Evolution of bone microanatomy of the tetrapod tibia and its use in palaeobiological inference

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Abstract

Bone microanatomy appears to track changes in various physiological or ecological properties of the individual or the taxon. Analyses of sections of the tibia of 99 taxa show a highly significant ($P \leq 0.005$) relationship between long-bone microanatomy and habitat. Randomization tests reveal a highly significant ($P \leq 0.005$) phylogenetic signal on several compactness profile parameters and lifestyle. Discriminant analyses yield an inference model which has a success rate of 63% when lifestyle is coded into three states (aquatic, amphibious and terrestrial) or 83% for a binary model (aquatic vs. amphibious to terrestrial). Lifestyle is inferred to have been terrestrial for the stem-tetrapod *Discosauriscus* (Early Permian), the basal synapsid *Dimetrodon* (Early Permian), the dicynodont therapsid *Dicynodon* (Late Permian), an unindentified gorgonopsian (Late Permian); the parareptile *Pareiasaurus* (Middle or Late Permian) is modelled as being aquatic, but was more likely amphibious.

Introduction

Bone microstructure exhibits many characteristics that reflect physiological or ecological properties of taxa such as locomotor parameters (Casinos, 1996), growth trajectory (Castanet *et al.*, 1993; Rimblot-Baly *et al.*, 1995), lifestyle (de Buffrénil *et al.*, 1987, 1990a, b; Laurin *et al.*, 2004, 2007; Germain & Laurin, 2005) and metabolism (de Ricqlès, 1983). Although the determinism of bone microstructure is complex (Cubo *et al.*, 2005), differences between bone microanatomy of aquatic and most terrestrial vertebrates have been known for a long time (Nopcsa, 1923). Several studies (reviewed by de Ricqlès & de Buffrénil, 2001; Laurin *et al.*, 2004, 2006; Germain & Laurin, 2005) have shown that long bones of terrestrial vertebrates have a large medullary cavity associated with a moderately thick compact cortical area and that most aquatic vertebrates have massive bones with a small medullary cavity, which act as ballast, or spongy bones

without a medullary cavity, which minimize inertia (Wall, 1983; Stein, 1989; Fish & Stein, 1991; Taylor, 1994). The increased skeletal mass may result from pachyostosis (a thickening of the cortex by increased deposition of periosteal bone), osteosclerosis (a reduction in resorption of medullary bone) or a combination of both processes (de Ricqlès & de Buffrénil, 2001). However, these relationships may not hold for all taxa or all bones, and may depend on the evolutionary time available for taxa to adapt to a new ecological niche (Laurin *et al.*, 2004, 2006). Furthermore, most studies on this topic were performed before the advent of modern comparative techniques such as phylogenetically independent contrasts (Felsenstein, 1985) and variance partitioning using phylogenetic eigenvector regression (PVR; Desdevises *et al.*, 2003), and did not take phylogenetic effects into consideration. Thus, it is not clear if relationships that were thought to be statistically significant will remain so when subjected to more rigorous tests of such methods.

The first studies in this field which used comparative techniques incorporating phylogenetic data were based on data sets of femora of 48 species of lissamphibians (Laurin *et al.*, 2004) and 51 species of amniotes (Germain

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& Laurin, 2005). They confirmed the link between bone microanatomy and lifestyle and inferred the lifestyle of Permian and Triassic taxa. They also showed that a strong phylogenetic signal is present in bone microanatomy, contrary to what earlier studies suggested (Castanet *et al.*, 2001; Cubo *et al.*, 2001); this was corroborated by other recent analyses (Cubo *et al.*, 2005).

The present study is based on a database on tibial microanatomy and body size, and attempts to show how bone microanatomy evolves to track changes in habitat of vertebrates. It has the most exhaustive taxonomic sampling (99 species) of any quantitative study on this topic published so far. Several reasons led us to study the tibia. Our previous analyses (Laurin *et al.*, 2004; Germain & Laurin, 2005) show that inference models obtained from one bone (e.g. the femur) cannot be used to obtain inferences based on another bone (e.g. the radius). Furthermore, the proximodistal gradient of compactness in bones of sea cows (de Buffrénil & Schoevaert, 1989) suggests that some bones may yield a stronger ecological signal than others. Therefore, comparable studies need to be performed on other long limb bones to determine which yields the most reliable ecological signal. As the main weight-bearing bone of the zeugopod of the hind limb, the tibia can be expected to reflect the mode of locomotion and habitat of tetrapods. Furthermore, given that fossil material is often extremely rare, it would be advantageous to be able to use several kinds of long bones to infer lifestyle of extinct taxa. This would ultimately enable the evolution of lifestyle and the conquest of land in vertebrates to be much better understood. Preliminary results on this topic are presented below, to illustrate the interest of this approach in palaeobiology.

Materials and methods

Biological sample

All cross-sections of the tibia were made at the mid-diaphyseal level, because compactness profile can vary along a single bone (the metaphysis is spongier than the diaphysis) and the mid-diaphysis is thought to maximize the ecological signal (Laurin *et al.*, 2004). The sections come from adult animals (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) to avoid ontogenetic variations in actively growing bones. Cross-sections were drawn with a camera lucida, digitized and transformed into binary images using Adobe Photoshop 7.0. Bone was marked with one colour and all other surfaces (medullary cavity, resorption lacunae and vascular spaces) with another. The cross-sections were then analysed using Bone Profiler (Girondot & Laurin, 2003).

Bone Profiler can extract several parameters of a compactness profile from a cross-section. A detailed description of the software is provided in Girondot & Laurin (2003) and Laurin *et al.* (2004). Thus, only a brief summary is presented here. First, Bone Profiler automatically finds the section centre. Next, it divides the cross-section into 60 radial sectors of 6° width. Each sector is further divided into 51 concentric areas (Fig. 1a). Bone compactness (the ratio between the surface occupied by bone tissues and the total bone surface) is measured in each of the 3060 resulting cells and in the whole section (global compactness). Because a sigmoid equation can usually describe bone compactness as a function of distance from the section centre, a sigmoid equation has been chosen to represent compactness profile. The parameters S, P, Min and Max are extracted from the sigmoid equation (Fig. 1b). S is the reciprocal of the slope at the sigmoid inflection point; it is proportional to the relative width of the transition zone between the medullary and the cortical regions. P is the position of the sigmoid inflexion point on the X-axis; it generally represents the position of the transition zone between medulla and the cortical area. Min is the minimal asymptotic value and corresponds to compactness in the centre of the medullary area. Max is the maximal asymptotic value and corresponds to the compactness in the superficial cortex. For each radial sector, a sigmoidal function and all its parameters are calculated. Values used here are the algebraic mean values of the 60 radial values, excluding outliers that are automatically removed by Bone Profiler.

Our sample includes 96 extant (38 lissamphibians, 33 mammals and 25 sauropsids) and eight extinct species. A recent investigation of the compactness profile in the femur of lissamphibians showed that intraspecific variability was much less than interspecific variability (Laurin *et al.*, 2004); so, all species in this study are represented by a single individual (Appendix 1).

The lifestyle is considered to be known for three extinct taxa based on morphological and taphonomic data (Carroll, 1988). *Eusthenopteron* was a primitively aquatic Devonian (Frasnian, about 375–385 Ma) sarcopterygian tetrapodomorph (a stem-tetrapod that retained paired fins) very common in the Escuminac Formation of Miguasha (Québec, Canada) (Laurin *et al.*, 2007). *Platecarpus* was a Cretaceous (Maastrichtian, 65–70 Ma) mosasaur (an aquatic squamate whose closest extant relatives are probably the snakes) and *Omphalosaurus* was a Triassic (Anisian, 237–245 Ma) marine reptile often considered to be an ichthyosaur (an extinct clade of aquatic diapsids of uncertain affinities), although this identification has been questioned (Motani, 2000). The lifestyle of five of the extinct taxa (one stem-tetrapod, one reptile and three synapsids) included in this study cannot be determined with confidence on the basis of such data. *Discosauriscus*, an Early Permian (Asselian, 295–299 Ma) stem-tetrapod that lived in Bohemia, has
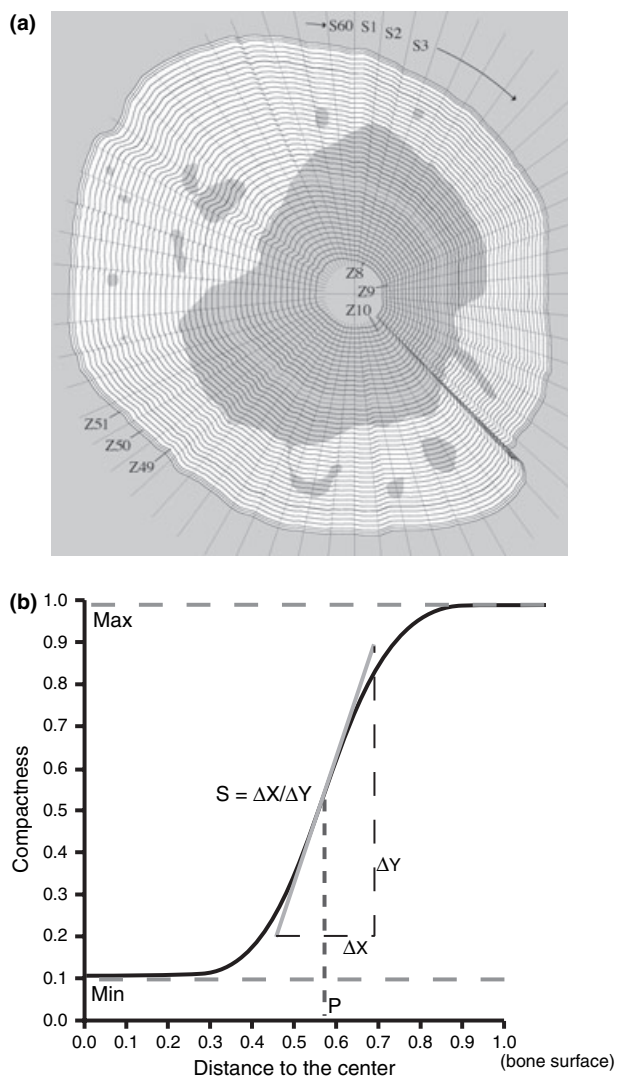
Fig. 1 (a) Sampling method for establishing the compactness profile in Bone Profiler. The bone section is divided into 60 sectors (S 1–60; only a few are identified), each of which measures 6° in width. Each sector is further divided into 51 zones (zones 1–7, near the centre, were not drawn for lack 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 urodele *Ambystoma andersoni*. (b) Mathematical model that shows the distribution of mineralized tissues as a function of distance to the centre of the bone. 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 or in the section shown above). See text for an explanation of *S*, *P*, Min and Max. Reproduced from Laurin *et al.* (2004).

several specimens from North America (Red Beds of Texas) and by more fragmentary european material. Its lifestyle has been considered amphibious or terrestrial (Romer, 1927; Haack, 1986; Florides *et al.*, 2001). Some taxa closely related to the Late Permian (Lopingian, 251–260 Ma) therapsid *Dicynodon* from South Africa have been considered amphibious or terrestrial (King, 1990; Germain & Laurin, 2005). Gorgonopsians, carnivorous Late Permian (Lopingian) therapsids found in South Africa and Russia, are considered terrestrial (Cruickshank, 1973). *Pareiasaurus* is a Late Permian (Lopingian) parareptile that was very common in Russia and South Africa. It has been considered amphibious (Piveteau, 1955).

