The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2010, **100**, 384–406. With 8 figures

Evolution of humeral microanatomy and lifestyle in amniotes, and some comments on palaeobiological inferences

AURORE CANOVILLE and MICHEL LAURIN*

UMR CNRS 7207 'Centre de Recherches sur la Paléobiodiversité et les Paléoenvironnements',
Muséum National d'Histoire Naturelle, Département Histoire de la Terre, Bâtiment de Géologie,
Case Postale 48, 43 rue Buffon, F-75231 PARIS Cedex 05, France

Received 26 October 2010; revised 14 December 2009; accepted for publication 15 December 2009

A study on the most exhaustive taxonomic sample of amniotes (75 extant and nine extinct taxa) of any quantitative work on this topic published so far demonstrates a strong relationship between lifestyle (aquatic, amphibious or terrestrial) and humeral microanatomy. We suggest that corrections for multiple testing be used to check for statistical artefacts in the context of a phylogenetic independent contrast analysis, and we use the false discovery rate procedure for this. Linear discriminant models segregate the various lifestyles with excellent success rate of up to 98.5%. Lifestyle was thus inferred for six extinct taxa of uncertain habitat. The results obtained suggest that *Captorhinus*, *Claudiosaurus*, and *Placodus* were amphibious, whereas *Neusticosaurus* and *Mesosaurus* were aquatic. *Lystrosaurus* may have been more aquatic than previously suggested, although the results of our inference models have to be integrated with other sources of data, which suggest that it may have been amphibious, rather than aquatic (as a literal interpretation of the models would suggest). Finally, we propose an alternative method of palaeobiological inference for hypothetical ancestors. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 384–406.

ADDITIONAL KEYWORDS: bone microanatomy – comparative biology – habitat – hypothetical ancestors – inference models – palaeobiology – phylogeny.

INTRODUCTION

Amniota is one of the most successful and diverse group of vertebrates. The origin of amniotes, in the Late Carboniferous, is regarded as a major event in vertebrate evolution because the amniotes are often considered to be the first fully terrestrial tetrapods (Carroll, 1988; Benton, 2005; Laurin, 2008). Amniotes are present in all major environments and thus show a wide range of lifestyle from fully aquatic to fully terrestrial. Palaeozoic terrestrial habitats opened great possibilities for the early amniotes, and they diversified extensively on land, although some repeatedly reinvaded the aquatic environment. The oldest known clearly aquatic amniotes are represented by the Early Permian mesosaurs (Carroll, 1988). The early history of habitat shifts of amniotes is unclear and under debate, and our ideas on this topic have changed drastically in the last decades.

According to Romer (1957, 1958), the ancestors of amniotes started laying their eggs on land before adults became terrestrial. This hypothesis was based on his interpretation of the Permo-Carboniferous amniote *Ophiacodon*, which he considered most representative of the first amniotes, and of the diadectomorph *Limnoscelis*, a close relative of the first amniotes. Romer (1957, 1958) considered both genera as primitively aquatic to amphibious animals.

Restudy of the oldest known amniotes (Carroll 1964, 1969) led to the suggestion that Amniota is a primitively terrestrial taxon, a hypothesis that is now widely accepted (Carroll, 1988; Benton, 2005; Laurin, 2008: 44; Shedlock & Edwards, 2009). The earliest

---

*Corresponding author. E-mail: aurore.canoville@free.fr and michel.laurin@upmc.fr

known amniotes, *Hylonomus*, *Paleothyris* and *Protoclepsydrops* from the Upper Carboniferous of Joggins, Nova Scotica, were probably agile terrestrial insectivores resembling squamates. Their high degree of ossification, well-developed limbs, and association with a predominantly terrestrial fauna attest to their terrestrial lifestyle.

More recent studies raise doubts about terrestriality as an ancestral feature of amniotes. Joggins may present an unusual taphonomic bias in which terrestrial forms may have had a better preservation potential than aquatic ones. Thus, the presence of two apparently terrestrial amniotes (*Protoclepsydrops* and *Hylonomus*) at Joggins does not imply that amphibious or aquatic amniotes did not exist elsewhere. Interpretations based on bone microanatomy (de Ricqlès, 1974; Germain & Laurin, 2005) have provided additional support for Romer's suggestions that *Ophiacodon* was amphibious to aquatic. However, optimizations of lifestyle of extant and extinct stegocephalians on phylogenies of amniotes suggest that the first amniotes were amphibious or terrestrial, which raises the possibility that ophiacodontids returned to a more aquatic lifestyle (Germain & Laurin, 2005; Kriloff *et al*., 2008). Thus, the time at which amniotes (and indeed, vertebrates) became truly terrestrial remains a thorny issue.

Lifestyle appears to be reflected in the skeleton at various levels ranging from gross morphology to histological microstructure (Bininda-Emonds, Gittleman & Kelly, 2001; de Buffrénil, Sire & Schoevaert, 1986). However, not all aquatic or amphibious vertebrates exhibit obvious morphological modifications (Carroll, 1985); it is thus often difficult to assess the ecology of a taxon on the basis of morphology alone and bone microanatomy may provide additional ecological data. Differences in the bone structure between aquatic and terrestrial vertebrates have been known for a long time (Nopcsa, 1923), and the relationship between bone microanatomy and lifestyle in extant tetrapods has been intensively studied (Scheyer & Sander, 2007; Kriloff *et al*., 2008). Generally, the long bones of terrestrial taxa have a large medullary cavity and a compact cortical region. By contrast, long bones of aquatic vertebrates generally have a smaller medullary cavity (if any), and the skeleton is either heavier or lighter than in their terrestrial relatives (de Ricqlès & de Buffrénil, 2001). However, these generalizations have been rigorously tested on few datasets.

One of the main purposes of the present study is to provide statistical validation to previous conclusions about how bone microanatomy evolves in response to returns to an aquatic lifestyle. The present study, based on the humerus, is complementary to previous work by Germain & Laurin (2005), which was based on a data set of radial sections of 51 species of amniotes, and Kriloff *et al*. (2008), which was based on a data set of tibial sections of approximately sixty species of amniotes (and of some lissamphibians). We have obtained new data on the humerus and body size on the most exhaustive taxonomic sample of amniotes (78 species of known lifestyle) of any quantitative study on this topic published so far. The present study, similar to the previous ones carried out by our laboratory on this topic, excludes flying taxa because flight imposes strong mechanical constraints different from those of terrestrial and aquatic taxa; their inclusion would needlessly complicate analysis of our data and interpretation of our results.

Several reasons led us to study the humerus. First, previous analyses (Germain & Laurin 2005; Kriloff *et al*., 2008; Laurin, Girondot & Loth, 2004) demonstrate that the reliability of inference models obtained from one bone (e.g. the radius) is lower when the model is applied to another bone (e.g. the tibia). Because fossils of early amniotes are rare and precious, and because producing histological sections is a destructive process, it would be advantageous to be able to base inferences on as many kinds of bones as possible, to take advantage of any fossil material. Indeed, the results obtained enable us to draw palaeobiological inferences about the lifestyle of five early amniote taxa, including three (*Captorhinus*, *Claudiosaurus*, and *Placodus*) for which no such inference had previously been made on the basis of mathematical models based on bone microanatomy. A second reason to study the humerus is to check for consistency with inferences obtained on the basis of other bones (Germain & Laurin, 2005; Kriloff *et al*., 2008) for the two other taxa (*Neusticosaurus* and *Lystrosaurus*). Third, the proximodistal gradient of compactness in bones of sea cows (de Buffrénil & Schoevaert, 1989) suggests that proximal limb bones may yield a stronger ecological signal than more distal bones. Therefore, obtaining data on the humerus enables us to test this hypothesis because the radius and tibia are more distal bones. Comparisons of the responses of various bones to changes in habitat are also useful because they might respond differently to a change in habitat as a result of the unique mechanical stresses to which each is subjected (de Margerie *et al*., 2005).

Statisticians have long recognized the need to correct for multiple tests. The main problem with multiple test correction is that these procedures typically reduce power (thus increasing the probability of making a type II error), although substantial progress on accounting for multiple tests was made with the development of the false discovery rate procedure (FDR), which is simple to implement and does not drastically reduce power (Benjamini & Hochberg, 1995; Curran-Everett, 2000). However, such procedures are still rarely used in comparative biology and our laboratory has only recently started using them (Laurin, Canoville & Quilhac, 2009). In the present study, we apply the FDR to tests that can determine whether the phylogenetically independent contrasts (PIC) are adequately standardized. Because up to four tests can be performed on each character (Midford, Garland & Maddison, 2008), and because several characters are normally analysed in each study, dozens of tests are often performed, and the probability of rejecting a tree as inadequate to standardize the contrasts is quite high, even if the tree is actually correct and the characters have evolved according to a Brownian motion model. Thus, we argue that correction for multiple testing such as the FDR should be used in this context, as we do in the present study. We believe that it is the first time that FDR (or any correction for multiple tests) has been performed to assess the suitability of a tree for PIC analysis.

The last methodological innovation that we propose below is a new method of palaeobiological inference. Typically, inference models are applied to extinct taxa (observed taxa, represented by fossils), and these inferences are then optimized onto a tree using parsimony or, more rarely, other methods (maximum likelihood, Bayesian analysis) to reconstruct character history. One of the main purposes of such exercises is to infer properties of hypothetical ancestors. For example, Kriloff *et al*. (2008) inferred the lifestyle (aquatic to terrestrial) of five early stegocephalian taxa and optimized these inferences, along with the observed lifestyle of extant tetrapods, on a tree to infer the primitive lifestyle for Amniota (represented by a node on the tree).

There is an alternative way to obtain such inferences on hypothetical taxa. The nodal value of the characters used by the inference models can be estimated using squared-change parsimony (or other methods, such as maximum likelihood, phylogenetic generalized least squares, etc.), and these values can be used to directly infer the character of interest (i.e. lifestyle) of a hypothetical ancestor using standard inference models (such as linear discriminant models). Optimizations are used in both cases but, in the new method proposed here, the optimization is performed earlier in the analysis and it typically involves continuous characters. This has the advantage of retaining all the quantitative data until the final step of the analysis. When the inferred character is discrete (as is the case with lifestyle), the discretization required by the classical approach discards much data, which should reduce reliability.

All the methodological innovations described above and applied to our data enable us to get a preliminary inference about the primitive way of life of amniotes, and to reassess the evolution of lifestyle and the conquest of land in vertebrates.

MATERIAL AND METHODS

BIOLOGICAL SAMPLE

The analysis carried out in the present study bears on detailed anatomical drawings of humeral mid-diaphyseal cross-sections, both because compactness profiles can vary within a single bone (the metaphysis is generally spongier than the diaphysis) and because the mid-diaphyseal level should yield the strongest ecological signal (Laurin *et al*., 2004). All bones represent adults (assessed based on size, fusion of epiphyseal secondary centres of ossification, degree of ossification of the endoskeleton and aspect of the sections, including the type of tissue and the distribution of lines of arrested growth) in order to avoid ontogenetic variations.

Our sample includes 75 extant species (48 of mammals; see Supporting Information, Appendix S1A; 13 of turtles; see Supporting Information, Appendix S1B; 13 of squamates, and one crocodilian; see Supporting Information, Appendix S1C) and nine extinct taxa determined to generic level (see Supporting Information, Appendix S1D), representing the main groups of amniotes relevant to the present study. The lifestyle is considered to be known (aquatic) for three extinct taxa based on morphological, taphonomic, and phylogenetic data (Carroll, 1988; Benton, 2005). This concerns *Muraenosaurus*, a Late Jurassic plesiosaur (Elasmosauridae; Fig. 1A) (Carroll, 1988; Benton, 2005), an unindentified specimen from the Miocene Cethoteriidae (Cetacea, Mysticeti; Fig. 1B), and the Early Jurassic ichthyosaur *Stenopterygius* (Fig. 1C).

The lifestyle of six of the extinct taxa included in the present study cannot be determined with great confidence on the basis of gross morphological or taphonomic data. The genus *Captorhinus* (Fig. 2A) is an Early Permian sauropsid from Texas and Oklahoma (de Ricqlès, 1980). This taxon is generally described as terrestrial in the literature because of its well-ossified endoskeleton and its abundance in the terrestrial locality of Fort Sill (Peabody, 1951; Sullivan & Reisz, 1999); however, de Ricqlès (1980) observed that Captorhinus long bones had thick cortices, which is characteristic of an amphibious or aquatic lifestyle (Fawcett, 1942; de Buffrénil & Schoevaert, 1989; de Ricqlès & de Buffrénil, 2001; Laurin *et al*., 2004). The Middle Triassic *Neusticosaurus* (Fig. 2B) from the Alps is a basal sauropterygian, thus related to the more recent plesiosaurs (Carroll & Gaskill, 1985; Sander, 1989; but see also Holmes,

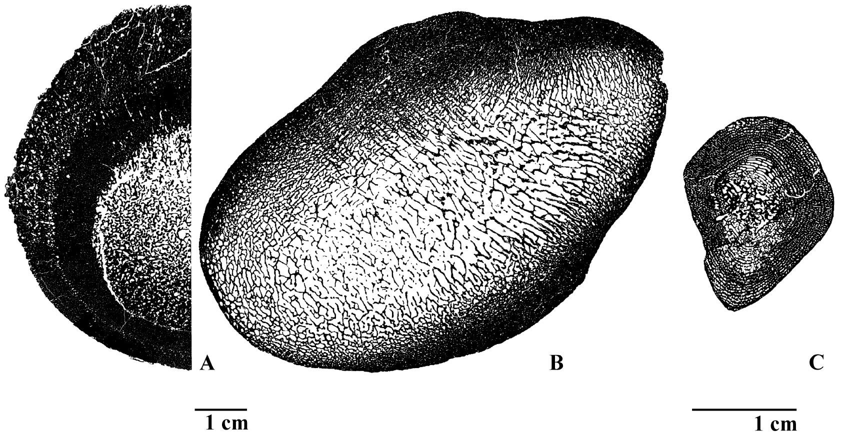

Figure 1. Drawings of mid-diaphyseal cross-sections of humeri of extinct taxa of known lifestyle. A, the plesiosaur *Muraenosaurus* (Upper Jurassic; scale bar = 1 cm). B, an unidentified specimen of Cetotheriidae (Cetacea, Mysticeti, Miocene, Tortonian; scale bar = 1 cm). C, the ichthyosaur *Stenopterygius* (Lower Jurassic, Toarcian; scale bar = 1 cm).

