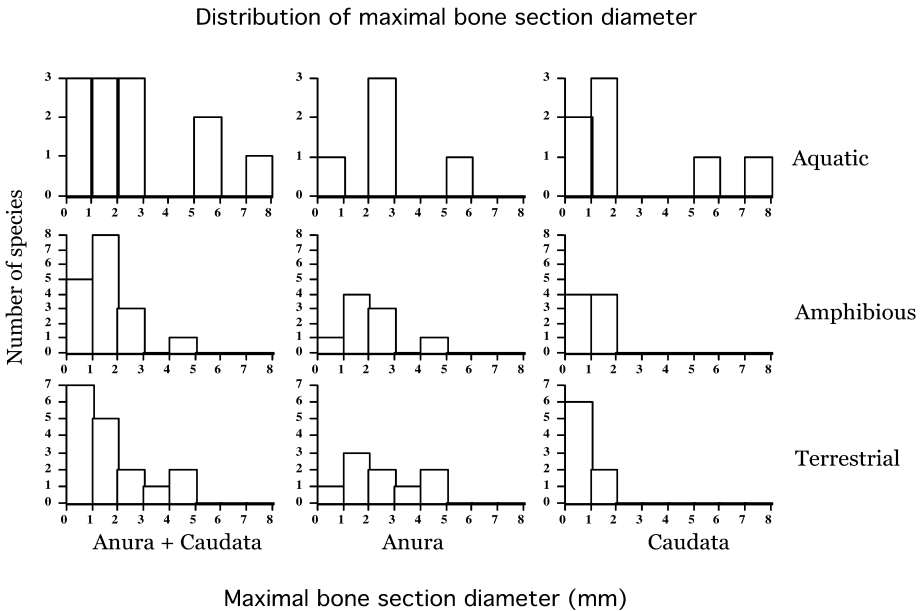Distribution of maximal bone section diameter

FIGURE 7. Histogram showing the distribution of bone size as a function of lifestyle.

TABLE 1. Probability that the observed number of steps (or squared length, for continuous characters) of various characters on the reference tree is the result of chance alone. These probabilities were determined by performing 10,000 random reshufflings of the terminal taxa (and their associated character values) onto the reference tree. For the compactness profile parameters, only the p-values for the angular parameters (calculated on 6° sectors) are shown because the p-values of the merged parameters were similar. Legend: * significant at a 0.05 threshold; ** significant at a 0.01 threshold; *** significant at a 0.001 threshold.

Character	p
Axial length (cm)	0.0096**
Axial length (ln of the length in cm)	0.0441*
Femoral diameter (mm, not transformed)	0.0877
Femoral diameter (ln of 10 × diameter, in mm)	0.0005***
Global compactness	0.0462*
Compactness profile parameter S (angular)	0.7127
Compactness profile parameter P (angular)	0.0008***
Compactness profile parameter Min (angular)	0.7945
Compactness profile parameter Max (angular)	0.4969
Lifestyle	0.0000***

might suggest that the amphibious taxa are not intermediate between aquatic and terrestrial taxa, but the difference in R^2 between 2.1 and 2.0 is so slight that we conclude conservatively that we cannot discriminate between amphibious and terrestrial taxa.

Concentrated-Changes Tests

Concentrated-changes tests showed a marginally significant correlation in the evolution of compactness parameter P or compactness with lifestyle ($p = 0.042$). This result was obtained if the threshold between the binary states was set at a compactness of 0.97 and at a parameter P value of 0.1. These state definitions segregated the aquatic taxa with the most compact bones from all others; these conditions appeared only twice on the tree, both in aquatic taxa. Setting the threshold at other, more moderate values failed to yield a significant relationship. The relationship between lifestyle and axial length appears to be stronger ($p = 0.010$). This result was obtained by setting the limit between both states at 7.5 cm. Such a large size (for lissamphibians) ap-

peared eight times in our tree, and five of these appearances were in aquatic taxa. The product of compactness and the logarithm (ln) of axial length (cm) appears to evolve even more closely with lifestyle ($p = 0.001$; Fig. 1). For this test, the limit between both states was set at 1.8, a value slightly higher than the highest terrestrial lissamphibian. These results suggest that bone compactness evolves with lifestyle, but that it is not as closely correlated with lifestyle as axial length is. Other thresholds also yield significant results ($p = 0.0074$ at a threshold of 2 or 2.5), but lowering the threshold to 1.5 yields nonsignificant results ($p = 0.1122$).

Logistic Regression

The logistic equation for Lissamphibia infers a correct lifestyle for 85% of the 105 individuals and 82% of the 46 species included in this analysis. In all cases, the errors inferred an aquatic lifestyle for taxa that are amphibious or terrestrial, and in all cases, this included the largest terrestrial taxa: *Bufo bufo*, *Bufo marinus*, *Pachymedusa dacnicolor*, *Rana catesbei-*

TABLE 2. Factors that influence compactness profile parameter P. These results were obtained by performing 9999 replicates of random permutations using the matrix option in Permute. $R^2 = 0.16$, $p = 0.0001$, globally. Legend: * significant at a 0.05 threshold; *** significant at a 0.001 threshold.

Variable	b coefficient	p
Lifestyle (coded as 0 for aquatic and 2 for amphibious and terrestrial)	0.125	0.0311*
Phylogeny	0.267	0.0001***
Axial length (ln of length in cm)	0.207	0.0098*

TABLE 3.   Factors that influence body size (axial length). These results were obtained by performing 9999 replicates of random permutations using the matrix option in Permute. $R^2 = 0.13$, $p = 0.0003$, globally. The calculations were performed by using the logarithm (ln) of the presacral body length (in cm). Using a backward elimination procedure to eliminate variables that have a nonsignificant effect (here, the phylogeny) alters only marginally the other R^2, b coefficient, and p values. Legend: ** significant at a 0.01 threshold.

Variable	b coefficient	p
Lifestyle (coded as 0 for aquatic and 2 for amphibious and terrestrial)	0.254	0.0023**
Phylogeny	−0.012	0.3795
Parameter P	0.214	0.0079**

ana, *Rana temporaria*, *Salamandra lanzai*, and *Salamandra salamandra* (one out of five individuals). The lifestyle of all 12 aquatic taxa in our data set is correctly inferred, whereas it is erroneously inferred in 8 of the 34 amphibious or terrestrial taxa.