Statistical analyses were carried out on the 99 species (96 extant and three extinct) whose lifestyle is known. The five extinct species whose lifestyle is uncertain were used to provide examples of application of the discriminant function that can infer the lifestyle using bone microanatomy and body size data. The resulting inferences were combined with those of Laurin *et al.* (2004) and Germain & Laurin (2005) and optimized on a time-calibrated phylogeny.

Body size is estimated by the presacral length (cm, from cranial margin of atlas to cranial margin of sacrum), cranial length (cm, from snout to occipital condyle) and bone section maximal diameter (mm). Lifestyle is coded in four states as follows: 0 = fully aquatic (taxa that never venture onto land, such as cetaceans), 1 = mostly aquatic (taxa which are active mostly in water, but have limited locomotor ability on land, such as marine turtles, seals and sea lions), 2 = partially aquatic (taxa active both on land and in water, such as many lissamphibians which live near the water edge and frequently move in and out of the water, otters and some of the most aquatic varanids), 3 = terrestrial (taxa which seldom swim, such as primates, canids and most felids); or in three states as follows: 0 = aquatic (which includes fully and mostly aquatic taxa), 1 = amphibious (partially aquatic), 2 = terrestrial (as defined above); or as a binary character as follows: 0 = aquatic (fully to mostly aquatic), 1 = amphibious to terrestrial. Ideally, lifestyle should be quantified as a proportion of time of activity spent in water (and more specifically, under the water surface), but such detailed information is seldom available in the literature. Thus, we have fitted all species into these two to four categories using both primary literature and compilations, such as Goin *et al.* (1978) and Duellman & Trueb (1986) for lissamphibians, and personal communications from curators and colleagues who provided the material (see Acknowledgments).

Reconstructing the phylogeny enables determination of the ancestral character states. The phylogeny (Fig. 2) used here is a compilation of previously published phylogenies cited by Laurin *et al.* (2004) and Germain & Laurin (2005). Incompatibilities between various phylogenies were resolved by giving preference to the

aquatic larvae, but the adult is considered terrestrial (Laurin, 2000). *Dimetrodon* is an Early Permian (Sakmarian, 284–295 Ma) synapsid (Synapsida is the largest clade which includes mammals but not reptiles) known from
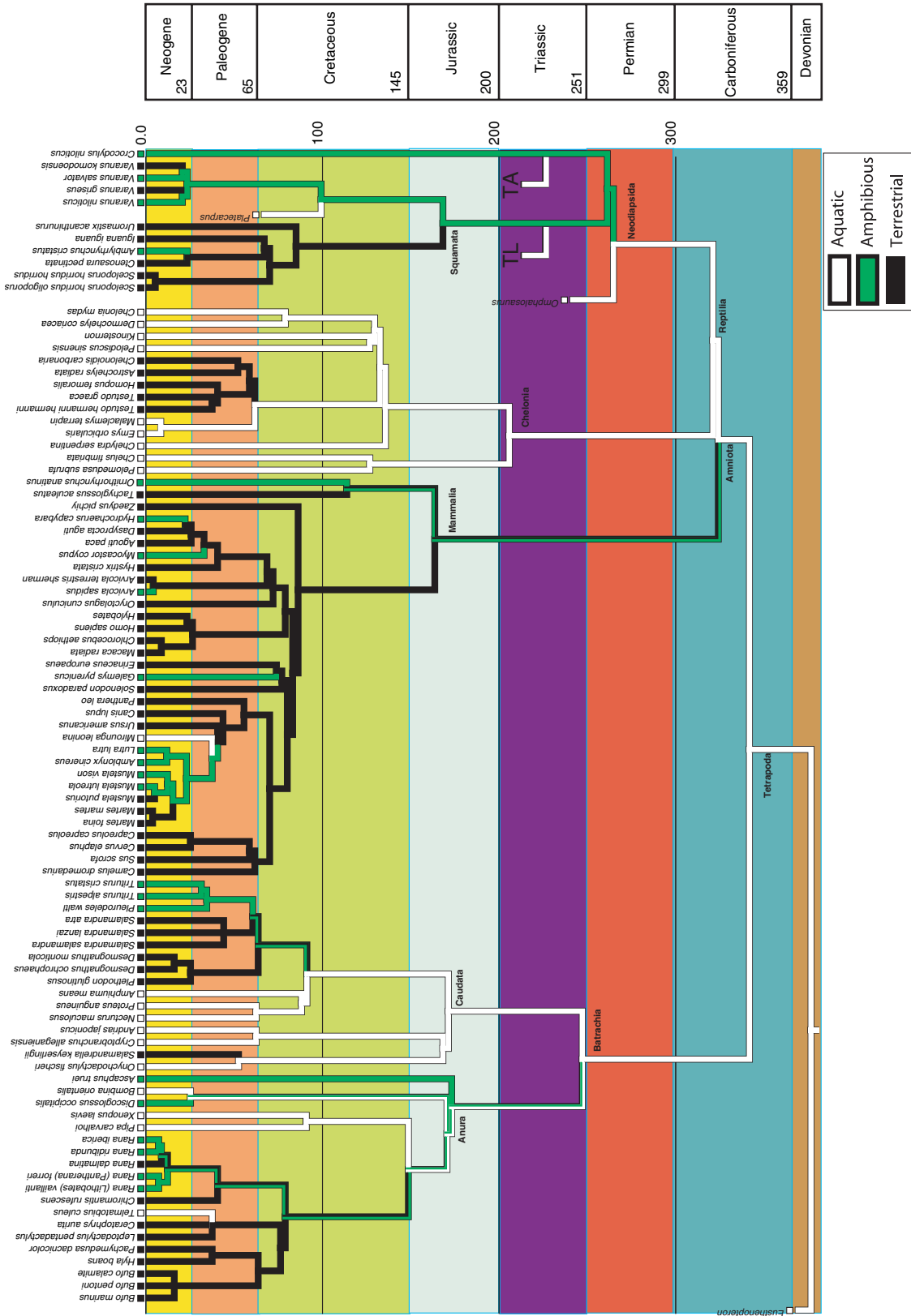
Fig. 2 Phylogeny used for statistical tests. This tree is a compilation of previously published phylogenies cited by Laurin *et al.* (2004) and Germain & Laurin (2005). The lifestyle is optimized as an ordered character using parsimony. The geological timescale follows Gradstein *et al.* (2004). White: aquatic, green: amphibious, black: terrestrial. Divergence times are in millions of years before present. The alternative positions in which turtles could be placed are denoted by TL (turtles among lepidosauromorphs) and TA (among archosauromorphs).

most recent ones, and to those which include the most taxa and informative characters. Minimal divergence times are given in millions of years (Ma) and are estimated by the oldest known fossil of the concerned clade. When information in the fossil record was insufficient and would clearly have yielded implausibly recent data, divergence times were established by previously published molecular estimates.

Reference phylogeny

A tree was compiled from previously published phylogenies cited by Laurin *et al.* (2004) and Germain & Laurin (2005). Clades were dated using the first date of occurrence of taxa from the fossil record, as explained in greater detail by Laurin *et al.* (2004, p. 593).

Turtles pose a special problem because their position is currently controversial; we have considered that they are parareptiles, as suggested by Reisz & Laurin (1991), Laurin & Reisz (1995) and Lee (1995, 2001). Several palaeontological (deBraga & Rieppel, 1997; Rieppel & Reisz, 1999; Müller, 2003), most molecular (Zardoya & Meyer, 1998, 2001; Hedges & Poling, 1999) and some mixed neontological and paleontological (Hill, 2005) studies have placed turtles within diapsids, either among lepidosauromorphs (as in several palaeontological studies) or among archosauromorphs (as in several molecular studies). A recent molecular study found turtles to be the sister group of diapsids among extant taxa (Frost *et al.*, 2006), and this finding is compatible with a position of turtles within parareptiles. Thus, turtles could have been placed in at least three positions in the phylogeny (among parareptiles, lepidosauromorphs and archosauromorphs).

Phylogenetic signal

We tested the presence of a phylogenetic signal by randomly reshuffling terminal taxa on a phylogeny whose topology and branch lengths were held constant as proposed by Laurin *et al.* (2004). This was performed in Mesquite (Maddison & Maddison, 2006). The character length for each tree was calculated by squared-change parsimony (Maddison, 1991) for continuous characters or standard parsimony for the discrete character (lifestyle). The phylogenetic signal is significant if fewer than 5% of the random trees require no more change than the reference tree.

A second test of phylogenetic signal was performed using a Mantel test. A phylogenetic distance matrix was obtained by stratigraphic tools (Josse *et al.*, 2006) and Euclidean phenotypic distances were obtained by *R* (Casgrain *et al.*, 2004). Regressions of the phenetic distance matrix against phylogenetic distance matrices are tested for significance using permutations in Permute (Legendre *et al.*, 1994). Random permutations of the dependent variable (using the matrix method of Per-

mute) generate a null distribution of the regression coefficients. For each parameter considered as the dependent variable that is permuted, the independent variable is the time of independent evolution. Correlation coefficients are underestimated with this method (Legendre, 2000); so, another test (PVR) is used to partition the influence of the different characters on data (Desdevises *et al.*, 2003).

Ecological signal

We performed variance partioning using PVR. This method uses multiple regressions on vectors of data and partitions variance of the explained character (habitat) into portions attributed to the phylogeny, to another factor (here, phenotype as represented by bone microanatomy and body size) and into a portion which represents covariation between both (Desdevises *et al.*, 2003).