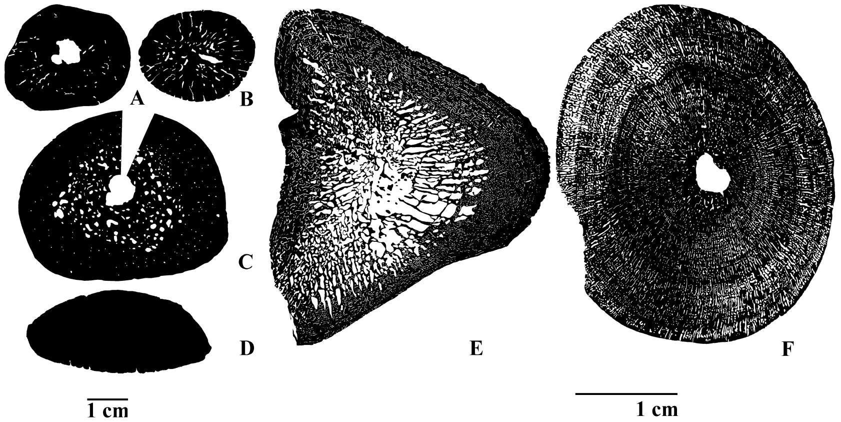

Figure 2. Drawings of mid-diaphyseal cross-sections of humeri of extinct taxa of unknown lifestyle. A, the basal eureptile *Captorhinus* (Lower Permian, Artinskian). B, the sauropterygian *Neusticosaurus* (Middle Triassic, Ladinian). C, the stem-diapsid *Claudiosaurus* (Late Permian, Changxingian). D, the parareptile *Mesosaurus* (Lower Permian). Scale bar = 1 cm for (A) to (D). E, the dicynodont therapsid *Lystrosaurus* (Upper Permian). F, the euryapsid *Placodus* (Middle Triassic, Anisian). Scale bar = 1 cm for (E) and (F).

Cheng & Wu, 2008), comprising one of the most important groups of marine amniotes of the Mesozoic. Thus, *Neusticosaurus* and its relatives (the pachypleurosaurs) are generally considered to have been completely aquatic (Carroll & Gaskill, 1985; Carroll, 1988), although some studies argue that they may have retained some terrestrial locomotion abilities (Schmidt, 1984; Storrs, 1993; Germain & Laurin, 2005). According to Carroll (1981, 1988), *Claudiosaurus* (Fig. 2C), a Late Permian diapsid from Madagascar, may be close to sauropterygians (marine diapsids including nothosaurs and plesiosaurs), but more recent phylogenetic analyses place it outside the diapsid crown group (Müller, 2003). Most skeletal features of this animal resemble those of early terrestrial diapsids, although some osteological characters, such as the long neck and the loss of ossification of the sternum, could be interpreted as an early stage of aquatic adaptation. Mesosaur, from the Lower Permian, are one of the first amniotes lineages that reinvaded aquatic environments during the Palaeozoic. They are probably parareptiles as suggested by Modesto (2006). *Mesosaurus* (Fig. 2D) has long been regarded as an aquatic form in the literature but its small size is compatible with limited terrestrial locomotor abilities, and divergent opinions about its habitat have been proposed (Carroll, 1982; Oelofsen & Araújo, 1983; Modesto, 2006). The therapsid *Lystrosaurus* (Fig. 2E) was abundant in the Late Permian and the Early Triassic. Some studies have considered it as terrestrial and perhaps fossorial on the basis of skeletal features such as a broad scapula or short and robust distal bones of the forelimbs (King, 1991; King & Cluver, 1991; Retallack, Smith & Ward, 2003). Other studies hold an amphibous or mostly aquatic lifestyle more probable on the basis of morphological (e.g. nostrils at the top of the skull; Broom, 1903; Cluver, 1971; Ray, 2006) and microanatomical (Germain & Laurin, 2005; Ray, Chinsamy & Bandyopadhyay, 2005) characters, and on its abundance in the fossil record. Likewise, the palaeoecology of *Placodus* (Fig. 2F), a Triassic placodont and thus a close relative of the sauropterygians, is somewhat uncertain. This taxon probably fed on hard-shelled preys such as molluscs and is generally considered to have lived in shallow costal waters (de Buffrénil & Mazin, 1992). It is often considered completely aquatic, although its skeleton shows few clear adaptations to an aquatic lifestyle (Carroll, 1988) apart from a pachyostotic rib cage and poorly ossified limb bones (de Buffrénil & Mazin, 1989; Rieppel, 1995). It has been demonstrated that pachyostosis can appear in amphibious animals, such as the early whales (Pakicetidae) and sirenians (Prorastomidae), and that microstructural change predates gross anatomical shift (Domning, 2001; Gray *et al.*, 2007).

All cross-sections were drawn with a camera lucida, digitized and transformed into binary images using PHOTOSHOP 7.0 (Adobe Systems Inc.). All cross-sections were analysed using BONE PROFILER (Girondot & Laurin, 2003).

DATA ACQUISITION

Cross-section analysis using BONE PROFILER

BONE PROFILER, already used by Steyer *et al.* (2004), Laurin *et al.* (2004, 2006, 2009), Germain & Laurin (2005), Kriloff *et al.* (2008), Canoville & Laurin (2009), and Scheyer & Sander (2009), allows extracting several compactness profile parameters of a sigmoid mathematical model, which shows the distribution of the bony tissue on a section. This method could reveal differences in the distribution of the bony tissue between aquatic and terrestrial taxa, even if the global compactness were equal in both groups. Four main parameters are extracted from this model and the values can be computed from whole sections (*S*, *P*, *Min* and *Max*), or from small, 6°-wide 'pie-chart' sections: *Srad*, *Prad*, *Minrad*, and *Maxrad*. Other parameters calculated by the software are included in our analyses: *Cc*, *Cp* and *Cg*. These parameters are defined in the Supporting Information (Appendix S2) and their associated values are also compiled in the Supporting Information (Appendix S1). More details about this model are provided in Girondot & Laurin (2003) and Laurin *et al.* (2004).

Body size and lifestyle

We also incorporated body size parameters (presacral length: *PLg*, and bone maximum diameter: *MD*, log-transformed or not) into the analyses (see Supporting Information, Appendix S1) because an initial examination of many sections revealed that the bones of small animals usually have a simpler structure than those of large animals. Furthermore, body size may contain ecological information because it has already been shown to differ between aquatic and terrestrial taxa (Bininda-Emonds *et al.*, 2001; Laurin *et al.*, 2004; Canoville & Laurin, 2009). Presacral length is measured from the atlas to the sacrum (cm) and bone diameter refers to the maximum diameter of the cross-section (mm).

Lifestyle is coded as follows: ternary coding, with 0 = aquatic, 1 = amphibious, and 2 = terrestrial (see Supporting Information, Appendix S1) for most analyses, and binary coding, with 0 = aquatic, 1 = amphibious or terrestrial, for a few analyses. 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. We have coded the habitat using both primary literature and databases such as Animal Web Diversity (Myers *et al.*, 2006) or the IUCN Red List of Threatened Species (IUCN, 2008).

Building the reference phylogeny

A time-calibrated supertree (Fig. 3) was produced using the method outlined by Laurin *et al.* (2004) for topology, and Laurin *et al.* (2009) for divergence times (for more details about the supertree construction, see Supporting Information, Appendices S3, S4A,B), rather than using MRP (Bininda-Emonds *et al.*, 2003; Bininda-Emonds, 2004). Turtles pose a special problem because their position is still controversial. We have considered that they are parareptiles and thus that they are excluded from Diapsida, as suggested by Reisz & Laurin (1991), Laurin & Reisz (1995) and Lee (1995, 1997, 2001). More recent molecular and developmental studies on extant taxa also support placement of turtles outside Diapsida (Frost *et al.*, 2006; Werneburg & Sánchez-Villagra, 2009), although this represents a minority point of view.

STATISTICAL ANALYSIS

Detecting phylogenetic and ecological signals in compactness profile and body size data

To detect the phylogenetic signal, we performed two distinct analyses. First, we performed variance partitioning using PVR (Desdevises *et al.*, 2003; for more details about this method, see Supporting Information, Appendix S5).

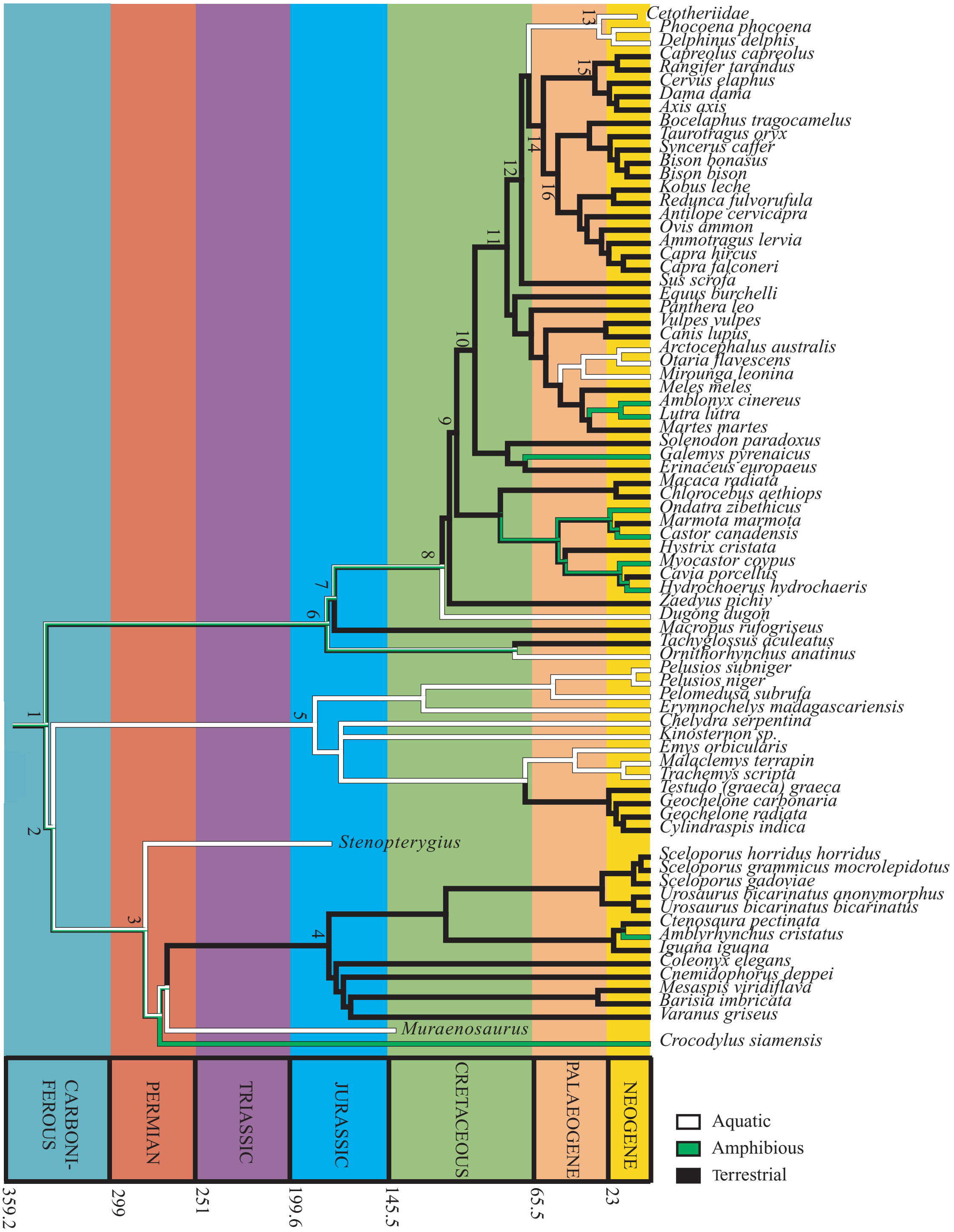

Figure 3. Phylogeny of taxa of known lifestyle used in the present study (taxa whose lifestyle is inferred are included in Fig. 8, but not here). This supertree was compiled using Stratigraphic Tools for MESQUITE (Josse, Moreau & Laurin, 2006) from previously published phylogenies (see Material and Methods). The lifestyle is optimized as an ordered character using parsimony. The geological time is expressed in million years before present. White, aquatic; green, amphibious; black, terrestrial. Some of the principal clades: 1, Amniota; 2, Sauropsida; 3, Neodiapsida; 4, Squamata; 5, Testudines; 6, Mammalia; 7, Theria; 8, Eutheria; 9, Boreoeutheria; 10, Laurasiatheria; 11, Cetferungulates; 12, Cetartiodactyla; 13, Cetacea; 14, Pecora; 15, Cervidae; 16, Bovidae.