We suspected that the relatively high error rate (15–18%) of the model based on all lissamphibians resulted from differences in body proportions between Anura and Caudata. Furthermore, the body shape of Anura is atypical and does not match that of early stegocephalians. Thus, we have produced a model based on Caudata, whose proportions match much better those of early stegocephalians. The logistic equation on Caudata using 11 parameters infers correctly the lifestyle in all (41) individuals and species. This might reflect the limited amount of interspecific phenotypic variation (in axial length and compactness profile) in Caudata than in Lissamphibia. Using backward elimination procedure, we withdrew the constants linked to the parameters that contributed the least information about lifestyle. The reduced model (based on Caudata) has only four constants and only uses information on maximal bone diameter, compactness profile parameter P, and axial length (Table 6). This model yields a correct lifestyle in 39 of the 41 individuals of extant Caudata, and in 21 of the 23 species.

One aquatic (*Ambystoma andersoni*) and one terrestrial (*Salamandrella keyserlingii*) species are modeled incorrectly. This is not unexpected because both are somewhat atypical; *A. andersoni* has a large medullary cavity and represents a very recent return to an aquatic lifestyle, whereas *S. keyserlingii* possesses one of the most compact femora among terrestrial Caudata (Appendix 2).

The results of these analyses can be used to infer the lifestyle of some early stegocephalians (Table 7, Appendix 3).

Discussion

Phylogenetic Effects on Size and Bone Microstructure

The permutational linear regression analysis failed to detect a phylogenetic effect on axial length (Table 3), but the distribution of random trees shows that this character is phylogenetically informative (Table 1). This apparent contradiction may be explained by the fact that the first analysis requires a linear relationship between phylogenetic distance and difference in axial length, and this requirement may not be met, even after a log-transformation of body lengths. Presumably, transforming branch lengths could linearize the relationship but this was not done because the random-trees test already revealed a phylo-

TABLE 4.   Factors that reflect the lifestyle (habitat). These results were obtained by performing 9999 replicates of random permutations using the matrix option in Permute. $R^2 = 0.11$, $p = 0.0007$, globally. Legend: * significant at a 0.05 threshold; ** significant at a 0.01 threshold.

Variable	b coefficient	p
Axial length (ln of length in cm)	0.262	0.0026**
Section diameter (ln[10 × diameter in mm])	−0.0080	0.4772
Phylogeny	0.062	0.0611
Compactness profile parameter P	0.132	0.0225*

TABLE 5. Proportion of the observed variance in the lifestyle explained by compactness profile parameter P, the phylogeny and axial length as a function of the coding of lifestyle amphibious taxa (aquatic taxa coded 0, terrestrial ones, 2).

Coding for amphibious taxa	R^2
0.5	0.0039
1	0.0274
1.5	0.0791
1.6	0.0876
1.7	0.0950
1.8	0.1011
1.9	0.1061
2	0.1101
2.1	0.1102
2.2	0.1098
2.3	0.1089
2.6	0.1037
3	0.0929

genetic signal in axial length. A more surprising result is that even the random-trees test appears to be sensitive to data transformation, as shown by the different probabilities obtained with or without axial length or femoral diameter logarithmic transformations (Table 1).

The systematic value of bone histological characters has been debated since the nineteenth century (Ricqlès 1993). Using squared-change parsimony (Maddison 1991), Padian et al. (2001) studied the evolution of growth rate by using data obtained from histological characters, thus assuming that these data were phylogenetically informative. This assumption is implicit in the use of squared-change

parsimony optimization because there is no reason to believe that optimization gives reliable information about character evolution if there is no phylogenetic signal (Laurin 2004). In this study, the word ''optimization'' always refers to a procedure that consists of minimizing character change throughout a tree of a given topology and set of branch lengths (Swofford and Maddison 1987; Maddison 1991). In contrast, Castanet et al. (2001) concluded, after performing some quantitative tests, that bone histological characters primarily reflected the ecology and life history of a taxon rather than its phylogenetic affinities. Our analysis does not extend to a truly histological level, but our compactness profile parameters correspond to a microanatomical level intermediate between the gross morphological and the histological levels. Many gross morphological characters include phylogenetic information, as the large number of published phylogenies using such characters suggests. Thus, we expected that at least some microanatomical characters would include phylogenetic information. Our results confirm this hypothesis because compactness profile parameter P appears to be phylogenetically informative. The absence of a phylogenetic signal in other compactness profile parameters (S, Min, and Max) may result from the taxonomic sampling of our study; lissamphibian bones are usually simple tubular structures, as is typical of small bones (Ricqlès

TABLE 6. Values of the constants of the logistic equations used to infer the lifestyle of early stegocephalians. See ''Materials and Methods,'' subsection ''Logistic regression and inference of lifestyle in extinct stegocephalians'' for the equation. Excel spreadsheets including these equations that can be used to infer lifestyle of extinct taxa are available from the authors upon request.

Compactness profile parameter	Associated constants	Value in model incorporating all Lissamphibia	Value in model incorporating only Caudata, simple model
Maximal diameter	C_1	−169.313	1154.0251
	C_2	−0.2619	−0.567522
S	C_1	−851.0145	—
	C_2	0.1758	—
P	C_1	−3641.1086	−8130.737
	C_2	3.2694	0.7979224
Min	C_1	134.9539	—
	C_2	27.8775	—
Max	C_1	103.8138	—
	C_2	−4.4379	—
C_0		119.0393	—

TABLE 7. Lifestyle inference of Paleozoic taxa based on logistic regression (the simplified Caudata model). The lifestyle estimate was binary; we can discriminate only between aquatic taxa (state 0) and amphibious or terrestrial ones (state 1). Interpretations discordant with the prevailing interpretation on lifestyle are in bold type. The axial lengths were estimated from the literature, by comparisons with close relatives or individuals of the same species. Ranges of axial length values were tested, but this never altered the conclusions. Sources of the length: for *Doleserpeton*, Bolt 1969, 1977, 1979; Werneburg 1991; for *Chroniosaurus*, Ivakhnenko et al. 1997; Golubev 1998; for *Cardiocephalus*, Carroll and Gaskill 1978; for *Phaanthosaurus*, Ivakhnenko et al. 1997; Watson 1914. Legend: Am/T, amphibious or terrestrial.

Taxon	Axial length estimates	Axial length values (cm)	Lifestyle inference, based on extant Caudata only	Prevailing interpretation in the literature
Doleserpeton	Minimal	2.5	Am/T	Terrestrial
	Best estimate	5.0	Am/T	
	Maximal	6.0	Am/T	
Chroniosaurus	Minimal	18	Am/T	**Aquatic**
	Best estimate	25	Am/T	
	Maximal	35	Am/T	
Cardiocephalus	Minimal	4	Am/T	Terrestrial
	Best estimate	7	Am/T	
	Maximal	9	Am/T	
Phaanthosaurus	Minimal	5	Am/T	Terrestrial
	Best estimate	7	Am/T	
	Maximal	9	Am/T	

1976: p. 15). Thus, S, Min, and Max exhibit little variation in this group. Typically, S has a low value that corresponds to a narrow transition zone, Min equals 0.0, and Max is 1.0. A similar study performed on amniotes, which possess a more complex bone microanatomy, might yield different results.