Phylogenetic eigenvector regression analyses involve several steps. First, a principal coordinates analysis is performed on a phylogenetic distance matrix to obtain coordinates that give the position of each taxon in an $(n - 1)$-dimensional space (where n is the number of taxa). This step is necessary to convert the phylogenetic distance matrix into linear values. Only a subset of the resulting $n - 1$ axes can be used in the analyses because otherwise no degree of freedom would be left. We selected phylogenetic axes which exhibit more variation than the random broken-stick model (Diniz-Filho *et al.*, 1998). Second, a multiple regression of the dependent variable (lifestyle) on independent (phenotypic) variables is carried on. The R^2 of this regression corresponds to the fraction of the lifestyle variance explained by the phenotype (Fig. 3a, b). Third, a multiple regression of lifestyle on principal coordinate axes of the phylogeny is performed. The R^2 of this regression corresponds to the fraction of lifestyle variance explained by the phylogeny (Fig. 3b, c). Fourth, a multiple regression of lifestyle on phenotypic variables and principal coordinate axes is carried out. The R^2 of this regression corresponds to the
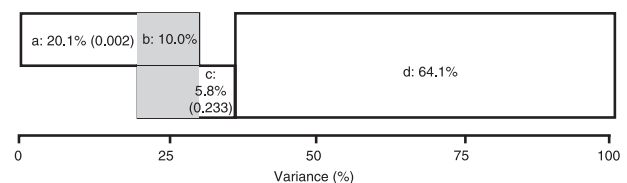
Fig. 3 Proportion of variance explained by phenotypic parameters, the phylogeny, both or neither, as established by a PVR analysis. Fraction *a* is linked only to phenotypic parameters; fraction *b* is linked both to phenotypic parameters and phylogeny; fraction *c* is linked only to the phylogeny; fraction *d* is the unexplained variance. The proportion of variance follows the letter which identifies each portion (*a–d*), followed, in parentheses (when this can be computed) by the associated probability.

fraction of lifestyle explained by all the studied variables (Fig. 3a–c). The lifestyle variance is split up into different parts *a*, *b*, *c*, *d*, where *a* is the lifestyle variance explained by phenotypic variables only, *b* is the part explained by both phylogeny of phenotypic variables, *c* is the part explained only by the phylogeny and *d* is the part unexplained by the studied variables. Fifth, a residual analysis is performed to test the significance of the parts *a* and *c* (Desdevises *et al.*, 2003) using permutations (in Permute).

Inference model

Discriminant analysis makes it possible to infer the lifestyle of extinct taxa. This method gives the probabilities of the inferred lifestyle and does not require linearity between categories of discrete variables (here, the lifestyle). The linear discriminant analysis was performed using *R* (R Development Core Team, 2004). A cross-validation on taxa of known lifestyle is realized to test the model efficiency. The principle is to remove a taxon and to infer its lifestyle with the discriminant function built from phenotypic data from all other taxa, and to apply this principle for each taxon. Inferred lifestyles obtained by this method are compared with observed lifestyles. The discriminant function applied to extinct taxa is built with all extant taxa and the three extinct taxa of known lifestyle. Several lifestyle codings have been tested: a binary coding, a coding into three states and a coding into four states; all of them were applied to all the sampled taxa.

To compare the relative performance of the discriminant function obtained on the tibia with a similar function obtained from other bones, three additional discriminant analyses have been performed: one on the femur of the lissamphibians studied by Laurin *et al.* (2004), another on the tibia of the lissamphibians included in this study and a discriminant analysis on the tibia of the amniotes included in this study; all of them with a coding into three states for the lifestyle. Germain & Laurin (2005) have already performed a linear discriminant analysis of the radius of amniotes; this analysis is not repeated here.

Optimization of habitat in early tetrapods

A preliminary optimization of habitat in early stegocephalians is based on the palaeobiological inferences obtained in this study (five taxa), as well as those obtained from comparable data by three previous studies. Germain & Laurin (2005) inferred the habitat of three taxa: a Triassic sauropterygian identified as *Pachypleurosaurus*, which is a junior synonym of *Neusticosaurus* (Sander, 1989), the Permian synapsid *Ophiacodon* and the Permo-Triassic therapsid *Lystrosaurus*. Steyer *et al.* (2004) assessed the habitat of the Triassic stereospondyl *Dutuitosaurus*, and Laurin *et al.* (2004) performed similar inferences on four additional taxa (the temnospondyl *Doleserpeton*, the embolomere *Chroniosaurus*, the 'microsaur' *Cardiocephalus* and the parareptile *Phaanthosaurus*). The Frasnian sarcopterygian *Eusthenopteron foordi*, which was certainly primitively aquatic and whose long bone microanatomy was recently studied (Laurin *et al.*, 2007), is also included. These data are complemented by inferences of the ancestral habitat of lissamphibians and crown-group turtles based on parsimony optimization of habitat on extant taxa performed here (Fig. 2). Data on the habitat of extant taxa extracted from the literature are incorporated: Mammalia, Lepidosauromorpha and Archosauromorpha are considered to be terrestrial (Carroll, 1988) and lissamphibians are considered to be amphibious (Laurin *et al.*, 2004). The stem-turtle *Proganochelys* is considered to have been terrestrial (Joyce & Gauthier, 2004; Scheyer & Sander, 2007). Thus, 15 extinct and five extant terminal taxa (Lissamphibia, Mammalia, Chelonia, Lepidosauromorpha and Archosauromorpha) are used to provide a preliminary optimization of lifestyle on a time-calibrated phylogeny. Habitat is considered to be an ordered character with three states.

Results

Evolution of habitat according to taxa of known lifestyle

On the basis of taxa of known lifestyle (all extant and three extinct taxa), there is ambiguity for the ancestral character state (amphibious or terrestrial) on the branch leading to Mammalia; Neodiapsida (a clade of diapsids which inclues the crown, younginiforms and Mesozoic marine reptiles) appears to be primitively aquatic because of the presence of *Omphalosaurus* (Fig. 2). However, these results illustrate the importance of an exhaustive taxonomic sampling for optimizations because palaeontological data strongly suggest that the mammalian and neodiapsid ancestral lifestyle was terrestrial (Carroll, 1988). The ancestral lifestyle of crown turtles is aquatic according to the optimization of lifestyle of the extant turtles included in this study (Fig. 2) and this inference is used for the optimization of habitat in early stegocephalians performed below. However, Germain & Laurin (2005) suggested that it was amphibious. These contradictory results probably come from different coding of lifestyle. In this study, shallow-water turtles have been considered as aquatic, whereas in Germain & Laurin (2005) they were considered amphibious, even though they do not move much on land.

Phylogenetic signal

The Mantel test finds a phylogenetic signal for the phenotypic variables (but only after a logarithmic transformation on the size variables), except in some compactness parameters like *S* and Max. The random taxon

Table 1 Phylogenetic signal in the characters, assessed by the probability that the variation in various characters is random with respect to the phylogeny.

Parameter	Random trees	Mantel test
Lifestyle		
Two states	0.001***	0.002**
Three states	0.001***	0.004**
Four states	0.001***	0.005**
Presacral length	0.018*	0.393
Ln presacral length	0.001***	0.001***
Maximal diameter	0.001***	0.425
Ln maximal diameter	0.001***	0.001***
Compactness	0.001***	0.002**
S	0.244	0.051
P	0.001***	0.002**
Min	0.001***	0.001***
Max	0.179	0.385

Two methods are used: optimization on random trees and Mantel test. Values are significant at a *0.05, **0.01 and ***0.001 thresholds.

reshuffling method also finds a phylogenetic signal for phenotypic variables (with or without logarithmic transformation), except in some compactness parameters like S and Max (Table 1).

Ecological signal

Multiple linear regressions on distance matrices with a backward elimination procedure show the presence of an ecological signal in Min, P and the phylogeny (Table 2). Aquatic taxa seem to have a greater compactness than amphibious or terrestrial taxa except the turtles which show the reverse trend (Table 3). The parameter P is inversely correlated with compactness, as expected. The

Table 2 Characters that reflect the lifestyle.

Parameter	Raw body size parameters		Log-transformed body size parameters	
	Simple test	Backward elimination	Simple test	Backward elimination
Phylogeny	0.016*	0.019*	0.017*	0.019*
Presacral length	0.060	**3**	0.423	**2**
Maximal diameter	0.059	**2**	0.438	**1**
Compactness	0.038*	**4**	0.031*	**4**
S	0.038*	**5**	0.067*	**5**
P	0.002**	0.002**	0.002**	0.002**
Min	0.001***	0.001***	0.001***	0.001***
Max	0.166	**1**	0.186	**3**

Probabilities (established through randomization procedures) that the variation of characters are randomly distributed with respect to the lifestyle are obtained by multiple linear regressions with simple test and backward elimination procedures. The phylogeny is represented by principal coordinate axes. Bold numbers indicate the step at which the parameter has been removed from the analysis. Values are significant at *0.05, **0.01 and ***0.001 thresholds.

parameter Max is very stable (always around 1). The parameter Min is higher for aquatic taxa (a spongiosa often fills the medullary cavity) than for amphibious taxa which have a small Min (Table 3).

Variance partitioning with PVR shows that about 20% of the lifestyle variance is explained only by phenotypic variables ($P = 0.002$), and an additional 10% is explained by the covariation between phenotype and phylogeny. The part of lifestyle variance explained by the phylogeny alone (5.8%) is not statistically significant ($P = 0.233$). Four principal coordinate axes (axes 1–4) were selected by the broken-stick model, and together they represent 62.35% of the phylogenetic variance of the 99 species. An important fraction remains unexplained by the variables studied (Fig. 3).

Inference model

The discriminant function obtained using all sampled taxa (Table 4) correctly attributes the lifestyle of 63 taxa (success rate: 63%) with the three-state coding (Appendix 2). The errors are not randomly distributed. The lifestyle of almost all aquatic and terrestrial taxa is correctly inferred. The amphibious lifestyle is often the origin of errors: amphibious taxa are often inferred to be terrestrial. Turtles are often problematic; amphibious turtles are often modelled as terrestrial and vice versa. Scheyer & Sander (2007) also noted problematic cases in which histological characters of the turtle shell does not reflect the habitat. A discriminant analysis has been tried on a lifestyle coded under four states (fully aquatic, mostly aquatic, amphibious and terrestrial). As expected, the success rate was somewhat lower (57%). Conversely, if lifestyle is coded as a binary character (aquatic vs. amphibious to terrestrial), the success rate reaches 83%.

The discriminant analysis on the lissamphibian tibia (Table 4) often models amphibious taxa and some aquatic taxa as terrestrial (Appendix 2). The discriminant analysis carried out on the lissamphibian femora (Laurin et al., 2004) with a coding into three states for the lifestyle gives a success rate of 42%, whereas the discriminant analysis performed on the lissamphibian tibiae of that study gives a success rate of 47%.

The discriminant analysis on amniote tibiae with a ternary coding (Table 4) shows about the same problems as the model obtained by the discriminant analysis performed on all sampled taxa (Appendix 2). The discriminant analysis carried on amniote radii (Germain & Laurin, 2005) and using a three-state coding gives a success rate of 70.6% which is comparable with the 68% success rate of the discriminant analysis on amniote tibiae (Appendix 2).

Lifestyle inferences for extinct taxa

Two linear discriminant models based on the total sample (99 species) have been used (Table 5): binary coding

Table 3 Mean values of parameters showing an ecological signal for each lifestyle.