Second, we generated 10000 trees by randomly permuting the terminal taxa (Laurin, 2004; see Supporting Information, Appendix S5) in MESQUITE.

To detect the ecological signal we performed two tests. First, we performed a variance partitioning analysis with PVR where the dependent variable was the lifestyle (ternary and binary codings) and the independent variables were the compactness profile parameters, the body size parameters, and the principal coordinates (PCs) representing the phylogeny. We used a forward selection procedure (with p to enter = 0.05) in PERMUTE (Casgrain, 2005) to determine which variables show the most important ecological signal.

Second, we performed a PIC analysis of the characters whose contrasts where adequately standardized on our tree (Fig. 3) in the PDAP Module of MESQUITE (Midford *et al*., 2008; Maddison & Maddison, 2008). We used a binary coding of lifestyle because previous studies (Canoville & Laurin, 2009; Laurin *et al*., 2009) showed that PIC is less powerful than variance partitioning with PVR to detect an ecological signal in bone microanatomy, because amphibious taxa are not phenotipically intermediate between aquatic and terrestrial ones, and because the greatest difference is between aquatic and other taxa (Laurin *et al*., 2004).

To test the hypothesis that the relationship between the three lifestyles is not linear and that the ternary coding 0 (aquatic), 1 (amphibious), and 2 (terrestrial) is not optimal for linear regressions, we performed multiple linear regressions with the lifestyles as dependent variables and the parameters S, P, Min, $Maxrad$, Cc, and Cp as independent variables. To determine the best coding for amphibious taxa (in the context of linear regressions), regressions using several coding alternatives of amphibious taxa were performed.

Linear discriminant analyses and inference models

Linear discriminant analyses, which make it possible to infer the lifestyle of extinct taxa, were carried out using STATISTICA, version 6 (StatSoft France, 2003; see Supporting Information, Appendix S5) among characters with a phylogenetic signal and whose contrasts were adequately standardized (to be usable on hypothetical ancestors). The discriminant functions applied to extinct taxa are based on the extant taxa and on the three extinct taxa of known lifestyle (the cetotheriid specimen, *Muraenosaurus*, and *Stenopterygius*). Turtles were included in one model, but not in the second because they have been shown to have atypical long bone compactness profiles (Germain & Laurin, 2005; Kriloff *et al*., 2008).

Optimization of habitat and lifestyle inference of hypothetical ancestors

An optimization of habitat in early stegocephalians is based on the palaeoecological inferences obtained in the present survey (six terminal taxa), as well as those obtained from comparable data by four previous studies (Laurin *et al*., 2004; Steyer *et al*., 2004; Germain & Laurin, 2005; Kriloff *et al*., 2008), confronted with other (morphological, taphonomic) sources of data on the habitat of the same taxa. The Frasnian sarcopterygian *Eusthenopteron foordi* Whiteaves, 1881, whose long bone microanatomy was recently studied (Laurin *et al*., 2007), was certainly aquatic: it is also included in this optimization. Moreover, for other taxa, data on the habitat extracted from the literature are incorporated: Dipnoi and Actinistia are aquatic (Laurin, 2008) and lissamphibians are considered to be amphibious (Laurin *et al*., 2004; Vallin & Laurin, 2004). Lastly, data are complemented by inferences of the ancestral habitat of crown-group Mammalia, Lepidosauria, Testudines based on parsimony optimization of lifestyle on extant taxa performed in the present study (Fig. 3). The stem-turtle *Proganochelys* is considered to have been terrestrial (Joyce & Gauthier, 2004; Scheyer & Sander, 2007). Thus, observed and inferred lifestyle of 21 extinct taxa (including 15 taxa from previous studies and six additional extinct taxa from our study) and seven extant taxa (Lissamphibia, Mammalia, Testudines, Lepidosauria, *Crocodylus siamensis*, Dipnoi and Coelacanthomorpha) are used to provide a new optimization of habitat on a time-calibrated phylogeny. Lifestyle, which is optimized using standard parsimony, is considered to be an ordered character with three states.

In addition to optimizing observed and inferred lifestyle and to infer the ancestral habitat for Amniota, Reptilia, and Mammalia, we optimized body size and microanatomical characters that were adequately standardized on our reference tree (Fig. 3): (1) without extinct taxa (75 extant species) or (2) with nine extinct taxa (three of known lifestyle: Cethoteriidae, *Muraenosaurus*, and *Stenopterygius*; six of inferred lifestyle: *Mesosaurus*, *Captorhinus*, *Claudiosaurus*, *Neusticosaurus*, *Lystrosaurus*, and *Placodus*) to determine the extent to which the inclusion of fossils affects the nodal estimates and the width of the confidence intervals (CIs). Thus, we obtained inferences of the lifestyle of the basal nodes of these taxa. Because the nodal estimates of continuous characters have an associated CI, we performed inferences using three sets of values to assess the uncertainty of our inferences: the best estimate of the nodal values for each character, plus the lower and upper limits of the 95% CIs of the characters calculated, using PIC (Garland, Midford & Ives, 1999).

This is only one of several sensitivity tests that could be implemented, although testing several combinations of parameter values within the CIs would take too much space and time, and sampling character distribution based on the means and intervals would require software developments beyond the scope of the present study.

RESULTS

PHYLOGENETIC SIGNAL

Two principal coordinate axes (PCs 1 and 2) were selected by the broken stick model. Together they represent 76.36% of the phylogenetic variance of the 78 species. The partitioning method (PVR) performed using PERMUTE (Casgrain, 2005) demonstrates that most bone compactness and body size data contain phylogenetic information (Table 1; see also Supporting Information, Appendix S6). We also used backward-elimination procedures to determine which PCs contributed significantly to the explanation of each variable (thus, 15 backward-elimination procedures were performed); it demonstrates that all parameters are phylogenetically informative (Table 1).

We succeeded in adequately standardizing contrasts for seven characters using data and branch length tranformations (Bartlett, 1947; Box & Cox, 1964; Hoyle, 1973). The reference tree has minimum length of terminal branches set to 8 Myr and minimal internal branch length of 4 Myr (Fig. 3). The following characters (some of which were transformed as shown) can thus be analysed using PIC and squared-change parsimony: natural logarithm of presacral length [$LN(PLg)$], natural logarithm of 10 × maximal section diameter [in mm; $LN(10 \times MD)$], global compactness (Cg), P based on global values (P), P based on radial values ($Prad$), $S^{0.5}$ and log [$Srad/(1 - Srad)$]. The random tree generation analysis in MESQUITE confirmed that all parameters, with the exception of log [$Srad/(1 - Srad)$] contain phylogenetic information (Table 2).

ECOLOGICAL SIGNAL

Ecological signal: complete data set

The forward selection procedure, implemented in Permute in a PVR analysis, retained the parameters S, P, Cc, and Cp as the most significant variables (ternary coding of the lifestyle; Table 3). Variance partitioning with PVR shows that these parameters exhibit an ecological signal ($P = 0.0001$; Table 4) and explain 42.88% of the lifestyle variance. An additional 19.64% is explained by the covariation between phenotype and phylogeny. The part of lifestyle variance explained by the phylogeny alone (0.07%) is not statistically significant ($P = 0.9760$). An important fraction (37.4%) remains unexplained by the variables studied (Fig. 4A). Using a binary coding changes these variance values little (Fig. 4B).

These first results suggest that bone compactness evolves with lifestyle. Aquatic taxa tend to have a smaller medullary cavity than their terrestrial and amphibious relatives (Table 5, column P). Furthermore, Cp, which reflects the compactness in the outermost cortex of the cross-section, has a lower value in aquatic taxa than in other (Table 5). Aquatic amniotes have higher values of parameter Cc than terrestrial animals (Table 5) because of the presence of a medullary spongiosa (Fig. 5), they are larger than terrestrial and amphibious taxa and have higher value of parameter S (the transition zone between the cortical compacta and the medulla is broadest in aquatic taxa; Table 5). The PIC analysis confirms that the parameter S is correlated with lifestyle (binary coding; Table 6).

Turtles are known to exhibit an atypical pattern in long bone microanatomy (Germain & Laurin, 2005). For example, turtles of all lifestyles show high tibial compactness (Kriloff *et al*., 2008). A similar pattern occurs in the humerus (Fig. 5 and Table 5). Moreover, turtles show a reverse trend for some characters by comparison with other amniotes. Thus, terrestrial species seem to be larger, to have stronger humeri, and to have broader transition zone (greater S) than aquatic species (Table 5). Lastly, this taxon presents no significant differences for Min and Cc between aquatic and terrestrial taxa, whereas other amniotes exhibit considerable variation (Fig. 5 and Table 5). Thus, we performed another variance partitioning analysis using PVR, excluding the turtles to estimate the relationship between humeral bone microanatomy and lifestyle in other amniotes.

Ecological signal: data excluding turtles

A new phylogenetic distance matrix was produced and only the first PC was selected by the brocken stick model. It represents 69.72% of the phylogenetic variance of the 65 species. The backward-elimination procedure retained S, P, Min, $Maxrad$, Cc, and Cp as the most significant variables (Table 3). These characters exhibit an ecological signal ($P = 0.0001$; Table 4), and they explain 81.6% of the lifestyle variance. The phylogeny by itself still does not explain habitat. Nevertheless, the fraction unexplained by the variables studied is reduced (17.3%; Fig. 4C, Table 4). Using a binary coding reduces further the unexplained variance to only 9.6% (Fig. 4D).

In this case, the PIC analysis suggests that body size (not retained by forward or backward-selection procedures) and S are significantly correlated with lifestyle (Table 6). These results confirm that aquatic

Table 1. Proportion of the observed variance in each character explained by the phylogeny and probability that this variance is unrelated to this phylogeny. To detect the phylogenetic signal, each compactness profile and body size character was considered as a dependent variable and the lifestyle and some principal coordinate axes (PCs) were considered as the independent variables

	Broken stick model		Backward-elimination procedures	
Dependent variable	Fraction related to phylogeny	*P* (rank)	Fraction related to phylogeny (identity of the PCs in parentheses)	*P* (rank)
Ln(*PLg*)	0.3418	**0.0001** (7–13)	0.4019 (PC5, PC6, PC10, PC16, PC18)	**0.0001** (7–13)
LN(10 × *MD*)	0.5253	**0.0001** (7–13)	0.2787 (PC2, PC10, PC18, PC32)	**0.0002** (14–15)
S	0.1299	**0.0061** (25)	0.2830 (PC2, PC18, PC 21, PC23, PC27, PC28, PC32)	**0.0009** (19)
P	0.2419	**0.0004** (17–18)	0.2975 (PC2, PC18, PC21, PC33)	**0.0001** (7–13)
Min	0.0183	0.5090 (35)	0.5862 (PC2, PC9, PC16, PC17, PC19, PC21, PC23, PC24, PC26, PC27, PC32, PC59)	**0.0003** (16)
Max	0.0285	0.3356 (33)	0.2896 (PC23, PC53)	**0.0127** (27)
Cg	0.2216	**0.0004** (17–18)	0.3050 (PC2, PC18, PC33, PC40)	**0.0001** (7–13)
Cc	0.0640	0.0790 (30)	0.4389 (PC2, PC9, PC17, PC19, PC21, PC24, PC26, PC27, PC32)	**0.0014** (21)
Cp	0.0728	0.0534 (29)	0.3060 (PC20, PC23, PC27, PC32)	**0.0010** (20)
Srad	0.0035	0.8926 (38)	0.5907 (PC23, PC26, PC68)	**0.0083** (26)
Prad	0.1700	**0.0017** (22)	0.3071 (PC2, PC18, PC33, PC36)	**0.0002** (14–15)
Minrad	0.0339	0.2787 (32)	0.6794 (PC2, PC9, PC17, PC19, PC21, PC22, PC23, PC24, PC26, PC27, PC32, PC40, PC41, PC49, PC59, PC66)	**0.0001** (7–13)
Maxrad	0.0176	0.5158 (36)	0.4735 (PC23, PC53, PC57)	**0.0277** (28)
LN[*Srad*/(1 – *Srad*)]	0.0195	0.4786 (34)	0.4808 (PC14, PC23, PC26, PC49, PC59, PC66, PC68)	**0.0001** (7–13)
$S^{0.5}$	0.1364	**0.0040 (24)**	0.2500 (PC2, PC18, PC23, PC27, PC28, PC32)	**0.0019** (23)

We used a broken stick model, which retained PCs 1 and 2, and backward-elimination procedures to determine which PCs contributed significantly to the explanation of each parameter. *P*-values marked in bold are significant according to the false discovery rate analysis (see Supporting Information, Appendix S6); their rank order of significance is given in parentheses after the probabilities.