The presence of a phylogenetic signal in the characters included in the model that enable us to infer the lifestyle of extinct taxa (compactness profile parameter P, axial length and section diameter) suggests that they could be optimized onto the phylogeny to infer the lifestyle of hypothetical ancestors. This approach could be advantageous because the optimization of discrete characters is often ambiguous. Optimizing the compactness profile parameters and size, and using the inferred values to infer the lifestyle of hypothetical ancestors, could provide a way of resolving ambiguity in the optimization of the lifestyle of both extant and extinct terminal taxa.

General Pattern of Adaptation to an Aquatic Lifestyle in Lissamphibians

The femora of aquatic lissamphibians have lower values of compactness profile parameter P, which means that they have smaller medullary cavities than the femora of amphibious or terrestrial lissamphibians. This finding is not unexpected and may reflect a widespread trend toward increasingly compact long bones in secondarily aquatic taxa that live in shallow water or recently returned to an aquatic lifestyle (Ricqlès and Buffrénil 2001). This character is usually interpreted as an adaptation to reduce buoyancy; the extra weight generated by the increase in mass of the skeleton prevents the animal from floating (Taylor 1994). The only secondarily aquatic vertebrates that do not exhibit such a trend and that possess a spongy, light skeleton are active, deep divers such as extant cetaceans (Madar 1998) and ichthyosaurs (Nopsca 1923; Buffrénil et al. 1987; Buffrénil and Mazin 1990, 1993). In cetaceans, the rib cage collapses when the animal reaches a certain depth; thus, the buoyancy generated by the lungs is greatly reduced. This lessens the need for compact, heavy bones, which counteract the lung buoyancy in several aquatic tetrapods (Buffrénil and Schoevaert 1989). In all active swimmers, reducing the weight and inertia of the bones should save energy. Aquatic lissamphibians are neither very active nor deep divers. Thus, the observed increase in compactness in the most aquatic members of this group is not surprising.

It is less obvious why aquatic taxa would not have bones of greater diameter than those of terrestrial taxa, because this would increase

their mass, in the same way an increase in compactness through reduction in size of the medullary cavity would. A potential reason may be that an increase in compactness does not increase hydrodynamic drag, whereas an increase in diameter would. This hypothesis could be tested by studying changes in diameter and compactness in aquatic and terrestrial taxa. The widespread pachyostosis of ribs of aquatic taxa (Buffrénil and Schoevaert 1989; Buffrénil et al. 1990) suggests that this hypothesis does not fully explain this phenomenon. Perhaps the low density of marrow tissues (often adipose) would make increasing bone diameter a very inefficient mechanism for increasing body density.

Inference of Lifestyle in Early Stegocephalians

Body Size.—Our data on the distribution of body size as a function of lifestyle (Fig. 6) suggests that the smallest known early amphibians (those less than 5 cm in axial length) were terrestrial or amphibious. This includes the early amphibians *Saxonerpeton* and *Brachystelechus* (Carroll and Gaskill 1978: Fig. 1). Among early amphibians, those that seem to have been aquatic, judging from other criteria (e.g., the presence of grooves for the lateral-line organ, or the low degree of ossification, or the presence of a high, long tail likely used for swimming), are all longer. This includes the following taxa: adelogyrinids (axial length not exactly known because they were limbless, but about 14 cm [Andrews and Carroll 1991]), nectrideans (e.g., axial length of 5.5 cm in *Ptyonius marshii*, 10 cm in *Urocordylus wandesfordii*, 13 cm in *Sauropleura scalaris*, 17 cm in *Sauropleura pectinata*, [Bossy 1976]), *Microbrachis* (about 8 cm in axial length [Carroll and Gaskill 1978: Fig. 1]), and lysorophians (from 10 to about 50 cm [Wellstead 1991]). The only apparent anomalies that we have detected in this relationship between body size and lifestyle among early amphibians concern the nectridean *Diceratosaurus brevirostris* and the poorly known taxon *Odonterpeton*. If the individual of *Diceratosaurus brevirostris* reconstructed by Milner (1980) is fully grown, its axial length is only about 4 cm. However, this species has a large head, a short trunk, and a

very long tail. Because it does not have typical proportions, the axial length does not give a proper estimate of its global size. The small size of *Odonterpeton* (axial length not exactly known, but approximately 4 cm) suggests a terrestrial lifestyle, but Carroll and Gaskill (1978: p. 147) suggested that it was aquatic, because they identified possible gill supports and because the elongate body with small limbs was reminiscent of *Amphiuma*. However, the presence of gill supports is not certain, and an elongate body and small limbs are found in some terrestrial squamates; therefore, *Odonterpeton* could have been terrestrial. Finally, the ontogenetic stage of the holotype and only known specimen of this taxon is not known; if it was not fully grown, the axial length of the adult may have exceeded 5 cm, and this length would be compatible with an aquatic lifestyle.

Logistic Regression using Extant Caudata.— Application of the equation obtained by phylogenetic logistic regression on the Caudata data (reduced model) yields results generally congruent with the prevailing lifestyle interpretations (Table 7). The general model based on all lissamphibians could be useful to infer the lifestyle of early anurans or early amphibians that are closely related to lissamphibians, or perhaps even to early stem-Caudata, but it would be inappropriate to infer the lifestyle of early stegocephalians, whose body proportions were much closer to those of Caudata than to those of Salientia. Thus, we only discuss the results of inferences about the lifestyle of early stegocephalians using the reduced Caudata model.