	PSL	MD	Compactness	*S*	*P*	Min	Max
Means of parameters for the total sample							
Aquatic	44.35	6.37	0.80	0.03173	0.40	0.15368	0.99
Amphibious	27.72	5.52	0.71	0.00589	0.52	0.00699	0.99
Terrestrial	31.03	7.51	0.71	0.02331	0.53	0.02048	0.97
Means of parameters for turtles only							
Aquatic	29.34	4.54	0.84	0.05923	0.39	0.25466	0.98
Terrestrial	17.98	3.85	0.93	0.15815	0.41	0.19338	0.80

Turtles are shown separately because they exhibit an atypical tendency. PSL, presacral length; MD, maximal diameter; *S*, reciprocal of the slope at the inflection point of the compactness profile; *P*, relative position of the inflection point of the compactness profile; Min, minimal compactness; Max, maximal compactness.

Table 4 Coefficients of the linear discriminant function for habitat inference using either a binary (aquatic vs. amphibious to terrestrial) or ternary coding (with three states: aquatic, amphibious and terrestrial).

	Total sample (binary)	Total sample (three states)		Lissamphibians only (three states)		Amniotes only (three states)	
	LD1	LD1	LD2	LD1	LD2	LD1	LD2
PSL	−0.0112	−0.0109	−0.0242	0.0506	−0.0363	−0.0031	−0.0295
MD	0.0711	0.0721	0.1753	0.2071	0.0834	0.0159	0.1796
Compactness	7.1402	6.8702	−1.8090	−2.4906	15.6187	10.0925	2.7216
S	−10.536	−10.273	12.9967	–	–	−18.2887	8.5176
P	9.9764	9.6392	−0.8080	−5.5137	7.9516	10.7707	3.2547
Min	−6.2126	−6.0581	2.0568	–	–	−7.8016	0.8443
Max	−1.5701	−1.6367	3.7831	–	–	−5.1107	3.2733

LD1, first discriminant axis; LD2, second discriminant axis. For other abbreviations, see Table 3.

Table 5 Probability of inferred lifestyle for the extinct taxa according to both models based on 99 species.

	Model A		Model B			
Taxon	Inferred lifestyle	Probability	Inferred lifestyle	Probability	Interpretations in the literature	Conclusion (this study)
Discosauriscus	Am/T	0.827	T	0.692	Aq–T	T
Pareiasaurus	Aq	0.808	Aq	0.647	Am–T	Am
Dimetrodon	Am/T	0.937	T	0.756	Am–T	T
Dicynodon	Am/T	0.541	T	0.655	T	T
Gorgonopsia	Am/T	0.534	T	0.463	T	T

Model A: binary coding; model B: three-state coding. Aq, aquatic; Am, amphibious; T, terrestrial.

(model A) and ternary coding (model B). The results of both models are generally congruent with the literature.

Discussion

Phylogenetic signal

A phylogenetic signal is detected in more parameters by the random tree generation method than by a Mantel test. This may be because the second method requires a linear relationship between the time of independent evolution and phenotypic distances, whereas the first method does not. Logarithmic transformations on body size parameters are enough to find a phylogenetic signal by the second method, but the random trees method seems to be more powerful and shows a phylogenetic signal in nearly all parameters even without a logarithmic transformation.

The presence of phylogenetic information in histological characters has been debated for a long time. Some studies concluded that such characters reflect ecology and life history (Castanet *et al.*, 2001; Cubo *et al.*, 2001), but Padian *et al.* (2001) optimized histological characters on a cladogram, which implies that they contain phylo-

genetic information. Here, we have shown that micro-anatomical characters contain both kinds of information (ecological and phylogenetic). They can indeed be included in phylogenetic analyses, and their evolution can be reconstructed through optimization on a phylogenetic tree. These results are congruent with those of Laurin *et al.* (2004), Cubo *et al.* (2005) and Germain & Laurin (2005). The tibia of our larger sample of tetrapods exhibits a phylogenetic signal in more parameters than the femur of lissamphibians (Laurin *et al.*, 2004) and the radius of amniotes (Germain & Laurin, 2005). This difference may be explained by the relatively simple structure of lissamphibian limb bones, that vary almost only in size and parameter *P*, and the more exhaustive sampling of this study than that of Germain & Laurin (2005).

Bone microanatomy and lifestyle

A low value of the *P* parameter indicates high bone compactness and is often found in aquatic taxa (Wall, 1983). Several studies (Wall, 1983; Taylor, 1994) have concluded that this high compactness is an adaptation to an aquatic environment: an increase in bone density reduces buoyancy. This phenomenon seems to be more apparent for aquatic taxa than for amphibious taxa (Wall, 1983), and in shallow divers than for deeper water, pelagic swimmers (de Ricqlès & de Buffrénil, 2001).

According to Taylor (1994), two buoyancy control systems exist. Active swimmers have a hydrodynamic control favoured by weight reduction resulting from low cortex compactness of long bones, whereas shallow-water divers have a passive control that relies on increased skeletal mass (pachyostosis, osteosclerosis).

Chelus fimbriatus (Fig. 4b), *Pelomedusa subrufa* or *Myocastor coypus* seem to belong to the shallow-water diver group.

Active deep-sea swimmers (e.g. large aquatic taxa like pinnipeds, mosasaurs and ichthyosaurs) have an extensive spongiosa (high values of S and Min). These values indicate that the spongiosa fills the medullary cavity. In animals, such as *Omphalosaurus nisseri*, *Dermochelys coriacea* (Fig. 5a) and *Mirounga leonina* (Fig. 4a), reduction in lung volume at great depths (> 20 m) may obviate the need for high bone compactness (Wall, 1983; Taylor, 1994). Furthermore, the general reduction in bone compactness in such taxa is probably advantageous in reducing inertia, which saves energy spent in locomotion (de Ricqlès & de Buffrénil, 2001).

Turtles of all lifestyles show high tibial compactness (Fig. 5). In fact, aquatic species have a lower compactness than terrestrial species in this group, contrary to what is observed in all other tetrapods. This had already been observed by Germain & Laurin (2005). But amphibious and some terrestrial turtles show similar compactness (e.g. *P. subrufa* and *Homopus femoralis*). This high compactness in the turtle tibia could be linked to biomechanical constraints (such as the presence of a carapace) or a phylogenetic effect. Moreover, 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).

The global compactness of the tibia of amphibious taxa does not differ significantly from that of terrestrial taxa. However, the high compactness values associated with a developed spongiosa observed in aquatic taxa are sometimes also present in amphibious taxa. For example, the marine iguana *Amblyrhynchus cristatus*, which can dive as deep as 10 m to feed on marine algae (Pough *et al.*, 2004), possesses a tibia whose
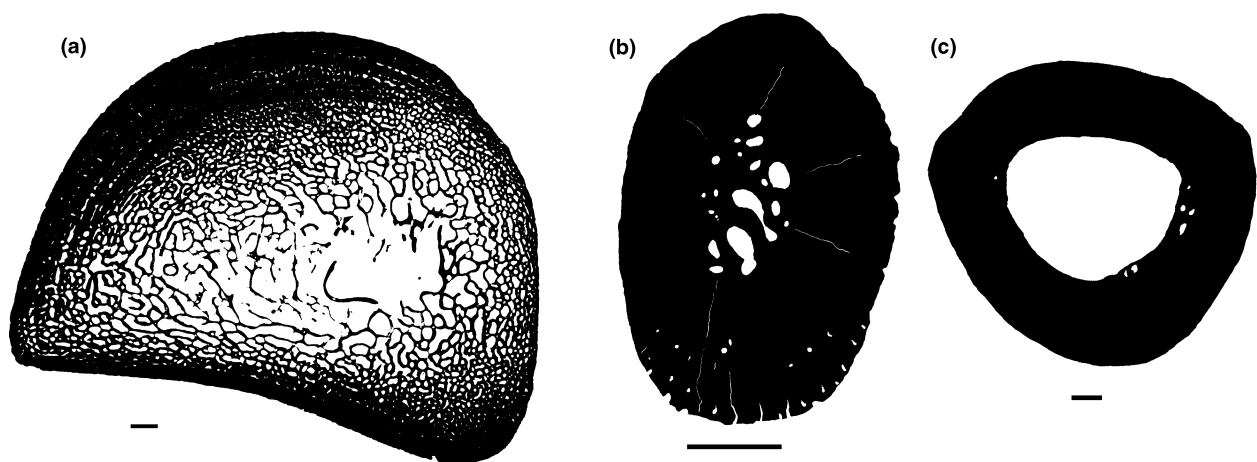
Fig. 4 Drawings of mid-diaphyseal cross-sections of tibiae of tetrapods of various lifestyles showing typical microstructural adaptations. (a) an aquatic mammal, *Mirounga leonina* (scale bar = 2 mm), with an extensive spongiosa which merges gradually into the cortical compacta; (b) a shallow-water aquatic turtle, *Chelus fimbriatus* (scale bar = 1 mm), with a very compact bone; and (c) a terrestrial mammal, *Canis lupus* (scale bar = 1 mm), with a large medullary cavity and a moderately thick cortical compacta.
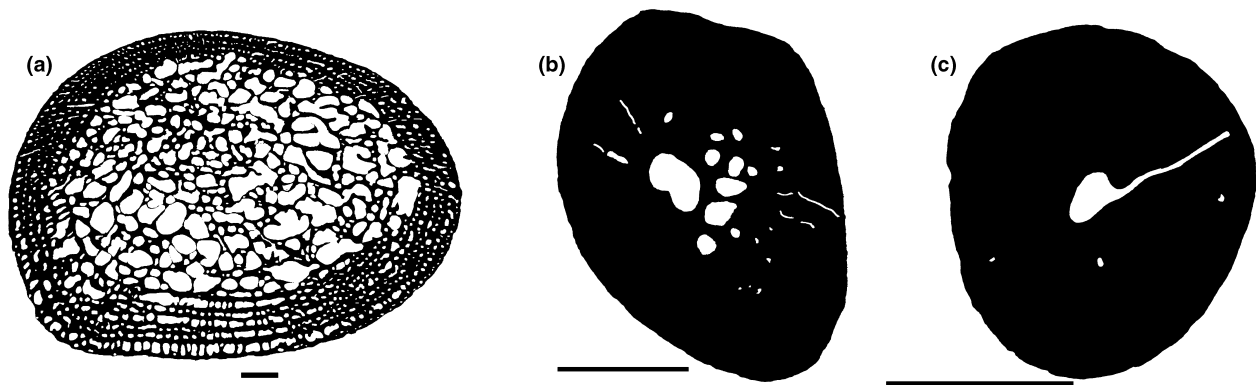
Fig. 5 Drawings of mid-diaphyseal cross-sections of tibiae of turtles showing the atypical microstructural adaptations to shallow-water and terrestrial lifestyles. (a) A deep-sea turtle, *Dermochelys coriacea*, showing a medullary spongiosa and very low global compactness; (b) an shallow-water aquatic turtle, *Pelomedusa subrufa*, with fairly compact bone; and (c) a terrestrial turtle, *Homopus femoralis*, with a still greater compactness, atypical of terrestrial taxa (scale bar = 1 mm).

compactness is higher (compactness 0.728) than that of its close terrestrial relative *ctenosaura pectinata* (compactness 0.434).