Table 2. Phylogenetic signal assessed through squared-change parsimony and a population of 10000 random trees produced by taxon reshuffling

Character	*P*-value
***LN*(*PLg*)**	**0.0000** (1–6)
***LN*(10 × *MD*)**	**0.0000** (1–6)
Cg	**0.0000** (1–6)
***S*$^{0.5}$**	**0.0000** (1–6)
P	**0.0000** (1–6)
Prad	**0.0000** (1–6)
***Log* [*Srad*/(1 – *Srad*)]**	0.1603 (31)

P-values marked in bold are significant according to the false discovery rate analysis (see Supporting Information, Appendix S6); they are followed by increasing probability rank (in parentheses)

amniotes are generally larger than their most terrestrial relatives.

All results that were initially statistically significant remain significant after applying FDR analyses (see Supporting Information, Appendix S6).

LINEAR DISCRIMINANT ANALYSIS

The discriminant function based on the data set of amniotes without turtles and the characters selected in the variance partitioning method and backward-elimination (*S*, *P*, *Min*, *Maxrad*, *Cc*, and *Cp*; Table 3) has a success rate of 90.8% with a ternary coding of lifestyle (see Supporting Information, Appendix S7A; Fig. 6). The lifestyle of 90% of the aquatic taxa and 97.8% of the terrestrial ones is correctly inferred. The errors are not randomly distributed and affect only adjacent states. The graphic representation of this discriminant function shows that the aquatic taxa are well separated from the amphibious and terrestrial ones (Fig. 6). However, taxa of amphibious and terrestrial lifestyles overlap partly.

Linear, nonphylogenetic regressions with permutations to test the significance show that aquatic taxa are well differentiated from the others on the basis of bone microanatomy, but there is less clear segregation between amphibious and terrestrial taxa (Table 7). In most cases, the proportion of explained variance is maximal when amphibious taxa are coded more than 1.2, with a maximum located near 2 (Fig. 7). This suggests that the amphibious taxa are very similar to terrestrial taxa as regards bone microanatomy. Thus, a binary coding of habitat was also performed (0 = aquatic; 2 = amphibious to terrestrial); the discriminant function, based on parameters selected in PVR, correctly attributed the lifestyle of 98.5% of the species (Fig. 6; see also Supporting Information, Appendix S7A). The error concerns only *Ornithorhynchus anatinus* (Shaw, 1799) which is inferred as amphibious to terrestrial, although it may actually be considered amphibious because it retains significant terrestrial locomotor capabilities, and was scored as such by Germain & Laurin (2005).

In a second model (for inferences on hypothetical ancestors), the parameters with adequate PIC standardization on our tree and with a phylogenetic signal [*LN*(*PLg*)], *LN*(10 × *MD*), *Cg*, *P*, *Prad*, *S*$^{0.5}$ and log [*Srad*/(1 – *Srad*)] are included as independent variables and the complete data set of amniotes is considered. When the lifestyle is coded as a ternary character, 77.3% of the lifestyles are correctly inferred. When the lifestyle is coded as a binary character, 85.3% of the lifestyles are correctly inferred. Nevertheless, errors are not only on adjacent states (see Supporting Information, Appendix S7B).

LIFESTYLE INFERENCE FOR EXTINCT TAXA

Two linear discriminant models based on the sample of amniotes without turtles (65 species) and including the parameters *S*, *P*, *Min*, *Maxrad*, *Cc*, and *Cp* have been used: ternary coding (Table 8, model A) and binary coding (Table 8, model B). The results of both models reveal interesting differences compared to the prevailing interpretations in the literature. Thus, the Early Permian *Captorhinus*, the Late Permian *Claudiosaurus*, and the Triassic *Placodus* are inferred to have been amphibious, whereas the literature suggests a terrestrial lifestyle for *Captorhinus*, and an aquatic lifestyle for *Claudiosaurus* and *Placodus*. The Late Permian and Early Triassic *Lystrosaurus*, the Early Permian *Mesosaurus*, as well as the Triassic *Neusticosaurus*, are all inferred to have been aquatic. For *Neusticosaurus* and *Mesosaurus*, there is a perfect congruence with the literature, whereas the habitat of *Lystrosaurus* has been controversial so far.

EVOLUTION OF HABITAT ACCORDING TO TAXA OF KNOWN LIFESTYLE

Optimization of observed lifestyle as an ordered character using standard parsimony on the reference phylogeny in MESQUITE (Fig. 3) reveals ambiguity for the ancestral character state on the branchs leading to Mammalia (aquatic, amphibious or terrestrial) and Neodiapsida (aquatic or amphibious). The ancestral lifestyle of crown turtles appears to be aquatic. Lastly, the ancestral lifestyle of amniotes remains ambiguous (aquatic, amphibious or terrestrial). These results show the necessity of extensive taxonomic sampling for optimizations. Indeed, palaeontological data suggest very clearly that the mammalian and neodiapsid ancestral lifestyle was terrestrial (Carroll, 1988).

Table 3. Characters that reflect the lifestyle (ternary coding) identified through a selection procedure in PERMUTE

	Removed from the backward-elimination analysis		Selected in the foreward selection analysis	
Parameters	With all the data set of amniotes	With the exclusion of turtles	With all the data set of amniotes	With the exclusion of turtles
Ln(*PLg*)	9	6		
Ln(10 × *MD*)	10	3		
S	**0.001** (vi–xix)	**0.001** (vi–xix)	**1 (0.001** (vi–xix)**)**	
P	4	**0.001** (vi–xix)	**4 (0.007 (xxi))**	**3 (0.001** (vi–xix)**)**
Min	7	**0.001** (vi–xix)		
Max	5	1		
Cg	1	8		
Cc	**0.001** (vi–xix)	**0.002 (xx)**	**2 (0.001** (vi–xix)**)**	
Cp	**0.009 (xxii-xxiiii)**	**0.001** (vi–xix)	**3 (0.009 (xxii-xxiii))**	**1 (0.001** (vi–xix)**)**
Srad	6	2		
Prad	12	4		
Minrad	2	7		**2 (0.001** (vi–xix)**)**
Maxrad	11	**0.001** (vi–xix)		**4 (0.001** (vi–xix)**)**
PC 1	8	5		
PC 2	3	–		

Numbers in bold indicate the step at which the parameter has been removed from the backward-elimination analysis or selected in the foreward selection analysis. The probability that the variation of characters is random with respect to lifestyle is obtained by multiple linear regressions (with permutations of the dependent variable to create a null distribution) for lifestyle as the dependent variable with a backward-elimination procedure. *P*-values marked in bold are significant according to the false discovery rate analysis (see Supporting Information, Appendix S6).

Table 4. Proportion of the habitat variance explained by the phenotype and by the phylogeny

	Performed on all amniote data*				Performed with the exclusion of turtles†			
	Ternary coding		Binary coding		Ternary coding		Binary coding	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
A	0.4288	**0.0001 (i–iii)**	0.4257	**0.0001 (i–iii)**	0.8159	**0.0001 (i–iii)**	0.8958	**0.0001 (i–iii)**
B	0.1964	–	0.2318	–	0.0108	–	0.0082	–
C	0.0007	0.9760 (39)	0.01121	0.6583 (37)	0	1.0000 (41)	5.36E–6	0.9846 (40)
D	0.3741	–	0.3312	–	0.1733	–	0.0959	–

*Performed on all amniote data with the habitat as the dependent (ternary or binary) variable and *S*, *P*, *Cc*, *Cp*, and the principal coordinate axis 1 and 2 as independent variables. †Performed with the exclusion of turtles and with the habitat as the dependent (ternary or binary) variable and *S*, *P*, *Min*, *Maxrad*, *Cc*, *Cp*, and the principal coordinate axis 1 as independent variables. In both cases, a broken stick model was used to select principal coordinate axes. *P*-values marked in bold are significant according to the false discovery rate analysis (see Supporting Information, Appendix S6). A, portion of the explained variance related to phenotype; B, related to phenotype and phylogeny; C, related to phylogeny; D, residual (unexplained).

EVOLUTION OF HABITAT INCORPORATING LIFESTYLE INFERENCES OF EXTINCT TAXA

Addition of extinct taxa resolves better the optimization of lifestyle (Fig. 8). The ancestral character states of Mammalia and Neodiapsida appear to be terrestrial and amphibious, respectively. Moreover, the ancestral lifestyle of Amniota (amphibious or terrestrial) is less ambiguous.

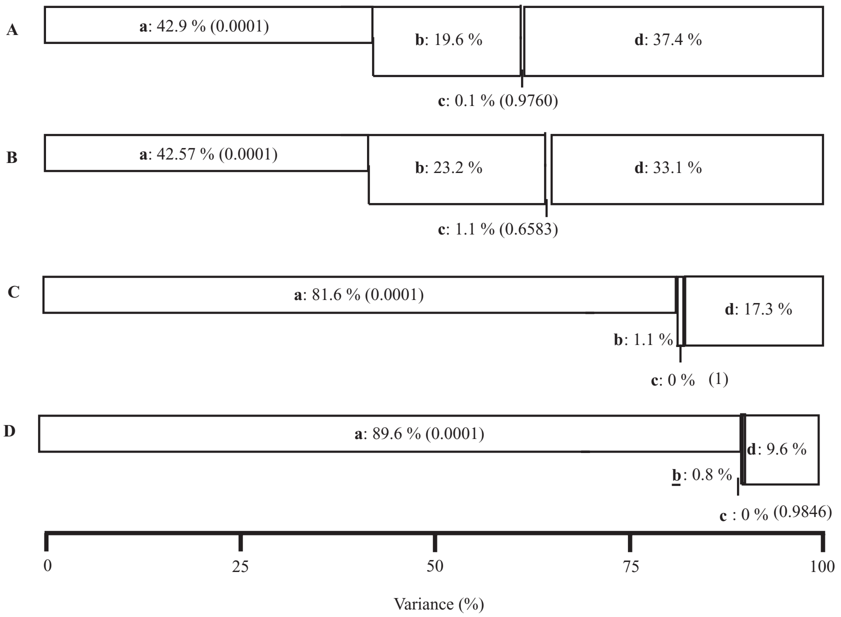

Figure 4. Portion of variance of the lifestyle (A, C, ternary coding; B, D, binary coding) explained by phenotypic parameters, the phylogeny, both, or neither, as established by a variance partition with PVR (Desdevises *et al.*, 2003) on the whole dataset (A, B) or without turtles (C, D). a: fraction linked only to phenotype; b: fraction linked both to phenotype and phylogeny; c: fraction linked only to phylogeny; d: fraction of unexplained variance. The proportion of variance follows the letter which identifies each fraction (a–d), followed (when this can be computed) by the associated probability in parentheses.

Table 5. Mean character value of humeral cross-sections as a function of lifestyle in: the total sample; the total sample with the exclusion of turtles; and turtles only

	LN(*PLg*)	*LN*(10 × *MD*)	*S*	*P*	*Min*	*Max*	*Maxrad*	*Cg*	*Cc*	*Cp*
	Total sample									
0	3.9343	4.8378	0.0893	0.4686	0.2554	0.9521	0.9418	0.7396	0.2984	0.9158
1	3.3454	4.0502	0.0517	0.4571	0.0053	0.9899	0.9771	0.7632	0.0090	0.9891
2	3.4775	4.4511	0.0350	0.5891	0.0268	0.9932	0.9858	0.6316	0.0306	0.9916
	Total sample excluding turtles									
0	4.9353	5.9064	0.0875	0.6286	0.3220	0.9215	0.9275	0.6000	0.3249	0.8537
1	3.3454	4.0502	0.0517	0.4571	0.0053	0.9899	0.9771	0.7632	0.0090	0.9891
2	3.5041	4.4291	0.0298	0.6076	0.0083	0.9943	0.9865	0.6141	0.0095	0.9931
	Turtles only									
0	2.8221	3.6504	0.0912	0.2909	0.1815	0.9861	0.9577	0.8946	0.2689	0.9848
2	3.1710	4.7040	0.0948	0.3766	0.2397	0.9804	0.9784	0.8333	0.2734	0.9744

Turtles are shown separately because they exhibit an atypical tendency. For lifestyle, 0 = aquatic, 1 = amphibious, 2 = terrestrial.

