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Fossils, Molecules, Divergence Times, and the Origin of Lissamphibians

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Abstract.—A review of the paleontological literature shows that the early dates of appearance of Lissamphibia recently inferred from molecular data do not favor an origin of extant amphibians from temnospondyls, contrary to recent claims. A supertree is assembled using new Mesquite modules that allow extinct taxa to be incorporated into a time-calibrated phylogeny with a user-defined geological time scale. The supertree incorporates 223 extinct species of lissamphibians and has a highly significant stratigraphic fit. Some divergences can even be dated with sufficient precision to serve as calibration points in molecular divergence date analyses. Fourteen combinations of minimal branch length settings and 10 random resolutions for each polytomy give much more recent minimal origination times of lissamphibian taxa than recent studies based on a phylogenetic analyses of molecular sequences. Attempts to replicate recent molecular date estimates show that these estimates depend strongly on the choice of calibration points, on the dating method, and on the chosen model of evolution; for instance, the estimate for the date of the origin of Lissamphibia can lie between 351 and 266 Mya. This range of values is generally compatible with our time-calibrated supertree and indicates that there is no unbridgeable gap between dates obtained using the fossil record and those using molecular evidence, contrary to previous suggestions. [Calibration point; fossil record; Lissamphibia; molecular dating; phylogenetics; stratigraphic fit; supertree.]

The origin of lissamphibians has been hotly debated in the last few years, and the number of hypotheses about their origin has not decreased (Carroll, 2001; Schoch and Carroll, 2003; Schoch and Milner, 2004; Lee and Anderson, 2006), despite several detailed phylogenetic analyses (Laurin, 1998; Anderson, 2001; Ruta et al., 2003; Vallin and Laurin, 2004). The currently competing hypotheses can be divided into three main categories. First, some investigations suggest that Lissamphibia is monophyletic and derived from the temnospondyls (Fig. 1a, b), in which case its sister-group may be *Doleserpeton* (Bolt, 1969), (*Doleserpeton* + *Amphibamus*; Ruta et al., 2003), Branchiosauridae (Milner, 1990, 1993b), or a subgroup of Branchiosauridae (Trueb and Cloutier, 1991). Second, in other hypotheses (Fig. 1c), Lissamphibia is monophyletic but derived from the "lepospondyls" (Laurin, 1998; Vallin and Laurin, 2004). Third, several studies have suggested diphyly or triphyly of extant amphibians (Fig. 1d), with an origin of anurans and sometimes urodeles within temnospondyls (sometimes different temnospondyls), and an origin of apodans and sometimes urodeles within "lepospondyls" (Carroll and Currie, 1975; Carroll and Holmes, 1980; Carroll et al., 1999; Anderson, 2001; Carroll, 2001; Schoch and Carroll, 2003; Lee and Anderson, 2006).

Zhang et al. (2005) recently assessed the relative merits of these various paleontological hypotheses about the origin of lissamphibians by estimating the dates of the main cladogeneses (splits) within Lissamphibia and comparing the minimal age of Lissamphibia with the time of origin of its presumed sister-groups. Their test rests on the plausible hypothesis that the lissamphibians (here taken as a crown-group; see Glossary) should have started differentiating around the time when their presumed extinct sister-group first appeared in the fossil record. However, the large number of autapomorphies of Lissamphibia (e.g., Trueb and Cloutier, 1991:285; Milner, 1993b:17; Laurin, 1998:6; Ruta et al., 2003:272) suggests that the lissamphibian stem must have persisted a fairly long time before the crown-group started differentiating. A literal interpretation of the fossil record suggests that lissamphibians started differentiating well after the origin of any of their suggested Paleozoic relatives (Ruta and Coates, 2003). However, a literal interpretation of the fossil record always underestimates the date of appearance of taxa because it can only give a latest possible date of appearance, not an earliest possible date of appearance; therefore, Zhang et al. (2005) tried to determine when the first lissamphibians actually appeared by dating a few relevant cladogeneses within that clade. They concluded that Lissamphibia arose about 337 million years ago (Mya), with a 95% confidence interval extending from 321 to 353 Mya. This means that the divergence between the last common ancestors of apodans and batrachians occurred between the Tournaisian and the Serpukhovian (thus spanning the Early Carboniferous), probably in the Viséan. Zhang et al. (2005) argued that this date suggests that a temnospondyl origin of lissamphibians is more credible than a lepospondyl origin. This is based on the time overlap between the prospective sister- or stem-groups and the inferred time of origin of Lissamphibia. Zhang et al. (2005) believed that Dissorophoidea had appeared in the Viséan and that Lysorophia had appeared in the Late Carboniferous. Because this date of appearance of the first lysorophians is close to the upper (most recent) end of the 95% confidence interval of the origin of lissamphibians, Zhang et al. (2005) concluded that this hypothesis was less credible than an origin of lissamphibians from dissorophoids.

The reasoning of Zhang et al. (2005) relies on the implicit assumption that the observed time of appearance of dissorophoids and lysorophians in the fossil record is not misleading. We make similar assumptions below because one of our aims is to show that the methods used by Zhang et al. (2005), when used with correct stratigraphic data, suggest that all recent hypotheses about a monophyletic lissamphibian origin are consistent with the timing of lissamphibian diversification. A time-calibrated supertree of lissamphibians suggests a much later time of diversification of this clade than

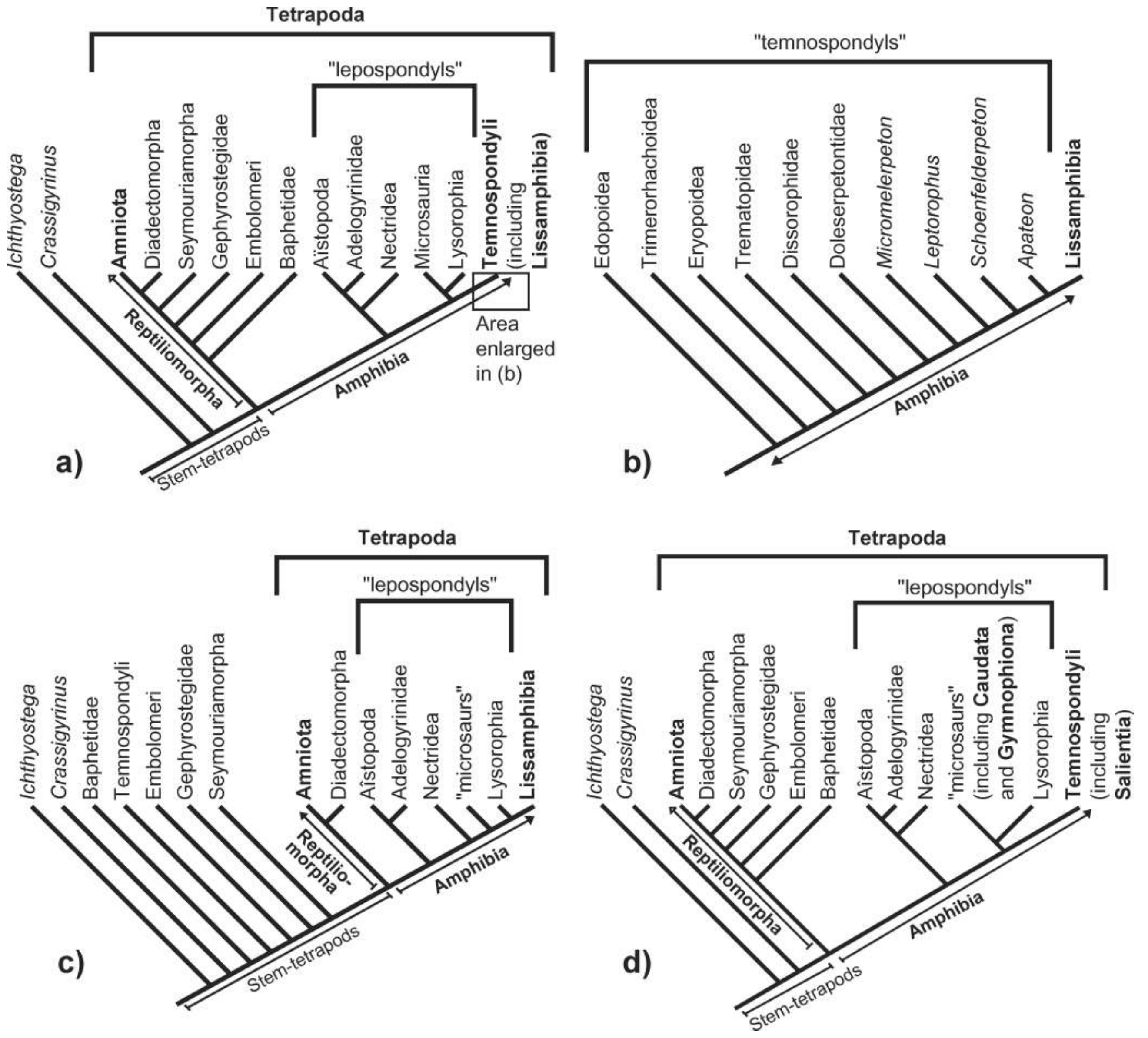

FIGURE 1. Competing phylogenetic hypotheses about the origin of extant amphibians showing (a and b) an origin of Lissamphibia within "temnospondyls"; (c) an origin of Lissamphibia within "lepospondyls"; (d) a polyphyletic origin of extant amphibians (the exact topology was not specified in most studies advocating this hypothesis; in some cases Caudata is placed among the temnospondyls). Names of extant taxa in bold.

argued by Zhang et al. (2005). Further, we date lissamphibian diversification by various methods, branch lengths obtained from several evolutionary models, and diverse combinations of calibration points, using the molecular data presented by Zhang et al. (2005). As such, our study attempts to be a fairly thorough analysis of both the fossil and molecular evidence about the timing of lissamphibian diversification.

MATERIALS AND METHODS

Stratigraphic Range of Paleozoic Stegocephalians

We assessed the stratigraphic range of potential relatives of Lissamphibia in the Paleozoic using the literature (Fig. 2). This survey was undertaken because the ranges used by Zhang et al. (2005) are clearly mistaken (see below).

Compilation of the Time-Calibrated Supertree

We suspected that the divergence dates within Lissamphibia inferred by Zhang et al. (2005) were too old when compared to the presumed sister-groups of Lissamphibia and to the lissamphibian fossil record. To test this idea, we have compiled a fairly extensive supertree of Lissamphibia and performed several sensitivity and statistical analyses.