Performance of inference models

Errors in inferences of amphibious mammals (rodents and mustelids) may be explained by their kind of locomotion: they live close to the water, and they swim at the surface, but they do not usually dive. Even if they spend a lot of time in the water, their surface-swimming behaviour means that their bone microanatomy characteristics are close to those of terrestrial animals because pachyostosis would be disadvantageous for them.

Crocodylus niloticus is also inferred as terrestrial because its bone compactness is relatively low for an amphibious animal. Instead, it increases its body density by gastroliths (Taylor, 1994) and a heavy dermal skeleton (Henderson, 2003).

A certain proportion of errors is unavoidable because bone microanatomy is not governed only by the environment. Many other factors may influence the construction of bones, such as phylogenetic inertia, growth rates (Amprino, 1947; de Margerie *et al.*, 2002) and mechanical stress (de Margerie *et al.*, 2005).

The success rates of the discriminant analyses carried on the tibia and the radius of amniotes are similar (68% and 71% respectively). These two bones are thus equally informative. The discriminant analysis of lissamphibian tibiae gives a slightly better success rate than the discriminant analysis performed on lissamphibian femora (Laurin *et al.*, 2004) with a coding into three states for the lifestyle (47% vs. 42% respectively). The various models established by these two discriminant analyses seem to suggest that the tibia contains more ecological information than the femur. The hind limb seems to be slightly less informative than the forelimb, possibly because some aquatic taxa have lost the hind limb (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. Moreover, the efficiency of models built with amniotes is higher than that of those built with lissamphibians, presumably because the bone microanatomy is simpler in lissamphibians than in amniotes. This, in turn, may to an extent reflect the small size of lissamphibians.

Palaeobiological inferences

The lifestyle of *Discosauriscus* is inferred as amphibious/terrestrial or terrestrial according to the various models. A few previous studies attributed an aquatic lifestyle to it, but adults are usually considered to be terrestrial (Klembara, 1995, 1996; Laurin, 2000). For optimization purposes, we consider *Discosauriscus* predominantly terrestrial because this conclusion is compatible with the results of both inference models.

The Permian synapsid *Dimetrodon* is inferred as terrestrial, corroborating earlier ideas (Romer, 1927). This does not necessarily refute the idea that it could sometimes venture into water (Berman, 1977; Berman *et al.*, 2001). Moreover, Haack (1986) and Florides *et al.* (2001) considered that the sail of *Dimetrodon* had a thermoregulatory use, more compatible with a terrestrial lifestyle.

In Germain & Laurin (2005), the lifestyle of *Ophiacodon* (one of the earliest known amiotes, a stem-synapsid older than *Dimetrodon*) was inferred as aquatic, which tends to confirm the interpretations based on morphology (Romer, 1957, 1958) and bone histology (de Ricqlès, 1974) that it spent a good proportion of its time in water. However, for character optimization (Fig. 6), we consider it amphibious, rather than truly aquatic, as the moderately well ossified limbs suggest.

Dicynodon is inferred as amphibious/terrestrial (model A) which is also suggested by King (1990). In model B,

Fig. 6 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 (this study and Germain & Laurin, 2005) or a phylogenetically weighted logistic regression (Laurin *et al.*, 2004). A few extant taxa are also included, but extinct taxa whose lifestyle was known from other types of evidence (morphological or anatomical) are not shown. For information on the source of the phylogeny and of the geological timescale, see Fig. 2. In this phylogeny, turtles could also be placed as a sister group of *Neusticosaurus* or within Archosauromorpha.
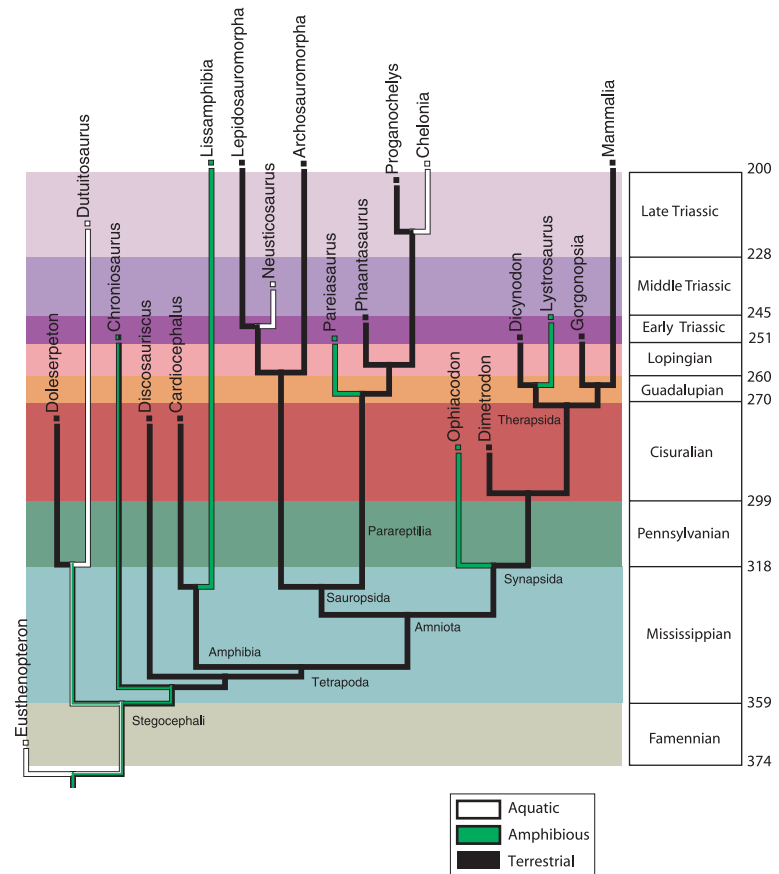
this taxon is inferred as terrestrial (Table 5). By contrast, Germain & Laurin (2005) inferred that the dicynodont *Lystrosaurus* was amphibious, although it has for a long time been considered terrestrial (King, 1991). This may reflect a diversity in habitat use in dicynodonts.

Gorgonopsians are considered to be terrestrial (Cruickshank, 1973), but few studies were performed on lifestyle of gorgonopsians. In model A, this taxon is inferred as amphibious/terrestrial. In model B, this taxon is inferred as terrestrial. Thus, all lines of evidence suggest a fairly terrestrial lifestyle.

Both models indicate an aquatic lifestyle for *Pareiasaurus*. Its skeleton was very heavy, and it was probably relatively slow on land. Therefore, we may suggest that this animal spent much of its time in the water, like the extant hippopotamus. The inference model may, however, mislead us somewhat in this case because its morphology (i.e. its well-ossified skeleton, and possibly graviportal adaptations) suggests adequate terrestrial locomotor abilities, and this taxon will be considered as amphibious (rather than aquatic) for the optimizations. Other facts are compatible with an amphibious habitat for pareiasaurs. Least conclusive is the taphonomic evidence. For instance, in Morocco, the pareiasaur *Arganacras vacanti* has been found in a level which has also yielded the aquatic amphibian *Diplocaulus minimus*,

as well as presumably more terrestrial taxa, such as the captorhinids *Acrodonta irerhi* and a moradisaurine (Jalil & Janvier, 2005). Similarly, the pareiasaur *Bunostegos akokanensis* from Niger is associated with the fairly aquatic temnospondyl *Nigerpeton* and more terrestrial taxa, such as the temnospondyl *Saharastega* and the captorhinid *Moradisaurus* (Sidor *et al.*, 2003; Steyer *et al.*, 2006). The ichnological data are a bit more conclusive. The ichnotaxon *Sukhonopus primus* from the *Proelginia permiana* Tetrapod Zone, which is probably a bradysaurid pareiasaur, shows that at least some pareiasaurs inhabited a lowland, fairly humid environment (Gubin *et al.*, 2003). Finally, morphological data have previously been used to suggest an amphibious or aquatic habitat for the pareiasaur *Scutosaurus* (Ivakhnenko, 2001; cited in Gubin *et al.*, 2003). The bones of the pareiasaur *Bunostegos* have been described as spongy and pachyostotic (Sidor *et al.*, 2003, p. 46), which is also compatible with an amphibious or aquatic habitat.

Evolution of habitat: a preliminary optimization

Our preliminary optimization is presented to summarize the results obtained so far using inference models developed by Laurin *et al.* (2004) and Germain & Laurin (2005) and above. These results may change as more results are

incorporated in subsequent studies because we have not yet sampled the earliest diapsids (araeoscelidians) and their closest relatives (captorhinids, 'protorothyridids'), and only a few stem-tetrapods are included.

The ancestral lifestyle of stegocephalians is ambiguous (aquatic, amphibious or terrestrial). Thus, the exact timing of the conquest of land by vertebrates cannot be resolved by our data (Fig. 6). This probably reflects the limited sampling of stem-tetrapods in this study and highlights the need for further work in this area. A terrestrial lifestyle seems to have appeared by the Mississippian because the ancestral lifestyle of crown tetrapods, Amphibia, Amniota, Synapsida and Sauropsida is terrestrial.

Insertion of the extinct stegocephalians *Discosauriscus*, *Doleserpeton*, *Dutuitosaurus*, *Chroniosaurus* and *Cardiocephalus* changes the optimization of the ancestral lifestyle of the crown group of tetrapods. On the tree based on extant species and extinct species whose lifestyle is known, the ancestral lifestyle of crown tetrapods is aquatic (Fig. 2). Adding the extinct species whose lifestyle has been inferred with bone microanatomy data makes the ancestral lifestyle of crown tetrapods terrestrial (Fig. 6). Clearly, parsimony based on extant taxa alone is insufficient to draw inferences about the habitat of Palaeozoic taxa, which is not surprising.

Several returns to an aquatic environment have occurred as soon as the late Palaeozoic. The oldest identified return to a more aquatic lifestyle in this study is *Ophiacodon* (by the Early Permian, at the latest), but earlier returns have been identified in Carboniferous amphibians and stem-tetrapods using a broader taxonomic sampling and other types of characters that provided preliminary assessment of the lifestyle (Vallin & Laurin, 2004: fig. 8). A larger sample of stem-tetrapods and extinct amniotes should provide a more reliable optimization that will better reflect the complexity of the history of the conquest of land by vertebrates.