ANCESTRAL VALUE RECONSTRUCTION AND INFERENCES OF HYPOTHETICAL ANCESTOR LIFESTYLE

In most cases, adding extinct taxa on the reference phylogeny allows to reduce the width of the 95% CIs of the nodal estimates of characters for Amniota, Reptilia, and Mammalia (see Supporting Information, Appendix S8). Inferences of the lifestyle of hypothetical ancestors based on these estimated values (see Supporting Information, Appendix S9) was performed using two inference models (based on a ternary or a binary coding of lifestyle) and two taxonomic samples for ancestral value inference (including or not extinct taxa). These inferences are

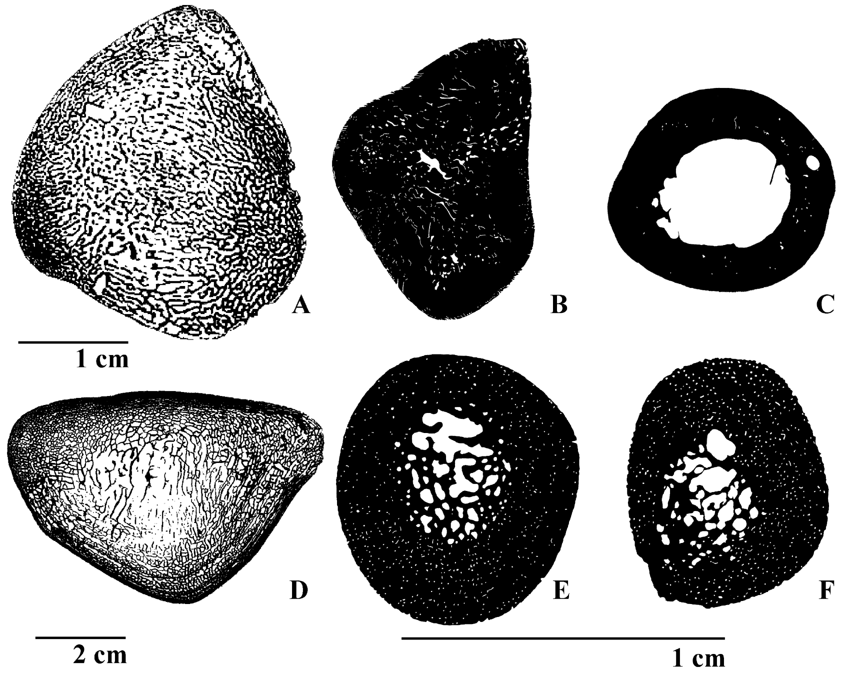

Figure 5. Drawings of mid-diaphyseal cross-sections of humeri of tetrapods of various lifestyles showing typical microstructural adaptations (A–E) or a microstructure atypical of a terrestrial tetrapod (F). A, an actively fast swimming mammal, *Delphinus delphis*. B, a shallow-water aquatic mammal, *Dugong dugong*, with very compact bone. C, a terrestrial mammal, *Canis lupus*, with a large medullary cavity and a moderately thick cortical compacta (scale bar = 1 cm). D, another fast swimming and actively deep-diving mammal, *Mirounga leonina* (scale bar = 2 cm). E, a shallow-water aquatic turtle, *Chelydra serpentina*, with a compact bone and a spongiosa in the medullary region. F, a terrestrial turtle, *Geochelone carbonaria*, with a still great compactness and no free medullary cavity, atypical of terrestrial taxa (scale bar = 1 cm).

Table 6. Relationship between lifestyle (binary coding) and phenotypic characters (body size and bone microanatomical characters adequately standardized on the tree) assessed through phylogenetically independent contrast analysis (Felsenstein, 1985)

Lifestyle (binary coding) versus:	With all the data set of amniotes			With the exclusion of turtles		
	Slope	*P*	Rank	Slope	*P*	Rank
LN(*PLg*)	–0.97812	0.0949	(xxvii)	–1.24416	**0.0255**	(xxv)
LN(10 × *MD*)	0.93880	0.0852	(xxvi)	–1.29782	**0.0133**	(xxiv)
Cg	–0.01765	0.8235	(xxxi)	–0.00396	0.9917	(xxxv)
$S^{0.5}$	–0.10254	**0.001**	(vi–xix)	–0.12045	**0.0001**	(i–v)
P	0.02003	0.9281	(xxxiii)	–0.00085	0.94	(xxxiv)
Log(*Srad* / 1 – *Srad*)	–0.51357	0.5896	(xxix)	–0.58646	0.1229	(xxviii)
Prad	0.04975	0.7453	(xxx)	0.01113	0.9275	(xxxii)

P-values marked in bold are significant according to the false discovery rate analysis (see Supporting Information, Appendix S6).

coherent when using the nodal estimates: Amniota, Reptilia, and Mammalia are inferred to have been amphibious to terrestrial by the binary models. The results obtained are more variable when using character values which represent the extreme values of the 95% CIs of the nodal estimates; although Mammalia is always inferred amphibious to terrestrial that way, one of the twelve estimates (four models times three values, reflecting the point estimate and both ends of the CI) each for Amniota and

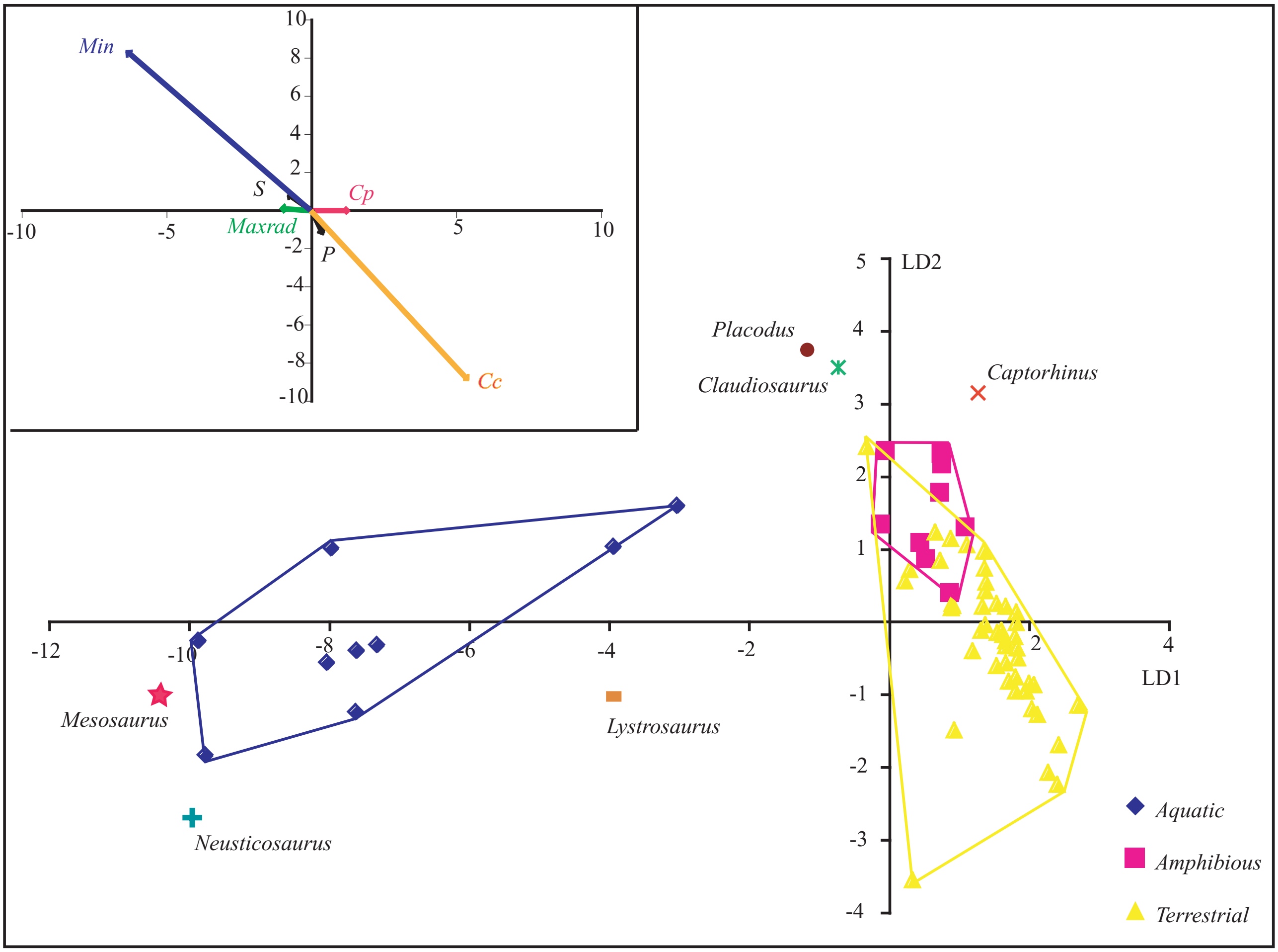

Figure 6. Distribution of taxa of various lifestyles on the first (LD1) and second (LD2) linear discriminant axes. The variables (*S*, *P*, *Min*, *Cp*, *Cc*, and *MD*) were selected by a backward-elimination procedure in Permute, and the discriminant function was obtained from STATISTICA. The polygons represent the limits of the position of taxa of various known lifestyles. The position of six extinct amniotes of uncertain lifestyle (*Mesosaurus*, *Captorhinus*, *Claudiosaurus*, *Placodus*, *Neusticosaurus*, and *Lystrosaurus*) is shown.

Table 7. Proportion of habitat variance explained by phenotypic characters (*S*, *P*, *Min*, *Maxrad*, *Cc*, and *Cp*) as a function of habitat coding

Habitats included	R^2	*P*
0, 1	0.8787	0.0002
0, 2	0.9122	0.0001
1, 2	0.3627	0.0036

Because the variance is assessed through linear regressions but the three states do not form a cline, states are compared two by two (with the exclusion of turtles). 0, aquatic; 1, amphibious; 2, terrestrial.

Reptilia yields an aquatic lifestyle, which is incongruent with other inferences.

DISCUSSION

Phylogenetic signal

The phylogenetic value of bone histological characters has long been debated (Castanet, Cubo & de Margerie, 2001; Cubo, de Margerie & Castanet, 2001; Padian, de Ricqlès & Horner, 2001). Recent studies suggest that bone histological and microanatomical characters can supply both functional and phylogenetic information and some studies have attempted to quantify these signals (Laurin *et al.*, 2004; Cubo *et al.*, 2005; Germain & Laurin, 2005; Cubo *et al.*, 2008; Kriloff *et al.*, 2008), and the results obtained in the present study confirm this. The humerus exhibits

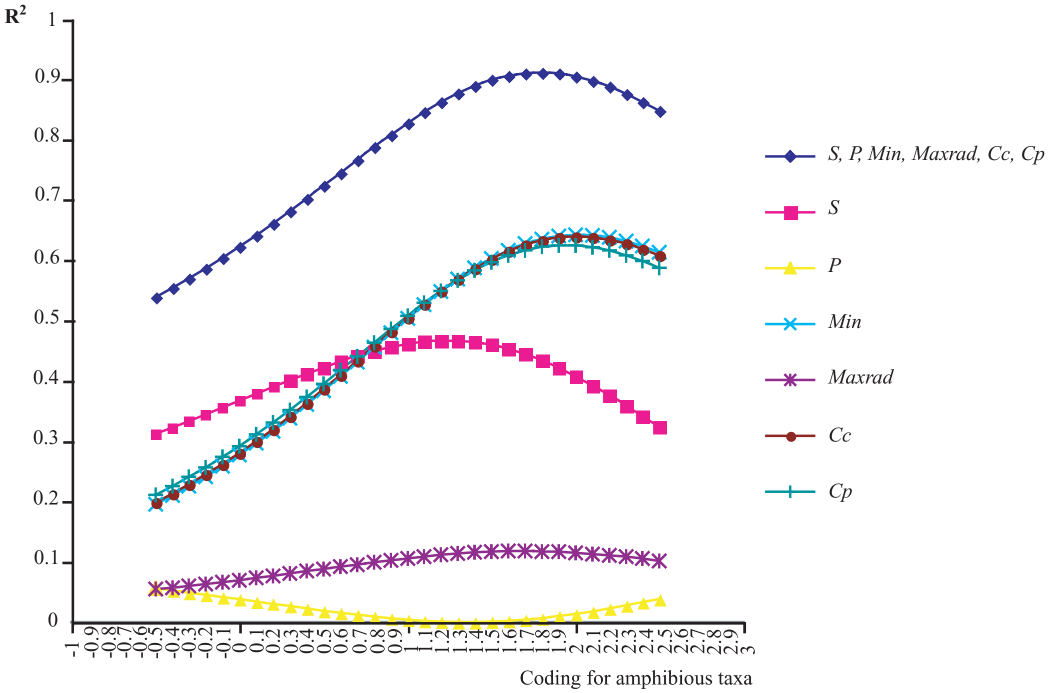

Figure 7. Proportion of the observed variance (R^2) in the lifestyle (ternary coding) explained by body size and compactness profile data (taken together or separately) as a function of the coding of lifestyle of amphibious taxa (turtles excluded).

Table 8. Probability of inferred lifestyle for the extinct taxa according to both models based on 65 species

	Discriminant function A		Discriminant function B			
Taxon	Inferred lifestyle	Probability	Inferred lifestyle	Probability	Interpretations in the literature	Conclusion (present study)
Captorhinus	Am	0.9296	Am–T	1	T	Am
Claudiosaurus	Am	0.9936	Am–T	1	Aq	Am
Lystrosaurus	Aq	0.9999	Aq	1	Aq/Am/T	Am
Neusticosaurus	Aq	1	Aq	1	Aq/Am	Aq
Placodus	Am	0.9974	Am–T	1	Aq	Am
Mesosaurus	Aq	1	Aq	1	Aq	Aq

Discriminant function A: based on the data set of amniotes without turtles and including the parameters selected by a backward-elimination procedure in STATISTICA (*S*, *P*, *Min*, *Maxrad*, *Cc*, *Cp*; ternary coding); Discriminant function B: binary coding. Aq, aquatic; Am, amphibious; T, terrestrial.

a phylogenetic signal in more parameters than the radius of amniotes (Germain & Laurin, 2005) and the tibia of tetrapods (Kriloff *et al*., 2008); however, these differences may result from the denser taxonomic sampling in the present study.