The Lower Permian temnospondyl *Doleserpeton* is usually thought to have been terrestrial (Bolt 1977: p. 245). Some authors consider this taxon to be closely related to lissamphibians (Bolt 1969, 1977), although others view it as a stem-tetrapod (Laurin 1998) (Fig. 8). The idea that *Doleserpeton* was terrestrial is in part based on its relative abundance at Fort Sill (Oklahoma), where most taxa (and the most abundant ones) are thought to have been terrestrial (Olson 1967; Sullivan and Reisz 1999). Thus, Fort Sill appears to represent a terrestrial community. The logistic-model estimates yield an amphibious to terrestrial life-
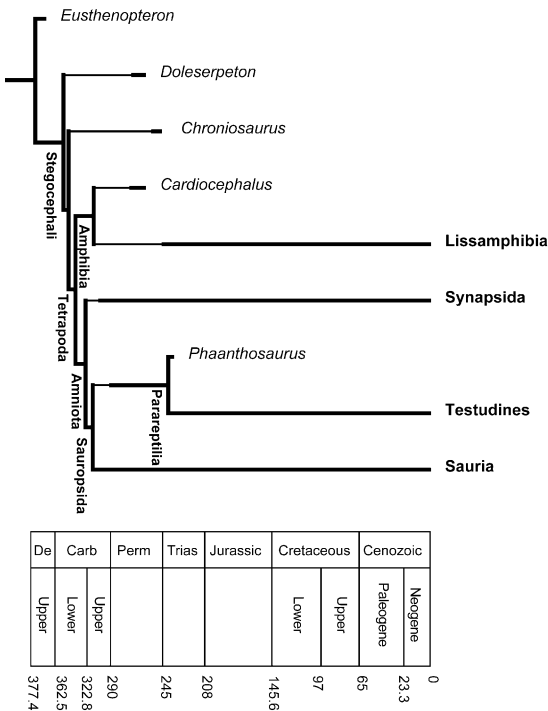
FIGURE 8. Phylogeny of the four taxa whose lifestyle is inferred using our logistic model, of the early sarcopterygian *Eusthenopteron*, and of other major tetrapod taxa. The phylogeny outside Amniota follows Laurin (1998), and inside Amniota, Laurin and Reisz (1995). The geological time is expressed in Myr B.P. Abbreviations: Carb, Carboniferous; De, Devonian; Perm, Permian; Trias, Triassic.

style for *Doleserpeton* for all axial lengths tested (Table 7). These results are also compatible with the widespread idea that other dissorophids and trematopsids were among the most terrestrial early stegocephalians (Berman et al. 1985; Sumida et al. 1998).

The logistic model yields an amphibious to terrestrial lifestyle for the embolomere *Chroniosaurus* from the Upper Permian of Russia. This is discordant with the opinion of Golubev (1998), who recently suggested that *Chroniosaurus* was among the most aquatic chroniosuchians. Thus, we raise doubts about the lifestyle of this taxon. The Upper Permian continental vertebrate fauna from Russia included several non-amniotic stegocephalians, some of which were presumably terrestrial, such as *Kotlassia* (Bystrow 1944). The lack of lateral-line canal grooves on the skulls of all chroniosuchians (although this is only negative evidence) suggests an amphibious or ter-

restrial lifestyle, rather than a truly aquatic one. This interpretation is supported further by the presence of dorsal scutes that are tightly articulated with the neural spines, and that bear accessory intervertebral articular surfaces. The presence of such scutes may have strengthened the axial skeleton and helped it support the weight of the animal on dry land. Some of the most terrestrial temnospondyls (according to prevailing interpretations of lifestyle), such as several dissorophoids (Carroll 1964), also possessed dorsal scutes that presumably reinforced their axial columns.

The Lower Permian amphibian *Cardiocephalus* is thought to have been terrestrial, partly because of its occurrence at Fort Sill. The logistic model yields an amphibious to terrestrial lifestyle, and this confirms previous ideas about the habitat of *Cardiocephalus*.

The amphibious or terrestrial lifestyle inferred by the logistic model for the Lower Triassic procolophonid *Phaanthosaurus* agrees with the terrestrial lifestyle that is usually attributed to this taxon, and to procolophonoids in general. Procolophonids and other parareptiles may be closely related to Chelonia (Laurin and Reisz 1995) (Fig. 8), a taxon whose extant representatives are mostly aquatic or amphibious; therefore, data on lifestyle in parareptiles may be useful to determine when the ancestors of Chelonia became amphibious or aquatic.

Validity of Our Inferences

The femora of the anurans *Bombina* and *Telmatobius* are not very compact for aquatic taxa, but they are more compact than those of their more terrestrial relatives. This suggests that incorporating data on the affinities of an extinct taxon would probably improve the reliability of an inference about its lifestyle, at least if the extinct taxon were within the clade of extant taxa that has been studied. Our logistic regression models (Table 6) currently do not incorporate the phylogenetic position of the extinct taxon whose lifestyle is to be inferred (although they were produced by using phylogenetic information about extant taxa), but such models probably could be developed. However, their conception and use would be

extremely complex and are beyond the scope of this study.

An important limitation of our study, and of any conceivable study using extant tetrapods to assess the lifestyle of early stegocephalians, is that the taxa for which we have inferred the lifestyle are not part of the clade of extant taxa (Lissamphibia and Caudata) that form the basis for that inference (Fig. 8). Thus, they are outside the extant phylogenetic bracket (Witmer 1995); inferences about their lifestyles are probably less reliable than inferences about the lifestyles of early Lissamphibia or early Caudata, which would be within the extant phylogenetic bracket. We plan to perform a similar study on extant amniotes, and this will bring *Cardiocephalus* and *Phaanthosaurus* into the extant phylogenetic bracket (Fig. 8). However, *Chroniosaurus* and *Doleserpeton* will remain outside that bracket (at least, under the phylogeny of Laurin 1998); thus, the inferences about the lifestyles of these taxa and several other early stegocephalians will remain slightly less reliable. The sampling of extant taxa cannot be expanded further out because the Dipnoi, the closest extant relatives of Tetrapoda, do not possess limbs; even though a femur (and humerus) is present, it is entirely cartilaginous, like most of the endoskeleton (Carroll 1988). Even in early dipnoans, which were much better ossified, the paired-fin endoskeleton was poorly ossified (Janvier 1996). The only remaining possibility would be to section a femur of an early, finned sarcopterygian with a better-ossified femur, such as *Eusthenopteron*, and we are currently attempting this. *Eusthenopteron* is not extant, but it had gill arches, fins, and several other characters indicative of an aquatic lifestyle. Thus, it would constitute an adequate substitute for an extant taxon. Obtaining such data would bring all early stegocephalians within the phylogenetic bracket (Fig. 8), but the sampling in finned sarcopterygians will remain restricted to one or two species, because of lack of material. Thus, the data for inferring the lifestyle of an early stegocephalian from a cross-section of a femur (and other long bones) will have to be based primarily on extant tetrapods, although it may be possible to add one or two species of finned sarcopterygians.