The inferred terrestrial habitat for synapsids disagrees with the results of Germain & Laurin (2005); this difference results from the addition of three extinct synapsids whose lifestyle has been inferred as terrestrial: *Dimetrodon*, *Dicynodon* and the gorgonopsian. According to the optimization, two synapsids (*Ophiacodon* and *Lystrosaurus*) became amphibious early in the history of this taxon. Romer (1957, 1958) thought that amniotes were primitively aquatic and that they only left the water to lay their eggs. This idea was mainly supported by the supposed amphibious or aquatic habits of *Ophiacodon*. However, even though this lifestyle has been confirmed by bone microanatomy, optimization shows that the amphibious lifestyle of *Ophiacodon* is probably secondary (Fig. 6).

Acknowledgments

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Appendix 1

Taxonomic identity and compactness profile parameters of the tibial sections of animals studied in the analysis. Each species is represented by a single individual. Values of *S*, *P*, Min and Max are the algebraic mean values of 60 radial values. Abbreviations: MD, maximal diameter; PSL, presacral length. Taxa of unknown lifestyle are not shown (see Table 5).

Taxon	Lifestyle	PSL (cm)	MD (mm)	Compactness	*S*	*P*	Min	Max
Osteolepiformes								
Eusthenopteron foordi	Aquatic	48.82	1.09	0.71307	0.00297	0.60334	0.12988	0.99991
Lissamphibia								
Urodela								
Salamandrella keyserlingii	Terrestrial	3.331	0.64389	0.61792	0.00329	0.65053	0.05827	0.99995
Onychodactylus fischeri	Aquatic	5.064	0.60178	0.91056	0.0047	0.27884	−0.00002	0.99981
Andrias japonicus	Aquatic	34	5.08073	0.97877	0.00151	0.12114	−0.00034	0.99858
Cryptobranchus alleganiensis	Aquatic	22.5	2.23586	0.8994	0.00213	0.41686	0.41876	0.99915
Amphiuma means	Aquatic	70.5	1.34495	0.59744	0.00131	0.24451	−0.00005	1.00016
Necturus maculosus	Aquatic	15.5	1.62019	0.96479	0.00191	0.19391	0	1.00002
Proteus anguineus	Aquatic	9.23	0.71977	0.76431	0.00397	0.50106	−0.00017	1.00012
Ambystoma andersoni	Aquatic	8	2.78684	0.86642	0.00275	0.37413	−0.00006	1.00001

Appendix 1

(*Continued*)

Taxon	Lifestyle	PSL (cm)	MD (mm)	Compactness	S	P	Min	Max
Ambystoma mexicanum	Aquatic	4.7	0.84692	0.53742	0.00523	0.68283	−0.00028	1.00014
Ambystoma opacum	Terrestrial	3.55	0.69912	0.40025	0.00313	0.77358	0.00044	0.99989
Salamandra atra	Terrestrial	4.055	0.99624	0.7111	0.0052	0.53396	−0.00029	1.00025
Salamandra lanzai	Terrestrial	5.7	1.20582	0.79563	0.0046	0.46768	−0.00034	1.00006
Salamandra salamandra	Terrestrial	6.4	1.28138	0.60451	0.00374	0.65938	−0.00009	1.00019
Pleurodeles waltl	Amphibious	5	0.96529	0.7407	0.00225	0.51111	−0.00001	1.00003
Triturus alpestris	Amphibious	3.806	0.26237	0.75895	0.00545	0.51947	−0.00031	1.00038
Triturus cristatus	Amphibious	3.822	0.92459	0.85333	0.00488	0.37851	−0.00025	1.00018
Plethodon glutinosus	Terrestrial	5.06	0.5705	0.73513	0.03536	0.50409	−0.00002	1.00021
Desmognathus ochrophaeus	Terrestrial	2.84	0.24871	0.64707	0.00424	0.57855	−0.00003	1.00006
Desmognathus monticola	Terrestrial	5.02	0.53041	0.64096	0.00384	0.59087	−0.00009	1.00017
Anura								
Rhinella marina (formerly known as *Bufo marinus*)	Terrestrial	18	4.15019	0.64754	0.00247	0.57493	−0.00007	0.96815
'Bufo' pentoni	Terrestrial	4.4	1.70914	0.72419	0.16873	0.42665	−0.08747	1.03745
Epidalea calamita (formerly known as *Bufo calamita*)	Terrestrial	3.787	3.89253	0.64264	0.00502	0.59174	−0.00018	0.99919
Boana boans (formerly known as *Hyla boans*)	Terrestrial	7.53	2.0573	0.52837	0.00321	0.68604	−0.00007	0.99994
Pachymedusa dacnicolor	Terrestrial	9	1.67693	0.75782	0.00202	0.4925	0	1.00001
Leptodactylus pentadactylus	Terrestrial	5.26	10.75468	0.75027	0.00424	0.49559	−0.00013	0.98817
Ceratophrys aurita	Terrestrial	6.652	27.85748	0.55605	0.00367	0.64515	−0.00005	0.97761
Telmatobius culeus	Aquatic	11.7	2.75602	0.73854	0.00265	0.51558	−0.00007	1.00003
Chiromantis rufescens	Terrestrial	3.4	0.91508	0.8156	0.00238	0.43117	−0.00004	1.00004
Rana vaillanti	Amphibious	4.639	2.81813	0.6133	0.00276	0.62384	−0.00002	0.99957
Rana forreri	Amphibious	2	1.13959	0.53454	0.00301	0.67905	−0.00008	0.97984
Rana dalmatina	Terrestrial	4.145	1.4094	0.74184	0.00284	0.50793	−0.00007	1.00007
Rana ridibunda	Amphibious	3.25	1.23711	0.68429	0.00332	0.55988	−0.0001	1.00003
Rana iberica	Amphibious	2.113	1.0494	0.68087	0.00222	0.55563	−0.00003	0.99987
Pipa carvalhoi	Aquatic	6.221	0.8878	0.96163	0.00128	0.2429	0.15376	0.98973
Xenopus laevis	Aquatic	4.1	2.82693	0.703	0.00273	0.37127	0.00003	0.90919
Discoglossus occipitalis	Amphibious	4.81	1.3421	0.5306	0.00331	0.68042	−0.00008	1.00006
Bombina orientalis	Aquatic	2.77	0.75746	0.72272	0.0038	0.52561	−0.00015	1.00009
Ascaphus truei	Amphibious	1.62	0.98296	0.89914	0.0035	0.31272	0.13191	0.99997
Chelonia								
Pelomedusa subrufa	Aquatic	16	3.0061	0.94549	0.00203	0.25947	0.35629	0.99441
Chelus fimbriatus	Aquatic	25	4.44438	0.96511	0.00075	0.24272	0.6201	0.99349
Chelydra serpentina	Aquatic	22.126	4.88215	0.82661	0.05158	0.36292	0.1614	0.95859
Emys orbicularis	Aquatic	13.81	2.51533	0.77045	0.23241	0.44243	−0.04453	1.10718
Malaclemys terrapin	Aquatic	17.38	2.95585	0.86946	0.00202	0.36867	0.33715	0.97526
Testudo hermanni	Terrestrial	30.65	1.90065	0.96982	0.68159	1.00013	0.05381	0.08421
Testudo graeca	Terrestrial	17.13	3.78009	0.8999	0.00125	0.33399	0.38885	0.96914
Homopus femoralis	Terrestrial	9.65	1.8747	0.9734	0.04173	0.1646	0.29898	0.99974
Astrochelys radiata	Terrestrial	19.314	6.46026	0.88838	0.05055	0.21605	−0.13778	0.95894
Chelonoidis carbonaria	Terrestrial	13.18	5.22107	0.89799	0.01562	0.35607	0.36304	0.97695
Pelodiscus sinensis	Aquatic	19.72	3.33597	0.85585	0.21448	0.10569	−0.61049	1.02547
Kinosternon sp.	Aquatic	18	1.86237	0.93397	0.00069	0.38962	0.64784	0.98406
Dermochelys coriacea	Aquatic	96	12.10516	0.57027	0.02654	0.73886	0.39379	0.84486
Chelonia mydas	Aquatic	36	5.77156	0.78644	0.00253	0.5927	0.43037	0.97913
Ichthyosauria								
Omphalosaurus nisseri	Aquatic	89.882	34.48	0.55886	0.00453	0.34767	0.36869	0.69604
Squamata								
Sceloporus horridus horridus	Terrestrial	5.6	1.20155	0.81232	0.00223	0.43509	−0.00002	1.00001
Sceloporus horridus oligoporus	Terrestrial	5.7	1.22308	0.69153	0.00343	0.54235	−0.00006	0.99989
Ctenosaura pectinata	Terrestrial	24	4.56065	0.43393	0.01147	0.75	0	1.00014

Appendix 1

(*Continued*)