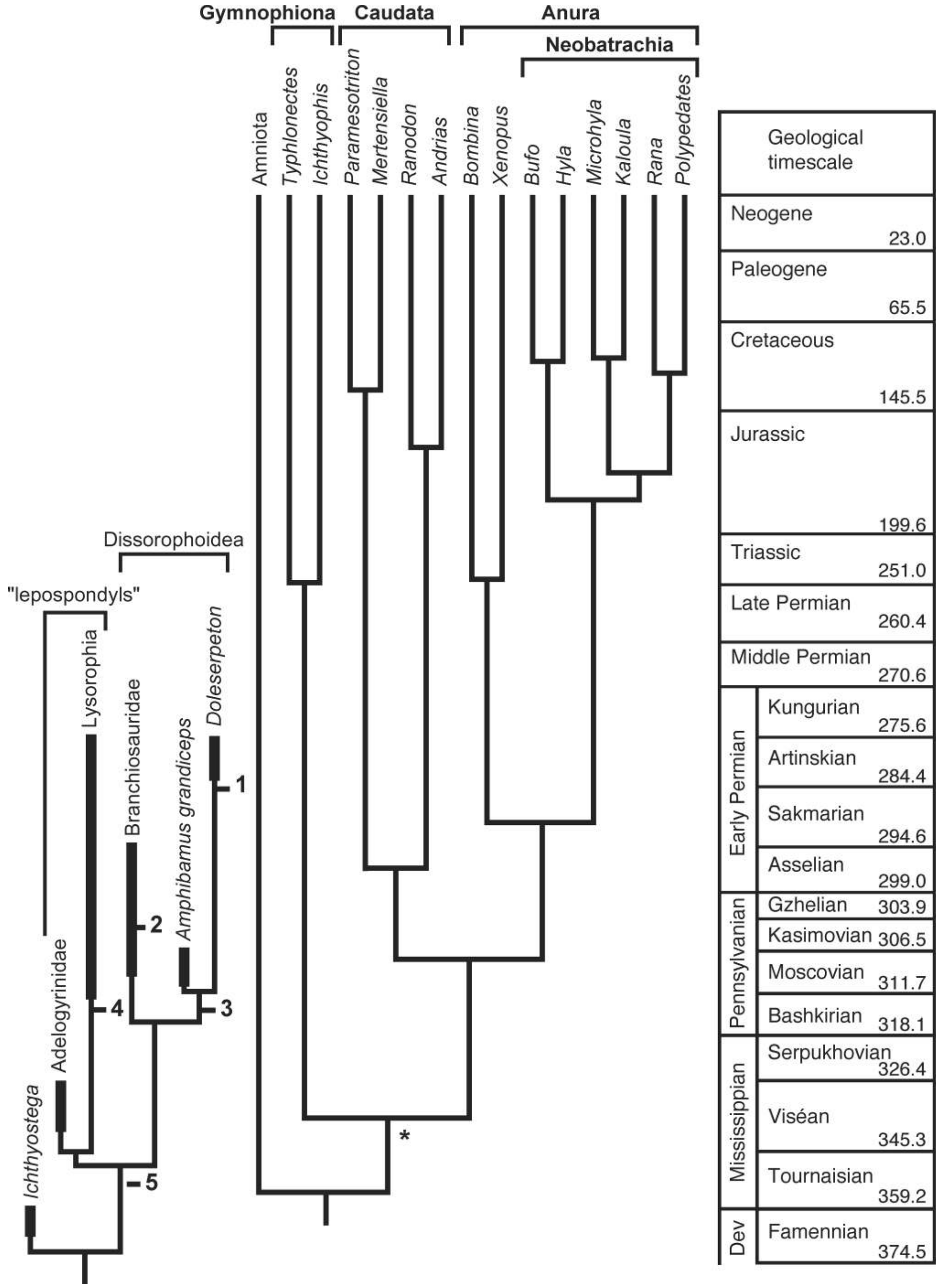

FIGURE 2. Stratigraphic range of proposed close relatives of Lissamphibia, possible positions of the lissamphibian stem, and main divergence dates within extant amphibians inferred by Zhang et al. (2005). Possible position of the lissamphibian stem (in all cases the placement of the stem has been put as high up into the geological section as seems plausible under the various phylogenetic hypotheses): 1, sister-group of *Doleserpeton* (Fig. 1a; Bolt, 1969); 2, within branchiosaurids (Fig. 1a, b; Trueb and Cloutier, 1991); 3, sister-group of a clade that includes *Doleserpeton* and *Amphibamus* (Fig. 1a; Ruta et al., 2003); 4, sister-group of lysorophians, within "lepospondyls" (Fig. 1c; Laurin, 1998); 5, polyphyletic origin in which some extant amphibians (gymnophiones, or gymnophiones and caudates) are nested within "lepospondyls" and the others are nested within temnospondyls (Fig. 1d; Carroll and Currie, 1975; Carroll and Holmes, 1980; Carroll et al., 1999). Known stratigraphic ranges (including uncertainties): thick lines; ghost ranges: thin lines. The latest possible time of divergence between the lissamphibian stem and its possible Paleozoic sister-group according to the dating of Zhang et al. (2005) is indicated by an asterisk (*). Note that this date is incompatible with the latest possible divergence date between the lissamphibian stem and its possible Paleozoic sister-groups (earliest possible divergence dates cannot be determined directly from the fossil record) but that it is compatible with the hypothesis that extant amphibians are polyphyletic with respect to Paleozoic stegocephalians (5). The geological time scale used is from Gradstein et al. (2004).

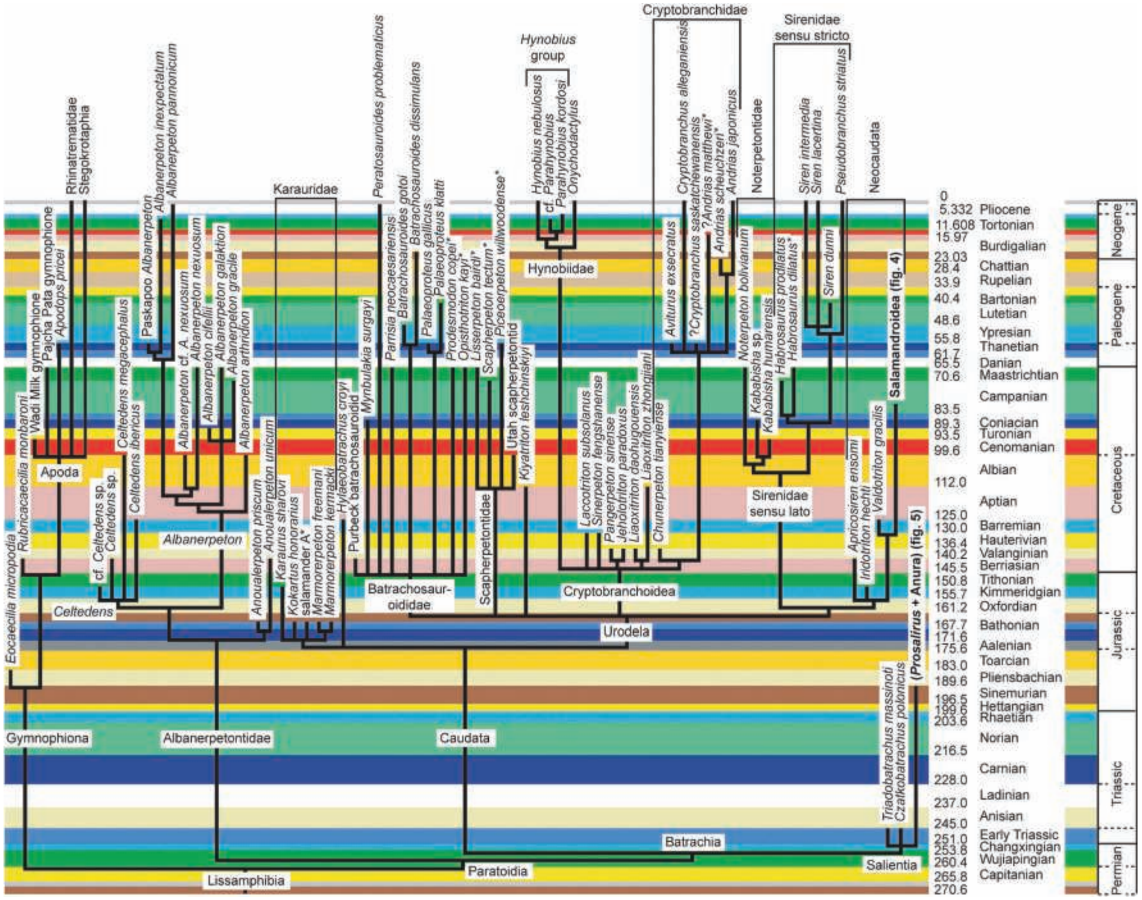

FIGURE 3. Time-calibrated supertree of extant and extinct lissamphibians, fitted to a geological timescale (Gradstein et al., 2004) in Mesquite (Maddison and Maddison, 2005). All extinct taxa that could be dated and placed with reasonable precision in the phylogeny are included; clades without a fossil record are collapsed or reduced to one representative species to save space. Taxa marked with an asterisk are known to extend at least into the following geological stage. All polytomies represent uncertainty (they are soft polytomies). The position of clade labels with respect to nodes, internodes, or terminal taxon labels is purely due to reasons of aesthetics and legibility, not, e.g., to the different types of phylogenetic definitions. See the text for more information. The sources are cited in Appendix 1. Continues in Figures 4 to 7. *Unlabeled stages from top to bottom:* "Quarternary" (Holocene + Pleistocene; beginning 1.806 Mya), Messinian (beginning 7.246), Serravallian (beginning 13.65), Langhian, Aquitanian (end 20.43), Priabonian (beginning 37.2), Selandian (end 58.7), Santonian (beginning 85.8), Callovian (beginning 164.7), Bajocian, Hettangian, Wordian (beginning 268.0), Roadian. The standard subdivisions of the periods are indicated by dashed lines. These are (*bottom to top*) "Guadalupian" and "Luopingian" for the Permian; "Early," "Middle," and "Late" for the Triassic and Jurassic; "Early" and "Late" for the Cretaceous; "Paleocene," "Eocene," and "Oligocene" for the Paleogene; and "Miocene" and "Pliocene" for the Neogene. The Early Triassic has now been divided into two stages (Gradstein et al., 2004), but we have not been able to apply this recent development to our data.

Taxa were selected and entered into a time-calibrated supertree (Figs. 3 to 7) in Mesquite (Maddison and Maddison, 2005) using the Stratigraphic Tools for Mesquite recently developed by our team (Josse et al., 2006). The terminal taxa were placed in the phylogeny and the stratigraphy according to several objective criteria (Appendix 1; all appendices of this paper can be downloaded from the Systematic Biology website at www.systematicbiology.org). In the absence of evidence to the contrary, all taxa (at all Linnaean ranks) were interpreted as clades. In some cases, like the Middle Jurassic to Early Cretaceous *Eodiscoglossus*, this might bias our conclusions towards earlier divergence dates, because paleoherpetologists have often attributed fragmentary fossils with few (if any) diagnostic characters to known (especially extant) taxa, sometimes based on unpolarized similarities. This practice may have resulted in the erroneous interpretation of paraphyletic taxa as clades. Thus, our approach could bias our results by yielding earlier diversification dates of several crown-groups, thereby decreasing the incongruence between our results and those of Zhang et al. (2005).

An effort was made to find the oldest known member of every clade, even if its phylogenetic position within that clade is unknown. We have expressed such uncertainties as polytomies (all polytomies in the supertree should be interpreted as soft polytomies). Again, this should generally bias our conclusions towards older divergence estimates because most possible resolutions of these polytomies would result in more recent appearances of crown-groups than shown here. As an extreme example, the divergence between the living *Bombina* and *Discoglossus* is Middle Jurassic in our tree (Fig. 5) because of the uncertain position of the Middle

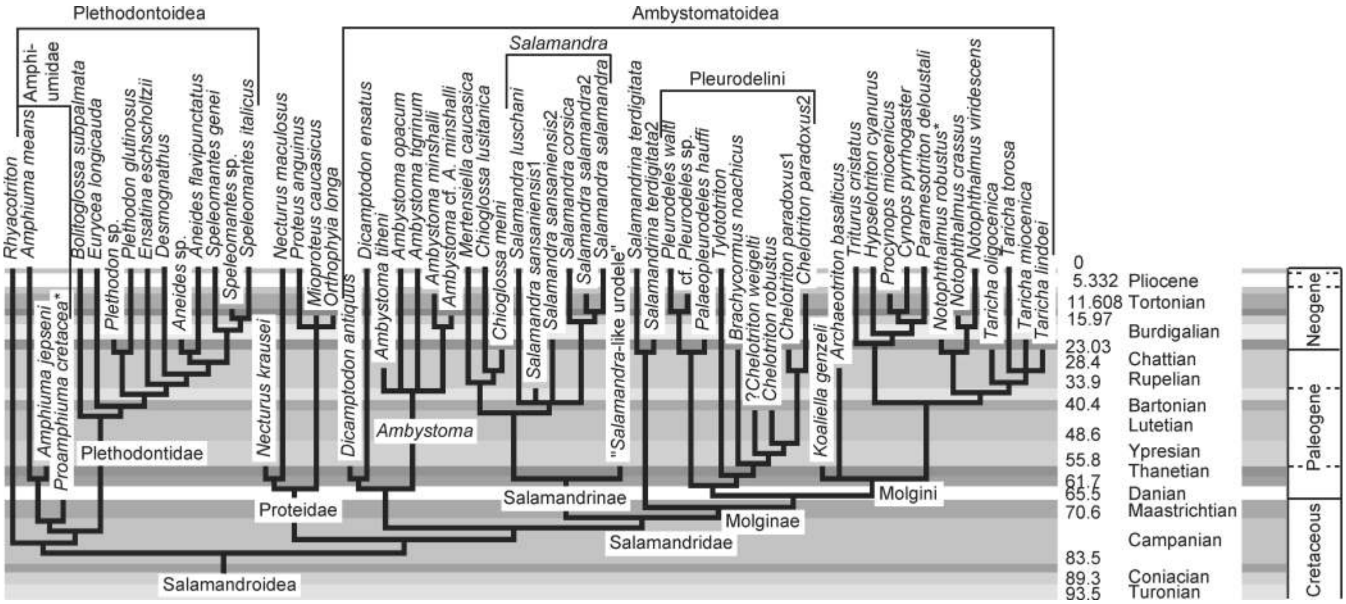

FIGURE 4. Time-calibrated supertree of extant and extinct Salamandroidea. See legend of Figure 3 for more information.