Another problem of using extant tetrapods to infer the lifestyle of early stegocephalians is that, as far as we know, there are no extant primitively aquatic tetrapods (i.e., all extant aquatic tetrapods had terrestrial or amphibious ancestors). Thus, it is conceivable that the adaptations to life in water that we see in extant tetrapods do not reflect the adaptations that allowed early stegocephalians to become terrestrial. We envision two partial solutions to this problem. First, we will include early, primitively aquatic sarcopterygians such as *Eusthenopteron* into the analysis. Second, we will include turtles, a taxon whose primitive lifestyle appears to be aquatic, judging by an optimization of the lifestyle of extant turtles on a phylogeny. (Thus, the primitive lifestyle for the crown group appears to be aquatic, but this does not mean that taxa outside the crown, like *Proganochelys*, were necessarily aquatic.) In turtles, there have been at least two transitions to a terrestrial lifestyle. These two extensions of our data set should enable us to determine how the limb bones evolved in transitions from an aquatic to a terrestrial lifestyle.

According to our mathematical equations, the match between observed and inferred lifestyle in our data set is good in extant Lissamphibia (85% of individuals, 82% of taxa) and excellent in Caudata (95% of individuals), and it coincides with traditional paleontological interpretations in most (three) of the four cases in which we have applied this method. The case of *Chroniosaurus*, for which our inference differs from previous interpretations, does not necessarily indicate a problem with our estimate because some gross morphological data suggest that our inference is plausible. Thus, we believe that this method can provide valuable insights into the lifestyle of early stegocephalians, and about the history of the conquest of land by vertebrates.

Acknowledgments

Literature Cited

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Andrews, S. M., and R. L. Carroll. 1991. The order Adelospondyli: Carboniferous lepospondyl amphibians. Transactions of the Royal Society of Edinburgh 82:239–275.

Báez, A. M. 1981. Redescription and relationships of *Saltenia ibanezi*, a late Cretaceous pipid frog from Northwestern Argentina. Ameghiniana 22:127–154.

Baez, A. M., and L. A. Pugener. 2003. Ontogeny of a new Palaeogene pipid frog from southern South America and xenopodimorph evolution. Zoological Journal of the Linnean Society 139:439–476.

Báez, A. M., and J.-C. Rage. 1998. Pipid frogs from the Upper Cretaceous of In Beceten, Niger. Palaeontology 41:669–691.

Báez, A. M., L. Trueb, and J. O. Calvo. 2000. The earliest known pipoid frog from South America: a new genus from the middle Cretaceous of Argentina. Journal of Vertebrate Paleontology 20:490–500.

Berman, D. S., R. R. Reisz, and D. A. Eberth. 1985. *Ecolsonia cutlerensis*, an early Permian dissorophid amphibian from the Cutler Formation of north-central New Mexico. Circular of the New Mexico Bureau of Mines and Mineral Resources 191:1–31.

Bininda-Edmonds, O. R. P., J. L. Gittleman, and C. K. Kelly. 2001. Flippers versus feet: comparative trends in aquatic and non-aquatic carnivores. Journal of Animal Ecology 70:386–400.

Bolt, J. R. 1969. Lissamphibian origins: possible protolissamphibian from the Lower Permian of Oklahoma. Science 166:888–891.

———. 1977. Dissorophoid relationships and ontogeny, and the origin of the Lissamphibia. Journal of Paleontology 51:235–249.

———. 1979. *Amphibamus grandiceps* as a juvenile dissorophid: evidence and implications. Pp. 529–563 *in* M. H. Nitecki, ed. Mazon Creek fossils. Academic Press, London.

Bossy, K. V. H. 1976. Morphology, paleoecology, and evolutionary relationships of the Pennsylvanian urocordylid nectrideans (subclass Lepospondyli, class Amphibia). Ph.D. dissertation. Yale University, New Haven, Conn.

Buffrénil, V. de, and J.-M. Mazin. 1990. Bone histology of the ichthyosaurs: comparative data and functional interpretation. Paleobiology 16:435–447.

———. 1992. Contribution de l'histologie osseuse à l'interprétation paléobiologique du genre *Placodus* Agassiz, 1833 (Reptilia, Placodontia). Revue de Paléobiologie 11:397–407.

———. 1993. Some aspects of skeletal growth in Triassic and post-Triassic ichthyosaurs as revealed by bone histology. Pp. 63–68 *in* G. Pinna and J.-M. Mazin, eds. Evolution, ecology and biogeography of the Triassic reptiles. Paleontologia Lombarda della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, nuova serie, Milan.

Buffrénil, V. de, and J.-C. Rage. 1993. La ''pachyostose'' vertébrale de *Simoliophis* (Reptilia, Squamata): données comparatives et considérations fonctionnelles. Annales de Paléontologie 79:415–335.

Buffrénil, V. de, and D. Schoevaert. 1988. On how the periosteal bone of the delphinid humerus becomes cancellous: ontogeny of a histological specialization. Journal of Morphology 198:149–164.

———. 1989. Données quantitatives et observations histologiques sur la pachyostose du squelette du dugong, *Dugong dugon* (Müller) (Sirenia, Dugongidae). Canadian Journal of Zoology 67:2107–2119.

Buffrénil, V. de, J. M. Mazin, and A. de Ricqlès. 1987. Caractères structuraux et mode de croissance du fémur d'*Omphalosaurus nisseri*, ichthyosaurien du Trias Moyen de Spitsberg. Annales de Paléontologie 73:195–216.

Buffrénil, V. de, A. de Ricqlès, D. Sigogneau-Russell, and E. Buffetaut. 1990. L'histologie osseuse des champsosauridés: données descriptives et interprétation fonctionnelle. Annales de Paléontologie 76:255–275.

Bystrow, A. P. 1944. *Kotlassia prima* Amalitzky. Geological Society of America Bulletin 55:379–416.

Carroll, R. L. 1964. Early evolution of the dissorophid amphibians. Bulletin of the Museum of Comparative Zoology 131:161–250.

———. 1985. Evolutionary constraints in aquatic diapsid reptiles. Palaeontology 33:145–155.

———. 1988. Vertebrate paleontology and evolution. W. H. Freeman, New York.

Carroll, R. L., and P. Gaskill. 1978. The order Microsauria. American Philosophical Society, Philadelphia.

Castanet, J., and M. H. Caetano. 1995. Influence du mode de vie sur les caractéristiques pondérales et structurales du squelette chez les amphibiens anoures. Canadian Journal of Zoology 73:234–242.

Castanet, J., K. Curry Rogers, J. Cubo, and J.-J. Boisard. 2000. Periosteal bone growth rates in extant ratites (ostriche and emu): implications for assessing growth in dinosaurs. Comptes Rendus de l'Académie des Sciences de Paris, Sciences de la vie/Life Sciences 323:543–550.

Castanet, J., J. Cubo, and E. de Margerie. 2001. Signification de l'histodiversité osseuse: le message de l'os. Biosystema 19:133–147.