BONE MICROANATOMY AND LIFESTYLE

The findings obtained in the present study are congruent with those of previous studies on this topic (Germain & Laurin, 2005; Kriloff *et al*., 2008). In amniote long bones (humerus, radius, and tibia), characters *P*, *S*, and *Min* exhibit an ecological signal. In the analyses based, respectively, on the femur (Laurin *et al*., 2004, 2009) and the humerus (Canoville & Laurin, 2009) of lissamphibians, characters *S* and *Min* did not exhibit statistically significant habitat-dependent variations. This may result from the relatively simpler structure of the long bones of small animals.

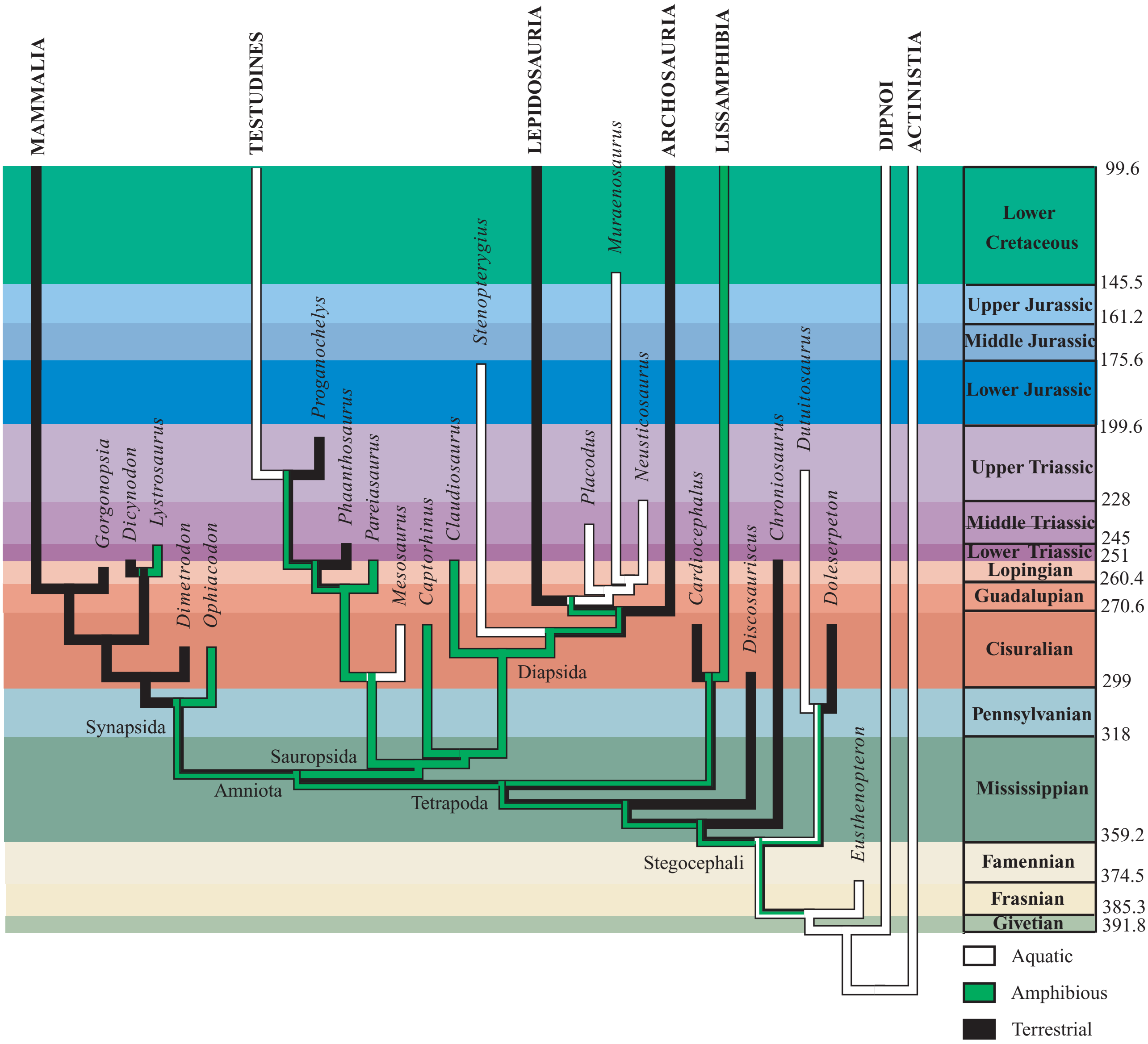

Figure 8. Optimization of the lifestyle (considered as an ordered character) on a phylogeny including extinct taxa whose lifestyle was inferred using microanatomical data (Steyer *et al.*, 2004) and a discriminant function (present study; Germain & Laurin, 2005; Kriloff *et al.*, 2008) or a phylogenetically weighted logistic regression (Laurin *et al.*, 2004). Habitat colours as in Figure 3 (aquatic, white; amphibious, green; terrestrial, black).

The humerus displays an ecological signal in more characters than in other limb bones. These humeral compactness profile and body size data contain a strong ecological signal because they explain 42.9% of the lifestyle variance (ternary coding, turtles included) when covariation with phylogeny excluded (62.5% when covariation with the phylogeny is included). This is by far greater than the results from the tibia, which explains only approximately 20% of the lifestyle variance (Kriloff *et al.*, 2008) when phylogenetic covariation is excluded. Using a binary coding of lifestyle yields higher values of explained variance (Table 4). The present study is also the first one to found a significant ecological signal in bone microanatomical data using PIC; Laurin *et al.* (2009) find a significant ecological signal only in body size, whereas no ecological signal was yielded by PIC analyses on our other datasets (Laurin *et al.*, 2004; Germain & Laurin, 2005; Kriloff *et al.*, 2008). Thus, the humerus appears ideally suited for lifestyle inference.

The atypical testudine pattern had already been observed by Germain & Laurin (2005) and Kriloff *et al.* (2008) in the radius and the tibia. This peculiarity may be linked to biomechanical constraints as a result of their singular bodyplan with a carapace, or a clade effect. In some turtles, adjustments in body density may occur through changes in the carapace rather than in the long bones, as recently suggested by Scheyer & Sander (2007) and Kriloff *et al*. (2008). However, the fact that, in this clade, terrestrial species seem to be larger than their aquatic relatives is most probably a result of the small size of our sample. Indeed, the largest known turtles are aquatic: *Stupendemys geographicus* Wood, 1976 inhabited in fresh water (de Lapparent de Broin, Bocquentin & Negri, 1993; Scheyer & Sánchez-Villagra, 2007) and *Archelon ischyros* Wieland, 1896 was a marine protostegid turtle (Scheyer & Sánchez-Villagra, 2007).

PERFORMANCE OF INFERENCE MODELS

The data obtained in the present study, similar to those of Laurin *et al*. (2004), Germain & Laurin (2005), Kriloff *et al*. (2008) Canoville & Laurin (2009), and Laurin *et al*. (2009), discriminate the aquatic lifestyle from the others. The models do not segregate nearly as well amphibious taxa from terrestrial taxa. The distribution of phenotypic characters of amniotes of these two lifestyles differs, although there is an overlap. In our most efficient discriminant model (Fig. 6; see also Supporting Information, Appendix S10), the first discriminant axis (LD1) perfectly segregates aquatic taxa from amphibious and terrestrial taxa on the basis of *S*, *Cp*, *Maxrad*, *Min*, and *Cc*. The second discriminant axis (LD2) attempts to differenciate amphibious taxa from terrestrial taxa on the basis of parameters *S*, *P*, Cc, and *Min*. As in Kriloff *et al*. (2008), errors of inferences based on variables selected by a PVR analysis affect more particularly those amphibious mammals (rodents and mustelids) that live close to the water and swim at the surface. Thus, the semi-aquatic mammals, such as *Galemys pyrenaicus* (E. Geoffroy Saint-Hilaire, 1811) (Pyrenean desman) and *Hydrochoerus hydrochaeris* (Linnaeus, 1766) (capybara) are excellent swimmers, and live in rivers, freshwater lakes or swamps. Even though they spend a lot of time in water, their surface-swimming behaviour means that pachyostosis would be disadvantageous for them. Thus, their amphibious lifestyle requires no microanatomical adaptations.

The success rates of the discriminant analyses carried on the humerus of amniotes (up to 90.8%, using a ternary coding and 98.5%, using a binary coding) are higher than those obtained from the radius and the tibia (71% and 68%, respectively; Germain & Laurin, 2005; Kriloff *et al*., 2008). Moreover, the binary discriminant model established with humeral compactness profile parameters of lissamphibians (Canoville & Laurin, 2009) is more powerful than the models of Laurin *et al*. (2004, 2009) that are based on the femur. Thus, this set of analyses suggests that the humerus contains more ecological signal than other long bones. These results are congruent with what we expected based on the decreasing proximo-distal gradient of compactness in sea cows (de Buffrénil & Schoevaert, 1989). The hindlimb appears to be slightly less informative than the forelimb, possibly because some aquatic taxa have lost the hindlimb (sirenians, sirenids) or retain only vestiges that are not functional in locomotion (cetaceans). Thus, these taxa cannot be incorporated into the analyses of tibial and femoral compactness profiles.

Finally, a certain proportion of error is inevitable because many factors influence bone microanatomy (Castanet *et al*., 2001). These include phylogenetic position (Cubo *et al*., 2005, Germain & Laurin 2005; Laurin *et al*., 2004), biomechanical constraints (de Margerie *et al*., 2005; Meers, 2002), ontogeny, pathology (Castanet *et al*., 1993; Castanet *et al*., 2001), metabolism (de Ricqlès, 1983) or reproductive status (de Buffrénil & Francillon-Vieillot, 2001).

Our new method of direct inference of hypothetical ancestors suggests that Amniota, Reptilia, and Mammalia were primitively amphibious to terrestrial. There remains significant uncertainty associated with these inferences because at least some values encompassed by the 95% confidence intervals of microanatomical character lead to an aquatic ancestral lifestyle inference for Amniota and Reptilia. Gathering additional data on early stegocephalians may narrow these confidence intervals and lead to lower lifestyle uncertainty.

Inference models based on the characters selected in PVR appear to be reliable (Table 8; see also Supporting Information, Appendix S7A). To make them accessible, spreadsheets allowing lifestyle inference solely from bone compactness parameters were produced (see Supporting Information, Appendix S10). However, because these models include some characters that could not be standardized adequately on our trees, they should not be applied to hypothetical taxa reconstructed through inferred nodal values on our tree.

PALAEOBIOLOGICAL INFERENCES

The lifestyle of *Placodus* is inferred as amphibious or amphibious/terrestrial according to the various models. Along LD1, *Placodus* is intermediate between aquatic and amphibious taxa, but closer to the latter. Its value of *Cp* (0.834) is reminiscent of aquatic taxa (Table 5) but low values of parameters *S*, and *Cc*, which reflect the presence of a narrow transition zone and a small but distinct medullary cavity (see Supporting Information, Appendix S2D) place it close to amphibious and terrestrial tetrapods. According to LD2, *Placodus* is closer to amphibious taxa than terrestrial ones because of low value of *P* (the small size of its medullary cavity). Placodont remains are known mainly from shallow-water marine sediments of Middle to Late Triassic age in Europe and the Middle East (Sues, 1987) and are usually considered mostly aquatic (Rieppel, 1995). The position of *Placodus*, well outside the three lifestyle clusters (Fig. 6), indicates that our inference must be interpreted cautiously. Nevertheless, Carroll (1988) stated that the limb skeleton shows only moderate specialization into aquatic paddles and reproduced Peyer's (1950) illustration of *Placodus* in a walking pose. This apparent contradiction in the various signals could be reconciled if *Placodus* fed partly on the abundant mollusk, echinoderm, and arthropod fauna found in intertidal zones and retained at least limited terrestrial locomotor abilities. Thus, for optimization purposes (Fig. 8), we consider *Placodus* amphibious, even though it must have been among the most aquatic of the taxa considered amphibious in the present study.

The early diapsid *Claudiosaurus* is inferred to have been amphibious or amphibious/terrestrial. Its position on the graphic representation (Fig. 6) is outside the three lifestyle clusters, but clearly closer to amphibious and terrestrial lifestyles than to the aquatic one for the same reasons as *Placodus*; it also has a small medullary cavity (Fig. 2C). Previous studies attributed a mostly aquatic lifestyle to it (Carroll, 1981), and de Buffrénil & Mazin (1989) showed the presence of an extensive pachyostosis, which is characteristic of an aquatic lifestyle. Nevertheless, Carroll (1981) noted that *Claudiosaurus* probably still possessed terrestrial locomotor abilities, and that it closely ressembles terrestrial diapsids in various skeletal features. For character optimization, we consider it amphibious rather than truly aquatic.