Jurassic *Eodiscoglossus oxoniensis* (Evans et al., 1990). Some resolutions of the polytomy (Discoglossoidea in Fig. 5, Discoglossoidea or Discoglossidae of most authors, Costata of Frost et al., 2006) in which these three taxa, the Early Cretaceous *Callobatrachus*, the Eocene and Oligocene *Opisthocoelellus*, and the Eocene (or Oligocene) to Pliocene *Latonia* lie, would yield much younger divergence date estimates for the living discoglossoids, as young as Oligocene in some cases. To study the impact of the polytomies on the age of appearance of various taxa, we have produced 10 random resolutions of all important polytomies in MacClade 4.06 (Maddison and Maddison, 2003) using the "equiprobable trees" algorithm, reproduced them manually into copies of our supertree, and redone the age adjustment of the tree using the Stratigraphic Tools (Josse et al., 2006), with the usual assumption that each species occupies at least an entire geological stage and that each internal branch is at least 3 My long. Ten random resolutions may seem low, but this procedure was time consuming because these topologies had to be manually entered into Mesquite and the stratigraphic adjustment had to be redone for each resolution of each polytomy; furthermore, many of the polytomies are trichotomies, for which every possible resolution occurs more often than once.

The huge phylogenetic analysis of Lissamphibia by Frost et al. (2006) appeared too late to be used as a source for the construction of our tree (with the exception of bufonid phylogeny). This opened the interesting possibility of testing if our analyses give different results for the tree by Frost et al. (2006) and our supertree (Appendices 2, 3). Therefore, we made a second supertree (Appendix 2) by rearranging our supertree to conform to the topology found by Frost et al. (2006).

A minimal internal branch length of 3 My was used when compiling the tree; this pushes speciose clades back in time, even when their fossil record is poor. A value greater than about 1 My is required to make the supertree legible (otherwise, many resolved clades would appear in the figures as if they were polytomies). The value of 3 My is, of course, arbitrary. If we had used 2 or 4 My, this would have modified the age of most clades only marginally; however, Laurin (2004) found that 3 My was a plausible minimal internal branch length in his study of early stegocephalian body size evolution. To test the impact of our branch length assumptions on the estimated age of the taxa, we have compiled the ages of 16 of the most relevant taxa yielded by 14 distinct assumptions (Appendix 4). Five of these assumptions differ only by the assumed minimal internal branch lengths (we tested values ranging from 0.1 to 5 My) and assumed, as shown in the supertree (Figs. 3 to 7), that each species occupied at least a whole geological stage. The value used to produce the supertree (3 My) was, for comparison, among those that we included. Another method that we used consists in assuming that each terminal branch (here represented by the included species) lasted at least a minimal amount of time (we tested values ranging from 0.1 to 5 My) and that each branch ends at the top of each geological stage; of course, minimal internal branch lengths must also be specified here (we tested values ranging from 0.1 to 5 My). Using three values each of internal and terminal branch lengths, nine cases were examined (Appendix 4). The 14 tested assumptions probably encompass all plausible values because it seems unlikely that the interval between most cladogenetic events included in our supertree was less than 0.1 My or more than 5 My. Using Stratigraphic Tools (Josse et al., 2006), these assumptions can be changed and the branch lengths of the supertree readjusted in seconds. However, the precision of the age is no better than about ±2 My if it is read by using the scale in Mesquite (as we did). Alternatively, accurate ages

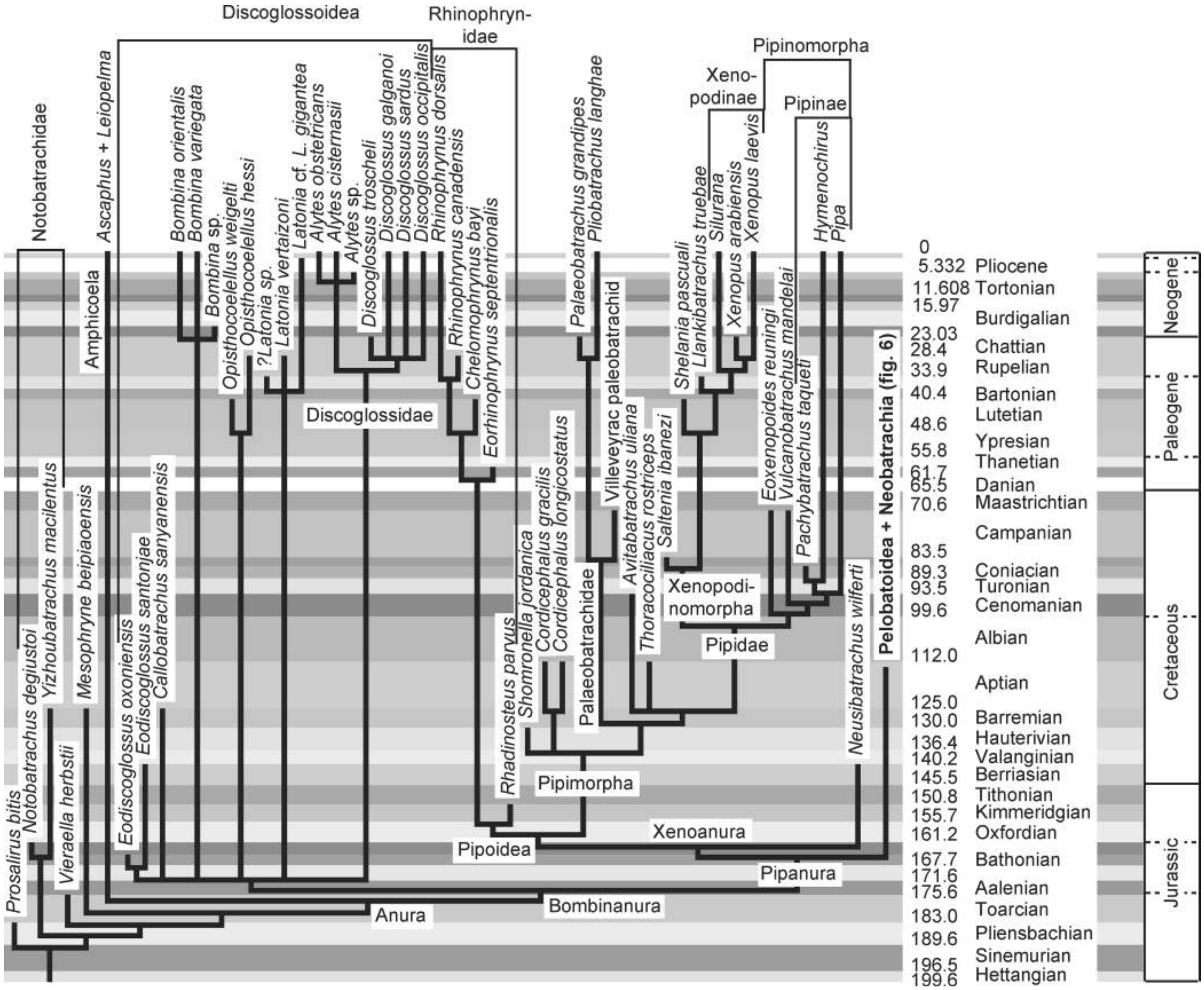

FIGURE 5. Time-calibrated supertree of extant and extinct Salientia (part). Continues in Figures 6 and 7. See legend of Figure 3 for more information. Note that *pliobatrachus*, the last palaeobatrachid, died out in the early Pleistocene.

could be computed by adding all the branches leading from the top of the tree to the various nodes, but given the several other imprecisions that affect the values obtained (real age of the various geological stage boundaries—up to ±4 My—location of the fossils compared to the stage boundaries, uncertainties about phylogenetic positions), the gain in precision would probably be more apparent than real. We have also tested the sensitivity of node ages to phylogenetic uncertainties by randomly resolving polytomies (Appendix 5).

Our supertrees include 223 extinct lissamphibian species, as well as several extant taxa that serve as a scaffold to ease the interpretation of the tree. Some of the extant species belong to large clades without a known fossil record.

More information about the supertree construction can be found in Appendix 1.

Because Frost et al. (2006) have produced the most comprehensive phylogeny of lissamphibians and proposed a detailed classification, we initially wished to use their nomenclature. However, several considerations led us to depart from this approach for most names (Appendix 6).

Fossils with uncertain stratigraphic positions (possible ranges spanning two or three stages) were interpreted as coming from the end of the lowest possible stage. In many cases, this results in a stratigraphic range that ends in the middle of the uncertainty interval; if this practice has introduced bias, it is towards older divergence estimates that should be more congruent with those of Zhang et al. (2005), especially because all terminal branches span at least an entire stage.

Test of the Stratigraphic Fit of the Supertree

To test if the lissamphibian fossil record is good enough for our subsequent calculations, we calculated the

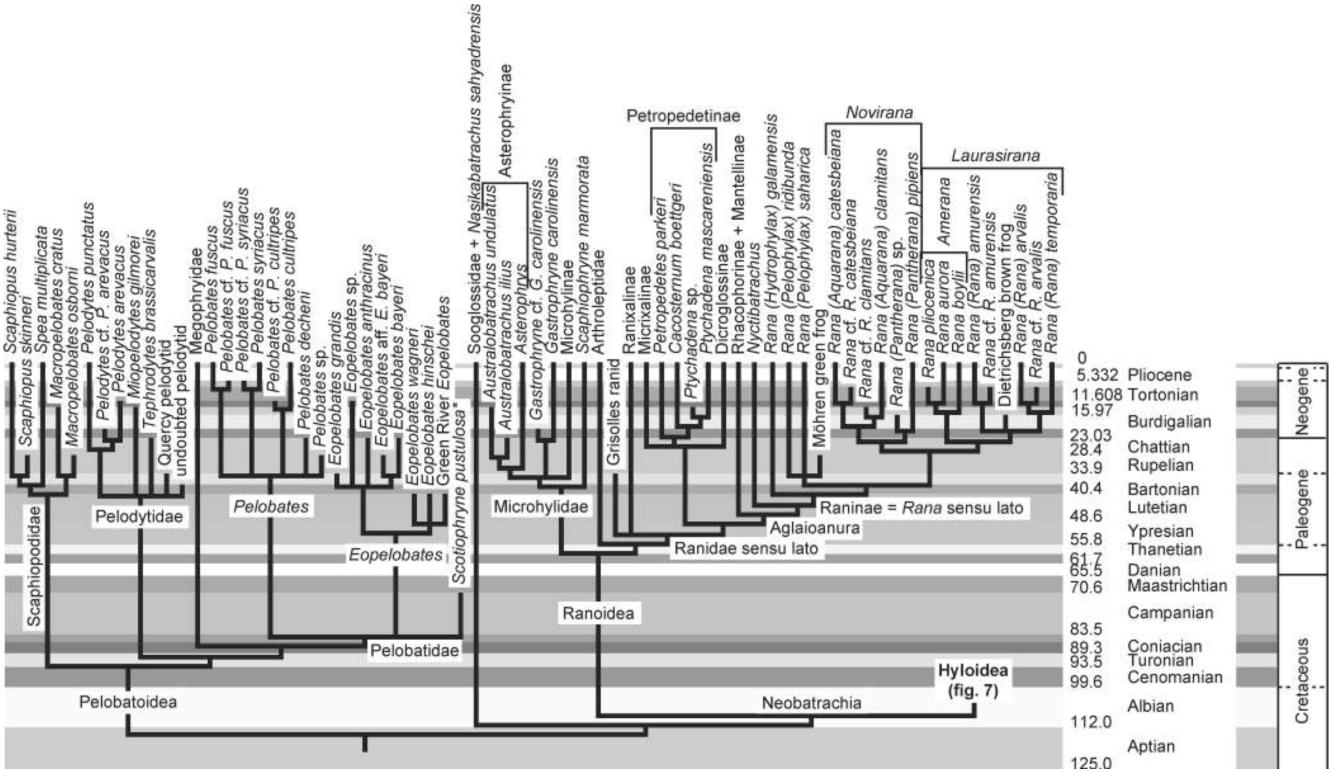

FIGURE 6. Time-calibrated supertree of extant and extinct Pelobatoidea and Neobatrachia (plus Sooglossidae & *Nasikabatrachus*). Continues in Figure 7. The name Aglaioanura comes from Frost et al. (2006). See legend of Figure 3 for more information.