Taxon	Lifestyle	PSL (cm)	MD (mm)	Compactness	S	P	Min	Max
Amblyrhynchus cristatus	Amphibious	40	6.15972	0.7278	0.00211	0.52172	0	1.00001
Iguana iguana	Terrestrial	5.5	4.92771	0.52362	0.00401	0.69023	0	0.99994
Uromastyx acanthinurus	Terrestrial	13	3.01441	0.73346	0.00256	0.5165	−0.00004	1.00002
Platecarpus sp.	Aquatic	120	21.69	0.87632	0.02689	0.51388	0.62654	0.98891
Varanus niloticus	Amphibious	42.913	4.77377	0.63194	0.00344	0.60639	−0.00011	1.00007
Varanus griseus	Terrestrial	27.5	4.46322	0.65147	0.00856	0.54946	0	0.99995
Varanus salvator	Amphibious	51	5.40609	0.65282	0.00323	0.58387	−0.00005	0.98925
Varanus komodoensis	Terrestrial	60	5.80211	0.64972	0.00326	0.59582	−0.00008	1.00011
Archosauria								
Crocodylus niloticus	Amphibious	175	30.61291	0.78038	0.00371	0.43195	−0.00016	0.9524
Mammalia								
Monotremata								
Tachyglossus aculeatus	Terrestrial	15.15	4.44249	0.53712	0.00384	0.67975	0.00019	0.99304
Ornithorhynchus anatinus	Aquatic	16.01	6.09	0.9545	0.00226	0.15796	−0.00027	0.97992
Xenarthra								
Zaedyus pichyi	Terrestrial	11.188	3.00699	0.8853	0.03283	0.30378	−0.06927	0.99724
Artiodactyla								
Camelus dromedarius	Terrestrial	316.96	42.99551	0.68558	0.00477	0.53722	−0.00043	0.96882
Sus scrofa	Terrestrial	57.2	17.91227	0.63785	0.00403	0.58674	−0.00022	0.97765
Cervus elaphus	Terrestrial	110	26.84739	0.62331	0.00348	0.58928	−0.00024	0.9519
Capreolus capreolus	Terrestrial	55	14.12589	0.81073	0.00211	0.42254	−0.00004	0.988
Carnivora								
Martes foina	Terrestrial	31	5.4	0.74389	0.00254	0.50935	−0.00003	0.99985
Martes martes	Terrestrial	28	3.72411	0.7293	0.00239	0.51568	−0.00002	0.99227
Mustela putorius	Terrestrial	35	4.50898	0.8298	0.00292	0.41333	−0.00008	0.99574
Mustela lutreola	Amphibious	35	3.59462	0.74389	0.00254	0.50935	−0.00003	0.99985
Mustela vison	Amphibious	37	2.14333	0.7574	0.00345	0.49885	−0.00013	1.00009
Amblonyx cinereus	Amphibious	24	4.87389	0.54728	0.03438	0.61357	−0.00849	0.90262
Lutra lutra	Amphibious	25	8.40335	0.85956	0.00363	0.37767	0.01994	0.99377
Mirounga leonina	Aquatic	420	38.94	0.60452	0.22127	0.70383	0.00738	1.26755
Ursus americanus	Terrestrial	95	19.76301	0.64238	0.00437	0.61689	0.03505	0.99689
Canis lupus	Terrestrial	71	21.57847	0.67235	0.02208	0.56221	0.00803	0.97951
Panthera leo	Terrestrial	120	27.46823	0.67235	0.02208	0.56221	0.00803	0.97951
Eulipotyphla								
Solenodon paradoxus	Terrestrial	15	3.97395	0.82793	0.0029	0.40221	−0.00019	0.99216
Galemys pyrenaicus	Amphibious	4	1.76368	0.74552	0.00251	0.50099	0	0.99924
Erinaceus europaeus	Terrestrial	16.01	7.26935	0.5294	0.00483	0.58654	0.08308	0.90313
Primates								
Macaca radiata	Terrestrial	28.5	8.07934	0.73739	0.0037	0.4936	−0.00026	0.96728
Chlorocebus aethiops	Terrestrial	32.5	10.76829	0.74805	0.00293	0.47512	−0.00002	0.95598
Homo sapiens	Terrestrial	65	20.73043	0.86563	0.00351	0.33747	−0.00011	0.97547
Hylobates sp.	Terrestrial	80	12.74663	0.72006	0.00351	0.52273	−0.00017	0.98504
Lagomorpha								
Oryctolagus cuniculus	Terrestrial	23.63	5.41202	0.6217	0.00293	0.60966	−0.00003	0.99598
Rodentia								
Arvicola sapidus	Terrestrial	6	1.80404	0.84939	0.0024	0.37449	−0.00012	0.9883
Arvicola terrestris	Terrestrial	6	1.02173	0.80901	0.00217	0.43511	−0.00002	1
Hystrix cristata	Terrestrial	40	13.54518	0.69782	0.00505	0.56053	0.01016	0.98427
Myocastor coypus	Amphibious	29.5	8.43407	0.86621	0.0027	0.34752	−0.00013	0.98145
Agouti paca	Terrestrial	33	9.3671	0.69124	0.00716	0.58323	0.07592	0.97027
Dasyprocta aguti	Terrestrial	24	6.42449	0.69699	0.0024	0.5441	−0.00006	0.98576
Hydrochoerus hydrochaeris	Amphibious	59.895	23.50703	0.60205	0.0254	0.64229	−0.00211	0.99743

Appendix 2

Linear discriminant analyses based on the tibia of 99 species. Probabilities are obtained after a cross-validation procedure. Correct inferences are in bold. Taxa of unknown lifestyle are not shown (see Table 5).

Taxon	Three states; total sample			Binary coding; total sample			Three states; lissamphibians only		
	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle
Osteolepiformes									
Eusthenopteron	0.539	Aquatic	Terrestrial	0.928	Aquatic	Amphibious/terrestrial			
Lissamphibia									
Urodela									
Salamandrella keyserlingii	**0.655**	Terrestrial	Terrestrial	**0.97**	Amphibious/terrestrial	Amphibious/terrestrial	**0.57**	Terrestrial	Terrestrial
Onychodactylus fischeri	0.496	Aquatic	Terrestrial	0.773	Aquatic	Amphibious/terrestrial	**0.466**	Aquatic	Aquatic
Andrias japonicus	**0.68**	Aquatic	Aquatic	**0.686**	Aquatic	Aquatic	**0.977**	Aquatic	Aquatic
Cryptobranchus alleganiensis	**0.802**	Aquatic	Aquatic	**0.809**	Aquatic	Aquatic	0.948	Aquatic	Terrestrial
Amphiuma means	**0.993**	Aquatic	Aquatic	**0.996**	Aquatic	Aquatic	**1**	Aquatic	Aquatic
Necturus maculosus	0.44	Aquatic	Aquatic	0.585	Aquatic	Amphibious/terrestrial	**0.841**	Aquatic	Aquatic
Proteus anguineus	0.631	Aquatic	Terrestrial	0.973	Aquatic	Amphibious/terrestrial	0.662	Aquatic	Terrestrial
Ambystoma andersoni	0.638	Aquatic	Terrestrial	0.944	Aquatic	Amphibious/terrestrial	0.389	Aquatic	Terrestrial
Ambystoma mexicanum	0.678	Aquatic	Terrestrial	0.987	Aquatic	Amphibious/terrestrial	0.636	Aquatic	Terrestrial
Ambystoma opacum	**0.641**	Terrestrial	Terrestrial	**0.971**	Amphibious/terrestrial	Amphibious/terrestrial	0.774	Terrestrial	Amphibious
Salamandra atra	**0.644**	Terrestrial	Terrestrial	**0.962**	Amphibious/terrestrial	Amphibious/terrestrial	**0.519**	Terrestrial	Terrestrial
Salamandra lanzai	**0.628**	Terrestrial	Terrestrial	**0.958**	Amphibious/terrestrial	Amphibious/terrestrial	**0.471**	Terrestrial	Terrestrial
Salamandra salamandra	**0.659**	Terrestrial	Terrestrial	**0.985**	Amphibious/terrestrial	Amphibious/terrestrial	**0.722**	Terrestrial	Terrestrial
Pleurodeles waltl	0.641	Amphibious	Terrestrial	0.962	Amphibious/terrestrial	Amphibious/terrestrial	0.531	Amphibious	Terrestrial
Triturus alpestris	0.643	Amphibious	Terrestrial	0.971	Amphibious/terrestrial	Amphibious/terrestrial	0.628	Amphibious	Terrestrial
Triturus cristatus	0.604	Amphibious	Terrestrial	0.898	Amphibious/terrestrial	Amphibious/terrestrial	0.421	Amphibious	Aquatic
Plethodon glutinosus	**0.627**	Terrestrial	Terrestrial	**0.907**	Amphibious/terrestrial	Amphibious/terrestrial	**0.647**	Terrestrial	Terrestrial
Desmognathus ochrophaeus	**0.636**	Terrestrial	Terrestrial	**0.958**	Amphibious/terrestrial	Amphibious/terrestrial	0.471	Terrestrial	Amphibious
Desmognathus monticola	**0.639**	Terrestrial	Terrestrial	**0.964**	Amphibious/terrestrial	Amphibious/terrestrial	**0.536**	Terrestrial	Terrestrial
Anura									
Rhinella marina	**0.661**	Terrestrial	Terrestrial	**0.968**	Amphibious/terrestrial	Amphibious/terrestrial	**0.643**	Terrestrial	Aquatic
'Bufo' pentoni	0.859	Terrestrial	Aquatic	0.879	Amphibious/terrestrial	Aquatic	**1**	Terrestrial	Terrestrial
Epidalea calamita	**0.707**	Terrestrial	Terrestrial	**0.978**	Amphibious/terrestrial	Amphibious/terrestrial	**0.623**	Terrestrial	Terrestrial
Boana boans	**0.669**	Terrestrial	Terrestrial	**0.977**	Amphibious/terrestrial	Amphibious/terrestrial	**0.637**	Terrestrial	Terrestrial
Pachymedusa dacnicolor	**0.632**	Terrestrial	Terrestrial	**0.958**	Amphibious/terrestrial	Amphibious/terrestrial	**0.518**	Terrestrial	Terrestrial
Leptodactylus pentadactylus	**0.786**	Terrestrial	Terrestrial	**0.988**	Amphibious/terrestrial	Amphibious/terrestrial	**0.696**	Terrestrial	Terrestrial
Ceratophrys aurita	**0.937**	Aquatic	Terrestrial	**1**	Aquatic	Amphibious/terrestrial	**0.998**	Terrestrial	Terrestrial
Telmatobius culeus	0.663	Aquatic	Terrestrial	0.977	Aquatic	Amphibious/terrestrial	0.724	Aquatic	Amphibious
Chiromantis rufescens	**0.612**	Terrestrial	Terrestrial	**0.939**	Amphibious/terrestrial	Amphibious/terrestrial	0.386	Terrestrial	Amphibious
Rana vaillanti	0.705	Amphibious	Terrestrial	**0.98**	Amphibious/terrestrial	Amphibious/terrestrial	**0.664**	Amphibious	Terrestrial

Appendix 2

(*Continued*)