Our inference of an amphibious (ternary coding) or amphibious/terrestrial (binary coding) for *Captorhinus* contrasts with the terrestrial lifestyle generally attributed to it (Olson, 1954; Modesto, 1998). The biocoenosis of Fort Sill, where *Captorhinus* is preserved, consisted primarily of terrestrial to amphibious amniotes, amphibians, temnospondyls, and seymouriamorphs (Kissel, Dilkes & Reisz, 2002). *Captorhinus* is relatively close to taxa of amphibious lifestyle on linear discriminant axes 1 and 2 (Fig. 6) because, similar to *Placodus* and *Claudiosaurus*, it has a small medullary cavity (Fig. 2A), and, we consider it amphibious for the preliminary optimization of habitat evolution. This assignment is compatible with the abundance of this taxon in Fort Sill because the presence, in the same locality, of temnospondyls and seymouriamorphs that had aquatic larvae with external gills (Boy & Sues, 2000; Laurin, 2000) suggests the proximity of aquatic environments. Furthermore, captorhinids are also known from several other localities, in which the aquatic influence was apparently much greater. For example, *Protocaptorhinus pricei* Clark & Carroll, 1973 occurs in the Admiral formation, along with the presumably aquatic to amphibious embolomere *Archeria* Case, 1918. Thus, captorhinids may not have been strictly terrestrial.

The diapsid *Neusticosaurus edwardsii* is considered to have lived in coastal surface waters, preying on small animals (Rieppel, 1989; Sander, 1989). Most fossils have been found in sediments deposited in quiet, marine water, but some isolated *Neusticosaurus* bones have been found together with potential indicators of near-shore conditions such as remains of aquatic temnospondyls (Sander, 1989). These animals exhibit relatively subtle aquatic adaptations such as the loss of ossified tarsal and carpal elements, the ventral expansion of the girdles, the laterally expanded tail, the flattening of fore- and hindlimbs and pachyostosis. They present an isodont dentition that is typical for piscivorous animals. Nevertheless, these sauropterygians still had a terrestrial middle-ear structure, and some studies have suggested that they were able to leave the water in order to breed and bask (Carroll & Gaskill, 1985). Germain & Laurin (2005) assigned them an amphibious lifestyle on the basis of radial compactness profile parameters. LD1 and our inference model suggest a truly aquatic lifestyle for *Neusticosaurus* (Fig. 6) because of its broad transition zone (high *S*) and great value of *Min* (Fig. 2B). This result, which is congruent with the most supported hypothesis, shows the reliability of our inference model. The present study provides an independent line of evidence about its habitat and is congruent with previous interpretations of a fully aquatic lifestyle. For optimization purposes, we consider *Neusticosaurus* predominantly aquatic (Fig. 8).

The lifestyle of the Permo-Triassic therapsid *Lystrosaurus* has long been debated. It was said to be aquatic, terrestrial or burrowing (King, 1991; King & Cluver, 1991; Ray *et al*., 2005). Specimens of *Lystrosaurus* have been reported from South Africa, India, Antarctica, China, Russia, and possibly Australia (King & Jenkins, 1997). In the South African Permo-Triassic Karoo Basin, this taxon was so abundant that it is used as a stratigraphic marker. LD1 yields an aquatic habitat for *Lystrosaurus* (Fig. 6) because of the broad transition zone (high *S*-value) and porous cortex (low *Cp*; Fig. 2E). For character optimization, to be coherent with previous interpretations and because of its morphology, we consider this genus amphibious. Microanatomy of the radius (Germain &

Laurin, 2005) and of several other bones (Ray *et al.*, 2005) also suggests an amphibious lifestyle. Such a lifestyle would explain the great number of its fossils by taphonomic processes: throughout geological history, the fossil record has always shown a bias toward aquatic animals. Moreover, Battail (1997) noted that *Lystrosaurus georgi* Kalandadze, 1975 was found in association with ostracods and branchiopods.

Finally, *Mesosaurus* is considered to have lived in water, filter-feeding on small, nektonic prey (Chiappe & Chinsamy, 1996; Modesto, 2006). Most fossils have been found in association with actinopterygians and crustaceans and exhibit many aquatic adaptations (Modesto, 2006). Although mesosaurs are always considered to have spent much time in water, there is some uncertainty about their lifestyle since Piñeiro (2008: 185) indicated that they were aquatic or semi-aquatic. LD1 and our inference model suggest a truly aquatic lifestyle for *Mesosaurus* (Fig. 6) because of its great value of *Min* (Fig. 2D).

PRELIMINARY OPTIMIZATION OF HABITAT EVOLUTION

Our preliminary optimization is generally congruent with the results obtained by Kriloff *et al*. (2008). One exception concerns early amniote evolution; our new results raise the possibility that Romer (1957, 1958) was correct in arguing for a primitive retention of an amphibious lifestyle in the earliest amniotes.

In the present study, we propose an alternative method to infer the lifestyle of hypothetical ancestors instead of terminal taxa. This method has the advantage of optimizing continuous characters only. However, it has the disadvantage of requiring standardizing the contrasts. Thus, some characters cannot be optimized on the reference phylogeny. Using our dataset, this decreased the performance of inference models on terminal taxa somewhat, but not necessarily the reliability of inferences on hypothetical taxa, which is more difficult to assess. Other datasets could yield better results.

ACKNOWLEDGEMENTS

All persons and institutions who have lent or given specimens or histological sections for this study are thanked. This includes J. Castanet (Université Paris 6), V. de Buffrénil (Université Paris 6), A. de Ricqlès (Collège de France), J.-S. Steyer (Muséum National d'Histoire Naturelle), E. Pellée, S. Godfrey, and the National Museum of Vienna. Furthermore, the authors are very grateful to M.-M. Loth, H. Lamrous, and M. Lemoine for the histological preparations and are indebted to D. Germain for his advice. We thank D. Marjanović and three anonymous referees for comments on an earlier draft of this paper, and the editor, J. A. Allen, for his efficient handling of the draft. This research was financed by the CNRS (UMR 7207) and two SYNTHESYS grants (AT-TAF-628 and 1437) to M. L.

REFERENCES

Bartlett MS. 1947. The use of transformations. *Biometrics* **3:** 39–52.

Battail B. 1997. Les genres *Dicynodon* et *Lystrosaurus* (Therapsida, Dicynodontia) en Eurasie: une mise au point. *GeoBios* **20:** 39–48.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B (Methodological)* **57:** 289–300.

Benton MJ. 2005. *Vertebrate Palaeontology*. Oxford: Blackwell Publishing.

Bininda-Emonds ORP. 2004. The evolution of supertrees. *Trends in Ecology and Evolution* **19:** 315–322.

Bininda-Emonds ORP, Gittleman JL, Kelly CK. 2001. Flippers versus feet: comparative trends in aquatic and non-aquatic carnivores. *Journal of Animal Ecology* **70:** 386–400.

Bininda-Emonds ORP, Jones KE, Price SA, Grenyer R, Cardillo M, Habib M, Purvis A, Gittleman J. 2003. Supertrees are a necessary not-so-evil: a comment on Gatesy *et al*. *Systematic Biology* **52:** 724–729.

Box GEP, Cox DR. 1964. An analysis of transformations. *Journal of the Royal Statistical Society, Series B* **26:** 211–252.

Boy JA, Sues HD. 2000. Branchiosaurs: larvae, metamorphosis and heterochrony in temnospondyls and seymouriamorphs. In: Heatwole H, Carroll RL, eds. *Amphibian Biology*. Chipping Norton: Surrey Beatty & Sons, 1150–1197.

Broom R. 1903. On the structure of the shoulder girdle in *Lystrosaurus*. *Annals of South African Museum* **4:** 139–141.

Canoville A, Laurin M. 2009. Microanatomical diversity of the humerus and lifestyle in lissamphibians. *Acta Zoologica* **90:** 110–122.

Carroll RL. 1964. The earliest reptiles. *Zoological Journal of the Linnean Society* **45:** 61–83.

Carroll RL. 1969. Origin of reptiles. In: Gans C, Bellairs AA, Parsons TS, eds. *Morphology A*. London: Academic Press, 1–44.

Carroll RL. 1981. Plesiosaur ancestors from the Upper Permian of Madagascar. *Philosophical Transactions of the Royal Society of London, Series B* **293:** 315–383.

Carroll RL. 1982. Early evolution of reptiles. *Annual Review of Ecology and Systematics* **13:** 87–109.

Carroll RL. 1985. Evolutionary constraints in aquatic diapsid reptiles. *Palaeontology* **33:** 145–155.

Carroll RL. 1988. *Vertebrate Paleontology and Evolution*. New York, NY: WH Freeman and Company.

Carroll RL, Gaskill P. 1985. The nothosaur *Pachypleurosaurus* and the origin of plesiosaurs. *Philosophical*

Transactions of the Royal Society of London, Series B **309:** 343–393.

Casgrain P. 2005. Permute! Montreal. Available from: http://www.bio.umontreal.ca/Casgrain/en/labo/permute/index.html.

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

Castanet J, Francillon-Vieillot H, Meunier FJ, de Ricqlès A. 1993. Bone and individual aging. In: Hall BK, ed. *Bone*. London: CRC Press, 245–283.

Chiappe LM, Chinsamy A. 1996. *Pterodaustro*'s true teeth. *Nature* **379:** 211–212.

Cluver MA. 1971. The cranial morphology of the dicynodont genus *Lystrosaurus*. *Annals of the South African Museum* **56:** 155–274.

Cubo J, de Margerie E, Castanet J. 2001. Homoplastic variation of bone vascularization in birds. *Journal of Vertebrate Paleontology* **21:** 43A.

Cubo J, Legendre P, de Ricqlès A, Montes L, de Margerie E, Castanet J, Desdevises Y. 2008. Phylogenetic, functional, and structural components of variation in bone growth rate of amniotes. *Evolution & Development* **10:** 213–223.

Cubo J, Ponton F, Laurin M, de Margerie E, Castanet J. 2005. Phylogenetic signal in bone microstructure of sauropsids. *Systematic Biology* **54:** 562–574.

Curran-Everett D. 2000. Multiple comparisons: philosophies and illustrations. *American Journal of Physiology–Regulatory Integrative and Comparative Physiology* **279:** 1–8.

de Buffrénil V, Francillon-Vieillot H. 2001. Ontogenic changes in bone compactness in male and female Nile monitors (*Varanus niloticus*). *Journal of Zoology* **253:** 539–546.

de Buffrénil V, Mazin JM. 1989. Bone histology of *Claudiosaurus germaini* (Reptilia, Claudiosauridae) and the problem of pachyostosis in aquatic tetrapods. *Historical Biology* **2:** 311–322.

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

de Buffrénil V, Schoevaert D. 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.

de Buffrénil V, Sire JY, Schoevaert D. 1986. Comparaison de la structure et du volume squelettiques entre un delphinidé (*Delphinus delphis* L.) et un mammifère terrestre (*Panthera leo* L.). *Canadian Journal of Zoology* **64:** 1750–1756.

de Lapparent de Broin F, Bocquentin J, Negri FR. 1993. Gigantic turtles (Pleurodira, Podocnemididae) from the Late Miocene–Early Pliocene of South Western Amazon. *Bulletin de l'Institut Français d'Etudes Andines* **22:** 657–670.

de Margerie E, Sanchez S, Cubo J, Castanet J. 2005. Torsional resistance as a principal component of the structural design of long bones: comparative multivariate evidence in birds. *Anatomical Record, Part A* **282A:** 49–66.

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

de Ricqlès A. 1980. Croissance périodique, ontogenèse, phylogenèse et stratégies démographiques: le cas des reptiles captorhinomorphes. *Bulletin de la Société Zoologique de France* **105:** 363–369.

de Ricqlès A. 1983. Cyclical growth in the long limb bones of a sauropod dinosaur. *Acta Palaeontologica Polonica* **28:** 225–232.

de Ricqlès A, de Buffrénil V. 2001. Bone histology, heterochronies and the return of tetrapods to life in water: where are we? In: Mazin JM, de Buffrénil V, eds. *Secondary Adaptation of Tetrapods to Life in Water*. Munich: Dr Friedrich Pfeil, 289–310.

Desdevises Y, Legendre P, Azouzi L, Morand S. 2003. Quantifying phylogenetically structured environmental variation. *Evolution* **57:** 2467–2652.

Domning DP. 2001. The earliest known fully quadrupedal sirenian. *Nature* **413:** 625–627.

Fawcett DW. 1942. The amedullary bones of the Florida manatee (*Trichechus latirostris*). *American Journal of Anatomy* **71:** 271–309.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* **125:** 1–15.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto B, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* **297:** 1–370.

Garland T Jr, Midford PE, Ives AR. 1999. An introduction to phylogenetically based methods, with a new method for confidence intervales on ancestral values. *American Zoologist* **39:** 374–388.

Germain D, Laurin M. 2005. Microanatomy of the radius and lifestyle in amniotes (Vertebrata, Tetrapoda). *Zoologica Scripta* **34:** 335–350.

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

Gray NM, Kainec K, Madar S, Tomko L, Wolfe S. 2007. Sink or Swim? Bone density as a mechanism for buoyancy control in early cetaceans. *Anatomical Record* **290:** 638–653.

Holmes R, Cheng YN, Wu XC. 2008. New information on the skull of *Keichousaurus hui* (Reptilia: Sauropterygia) with comments on sauropterygian interrelationships. *Journal of Vertebrate Paleontology* **28:** 76–84.

Hoyle MH. 1973. Transformations: an introduction and a bibliography. *International Statistical Review* **41:** 203–223.

IUCN. 2008. *2008 IUCN Red List of Threatened Species*. Available at: http://www.iucnredlist.org.

Josse S, Moreau T, Laurin M. 2006. Stratigraphic tools for Mesquite. Available from http://mesquiteproject.org/packages/stratigraphicTools/.