stratigraphic fit of our trees. A low stratigraphic fit would mean that either the fossil record or the trees or both are of low quality; but given the quality and general congruence of the references used to construct the supertrees, we assume here that the trees are correct enough that they can be used to test the quality of the fossil record.

The stratigraphic fit of our supertrees was tested using Ghost (Wills, 1999), which computes three of the main stratigraphic fit indices, the Stratigraphic Consistency Index (SCI; Huelsenbeck, 1994), the Relative Completeness Index (RCI; Benton, 1994), and the Gap Excess Ratio (GER; Wills, 1999), and performs randomization of the

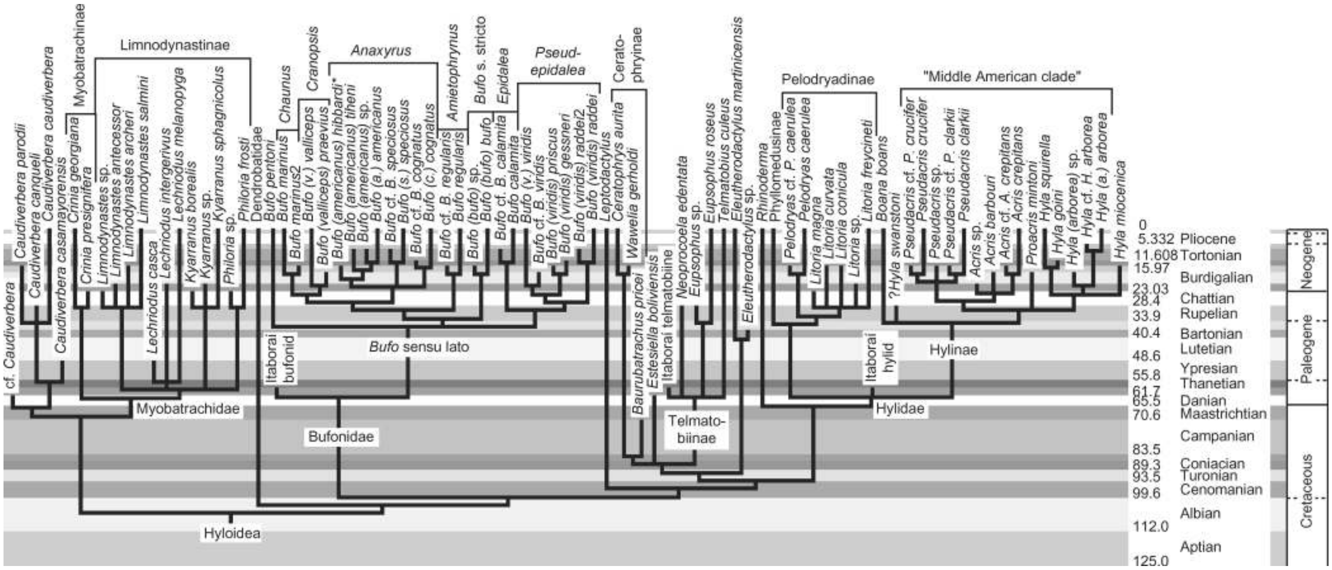

FIGURE 7. Time-calibrated supertree of extant and extinct Hyloidea. The resolution of *Bufo* sensu lato comes from Frost et al. (2006). See legend of Figure 3 for more information.

stratigraphic ranges to determine if the stratigraphic fit observed is better than random. We performed 9999 randomization replicates for each tree.

This test can assess both the fit of a tree to the fossil record, if the latter is considered sufficiently complete to be reliable, or (more importantly for our purpose) it can assess the completeness of the fossil record if the phylogeny is considered sufficiently reliable. This latter test is possible because if the fossil record of a group were very poor, we would expect a congruence between the order of cladogenesis and the order of appearance in the fossil record no better than random. Indeed, this method was used by Norell and Novacek (1992a, 1992b), Benton (1994, 1998, 2001), Benton and Storrs (1994), Benton and Simms (1995), Benton and Hitchin (1996), Benton et al. (1999, 2000), and Fara and Benton (2000), among others, to assess the quality of the known fossil record of various metazoans and its improvement in historical times.

The use of the SCI, RCI, and GER has been criticized because these indices are affected by the number of taxa in a tree, the number of stratigraphic ages of included taxa, and tree balance (Siddall, 1996, 1997; Pol et al., 2004). Thus, these indices cannot be used to compare the stratigraphic fit of trees that differ in any of these three factors. This is not a problem for our study because we only assess the stratigraphic fit of our trees by comparing these indices on our reference tree using the original (observed) stratigraphic ranges of taxa to the same indices on the same tree on which the stratigraphic ranges have been permuted. In this context, the use of these indices to assess the statistical significance of a stratigraphic fit should be unproblematic (Wills, 1999:567; Pol et al., 2004:70).

We have also tested the stratigraphic fit of the tree by Zhang et al. (2005) compared to that of our tree. As explained above, this is only meaningful if the trees are very similar; therefore, we pruned all taxa except for those used by Zhang et al. (2005) from our tree. Counting the branch lengths (in My) that lie between the first fossil of a clade and its estimated origin as ghost lineages, we calculated the total length of all ghost lineages (here termed the Actual Implied Gap, or AIG) for each tree. As the estimated date of origin, we used its molecular divergence date estimate without confidence intervals calculated by Zhang et al. (2005) and its divergence date based on a literal reading of our tree (with every terminal branch occupying at least an entire stage and minimal internal branch length set to 3 My). We interpreted the terminal branches as total clades and resolved polytomies in the way that gives the smallest gap; thus, we, e.g., counted the Wadi Milk Formation gymnophione as either Rhinatrematidae or Stegokrotaphia, which means that one of these two taxa is interpreted as having a continuous fossil record over the last 99.6 My.

Reanalysis of the Data Set of Zhang et al. (2005)

We calculated the most likely tree for the data set of Zhang et al. (2005: supplementary information) in PAUP* 4.0b10 (Swofford, 2003; 10 addition-sequence replicates, TBR swapping) using three different likelihood settings (evolutionary models) chosen by ModelTest 3.7 (see Posada and Crandall, 1998, for the first version) using the Akaike information criterion (following Posada and Buckley, 2004). We cannot replicate the model parameters Zhang et al. used (personal communication from P. Zhang), presumably because we used version 3.7 rather than 3.06.

Choice of models of evolution.—We would like to draw attention to some procedures followed by ModelTest that were not presented by the programmers (Posada and Crandall, 1998; Posada and Buckley, 2004). ModelTest begins by having PAUP* construct a tree from the data and then tests which parameters fit this tree best. That tree is a neighbor-joining tree calculated using the Jukes-Cantor model and is by default not displayed (Debruyne and Tassy, 2004). For the data of Zhang et al. (2005) this tree differs widely from all phylogenetic hypotheses proposed so far (Fig. 8a). We strongly disagree with this combination of approaches because it chooses the model parameters, which are subsequently used for the calculation of a phylogenetic tree, to fit a similarity diagram that is a poor representation of lissamphibian phylogeny. In this case, this is shown by the fact that Bombinanura (and by implication Anura) is polyphyletic, which is distressing because the monophyly of this taxon (in the context of this analysis: an anuran clade that excludes apodans and urodeles) is universally accepted and is supported by numerous apomorphies (Laurin, 1998; Frost et al., 2006). Accordingly, we modified the script of ModelTest 3.7 (which is a macro for PAUP*, that is, a NEXUS file) to calculate the most parsimonious tree (that is, a phylogenetic tree) and to display it. (This modified script is available from us upon request.) The tree (Fig. 8b) and model (Appendix 7) that result from this procedure are markedly different.

Still, the most parsimonious tree contains a monophyletic "Archaeobatrachia." Because we, like Zhang et al. (2005), consider this to be unlikely (see above), we inserted a constraint into the modified ModelTest script, requiring the topology (*Bombina* + (*Xenopus* + Neobatrachia)), which has so far been found by all studies that find "Archaeobatrachia" to be paraphyletic, except for that of Frost et al. (2006). The model calculated using this tree (see below) is very similar to the model chosen based on the unconstrained most parsimonious tree, presumably reflecting the fact that these two trees are much more similar to each other than to the neighbor-joining tree.

Divergence date estimates.—Zhang et al. (2005) used Multidivtime (Thorne and Kishino, 2002) to estimate the dates of the cladogeneses in their tree. For various reasons, including our unfamiliarity with UNIX commands, we found ourselves unable to use it. We had to resort to more user-friendly programs that use different dating algorithms, namely quartet dating as implemented in QDate 1.11 (Rambaut and Bromham, 1998), penalized likelihood (Sanderson, 2002) as implemented in r8s 1.71 (Sanderson, 2003, 2006), and a method recently presented by Anderson (2006), as implemented in PATHd8.