Coates, M. I., and J. A. Clack. 1991. Fish-like gills and breathing in the earliest known tetrapod. Nature 352:234–236.

Dubois, A. 1992. Notes sur la classification des Ranidae (amphibiens anoures). Bulletin Mensuel de la Société Linnéenne de Lyon 61:305–352.

Dubois, A., and A. Ohler. 1994. Frogs of the subgenus *Pelophylax* (Amphibia, Anura, genus *Rana*): a catalogue of available and valid scientific names, with comments on name-bearing types, complete synonymies, proposed common names, and maps showing all type localities. Zoologica Poloniae 39:139–204.

Duellman, W. E., and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, New York.

Estes, R. 1969. The fossil record of amphiumid salamanders. Breviora 322:1–11.

Fawcett, D. W. 1942. The amedullary bones of the Florida man-

atee (*Trichechus latirostris*). American Journal of Anatomy 71: 271–309.

Fish, F. E., and B. R. Stein. 1991. Functional correlates of differences in bone density among terrestrial and aquatic genera in the family Mustelidae (Mammalia). Zoomorphology 110:339–345.

Ford, L., and D. C. Cannatella. 1993. The major clades of frogs. Herpetological Monographs 7:94–117.

Gao, K.-Q., and N. H. Shubin. 2001. Late Jurassic salamanders from northern China. Nature 410:574–577.

———. 2003. Earliest known crown-group salamanders. Nature 422:424–4828.

Gao, K., and Y. Wang. 2001. Mesozoic anurans from Liaoning province, China, and phylogenetic relationships of archaeobatrachian anuran clades. Journal of Vertebrate Paleontology 21:460–476.

Girondot, M., and M. Laurin. 2003. Bone profiler: a tool to quantify, model and statistically compare bone section compactness profiles. Journal of Vertebrate Paleontology 23:458–461.

Golubev, V. K. 1998. Narrow-armored chroniosuchians (Amphibia, Anthracosauromorpha) from the late Permian of Eastern Europe. Paleontological Journal 32:278–287.

Hay, J. M., I. Ruvinsky, S. B. Hedges, and L. R. Maxson. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. Molecular Biology and Evolution 12:928–937.

Henrici, A. C., and A. M. Báez. 2001. First occurrence of *Xenopus* (Anura: Pipidae) on the Arabian peninsula: a new species from the upper Oligocene of Yemen. Journal of Paleontology 75:870–882.

Hua, S., and V. de Buffrénil. 1996. Bone histology as a clue in the interpretation of functional adaptations in the Thalattosuchia (Reptilia, Crocodylia). Journal of Vertebrate Paleontology 16:703–717.

Ivakhnenko, M. F., V. K. Golubev, Y. M. Gubin, N. N. Kalandadze, I. V. Novikov, A. G. Sennikov, and A. S. Rautian. 1997. Permian and Triassic tetrapods of Eastern Europe. Geos, Moscow.

Janvier, P. 1996. Early vertebrates. Clarendon, Oxford.

Larson, A., and W. W. Dimmick. 1993. Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters. Herpetological Monographs 7:77–93.

Laurin, M. 1998. The importance of global parsimony and historical bias in understanding tetrapod evolution, Part I. Systematics, middle ear evolution, and jaw suspension. Annales des Sciences Naturelles, Zoologie et Biologie Animale, 13e série, 19:1–42.

Laurin, M. 2004. The evolution of body size, Cope's rule and the origin of amniotes. Systematic Biology 53:594–622.

Laurin, M., and R. R. Reisz. 1995. A reevaluation of early amniote phylogeny. Zoological Journal of the Linnean Society 113:165–223.

Leclair, R., Jr., C. Lamontagne, and A. Aubin. 1993. Allométrie de la masse du squelette chez des amphibiens anoures. Canadian Journal of Zoology 71:352–357.

Legendre, P., F.-J. Lapointe, and P. Casgrain. 1994. Modeling brain evolution from behavior: a permutational regression approach. Evolution 48:1487–1499.

Lehman, J.-P. 1955. Rachitomi. Pp. 67–125 *in* J. Piveteau, ed. Traité de Paléontologie. Masson, Paris.

Madar, S. I. 1998. Structural adaptations of early archaeocete long bones. Pp. 353–378 *in* J. G. M. Thewissen, ed. The emergence of whales—evolutionary patterns in the origin of Cetacea. Plenum, New York.

Maddison, W. P. 1990. A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? Evolution 44:539–557.

———. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. Systematic Zoology 40:304–314.

Maddison, W. P., and D. R. Maddison. 2002. Mesquite: a modular system for evolutionary analysis, Version 0.992. http://mesquiteproject.org.

Maddison, W. P., D. R. Maddison, and P. Midford. 2002. TreeFarm package of modules for Mesquite, Version 0.992. http://mesquiteproject.org.

Martin, R. F. 1972. Evidence from osteology. Pp. 37–70 *in* W. F. Blair, ed. Evolution in the genus *Bufo*. University of Texas Press, Austin.

Milner, A. C. 1980. A review of the Nectridea (Amphibia). Pp. 377–405 *in* A. L. Panchen, ed. The terrestrial environment and the origin of land vertebrates. Academic Press, London.

Nopsca, F. B. 1923. Vorläufige Notiz über die Pachyostose und Osteosklerose einiger mariner Wirbeltiere. Anatomischer Anzeiger 56:353–359.

Olson, E. C. 1967. Early Permian vertebrates. Oklahoma Geological Survey Circular 74.

Padian, K., A. de Ricqlès, and J. R. Horner. 2001. Dinosaurian growth rates and bird origins. Nature 412:405–408.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. Cabios 11:247–251.

Rambaut, A., and M. Charleston. 2001. TreeEdit. University of Oxford. Available at http://evolve.zoo.ox.ac.uk/

Ricqlès, A. de. 1974a. Recherches paléohistologiques sur les os longs des tétrapodes. V. Cotylosaures et mésosaures. Annales de Paléontologie 60:171–216.

———. 1974b. Recherches paléohistologiques sur les os longs des tétrapodes. IV. Eothériodontes et pélycosaures. Annales de Paléontologie 60:1–39.

———. 1975a. Quelques remarques paléo-histologiques sur le problème de la néoténie chez les stégocéphales. Problèmes Actuels de Paléontologie: Evolution des Vertébrés. Colloque International du Centre National de Recherche Scientifique 218:351–363.

———. 1975b. Recherches paléohistologiques sur les os longs des tétrapodes. VII. Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes. Première partie. Annales de Paléontologie 61:51–129.

———. 1976. Recherches paléohistologiques sur les os longs des tétrapodes. VII. Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes. Deuxième partie. Annales de Paléontologie 62:71–126.