Joyce WG, Gauthier JA. 2004. Palaeoecology of Triassic stem turtles sheds new light on turtle origins. *Proceedings of the Royal Society of London, Series B, Biological Sciences* **271:** 1–5.

King GM. 1991. The aquatic *Lystrosaurus*: a palaeontological myth. *Historical Biology. An International Journal of Paleobiology* **4:** 285–321.

King GM, Cluver MA. 1991. The aquatic *Lystrosaurus*: an alternative lifestyle. *Historical Biology*. *An International Journal of Paleobiology* **4:** 323–341.

King GM, Jenkins I. 1997. The dicynodont *Lystrosaurus* from the Upper Permian of Zambia: evolutionary and stratigraphical implications. *Palaeontology* **40:** 149–156.

Kissel RA, Dilkes DW, Reisz RR. 2002. *Captorhinus magnus*, a new captorhinid (Amniota: Eureptilia) from the Lower Permian of Oklahoma, with new evidence on the homology of the astragalus. *Canadian Journal of Earth Sciences* **39:** 1363–1372.

Kriloff A, Germain D, Canoville A, Vincent P, Sache M, Laurin M. 2008. Evolution of bone microanatomy of the tetrapod tibia and its use in paleobiological inference. *Journal of Evolutionary Biology* **21:** 1–11.

Laurin M. 2000. Seymouriamorphs. In: Heatwole H, Carroll RL, eds. *Amphibian Biology*. Chipping Norton: Surrey Beatty & Sons, 1064–1080.

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

Laurin M. 2008. *Systématique, paléontologie et biologie évolutive moderne: l'exemple de la sortie des eaux des vertébrés*. Paris: Ellipses.

Laurin M, Canoville A, Quilhac A. 2009. Use of paleontological and molecular data in supertrees for comparative studies: the example of lissamphibian femoral microanatomy. *Journal of Anatomy* **215:** 110–123.

Laurin M, Germain D, Steyer JS, Girondot M. 2006. Données microanatomiques sur la conquête de l'environnement terrestre par les vertébrés. *Comptes Rendus Palevol* **5:** 603–618.

Laurin M, Girondot M, Loth MM. 2004. The evolution of long bone microanatomy and lifestyle in lissamphibians. *Paleobiology* **30:** 589–613.

Laurin M, Meunier FJ, Germain D, Lemoine M. 2007. A microanatomical and histological study of the paired fin skeleton of the Devonian sarcopterygian *Eusthenopteron foordi*. *Journal of Paleontology* **81:** 143–153.

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

Lee MSY. 1995. Historical burden in systematics and the interrelationships of 'parareptiles', *Biological Reviews of the Cambridge Philosophical Society* **70:** 459–547.

Lee MSY. 1997. Pareiasaur phylogeny and the origin of turtles. *Zoological Journal of the Linnean Society* **120:** 197–280.

Lee MSY. 2001. Molecules, morphology, and the monophyly of diapsid reptiles. *Contributions to Zoology* **70:** 1–18.

Maddison WP, Maddison DR. 2008. *Mesquite: a modular system for evolutionary analysis*, Version 2.5. Available at: http://mesquiteproject.org.

Meers MB. 2002. Cross-sectional geometric properties of the crocodylian humerus: an exception to Wolff's law? *Journal of Zoology* **258:** 405–418.

Midford P, Garland TJ, Maddison WP. 2008. *PDAP package for Mesquite*, Version 1.12. Available at http://mesquiteproject.org/pdap_mesquite/index.html.

Modesto SP. 1998. New information on the skull of the Early Permian reptile *Captorhinus aguti*. *PaleoBios* **18:** 21–35.

Modesto SP. 2006. The cranial skeleton of the Early Permian aquatic reptile *Mesosaurus tenuidens*: implications for relationships and palaeobiology. *Zoological Journal of the Linnean Society* **146:** 345–368.

Müller J. 2003. Early loss and multiple return of the lower temporal arcade in diapsid reptiles. *Naturwissenschaften* **90:** 473–476.

Myers P, Espinosa R, Parr CS, Jones T, Hammond GS, Dewey TA. 2006. The animal diversity web (online). Available at: http://animaldiversity.org.

Nopcsa FB. 1923. Vorläufige Notiz über die Pachyostose und Osteosklerose einiger mariner Wirbeltiere. *Anatomischer Anzeiger* **56:** 353–359.

Oelofsen B, Araújo DC. 1983. Palaeoecological implications of the distribution of mesosaurid reptiles in the Permian Irati Sea (Paraná Basin), South America. *Revista and Brasileira de Geociências* **13:** 1–6.

Olson EC. 1954. Fauna of the Vale and Choza: 9–Captorhinomorpha. *Fieldiana Geology* **10:** 211–218.

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

Peabody FE. 1951. The origin of the astragalus of reptiles. *Evolution* **5:** 339–344.

Peyer B. 1950. *Geschichte der Tierwelt*. Zürich: Büchergilde Gutenberg.

Piñeiro G. 2008. Los mesosaurios y otros fosiles de fines del Paleozoico. In: Perera D, ed. *Fosiles de Uruguay*. Montevideo: DIRAC, 179–205.

Ray S. 2006. Functional and evolutionary aspects of the postcranial anatomy of dicynodonts (Synapsida, Therapsida). *Palaeontology* **49:** 1263–1286.

Ray S, Chinsamy A, Bandyopadhyay S. 2005. *Lystrosaurus murrayi* (Therapsida, Dicynodontia): bone histology, growth and lifestyle adaptations. *Palaeontology* **48:** 1169–1185.

Reisz RR, Laurin M. 1991. *Owenetta* and the origin of turtles. *Nature* **349:** 324–326.

Retallack GJ, Smith RMH, Ward PD. 2003. Vertebrate extinction across the Permian-Triassic boundary in Karoo Basin, South Africa. *GSA Bulletin* **115:** 1133–1152.

Rieppel O. 1989. A new pachypleurosaur (Reptilia: Sauropterygia) from the Middle Triassic of Monte San Giorgio, Switzerland. *Philosophical Transactions of the Royal Society of London, Series B* **323:** 1–73.

Rieppel O. 1995. The genus *Placodus*: systematics, morphology, paleobiogeography and paleobiology. *Fieldiana (Geology), N.S.* **31:** 1–85.

Romer AS. 1957. Origin of the amniote egg. *Scientific Monthly* **85:** 57–63.

Romer AS. 1958. Tetrapods limbs and early tetrapod life. *Evolution* **12:** 365–369.

Sander PM. 1989. The pachypleurosaurids (Reptilia: Nothosauria) from the Middle Triassic of Monte San Giorgio (Switzerland) with the description of a new species. *Philosophical Transactions of the Royal Society of London, Series B* **325:** 561–670.

Scheyer TM, Sánchez-Villagra MR. 2007. Carapace bone histology in the giant pleurodiran turtle *Stupendemys geographicus*: Phylogeny and function. *Acta Palaeontologica Polonica* **52:** 137–154.

Scheyer TM, Sander PM. 2007. Shell bone histology indicates terrestrial palaeoecology of basal turtles. *Proceedings of the Royal Society of London, Series B, Biological Sciences* **274:** 1885–1893.

Scheyer TM, Sander PM. 2009. Bone microstructures and mode of skeletogenesis in osteoderms of three pareiasaur taxa from the Permian of South Africa. *Journal of Evolutionary Biology* **22:** 1153–1162.

Schmidt S. 1984. Paleoecology of nothosaurs. In: Reif WF, ed. *Third Symposium on Mesozoic Ecosystems, short papers*. Tübingen: Attempto Verlag, 215–218.

Shedlock AM, Edwards SV. 2009. Amniotes (Amniota). In: Hedges SB, Kumar S, eds. *The timetree of life*. Oxford: Oxford University Press, 375–379.

StatSoft France. 2003. *STATISTICA*, Version 6. www.statsoft.com.

Steyer JS, Laurin M, Castanet J, de Ricqlès A. 2004. First histological and skeletochronological data on temnospondyl growth; palaeoecological and palaeoclimatological implications. *Palaeogeography, Palaeoclimatology, Palaeoecology* **206:** 193–201.

Storrs GW. 1993. Function and phylogeny in sauropterygian (Diapsida) evolution. *American Journal of Science* **293A:** 63–90.

Sues HD. 1987. On the skull of *Placodus gigas* and the relationships of the Placodontia. *Journal of Vertebrate Paleontology* **7:** 138–144.

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

Vallin G, Laurin M. 2004. Cranial morphology and affinities of *Microbrachis*, and a reappraisal of the phylogeny and lifestyle of the first amphibians. *Journal of Vertebrate Paleontology* **24:** 56–72.

Werneburg I, Sánchez-Villagra MR. 2009. Timing of organogenesis support basal position of turtles in the amniote tree of life. *BMC Evolutionary Biology* **9:** 1–9.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. (A) Cross-section of humeri of extant mammals studied in this analysis (48 species, 51 individuals). When more than one specimen of a given species was used, the species name is not repeated. For the habitat: 0 = aquatic, 1 = amphibious, 2 = terrestrial. LS, lifestyle; *PLg*, presacral length; *MD*, maximal diameter of the cross-section; *Cg*, global compactness; *Cc*, compactness in the center of the cross-section; *Cp*, compactness in the periphery of the cross-section [L, private collection of Michel Laurin; B, private collection of Vivian de Buffrénil; MNHN, Muséum National d'Histoire naturelle (Paris); NMW, National Museum of Vienna (Austria)]. (B) Cross-section of humeri of testudines studied in this analysis (13 species, 14 individuals). When more than one specimen of a given species was used, these are numbered from 1 to *x* (up to 2). For the habitat: 0 = aquatic, 1 = amphibious, 2 = terrestrial. LS, lifestyle; *PLg*, presacral length; *MD*, maximal diameter of the cross-section; *Cg*, global compactness; *Cc*, compactness in the center of the cross-section; *Cp*, compactness in the periphery of the cross-section [L, private collection of Michel Laurin; C, private collection of Jacques Castanet; MNHN, Muséum National d'Histoire Naturelle (Paris)]. (C) Cross-section of humeri of diapsids studied in this analysis (14 species, 15 individuals). When more than one specimen of a given species was used, the species name is not repeated. For the habitat: 0 = aquatic, 1 = amphibious, 2 = terrestrial. LS, lifestyle; *PLg*, presacral length; *MD*, maximal diameter of the cross-section; *Cg*, global compactness; *Cc*, compactness in the center of the cross-section; *Cp*, compactness in the periphery of the cross-section [L, private collection of Michel Laurin; B, private collection of Vivian de Buffrénil; MNHN, Muséum National d'Histoire Naturelle (Paris)]. (D) Cross-section of humeri of extinct taxa studied in this analysis (nine taxa). For the habitat: 0 = aquatic, 1 = amphibious, 2 = terrestrial. LS, lifestyle; *PLg*, presacral length; *MD*, maximal diameter of the cross-section; *Cg*, global compactness; *Cc*, compactness in the center of the cross-section; *Cp*, compactness in the periphery of the cross-section [L, private collection of Michel Laurin; B, private collection of Vivian de Buffrénil; R, private collection of Armand de Ricqlès; MNHN, Muséum National d'Histoire naturelle (Paris)].

Appendix S2. Profil compactness parameters calculated in BONE PROFILER (Girondot & Laurin, 2003) and used in the present analysis.

Appendix S3. Method used to build the reference phylogeny.

Appendix S4. Time-calibrated supertree of the species sampled in this study and of paleontological and molecular calibrations of the divergence times. See Appendix S3 for more information.

Appendix S5. Details on statistical analyses performed in this study.

Appendix S6. False discovery rate analysis (Benjamini & Hochberg, 1995; Curran-Everett, 2000) of the statistical results concerning the phylogenetic and ecological signals. *P*-values marked in bold are significant. BE, backward-elimination procedure; FIC, Felsenstein independant contrasts analysis; FS, forward selection procedure; PC, principal coordinates; Prob., probability.

Appendix S7. (A) Probability of inferred lifestyle for each taxon obtained by the discriminant functions built with the characters selected by a backward-elimination procedure in PERMUTE. The lifestyle is coded: for ternary coding, 0 = aquatic, 1 = amphibious, 2 = terrestrial; for binary coding, 0 = aquatic, 2 = amphibious to terrestrial. All errors are marked in bold. SR corresponds to the success rate of the model. (B) Probabilities of inferred lifestyle for each taxon obtained by the discriminant functions built with the parameters without statistical artefacts. The lifestyle is coded: for ternary coding, 0 = aquatic, 1 = amphibious, 2 = terrestrial; for binary coding: 0 = aquatic, 2 = amphibious to terrestrial. All errors are marked in bold and errors between two non-adjacent states are marked by an asterisk (*). SR corresponds to the success rate of the model.

Appendix S8. Continuous character values of hypothetical ancestors.

Appendix S9. Direct habitat inference on hypothetical ancestors from microanatomical and size character values inferred from squared-change parsimony, with confidence intervals reconstructed using phylogenetically independent contrasts. The inference models were based exclusively on characters whose contrasts were adequately standardized on the reference trees. Prob, probability; Inf. LS, inferred lifestyle.

Appendix S10. A, discriminant function A. B, detailed formula A. C, discriminant function B. D, detailed formula B.