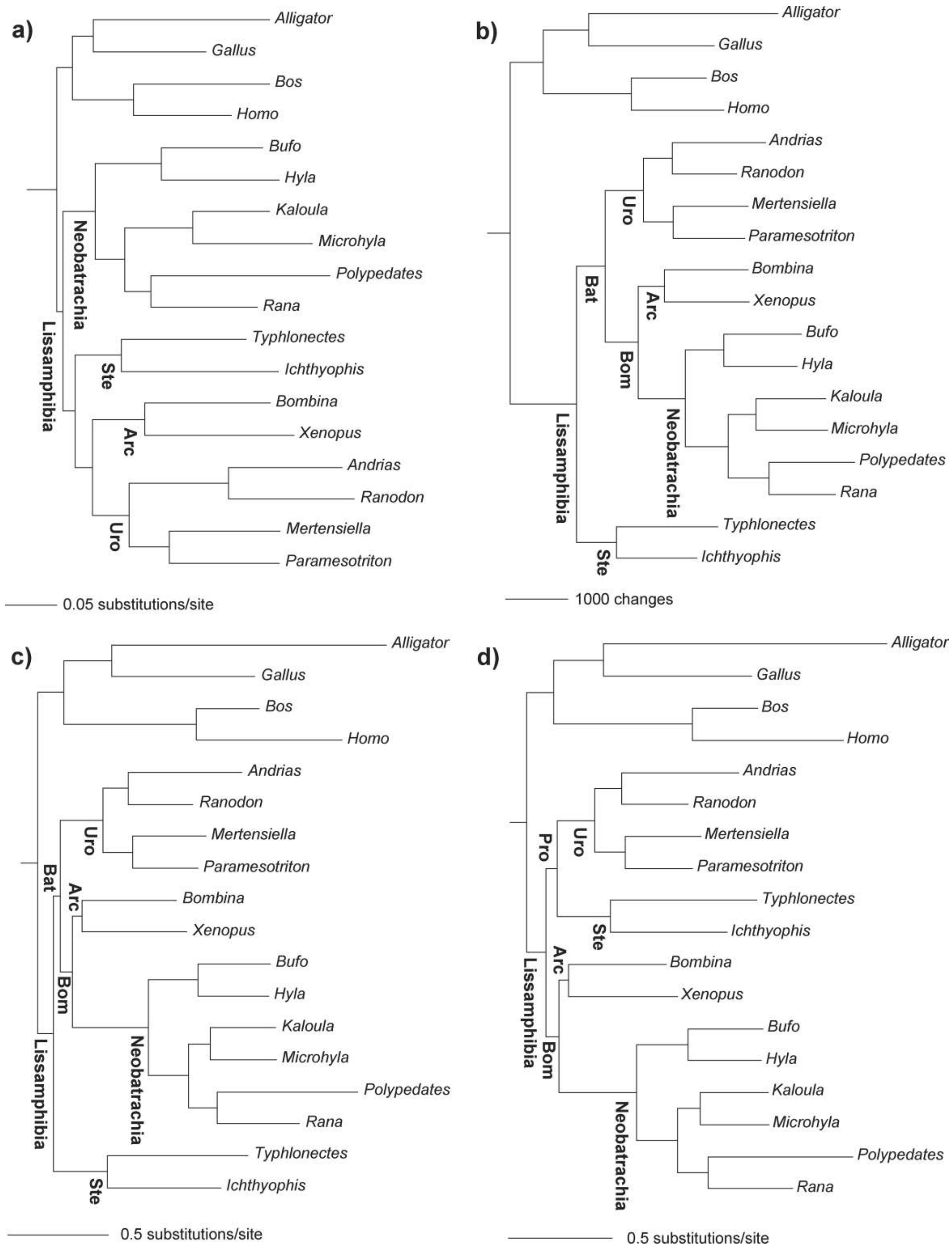

FIGURE 8. Trees produced from various analyses of the data set of Zhang et al. (2005). Dogfish (*Scyliorhinus canicula*), carp (*Cyprinus carpio*), and lungfish (*Protopterus dolloi*) not shown. (a) Neighbor-joining (phenetic) tree calculated using the Jukes-Cantor model, which is used by the stock version of ModelTest to choose the evolutionary model. Note the diphyly of Bombinanura. (b) Most parsimonious (phylogenetic) tree used by our modified ModelTest script to choose the model of evolution; the lungfish had to be constrained to be closer to the tetrapods than the carp. (c) Most likely (phylogenetic) tree calculated using a model chosen by the stock version of ModelTest 3.7 (Posada and Crandall, 1998) to fit the neighbor-joining (phenetic) tree from (a). Note the close similarity to the tree that Zhang et al. (2005: fig. 1) found; also note the difference in scale—0.5 substitutions per site on our tree are equal to 0.1 on theirs. (d) Most likely (phylogenetic) tree calculated using a model chosen by a slightly modified version of ModelTest 3.7 to fit the most parsimonious (phylogenetic) tree shown in (b). Note the similarity in branch lengths and the difference in topology to (c). Arc, Archaeobatrachia; Bat, Batrachia; Bom, Bombinanura; Pro, Procera; Ste, Stegokrotaphia; Uro, Urodela.

TABLE 1. Calibration points used in this study, or recommended (*), based on our supertree. The origin of Amniota had to be used because of the requirements of QDate; because of its poorly constrained upper bound, its use is likely to result in overly large confidence intervals in molecular divergence date estimation.

Taxon	Main taxonomic content	Minimal divergence age (Mya)	Maximal divergence age (Mya)
Tetrapoda*	Amphibia, Amniota	332	360
Amniota	Theropsida, Sauropsida	310	345
Anura*	Amphicoela, Bombinanura	170	185
Batrachia*	Salientia, Caudata	250	275
Bombinanura*	Discoglossoidea, Pipanura	170	185
Pipoidea*	Rhinophrynidae, Pipimorpha	155	175
Urodela*	Cryptobranchoidea, (Sirenidae sensu lato + Neocaudata)	155	170

However, unlike Zhang et al. (2005), we tested various combinations of internal and external calibration dates.

QDate can only use symmetric trees with four terminal taxa. Given the topology and point dates for the two most recent divergences, it calculates the age of the root node (with a confidence interval), assuming one constant rate of evolution for each half of the tree. Because few calibration points are available (Table 1), we were only able to date two divergences with QDate, namely the origin of Tetrapoda and the origin of Batrachia. Because QDate requires point estimates, we did the datings twice, using the upper and the lower bounds of the calibration points as point estimates. To avoid influence from the widely differing terminal branch lengths on the divergence date estimates (the molecular tree is far from ultrametric), we repeated each run of QDate 12 or 16 times with different combinations of terminal taxa. The exact quartets used are listed in Appendix 8.

The program r8s is more suitable for our purpose. Like Multidivtime, it can accept ranges as calibration points, multiple calibration points, and a complex phylogeny. Using the penalized-likelihood method and a range of smoothing parameters, we estimated a range of divergence dates (unfortunately, without confidence intervals). We were able to take two phylogenetic hypotheses into account, namely the monophyly of "Archaeobatrachia" as found by Zhang et al. (2005), and its paraphyly as (*Bombina* + (*Xenopus* + Neobatrachia)). The value of the smoothing parameter was selected using two selection procedures: one that minimizes the standardized squared difference between the predicted and the observed number of substitutions on each branch, as suggested by Sanderson (2002), and another that minimizes the relative error between the upper and the lower bounds of calibration dates on the one hand and the inferred dates on the other hand, as suggested by Near and Sanderson (2004).

Calibration points.—Zhang et al. (2005) used two external calibration points: the Dipnomorpha-Tetrapodomorpha (lungfish-tetrapod) split, assumed to have taken place 400 ± 10 Mya, and the Theropsida (Synapsida)-Sauropsida (mammal-bird/crocodile/lizard/turtle) split, supposedly 310 ± 10 Mya. The lower bounds on these ranges are almost certainly too young. The earliest known dipnomorph is late Lochkovian or early Pragian in age (Müller and Reisz, 2005); the Pragian (middle Early Devonian) ended 407.0 ± 2.8 Mya (Gradstein et al., 2004), so a lower bound of 410 rather than 390 Mya seems realistic for the uncertainty range of the dipnomorph-tetrapodomorph divergence. The presence and diversity of the earliest and basalmost known sarcopterygians and actinopterygians around the Silurian-Devonian boundary (Zhu et al., 1999, 2001, 2006) probably indicate that the split between dipnomorphs and tetrapodomorphs happened around this time at most (perhaps up to 420 Mya), but the very poor fossil record of gnathostomes (Cappetta et al., 1993; Gardiner, 1993a, 1993b), if not vertebrates in general (Blieck, 1984), in the Silurian precludes a more definitive statement on this.

The oldest known amniote, *Hylonomus lyelli*, apparently comes from the late Bashkirian (Calder, 1994), which ended 311.7 ± 1.1 Mya (Gradstein et al., 2004), so that the age of 310 My should be the lower (younger) bound rather than the midpoint of the date estimate of the theropsid-sauropsid divergence; an upper bound, on the other hand, is very difficult to estimate. In addition to the detailed argument provided by Reisz and Müller (2004), we wish to emphasize that the fossil record of stem-amniotes is not good enough to tell whether the apparent absence of amniotes before about 312 Mya is real. All undisputed stem-amniotes (*Solenodonsaurus* and Diadectomorpha; Vallin and Laurin, 2004, and references therein) are younger than *Hylonomus*. Tetrapods are very rare in the preceding stage (the Serpukhovian). The yet older Viséan stage has yielded the controversial *Westlothiana lizziae* which was originally interpreted as an amniote or close amniote relative but may not even be a tetrapod (Vallin and Laurin, 2004), the intriguing but poorly preserved *Casineria kiddi* for which similar suggestions have been made in the literature (Paton et al., 1999), and a few amphibians. The Tournaisian fossil record is so poor that the absence of tetrapods can again not be determined. Only in the Famennian (Upper Devonian) does the presence of several stem-tetrapods and the absence of any tetrapods enable us to conclude with reasonable confidence that the divergence between the lissamphibian and the amniote stems did not occur more than about 360 Mya, and that this divergence must significantly predate the origin of theropsids (synapsids) and sauropsids. In our QDate analyses we assumed an upper limit of 345 Mya (Appendix 9) based on the Viséan fossil record that has yielded several basal amphibians ("lepospondyls") and no amniotes, even though close relatives of the latter must have been present. As emphasized by Müller and Reisz (2005), this calibration is poorly constrained (in an interval that spans at least 35 My). Because of this, we expect the interval of inferred divergence dates based on molecular data to be correspondingly broad, but there is no reason to believe that they will be less reliable.

Zhang et al. (2005) did not use any internal calibration points. However, Brochu (2004) has found that, to get plausible divergence dates using quartet-dating, it is necessary to use both old and young calibration points. When only old divergence dates were used to calibrate the tree, the age estimates of other taxa were too old. This effect, if it extends beyond quartet-dating, may have overcompensated for the contrary effect produced by the underestimated age of the calibration points used by Zhang et al. (2005). Below, we suggest some calibration points within Lissamphibia for future molecular divergence time studies (Table 1); we have also used them to estimate divergence dates through penalized maximum likelihood in r8s (Sanderson, 2006).

RESULTS

Stratigraphic Range of Paleozoic Stegocephalians

The stratigraphic range of the dissorophoids reported by Zhang et al. (2005) is erroneous, and this invalidates their main conclusion about the origin of lissamphibians. Contrary to their statement, there is no evidence that Dissorophoidea appeared in the Viséan. The oldest known dissorophoids date from the Moscovian, in the Late Carboniferous (Milner, 1990, 1993a:672). Lysorophians and dissorophoids are abundant in the Moscovian locality of Linton, Ohio (Hook and Baird, 1986; Milner, 1993a:672). The oldest known dissorophoid (*Amphibamus grandiceps*) is only slightly older (Upper Westphalian C to Lower Westphalian D, both equivalent to Moscovian) and occurs in Mazon Creek, Illinois (Gregory, 1950; Milner, 1993a), where lysorophians (*Brachydectes*) are also present (Baird, 1964:14; Wellstead, 1991). Mazon Creek is a well-studied locality whose geological age is supported by several lines of evidence, including detailed studies of pollen and spores (Peppers, 1996, and references cited therein). The oldest undisputed lysorophian specimens come from Newsham (Boyd, 1980; Wellstead, 1991), date from the Westphalian B (equivalent to the late Bashkirian, the oldest stage of the Late Carboniferous), and are only slightly older than the oldest known dissorophoids. This locality is not as well known as Mazon Creek, but it is universally considered to be older (Wellstead, 1991; Turner et al., 2005; Jeffery, 2006). Lysorophians have even been reported from localities older than Newsham, such as Jarrow, that date from lower in the Westphalian B, but the specimens are poorly preserved and only tentatively attributed to Lysorophia (Boyd, 1980; Wellstead, 1991:71), so we will ignore them for the purposes of this study. To conclude, the latest possible date of appearance of Lysorophia is slightly earlier than that of Dissorophoidea, rather than much later as mistakenly reported by Zhang et al. (2005). Thus, the early divergence date inferred from the molecular date does not suggest an origin of lissamphibians among dissorophoids.