Taxon	Three states; total sample Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Binary coding; total sample Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Three states; amniotes only Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle
Rana forreri	0.702	Amphibious	Terrestrial	**0.977**	Amphibious/terrestrial	Amphibious/terrestrial			
Rana dalmatina	**0.645**	Terrestrial	Terrestrial	**0.963**	Amphibious/terrestrial	Amphibious/terrestrial			
Rana ridibunda	0.662	Amphibious	Terrestrial	**0.969**	Amphibious/terrestrial	Amphibious/terrestrial			
Rana iberica	0.659	Amphibious	Terrestrial	**0.966**	Amphibious/terrestrial	Amphibious/terrestrial			
Pipa carvalhoi	**0.65**	Aquatic	Aquatic	**0.644**	Aquatic	Aquatic			
Xenopus laevis	0.489	Aquatic	Terrestrial	0.715	Aquatic	Amphibious/terrestrial			
Discoglossus occipitalis	0.706	Amphibious	Terrestrial	**0.974**	Amphibious/terrestrial	Amphibious/terrestrial			
Bombina orientalis	0.652	Aquatic	Terrestrial	**0.963**	Amphibious/terrestrial	Amphibious/terrestrial			
Ascaphus truei	0.53	Amphibious	Aquatic	0.509	Amphibious/terrestrial	Aquatic			
Chelonia									
Pelomedusa subrufa	**0.939**	Aquatic	Aquatic	**0.95**	Aquatic	Aquatic	**0.949**	Aquatic	Aquatic
Chelus fimbriatus	**0.997**	Aquatic	Aquatic	**0.999**	Aquatic	Aquatic	1	Aquatic	Aquatic
Chelydra serpentina	**0.726**	Aquatic	Aquatic	0.739	Aquatic	Aquatic	**0.679**	Aquatic	Aquatic
Emys orbicularis	**0.643**	Aquatic	Aquatic	0.715	Aquatic	Aquatic	**0.999**	Aquatic	Aquatic
Malaclemys terrapin	**0.823**	Aquatic	Aquatic	0.834	Aquatic	Aquatic	**0.727**	Aquatic	Aquatic
Testudo hermanni	–	Terrestrial	Terrestrial	–	Amphibious/terrestrial	Aquatic	**0.93**	Terrestrial	Terrestrial
Testudo graeca	0.94	Terrestrial	Aquatic	0.951	Amphibious/terrestrial	Aquatic	0.899	Terrestrial	Aquatic
Homopus femoralis	0.996	Terrestrial	Aquatic	0.998	Amphibious/terrestrial	Aquatic	0.998	Terrestrial	Aquatic
Astrochelys radiata	**0.461**	Terrestrial	Terrestrial	**0.646**	Amphibious/terrestrial	Amphibious/terrestrial	**0.781**	Terrestrial	Terrestrial
Chelonoidis carbonaria	0.881	Terrestrial	Terrestrial	0.896	Amphibious/terrestrial	Aquatic	0.845	Terrestrial	Aquatic
Pelodiscus sinensis	0.808	Aquatic	Aquatic	0.938	Aquatic	Amphibious/terrestrial	**0.532**	Aquatic	Aquatic
Kinosternon	**0.981**	Aquatic	Aquatic	**0.986**	Aquatic	Aquatic	**0.997**	Aquatic	Aquatic
Dermochelys coriacea	0.486	Aquatic	Terrestrial	0.681	Aquatic	Amphibious/terrestrial	0.585	Aquatic	Terrestrial
Chelonia mydas	0.49	Aquatic	Terrestrial	0.675	Aquatic	Amphibious/terrestrial	0.696	Aquatic	Terrestrial
Ichthyosauria									
Omphalosaurus nisseri	**0.617**	Aquatic	Aquatic	**0.857**	Aquatic	Aquatic	**0.999**	Aquatic	Aquatic
Squamata									
Sceloporus horridus horridus	**0.613**	Terrestrial	Terrestrial	**0.94**	Amphibious/terrestrial	Amphibious/terrestrial	**0.726**	Terrestrial	Terrestrial
Sceloporus horridus oligoporus	**0.642**	Terrestrial	Terrestrial	**0.959**	Amphibious/terrestrial	Amphibious/terrestrial	**0.733**	Terrestrial	Terrestrial
Ctenosaura pectinata	**0.676**	Terrestrial	Terrestrial	**0.969**	Amphibious/terrestrial	Amphibious/terrestrial	**0.767**	Terrestrial	Terrestrial
Amblyrhynchus cristatus	0.647	Amphibious	Terrestrial	**0.961**	Amphibious/terrestrial	Amphibious/terrestrial	0.718	Amphibious	Terrestrial
Iguana iguana	**0.726**	Terrestrial	Terrestrial	**0.985**	Amphibious/terrestrial	Amphibious/terrestrial	**0.779**	Terrestrial	Terrestrial
Uromastyx acanthinurus	**0.654**	Terrestrial	Terrestrial	**0.966**	Amphibious/terrestrial	Amphibious/terrestrial	**0.731**	Terrestrial	Terrestrial
Platecarpus sp.	**0.848**	Aquatic	Aquatic	0.885	Aquatic	Aquatic	**0.987**	Aquatic	Aquatic
Varanus niloticus	0.636	Amphibious	Terrestrial	**0.962**	Amphibious/terrestrial	Amphibious/terrestrial	0.704	Amphibious	Terrestrial
Varanus griseus	**0.638**	Terrestrial	Terrestrial	**0.931**	Amphibious/terrestrial	Amphibious/terrestrial	**0.751**	Terrestrial	Terrestrial
Varanus salvator	0.619	Amphibious	Terrestrial	**0.954**	Amphibious/terrestrial	Amphibious/terrestrial	0.691	Amphibious	Terrestrial

Appendix 2

(*Continued*)

Taxon	Three states; total sample			Binary coding; total sample					
	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle
Varanus komodoensis	**0.587**	Terrestrial	Terrestrial	**0.954**	Amphibious/terrestrial	Amphibious/terrestrial	**0.68**	Terrestrial	Terrestrial
Archosauria									
Crocodylus niloticus	0.795	Amphibious	Terrestrial	**0.929**	Amphibious/terrestrial	Amphibious/terrestrial	0.741	Amphibious	Terrestrial
Mammalia									
Monotremata									
Tachyglossus aculeatus	**0.694**	Terrestrial	Terrestrial	**0.98**	Amphibious/terrestrial	Amphibious/terrestrial	**0.754**	Terrestrial	Terrestrial
Ornithorhynchus anatinus	0.467	Aquatic	Aquatic	0.545	Aquatic	Amphibious/terrestrial	0.758	Aquatic	Terrestrial
Xenarthra									
Zaedyus pichyi	**0.557**	Terrestrial	Terrestrial	**0.822**	Amphibious/terrestrial	Amphibious/terrestrial	**0.77**	Terrestrial	Terrestrial
Artiodactyla									
Camelus dromedarius	0.475	Terrestrial	Amphibious	**0.72**	Amphibious/terrestrial	Amphibious/terrestrial	**0.624**	Terrestrial	Terrestrial
Sus scrofa	**0.794**	Terrestrial	Terrestrial	**0.989**	Amphibious/terrestrial	Amphibious/terrestrial	**0.807**	Terrestrial	Terrestrial
Cervus elaphus	**0.802**	Terrestrial	Terrestrial	**0.989**	Amphibious/terrestrial	Amphibious/terrestrial	**0.804**	Terrestrial	Terrestrial
Capreolus capreolus	**0.717**	Terrestrial	Terrestrial	**0.962**	Amphibious/terrestrial	Amphibious/terrestrial	**0.776**	Terrestrial	Terrestrial
Carnivora									
Martes foina	**0.65**	Terrestrial	Terrestrial	**0.964**	Amphibious/terrestrial	Amphibious/terrestrial	**0.724**	Terrestrial	Terrestrial
Martes martes	**0.622**	Terrestrial	Terrestrial	**0.955**	Amphibious/terrestrial	Amphibious/terrestrial	**0.711**	Terrestrial	Terrestrial
Mustela putorius	**0.58**	Terrestrial	Terrestrial	**0.916**	Amphibious/terrestrial	Amphibious/terrestrial	**0.711**	Terrestrial	Terrestrial
Mustela lutreola	0.611	Amphibious	Terrestrial	**0.95**	Amphibious/terrestrial	Amphibious/terrestrial	0.697	Amphibious	Terrestrial
Mustela vison	0.578	Amphibious	Amphibious	**0.934**	Amphibious/terrestrial	Amphibious/terrestrial	0.677	Amphibious	Terrestrial
Amblonyx cinereus	0.69	Amphibious	Amphibious	**0.911**	Amphibious/terrestrial	Amphibious/terrestrial	0.762	Amphibious	Terrestrial
Lutra lutra	0.7	Aquatic	Aquatic	**0.932**	Aquatic	Amphibious/terrestrial	0.781	Aquatic	Terrestrial
Mirounga leonina	**0.9**	Aquatic	Aquatic	**0.943**	Aquatic	Aquatic	1	Aquatic	Aquatic
Ursus americanus	**0.755**	Terrestrial	Terrestrial	**0.983**	Amphibious/terrestrial	Amphibious/terrestrial	**0.781**	Terrestrial	Terrestrial
Canis lupus	**0.823**	Terrestrial	Terrestrial	**0.986**	Amphibious/terrestrial	Amphibious/terrestrial	**0.841**	Terrestrial	Terrestrial
Panthera leo	**0.808**	Terrestrial	Terrestrial	**0.981**	Amphibious/terrestrial	Amphibious/terrestrial	**0.826**	Terrestrial	Terrestrial
Eulipotyphla									
Solenodon paradoxus	**0.625**	Terrestrial	Terrestrial	**0.926**	Amphibious/terrestrial	Amphibious/terrestrial	**0.744**	Terrestrial	Terrestrial
Galemys pyrenaicus	0.656	Amphibious	Terrestrial	**0.962**	Amphibious/terrestrial	Amphibious/terrestrial	0.735	Amphibious	Terrestrial
Erinaceus europaeus	**0.593**	Terrestrial	Terrestrial	**0.813**	Amphibious/terrestrial	Amphibious/terrestrial	**0.776**	Terrestrial	Terrestrial
Primates									
Macaca radiata	**0.693**	Terrestrial	Terrestrial	**0.967**	Amphibious/terrestrial	Amphibious/terrestrial	**0.752**	Terrestrial	Terrestrial
Chlorocebus aethiops	**0.721**	Terrestrial	Terrestrial	**0.971**	Amphibious/terrestrial	Amphibious/terrestrial	**0.769**	Terrestrial	Terrestrial
Homo sapiens	**0.763**	Terrestrial	Terrestrial	**0.952**	Amphibious/terrestrial	Amphibious/terrestrial	**0.826**	Terrestrial	Terrestrial
Hylobates sp.	**0.653**	Terrestrial	Terrestrial	**0.96**	Amphibious/terrestrial	Amphibious/terrestrial	**0.717**	Terrestrial	Terrestrial
Lagomorpha									
Oryctolagus cuniculus	**0.686**	Terrestrial	Terrestrial	**0.976**	Amphibious/terrestrial	Amphibious/terrestrial	**0.745**	Terrestrial	Terrestrial

Appendix 2

(*Continued*)

Taxon	Three states; total sample			Binary coding; total sample					
	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle	Probability of inferred lifestyle	Observed lifestyle	Inferred lifestyle
Rodentia									
Arvicola sapidus	**0.586**	Terrestrial	Terrestrial	**0.9**	Amphibious/terrestrial	Amphibious/terrestrial	**0.737**	Terrestrial	Terrestrial
Arvicola terrestris	**0.607**	Terrestrial	Terrestrial	**0.936**	Amphibious/terrestrial	Amphibious/terrestrial	**0.725**	Terrestrial	Terrestrial
Hystrix cristata	**0.768**	Terrestrial	Terrestrial	**0.988**	Amphibious/terrestrial	Amphibious/terrestrial	**0.79**	Terrestrial	Terrestrial
Myocastor coypus	0.67	Amphibious	Terrestrial	**0.91**	Amphibious/terrestrial	Amphibious/terrestrial	0.767	Amphibious	Terrestrial
Agouti paca	**0.725**	Terrestrial	Terrestrial	**0.972**	Amphibious/terrestrial	Amphibious/terrestrial	**0.783**	Terrestrial	Terrestrial
Dasyprocta aguti	**0.69**	Terrestrial	Terrestrial	**0.974**	Amphibious/terrestrial	Amphibious/terrestrial	**0.745**	Terrestrial	Terrestrial
Hydrochaerus capybara	0.939	Amphibious	Terrestrial	**0.996**	Amphibious/terrestrial	Amphibious/terrestrial	0.869	Amphibious	Terrestrial