———. 1977. Recherches paléohistologiques sur les os longs des tétrapodes. VII. Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux des tétrapodes. Deuxième partie, suite. Annales de Paléontologie 63:33–56.

———. 1981. Recherches paléohistologiques sur les os longs des tétrapodes. VI. Stégocéphales. Annales de Paléontologie 67: 141–160.

———. 1989. Les mécanismes hétérochroniques dans le retour des tétrapodes au milieu aquatique. Geobios Mémoire Spécial 12:337–348.

———. 1993. Some remarks on palaeohistology from a comparative evolutionary point of view. Pp. 37–77 *in* G. Grupe and A. N. Garland, eds. Histology of ancient human bone. Springer, Berlin.

Ricqlès, A. de, and V. de Buffrénil. 2001. Bone histology, heterochronies and the return of tetrapods to life in water: were are we? Pp. 289–310 *in* J. M. Mazin and V. de Buffrénil, eds. Secondary adaptation of tetrapods to life in water. Pfeil, Munich.

Romer, A. S. 1957. Origin of the amniote egg. Scientific Monthly 85:57–63.

———. 1958. Tetrapod limbs and early tetrapod life. Evolution 12:365–369.

———. 1966. Vertebrate paleontology. University of Chicago Press, Chicago.

Ruta, M., M. I. Coates, and D. D. L. Quicke. 2003. Early tetrapod relationships revisited. Biological Reviews 78:251–345.

Skulan, J. 2000. Has the importance of the amniote egg been overstated? Zoological Journal of the Linnean Society 130: 235–261.

Sullivan, C., and R. R. Reisz. 1999. First record of *Seymouria* (Vertebrata: Seymouriamorpha) from Early Permian fissure fills at Richards Spur, Oklahoma. Canadian Journal of Earth Sciences 36:1257–1266.

Sumida, M., M. Ogata, and M. Nishioka. 2000. Molecular phylogenetic relationships of pond frogs distributed in the palearctic region inferred from DNA sequences of mitochondrial 12S ribosomal RNA and cytochrome b genes. Molecular Phylogenetics and Evolution 16:278–285.

Sumida, S. S. 1997. Locomotor features of taxa spanning the origin of amniotes. Pp. 353–398 *in* S. Sumida and K. Martin, eds. Amniote origins: completing the transition to land. Academic Press, London.

Sumida, S. S., D. S. Berman, and T. Martens. 1998. A new trematopid amphibian from the Lower Permian of central Germany. Palaeontology 41:605–629.

Swofford, D. L., and W. P. Maddison. 1987. Reconstructing ancestral character states under Wagner parsimony. Mathematical Biosciences 87:199–229.

Taylor, M. A. 1994. Stone, bone or blubber? Buoyancy control strategies in aquatic tetrapods. Pp. 151–161 *in* L. Maddock, Q. Bone, and J. M. V. Rayner, eds. Mechanics and physiology of animals swimming. Cambridge University Press, Cambridge.

Titus, T. A., and A. Larson. 1995. A molecular phylogenetic perspective on the evolutionary radiation of the salamander family Salamandridae. Systematic Biology 44:125–151.

———. 1996. Molecular phylogenetics of desmognathine salamanders (Caudata: Plethodontidae): a reevaluation of evolution in ecology, life history and morphology. Systematic Biology 45:451–472.

Trueb, L. 1999. The early Cretaceous pipoid anuran, *Thoraciliacus*: redescription, revaluation, and taxonomic status. Herpetologica 55:139–157.

Uzzell, T. 1982. Immunological relationship of Western Palearctic Water Frogs (Salientia: Ranidae). Amphibia-Reptilia 3:135–143.

Veith, M., S. Steinfartz, R. Zardoya, A. Seithz, and A. Meyer. 1998. A molecular phylogeny of ''true'' salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. Journal of Zoological Systematics and Evolutionary Research 36:7–16.

Wall, W. P. 1983. The correlation between high limb-bone density and aquatic habits in recent mammals. Journal of Paleontology 57:197–207.

Watson, D. M. S. 1914. *Procolophon trigoniceps*, a cotylosaurian reptile from South Africa. Proceedings of the Zoological Society of London 2022:735–747.

Wellstead, C. F. 1991. Taxonomic revision of the Lysorophia, Permo-Carboniferous lepospondyl amphibians. Bulletin of the American Museum of Natural History 209:1–90.

Werneburg, R. 1991. Die Branchiosaurier aus dem Unterrotliegend des Döhlener Beckens bei Dresden. Veröffentlichungen des Naturhistorisches Museum Schleusingen 6:75–99.

Wiffen, J., V. de Buffrénil, A. de Ricqlès, and J.-M. Mazin. 1995. Ontogenetic evolution of bone structure in late Cretaceous Plesiosauria from New Zealand. Geobios 28:625–640.

Williston, S. W. 1911. A new family of reptiles from the Permian of New Mexico. American Journal of Science 31:378–398.

Witmer, L. M. 1995. The extant phylogenetic bracket and the importance of reconstructing soft tissues in fossils. Pp. 19–33 *in* J. J. Thomason, ed. Functional morphology in vertebrate paleontology. Cambridge University Press, New York.

Yamaoka, K., T. Nakagawa, and T. Uno. 1978. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. Journal of Pharmacokinetics and Biopharmaceutics 6:165–175.

Appendix 1

Papers used to compile the phylogenies and divergence times

Lissamphibia: Hay et al. (1995).
Urodeles: Larson and Dimmick (1993: Fig. 3); Gao and Shubin (2001).
Cryptobranchoidea: Gao and Shubin (2003).
Amphiumidae: Estes (1969).
Salamandridae: Titus and Larson (1995); Veith et al. (1998).
Desmognathinae: Titus and Larson (1996).
Salientia: Ford and Cannatella (1993); Gao and Wang (2001).
Pipidae: Báez (1981); Báez and Rage (1998); Henrici and Báez (2001).
Ranidae: Uzzell (1982); Dubois (1992); Dubois and Ohler (1994); Sumida et al. (2000).
Bufo: Martin (1972).

Appendix 2.

Femoral sections of lissamphibians studied for this analysis. For the habitat, 0 = aquatic, 1 = amphibious, 2 = terrestrial. These states are defined by the relative amount of time spent in water: >90% for aquatic taxa, between 20% and 90% for amphibious taxa, and <20% for terrestrial taxa. Axial length is measured from the anterior edge of atlantal centrum to the anterior edge of the sacral centrum for Caudata, but from the atlantal centrum to the posterior end of the urostyle for anurans, to compensate for their short, stocky body. The maximal bone diameter is measured on the mid-diaphyseal section. The compactness was measured over the whole section, including the medullary cavity. Other parameter profile parameters (Min, Max, S) are not included because they are nearly constant in lissamphibians and are not relevant to this study.