The preceding discussion simplifies the presentation of the hypothesis that lissamphibians are nested within temnospondyls to make it comparable to the argument presented by Zhang et al. (2005). However, to our knowledge, no paleontologist has suggested that Dissorophoidea as a whole were the sister-group to Lissamphibia. Rather, subclades of Dissorophoidea have been proposed as the sister-group (*Doleserpeton,* or a clade composed of the latter and *Amphibamus*) or stem-group (Branchiosauridae) of the lissamphibians (Bolt, 1969; Trueb and Cloutier, 1991; Ruta et al., 2003). This distinction is important because these subclades appeared later than Dissorophoidea as a whole. The first branchiosaurids appeared only slightly later than the first dissorophoids, in the Westphalian D (equivalent to Moscovian; Milner, 1993a), but *Doleserpeton* is much more recent (Artinskian; Fig. 2). Thus, two of the three proposed alternative positions of the lissamphibian stem suggest a later date for the origin of Lissamphibia than if Dissorophoidea were the sister-group of Lissamphibia (Fig. 2).

Time-Calibrated Supertrees

A literal interpretation of our tree (Figs. 3 to 7) confirms that the diversification of lissamphibians is much more recent than hypothesized by Zhang et al. (2005). Furthermore, there is a very good congruence between geological age and phylogenetic position. The four oldest salientian species (Triassic to Early Jurassic) all belong to the stem rather than to the crown-group Anura. These are *Triadobatrachus massinoti* (Rage and Roček, 1989) and *Czatkobatrachus polonicus* (Borsuk-Białynicka and Evans, 2002) from the Early Triassic, and *Prosalirus bitis* (Shubin and Jenkins, 1995) and *Vieraella herbstii* (Reig, 1961) from the Early Jurassic. Similarly, at least the two oldest gymnophiones belong to the stem; these are the Early Jurassic *Eocaecilia micropodia* (Jenkins and Walsh, 1993) and the Early Cretaceous *Rubricacaecilia monbaroni* (Evans and Sigogneau-Russell, 2001). Likewise, several stem-caudates are older than the oldest crown-caudates; these include *Kokartus honorarius* (Nessov, 1988), *Marmorerpeton kermacki* and *M. freemani* (Evans et al., 1988), and "Salamanders A and B" (Evans and Milner, 1991), all from the Middle Jurassic. The oldest known crown-salientian (anuran) is *Eodiscoglossus oxoniensis* from the Middle Jurassic (Evans et al., 1990). The oldest known possible crown-gymnophione (apodan) is an unnamed form from the Wadi Milk Formation in Sudan, which dates from the beginning of the Late Cretaceous (Cenomanian; Evans et al., 1996); however, the phylogenetic position of this gymnophione, and that of the terminal Cretaceous fossil from Pajcha Pata in Bolivia (Maastrichtian; Gayet et al., 2001), are poorly established; they could be stem-gymnophiones. The oldest gymnophione that was proposed to be related to an extant subgroup of apodans (i.e., to be within the crown-group) is the Paleocene *Apodops pricei* (Estes and Wake, 1972) that was argued to be a caeciliid; but at the time of its description Caeciliidae was thought to include all apodans except Ichthyophiidae, Scolecomorphidae, and Typhlonectidae. Even Rhinatrematidae was included, so in effect the place of *Apodops* could be anywhere within the crown-group or even just outside it. The oldest crown-caudate (urodele) is *Iridotriton hechti* from the Late Jurassic (Evans et al., 2005). Albanerpetontids, which may be the sister-group of Batrachia (Gardner, 2001; McGowan, 2002), first appear in the Middle Jurassic (Gardner et al., 2003), at the same time as the oldest crown-salientian (anuran), and before any crown-gymnophione (apodan) or crown-caudate (urodele). Thus, there do not seem to be any major anomalies in the stratigraphic distribution of lissamphibian fossils, and this suggests that our time-calibrated trees can be used to assess the approximate time of origin of at least some lissamphibian clades.

Changing the assumptions about minimal branch lengths (Appendix 4) does not significantly alter our conclusions because for most clades the range of values of minimal computed age using our paleontological database encompasses less than 30 My. The age of the oldest nodes is especially stable, which is not surprising considering that this value is constrained by fossils separated from these nodes by only a few branches. For instance, the age of Salientia varies between 246 and 252 My (Appendix 4). Conversely, the age of relatively speciose clades with a poor fossil record is poorly constrained by our method. This is best exemplified by Ranoidea, whose minimal age varies between 34 and 74 My, depending on the chosen minimal branch length assumptions (Appendix 4). In all cases, even taking into account that they are estimates of minimal rather than actual divergence dates, our dates remain much younger than those suggested by Zhang et al. (2005). For instance, the age of Lissamphibia varies between 246 and 267 My (260 My in our reference supertree), and that of Bombinanura varies between 166 and 187 My (174 My in our reference tree). By comparison, Zhang et al. (2005) inferred ages of 337 and 290 My for these taxa, and the confidence intervals of these ages (321 to 353 and 268 to 313 My, respectively) exclude our range of paleontological ages. The paleontological age of nearly all clades of lissamphibians represented in the data of Zhang et al. (2005) is excluded from the 95% credibility interval computed by Zhang et al. (2005; Fig. 9). The only exception is the smallest clade that includes *Hyla* and *Bufo*, for which our age estimate is compatible with that of Zhang et al. (2005); the (*Kaloula* + *Microhyla*) clade does not have a known fossil record, so we are not able to infer a divergence date for it. Finally, the set of assumptions that we have used to build the supertree (Figs. 3 to 7) yields dates that are often among the oldest or at least average among the fourteen sets of assumptions that we have tested (Appendix 4).

Similarly, our use of polytomies generally biases our results towards congruence with the greater ages of taxa obtained by Zhang et al. (2005). This is shown by our random resolution test of polytomies that affect the age of 15 polytomies containing 23 taxa (Appendix 5). As our tests show, the average age on randomly resolved trees for the taxa surveyed is 77 My, whereas the average age of the same taxa on our reference tree (with polytomies) is 80 My (Appendix 5). Thus, the presence of polytomies in our supertree tends to slightly overevaluate the age of the taxa, making them more similar to the ages proposed by Zhang et al. (2005) than most random resolutions would.

Adopting the rather different topology presented by Frost et al. (2006) does not alter the age of most taxa significantly, to the extent that they can be compared (Appendix 3). The two trees are mostly congruent, but in the case of Pipidae we had to create a large polytomy because we are unable to fit most fossil pipoids into the unorthodox topology found by Frost et al. (2006). The whole tree is available in the online supplementary data (Appendix 2); here, we only compare the geological age of 16 taxa on our tree and that by Frost et al. (2006). This comparison shows that the average age for these 16 taxa differs only by 1 My (less than 1% of the absolute age). Thus, our results appear to be reasonably robust to changes in topology.

The stratigraphic fit for our lissamphibian tree is surprisingly good, with a RCI of −2.5161, a SCI of 0.4583, and a GER of 0. 8146. The probability that such values are generated by a random association between phylogeny and fossil record is about 0.0001. By implying that the fossil record is fairly complete, this suggests that the minimal divergence dates of our tree are not severely underestimated.

The tree based on that by Frost et al. (2006) has similar values, except for the RCI: RCI =−187.7249, SCI = 0.4861, GER = 0.8057. The probability for this being a random result is identical.

Inevitably, our tree (reduced to the taxa used by Zhang et al., 2005) has much better stratigraphic fit than the molecular tree of Zhang et al. (2005). Our tree has an Actual Implied Gap (AIG) of 857.1 My, the one by Zhang et al. (2005) has an AIG of 1906.7 My.

Calibration Points for Molecular Analyses

Raaum et al. (2005: fig. 2) have proposed criteria for identifying calibration points for molecular dating: “It is best to have fossils [. . .] attributed to one or the other, or both, of the extant lineages, as well as other fossil specimens from around the time of the split” which lie outside the crown-group in question (Raaum et al., 2005: fig. 2); the more there are, and the better the tree fits their ages, the more probable it is that the fossil record is well enough sampled around the divergence to allow a reliably accurate estimate of the latter’s date. This is arguably the case for a few divergences in our supertree, but the fossil record of lissamphibians is not ideal in this respect. We suggest the use of the dates discussed below (Table 1) as possible internal calibration points for determining a molecular timescale within Lissamphibia (to be used together with at least one external calibration point), but only the minimal divergence dates are well-constrained; maximal ages are much more difficult to determine.

Origin of Bombinanura.—The divergence between Discoglossoidea and Pipanura (or, according to Frost et al., 2006, that between Xenoanura and Sokolanura), and the preceding one between Bombinanura and Amphicoela, seem to have taken place between the middle Middle Jurassic and the middle Early Jurassic (Fig. 5), some 170 Mya (Gradstein et al., 2004) at the latest and probably not much more than 185 Mya. The lower bound for both divergences in both trees is provided by the oldest known discoglossoid, *Eodiscoglossus oxoniensis*,

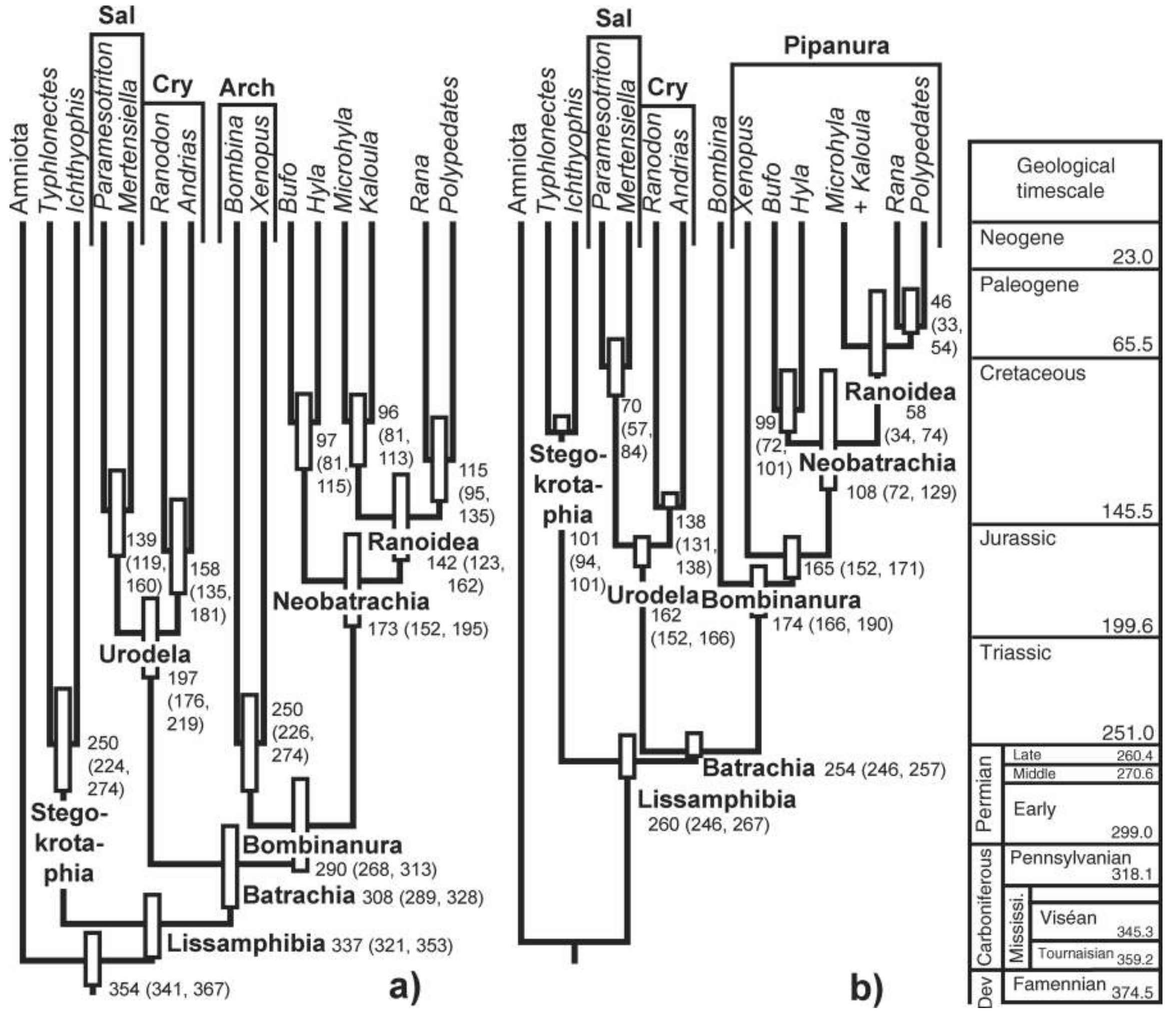

FIGURE 9. Comparison between lissamphibian divergence dates implied by (a) the analysis of molecular data by Zhang et al. (2005) and (b) paleontological data (from Figs. 3 to 7). In (a), credibility intervals (95%) are represented by boxes; the best estimates and lower and upper boundaries of the 95% credibility intervals (in My) are also indicated in parentheses. In (b), we are unable to date the origin of the smallest clade that contains *Microhyla* and *Kaloula* because it does not have a fossil record. The numbers given next to the nodes represent the estimated age given the assumptions used to build the supertree (each species occupies at least an entire geological stage, and the minimal internal branch length is set at 3 My), followed, in parentheses, by the minimal and maximal latest possible age obtained by using the various minimal branch length assumptions listed in Appendix 4. Thus, these numbers are not really confidence intervals but represent ranges of values of the minimal (rather than actual) divergence dates; still, they give an idea of the plausible range in the paleontological age of each taxon (also shown as boxes around each node). Paleontological ages are not given for the basal node (Tetrapoda) because it is not included in our supertree. Note that the paleontological estimates of minimal divergence dates (b) lie in most cases outside the 95% credibility interval of the molecular dates (a) by Zhang et al. (2005), with only the smallest clade, which includes *Bufo* and *Hyla*, being an obvious exception. Arch, Archaeobatrachia; Cry, Cryptobranchoidea; Mississi., Mississippian; Sal, Salamandridae.

which is Bathonian in age. An upper bound may be estimated from *Vieraella* and *Prosalirus*, two successive sister-groups of Anura, the ages of which are thought to be Toarcian or Aalenian (Roček, 2000) and Pliensbachian (Shubin and Jenkins, 1995), respectively. However, the much later appearance date of *Mesophryne* (creating a ghost lineage of at least 55 My), which is the sister-group to Anura, as well as the complete lack of known fossil amphicoelans, raises the possibility that Bombinanura and Anura are older than our supertree suggests, as may the wholesale absence of known salientians between the middle Early Jurassic and the Early Triassic. Accordingly, we have repeated those runs of r8s where the origin of Bombinanura was used as a calibration point, assuming that the distance between the minimal and the maximal age was twice or three times as large as proposed here (that is, a maximal age of 200 or 215 My). The divergence between Discoglossoidea and Pipanura (or Xenoanura and Sokolanura) obviously must have happened after the basal divergence of Anura, but the fossil record does not tell how much later. A great separation seems to be unlikely given the small number of synapomorphies that have been proposed for Discoglossoidea, Pipanura, and Sokolanura.

Origin of Pipoidea.—Rhinophrynids and pipimorphs may have diverged in the Middle or early Late Jurassic, between about 155 and 175 My. The earliest known rhinophrynid, *Rhadinosteus*, is Kimmeridgian or Tithonian in age (Henrici, 1998); an upper bound is only provided by the divergence between Discoglossoidea and Pipanura or Xenoanura and Sokolanura (see above). The only known stem-xenoanuran, *Neusibatrachus*, is from the Berriasian or younger (Roček, 2000; Chiappe and Lacasa-Ruiz, 2002; Padian, 2004) and thus younger than the xenoanuran crown-group (Pipoidea). Pelobatoidea and Neobatrachia, together the sister-group of Xenoanura in our main tree, do not appear in the fossil record before the Campanian (some 75 Mya).

Origin of Urodela.—A Middle or early Late Jurassic (∼155 to ∼170 Mya) origin seems plausible for Urodela (that is, Caudata of Frost et al., 2006). The earliest known urodelan is the basal neocaudate *Iridotriton* (Kimmeridgian or Tithonian: Evans et al., 2005; see also He et al., 2004, and Wang and Rose, 2005). Urodeles are so far not known from the Bathonian deposits in western Europe and central Asia, which have yielded relatively abundant karaurids, the only currently undisputed nonurodelan caudates. The complete absence of known older caudates is, however, not encouraging, given the size of the gap implied by the oldest known salientians. Accordingly, as with Bombinanura, we have repeated those runs of r8s where we used the origin of Urodela as a calibration point, under the assumption that the distance between the minimal and the maximal age was twice or three times as large as proposed here (that is, a maximal age of 185 or 200 My).

Some recent studies (San Mauro et al., 2005; Mueller, 2006; Bossuyt et al., 2006) have used the age of the oldest known cryptobranchid, *Chunerpeton tianyiense*, as the minimum age of the split between Cryptobranchidae and Hynobiidae. Originally (Gao and Shubin, 2003) this age was reported as 161 My (equivalent to the Middle-Late Jurassic boundary; Gradstein et al., 2004), but this rests on the idea that the Daohugou Beds, which have yielded *Chunerpeton*, belong to the Jiulongshan Formation, on top of which lies the ignimbrite that has yielded the radiometric date. Reportedly, however, they overlie rather than underlie the even higher Tuchengzi Formation (He et al., 2004). A radiometric date from somewhere in the upper part of this formation is 139.4 ± 0.2 My (Swisher et al., 2002), equivalent to the Berriasian-Valanginian boundary (Gradstein et al., 2004) in the early part of the Early Cretaceous. If this stratigraphic relationship is correct, the Daohugou Beds cannot be older than Valanginian, and Zhang et al. (2005) are mistaken in interpreting *Chunerpeton* as supporting their molecular divergence date estimate of Cryptobranchoidea (181 to 135 Mya, best estimate of 158 Mya). Based on the Barremian-Aptian boundary age of the Yixian Formation and on crude biostratigraphy, the Daohugou Beds could be as young as Barremian (and were assumed to be so by Wang and Rose, 2005). However, they underlie the Yixian Formation (He et al., 2005), so this is their youngest possible age. In keeping with our treatment of stratigraphic uncertainty explained above, we have assumed a Valanginian age in the construction of our tree and the calculation of its stratigraphic fit.

A Middle Jurassic age of the Daohugou Beds is defended by Gao and Ren (2006), who maintain that the beds underlie the ignimbrite (and the Tuchengzi Formation) and highlight several problems in the paper by He et al. (2004), but He et al. (2005) provide evidence, not addressed by Gao and Ren (2006), that the Daohugou Beds overlie the ignimbrite. A resolution to this debate does not appear to be in sight; to us, it seems that the correlation of the many outcrops assigned to the Daohugou Beds and other formations over a vast area of hilly terrain in Liaoning, Hebei, and Inner Mongolia might benefit from a review.

In any case, this problem only affects the ages of Cryptobranchoidea and Urodela, which is not a problem for our molecular dating. We only assume a minimal divergence date for Cryptobranchoidea (140 My). The age bracket of Urodela (minimum 155 My, maximum 170, 185, or 200 My) is also irrelevant because we never found a younger age than 170 My (older than any of the proposed ages of the Daohugou Beds) for that clade; whenever a constraint was active, it was the upper (older) one.

On a related note, He et al. (2006) date the Dabeigou Formation, which has yielded *Sinerpeton* and *Laccotriton*, as around 130 Mya (Hauterivian-Barremian boundary; Gradstein et al., 2004). Originally (Gao and Shubin, 2001), this layer was reported as Tithonian (late Late Jurassic) simply because it overlies the Zhangjiakou Formation, which is 151 My old (Kimmeridgian-Tithonian boundary).

Origin of Batrachia.—The oldest known batrachians are *Triadobatrachus* and *Czatkobatrachus*, both from the Early Triassic. Because their precise age within the Early Triassic is unknown, we think that the minimal age of Batrachia should be assumed around 250 My; that is, the Induan-Olenekian boundary (249.7 ± 0.7 Mya; Gradstein et al., 2004), which lies very close to the Permian-Triassic (Changxingian-Induan) boundary itself (251.0 ± 0.4 Mya). The maximal age is in principle as poorly constrained as that of Lissamphibia itself; however, we choose the rich Artinskian fossil record (284.4 ± 0.7 to 275.6 ± 0.7 Mya), which has yielded many basal amphibians but no lissamphibians, to establish the upper bound at 275 Mya, based on the consideration that it is more probable that two lineages of stem-lissamphibians are "hiding" in it than that four lissamphibian lineages (Gymnophiona, Albanerpetontidae, Salientia, and Caudata) await discovery in Artinskian sediments (Table 1).

Origin of Tetrapoda.—There is much confusion in the literature about the age of Tetrapoda because this taxon has been used as if it were defined by an apomorphy (the origin of the limb) in most paleontological studies, but as if it were a crown-group in most neontological studies that have used it in any precise sense (Laurin and Anderson, 2004). Thus, a brief discussion of the minimal and maximal age of Tetrapoda (the crown-group) may be useful, even though our supertree only includes lissamphibians. The oldest undoubted tetrapod, the amphibian *Lethiscus stocki*, comes from the Wardie shales of

Scotland. These lie in the Lower Oil Shale Group and are mid-Viséan (Holkerian) in age (Wellstead, 1982; Carroll, 2000). The Holkerian dates from about 332 to 339 Mya (Gradstein et al., 2004), so Tetrapoda cannot have originated less than 332 Mya. This is the minimal age of Tetrapoda under any recent phylogeny (Ruta and Coates, 2003). The upper bound is as always less secure, but the presence of several species of stem-tetrapods in the Famennian (375 to 359 Mya) suggests that an upper bound of 360 Mya is realistic. This bound is plausible because most Famennian stegocephalians come from fairly high up in that stage (Blom et al., 2005) and because of the large number of apomorphies shared by tetrapods but not by Devonian stegocephalians (Laurin, 1998).

Molecular Phylogenetic Analysis

Because the neighbor-joining tree (Fig. 8a) is a phenogram rather than a cladogram, we do not discuss its peculiarities in detail even though this is the tree to which the stock version of ModelTest tries to fit the model.

The most likely tree calculated using this model (Fig. 8c) is much more congruent with published phylogenetic hypotheses than the neighbor-joining tree. The topology is identical to that found by Zhang et al. (2005: fig. 1), and the branch lengths are quite similar, despite the major differences in the models (Appendix 7). The especially short internal branches, which we also note in Zhang et al. (2005: fig. 1) appear implausible by comparison with morphological and paleontological data.

The unconstrained most parsimonious tree (Fig. 8b) contains the same topology, but the internal branch lengths appear more plausible; the branch subtending Lissamphibia is the longest of all internal branch lengths (consistent with morphological and paleontological data), and the other nodes are almost evenly spaced, with internal branch lengths almost half as long (on average) as the terminal ones.

Surprisingly, when the unconstrained most parsimonious tree is used as the guide tree for ModelTest and the resulting model used for a maximum-likelihood analysis, the most likely tree (Fig. 8d) shows Gymnophiona and Caudata, rather than Salientia and Caudata, as sister-groups (rendering Batrachia redundant with Lissamphibia), coherent with the so-called "Procera hypothesis" (but the branch lengths are very similar to those in Fig. 8c). Zhang et al. (2005) have summarized the history of both phylogenetic hypotheses. In both maximum likelihood trees the branches in this region of the tree are very short (not, however, in the most parsimonious tree), much shorter than the terminal branches. Indeed, Zhang et al. (2005: fig. 1) have found a low bootstrap value for Batrachia (as well as for Lissamphibia, "Archaeobatrachia", and Bombinanura). Alternatively, this lack of robustness in the position of anurans, urodeles, and apodans may reflect the low density of the taxon sampling (Hedtke et al., 2006).