Taxon	Habitat	n	Presacral length (cm)	SD of presacral length	Maximal bone diameter (mm)	SD of maximal bone diameter	Compactness	SD of compactness	Parameter P	SD of parameter P
Hynobiidae										
Salamandrella (Hynobius) keyserlingii	2	1	3.331	—	0.642	—	0.961	—	0.109	—
Onychodactylus fischeri	0	1	5.064	—	0.712	—	0.926	—	0.276	—
Cryptobranchidae										
Andrias japonicus	0	1	34.000	—	7.324	—	0.966	—	0.043	—
Cryptobranchus alleganiensis	0	1	22.500	—	5.061	—	0.979	—	0.106	—
Amphiumidae										
Amphiuma means	0	1	70.500	—	1.275	—	0.979	—	0.109	—
Proteidae										
Necturus maculosus	0	1	15.500	—	1.777	—	0.933	—	0.258	—
Proteus anguineus	0	1	9.230	—	0.440	—	0.910	—	0.301	—
Ambystomatidae										
Ambystoma andersoni	0	1	8.000	—	1.754	—	0.645	—	0.596	—
Ambystoma opacum	2	1	3.550	—	0.789	—	0.720	—	0.521	—
Salamandridae										
Salamandra atra	2	1	4.055	—	0.913	—	0.909	—	0.272	—
Salamandra lanzai	2	1	5.700	—	1.620	—	0.899	—	0.304	—
Salamandra salamandra	2	5	5.895	1.861	1.547	0.773	0.774	0.083	0.475	0.093
Pleurodeles waltl	1	1	5.000	—	1.430	—	0.794	—	0.453	—
Pleurodeles poireti	1	13	2.827	0.511	0.809	0.146	0.833	0.052	0.419	0.070
Triturus marmoratus	1	3	5.899	0.524	1.247	0.111	0.893	0.047	0.325	0.092
Triturus cristatus	1	1	3.822	—	0.774	—	0.917	—	0.285	—
Triturus alpestris	1	1	3.806	—	0.750	—	0.890	—	0.337	—
Triturus helveticus	1	1	2.145	—	0.398	—	0.883	—	0.348	—
Triturus montandoni	1	1	5.060	—	1.070	—	0.958	—	0.230	—
Plethodontidae										
Desmognathus ochrophaeus	2	1	2.840	—	0.366	—	0.745	—	0.504	—
Desmognathus monticola	2	1	5.020	—	0.620	—	0.826	—	0.420	—
Desmognathus quadramaculatus	1	1	6.450	—	1.122	—	0.703	—	0.547	—
Plethodon glutinosus	2	1	5.060	—	0.553	—	0.761	—	0.489	—

Appendix 2. Continued.

Taxon	Habitat	n	Presacral length (cm)	SD of presacral length	Maximal bone diameter (mm)	SD of maximal bone diameter	Compactness	SD of compactness	Parameter P	SD of parameter P
Ascaphidae										
Ascaphus truei	1	1	1.620	—	0.993	—	0.898	—	0.315	—
Discoglossidae										
Discoglossus occipitalis	1	1	4.810	—	1.125	—	0.490	—	0.713	—
Bombina orientalis	0	1	2.770	—	0.988	—	0.723	—	0.542	—
Pipidae										
Xenopus laevis	0	1	4.100	—	2.203	—	0.864	—	0.045	—
Pipa pipa	0	3	10.667	0.764	5.097	0.236	0.892	0.048	0.297	0.076
Pipa carvalhoi	0	1	6.221	—	2.472	—	0.692	—	0.544	—
Pelobatidae										
Pelobates fuscus	2	1	1.760	—	0.880	—	0.435	—	0.752	—
Leptodactylidae										
Ceratophrys aurita	2	1	6.652	—	3.532	—	0.510	—	0.690	—
Telmatobius culeus	0	4	10.100	1.068	2.472	0.167	0.797	0.049	0.451	0.057
Leptodactylus pentadactylus	2	1	5.260	—	4.383	—	0.653	—	0.593	—
Ranidae										
Rana catesbeiana	1	1	11.504	—	4.807	—	0.654	—	0.538	—
Rana esculenta	1	5	4.163	0.763	1.740	0.319	0.668	0.127	0.571	0.115
Rana temporaria	1	2	5.915	2.025	2.472	0.846	0.859	0.137	0.358	0.221
Rana dalmatina	2	3	4.024	0.137	1.491	0.051	0.794	0.017	0.459	0.020
Rana ridibunda	1	2	2.536	1.010	1.445	0.576	0.672	0.034	0.574	0.031
Rana iberica	1	2	1.897	0.305	1.081	0.174	0.689	0.021	0.557	0.021
Rana (Lithobates) vaillanti	1	1	4.639	—	2.643	—	0.573	—	0.654	—
Rana (Pantherana) forreri	1	1	6.000	—	2.507	—	0.688	—	0.564	—
Bufonidae										
Bufo bufo	2	3	10.087	1.170	2.530	0.294	0.769	0.014	0.456	0.028
Bufo marinus	2	3	14.000	3.464	4.159	1.285	0.622	0.053	0.603	0.045
Bufo pentoni	2	21	4.576	0.357	1.465	0.114	0.620	0.095	0.611	0.081
Hylidae										
Pachymedusa dacnicolor	2	1	9.000	—	1.593	—	0.749	—	0.502	—
Hyla boans	2	4	7.135	0.668	2.133	0.201	0.569	0.038	0.659	0.030

Appendix 3

Femoral sections of early stegocephalians used to infer the lifestyle. MSD = maximal section diameter.

Taxon	MSD (mm)	Compactness profile parameter				Source of the material
		S	*P*	*Min*	*Max*	
Doleserpeton	0.90	0.00216	0.5474	−3.69E-05	1.000	Section 819.5.3.T from collection of A. de Ricqlès, illustrated in Ricqlès 1975b: Plate I, Fig. 1
Chroniosaurus	5.03	0.0189	0.4922	0.0564	0.923	Section made from specimen donated by V. K. Golubev
Cardiocephalus	1.18	0.00163	0.4249	3.71E-5	0.973	Section made from specimen donated by Robert R. Reisz
Phaanthosaurus	2.76	0.00439	0.6176	−2.21E-5	1.000	Section 322.2.1.T from collection of A. de Ricqlès, illustrated in Ricqlès 1974a: Plate IV, Figs. 8, 9