According to the simulation study by Kolaczkowski and Thornton (2004), maximum parsimony fares better than maximum likelihood (and Bayesian analysis) under a wide range of realistic conditions, in spite of its undeniably greater susceptiveness to long-branch attraction (which is confirmed by Kolaczkowski and Thornton, 2004). This is because parsimony does not need an assumption on how many rate categories there are; in many real cases more or less each nucleotide position evolves at its own speed, causing potential problems for approaches that include evolution models (maximum likelihood and Bayesian analysis) but not for maximum parsimony. Thus, we do not think that the most parsimonious tree, which finds Bombinanura and Urodela as sister-groups (Fig. 8b), is necessarily less probable or a worse explanation for the data of Zhang et al. (2005) than the most likely tree (Fig. 8d). Furthermore, the branch lengths of the most parsimonious tree fit morphological data better than the most likely trees, in which many internal branches are disquietingly short.

Finally, we note that the "Procera hypothesis" would remove a large stratigraphic gap from our tree (the entire basal ghost lineage of Gymnophiona, about 70 My; Fig. 3). However, the position of Albanerpetontidae, which has its own long ghost lineage on our tree, is unclear under that topology.

All trees show *Bombina* and *Xenopus* as sister-groups. The consistency of this result still cannot rule out long-branch attraction, however.

Molecular Divergence Date Estimates

Quartet dating using QDate.—The age of the calibration points has more influence on the inferred divergence dates than the model of evolution (Appendix 9). The age of Tetrapoda estimated by Zhang et al. (2005) is contained within the range of our estimates regardless of which model is used, except when the upper (younger) bounds of the calibration points are used under the model that fits the neighbor-joining tree best. The age estimates for Batrachia, on the contrary, are always much younger than the entire confidence interval found by Zhang et al. (2005)—in fact, they are all younger than the oldest batrachian fossils, the Early Triassic (245 to 251 Mya) salientians *Triadobatrachus* and *Czatkobatrachus*. Because the morphology of the fairly well-preserved *Triadobatrachus* leaves little doubt about its salientian affinities (Rage and Roček, 1989), the age of Batrachia as estimated by QDate is clearly erroneous.

Penalized-likelihood dating using r8s.—Contrary to our expectations, the topology has very little influence on the divergence dates (Table 2, Appendix 10), possibly because the branch lengths are so similar. Thus, long-branch attraction, if it explains the archaeobatrachian monophyly recovered in our analyses, has little impact on the molecular dates using model 1 (Table 2, Appendix 10). Similarly, the smoothing parameter, which was selected using two cross-validation procedures (see above), only moderately influences the results (Table 2). In contrast, the choice of calibration points has by far the most impact. Using only external calibration points yields

TABLE 2. Summary of the divergence dates obtained from penalized likelihood as implemented in r8s (full data and calculations in Appendix 10). Rows 1 and 2 are included for comparison purposes. Row 1 consists of the point estimates and 95% confidence intervals found by Zhang et al. (2005). Row 2 shows the results from our supertree where the "point estimate" was derived from setting all internal branches to 3 My or longer and having all terminal branches occupy minimally an entire geological stage, and the range represents values obtained from other combinations of branch lengths (listed in Appendix 4). The values in rows 3 to 7 are based on the dates obtained using several combinations of calibration dates, evolutionary models, topologies, penalty functions, and smoothing factors (selected by cross-validation out of 92 analyses in rows 3 to 6). Other settings of smoothing factors examined to assess the impact of this parameter on dates and found in Appendix 10 are not considered here, except in row 7. Row 3 shows the total range of values achievable when the smoothing factor is selected by cross-validation (when the two cross-validation procedures selected different values, both were used); each line in rows 4 to 7 holds one of these factors constant and shows the results from analyses varying all other factors. In each cell of rows 3 to 7, the first number represents the average age of several tests; the minimal and maximal values given by various analyses are shown in parentheses. Bat, Batrachia; Bom, Bombinanura; Cry, Cryptobranchoidea; Liss, Lissamphibia; Neo, Neobatrachia; Pro, Procera; sm, smoothing factor; Uro, Urodela.

	Liss	Bat or Pro	Bom	Neo	Uro	Cry
1: Zhang et al. (2005)	337 (321–353)	308 (289–328)	290 (268–313)	173 (152–195)	197 (176–219)	158 (135–181)
2: Our time-calibrated supertree	260 (246–267)	254 (246–257)	175 (166–185)	108 (72–124)	162 (152–166)	143 (138–150)
3: All analyses with optimal smoothing factors ($n = 38$)	282 (250–356)	263 (227–347)	223 (185–338)	167 (106–296)	195 (170–273)	146 (140–184)
4.1: External calibration dates only or external and internal without upper bound ($n = 8$)	340 (321–356)	325 (300–347)	317 (288–338)	237 (173–296)	250 (224–273)	165 (150–184)
4.2: Internal calibration points with upper bounds (with or without external calibration points) ($n = 30$)	267 (250–291)	246 (227–263)	198 (185–215)	149 (106–192)	180 (170–200)	141 (140–150)
5.1: Based on model 1 ($n = 19$)	291 (255–356)	270 (250–347)	226 (185–338)	200 (163–296)	201 (170–273)	143 (140–154)
5.2: Based on model 3 ($n = 19$)	273 (250–342)	255 (227–320)	220 (185–316)	134 (106–196)	189 (170–239)	149 (140–184)
6.1: With monophyletic Archaeobatrachia ($n = 20$)	281 (250–356)	262 (232–347)	223 (185–338)	168 (110–296)	194 (170–272)	145 (140–184)
6.2: With topology (*Bombina*, (*Xenopus*, Neobatrachia)) ($n = 18$)	284 (250–356)	264 (227–347)	224 (185–338)	166 (106–294)	196 (170–273)	146 (140–179)
7.1: sm = 1 ($n = 21$)	292 (250–345)	266 (227–335)	228 (185–326)	170 (97–284)	197 (170–259)	142 (129–179)
7.2: sm = 3000–10000 (lowest value available) ($n = 21$)	289 (250–356)	272 (234–347)	231 (185–358)	187 (120–320)	202 (170–273)	146 (140–183)

very old divergence date estimates (Table 2); using only internal ones yields some that are younger than the fossil record allows (Appendix 10). Both must be used to obtain realistic estimates for most clades, as Brochu (2004) found for quartet-dating using a crocodilian data set. By using internal and external calibration points together, we find divergence dates (Table 2) much more recent than those estimated by Zhang et al. (2005) and more compatible with paleontological evidence for all considered clades (within and outside Lissamphibia). Doubling or even tripling the distance between the minimal and maximal ages of Bombinanura and Urodela when they were used as calibration dates had rather little effect; the dates of divergences within Lissamphibia became older, those outside became younger (Appendix 10).

The choice of the penalty function altered the results only moderately. A new log penalty function penalizes differences in the logarithm of rates on neighboring branches; the older, additive function penalizes the squared differences in rates across neighboring branches in the tree. Most analyses were performed with the log penalty function, which is supposed to yield more realistic deep divergence time estimates from shallow calibration points, but an analysis using the additive (older) function yielded only slightly to moderately older ages for Tetrapoda, Lissamphibia, and Neobatrachia (Appendix 10, setting 2.6b).

Dating using PATHd8.—This method, presented by Anderson (2006), did not yield plausible results (Appendix 11). These results will not be discussed further.

DISCUSSION

Time of Origin of Lissamphibia, Topology, and the Previously Suggested Amphibian Stem-Groups

Of all the main paleontological hypotheses on lissamphibian origins, the one most compatible with the early lissamphibian appearance date inferred by Zhang et al. (2005) is the hypothesis of a polyphyletic origin (Figs. 1d, 2; Lee and Anderson, 2006). However, because all recent phylogenetic analyses of early stegocephalians have found that the "lepospondyls" are more closely related to the amniotes than to any temnospondyls (Carroll, 1995; Laurin, 1998; Anderson, 2001; Ruta et al., 2003; Vallin and Laurin, 2004), this would result in Lissamphibia being paraphyletic with respect to Amniota, but all molecular phylogenies of Tetrapoda have found a monophyletic Lissamphibia (Hedges et al., 1990; Hedges and Maxson, 1993; Hay et al., 1995; Feller and Hedges, 1998; Zardoya and Meyer, 2001; Zhang et al., 2005). Thus, despite the good match in timing, a polyphyletic origin of lissamphibians is incompatible with the topology recovered by all recent molecular phylogenetic studies of amphibians and can be rejected on that basis (Laurin, 2002). The stratigraphic range of Paleozoic stegocephalians does not help to identify the presumed sister-group of the lissamphibians because dissorophoids and lysorophians first appeared at approximately the same time.

Diversification of Lissamphibia According to the Fossil Record

Our time-calibrated supertree of lissamphibians (Figs. 3 to 7) suggests much more recent dates of lissamphibian diversification (Fig. 9). Instead of a basal lissamphibian divergence in the Viséan (337 Mya), the absence of any lissamphibian fossil before the Early Triassic (251 to 245 Mya) suggests that this crown-group arose in the Permian, probably less than 300 Mya. This impression is reinforced by the excellent and highly significant stratigraphic fit of our tree (tested using three indices in conjunction with randomization tests), which indicates that the fossils occur in an order coherent with the topology. Thus, the fossil record of Lissamphibia is probably not too incomplete to be suitable for our purpose. This record implies dates of lissamphibian diversification that are consistently more recent than those inferred by Zhang et al. (2005) using molecular data (Fig. 9); this applies to all lissamphibian divergences dated by Zhang et al. (2005) and present in our tree except one (*Bufo*/*Hyla*).

Molecular Data on the Origin and Diversification of Lissamphibia

Zhang et al. (2005:391) wrote: "The amphibian species were carefully selected so that every major amphibian group contained at least two species (in an effort to reduce long-branch attraction artefacts)." Yet they only selected two "archaeobatrachians" (*Xenopus* and *Bombina*), even though they explicitly consider "Archaeobatrachia" to be paraphyletic (Zhang et al., 2005:394, and the footnote to their table 2). In their tree (our Fig. 9a), the two "archaeobatrachians" are sister-groups that have a very early divergence date. San Mauro et al. (2004) find the same topology and attribute it to long-branch attraction; Vences et al. (2003), San Mauro et al. (2005), Roelants and Bossuyt (2005), and Frost et al. (2006) find "Archaeobatrachia" to be paraphyletic, as does the morphological analysis of Gao and Wang (2001); Hoegg et al. (2004), who likewise find "Archaeobatrachia" as paraphyletic, find high numbers of unique substitutions in two genes (although none used by Zhang et al., 2005) in *Bombina*, *Pipa*, and *Xenopus*. Consequently, we suspect that the monophyletic Archaeobatrachia that both Zhang et al. (2005) and we have found is a good example of long-branch attraction. However, we have shown that the impact of this potential artefact on the dates of all divergences ancestral to (*Xenopus* + *Bombina*) is minimal (Table 2; Appendices 9 to 11).

Molecular divergence date estimates are highly sensitive to the choice of calibration points, as well as to the assumed model of evolution (and general method of estimating the dates) and the branch lengths of the tree used. As Brochu (2004) found for quartet-dating, using only ancient external calibration points yields very old divergence date estimates under a penalized-likelihood method, whereas using only internal calibration points yields divergence dates that are sometimes younger than the fossil record allows. Both must be included to obtain realistic estimates. Zhang et al. (2005) used only external calibration points; this may be the reason for their generally very old divergence date estimates, overcompensating for the effects of the unrealistically young dates they assumed for their calibration points. Another possible factor is the tendency of Bayesian methods to overestimate divergence dates reported by Anderson (2006). Regardless of the explanation, it is clear that the molecular data used by Zhang et al. (2005) are compatible with the paleontological dates derived from our time-calibrated supertree. Thus, contrary to previous suggestions, there is no strong opposition between molecular and paleontological dates, at least in this case. We suggest that discrepancies between these types of dates may often arise from an inadequate choice of calibration points.

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GLOSSARY

The definitions below are not to be understood as formally proposed definitions. They are only intended to make our present use of taxon names explicit.

Amniota: the smallest clade that includes birds and mammals (a crown-group).

Amphibia: the largest clade that includes Lissamphibia but not Amniota.

Anura: the smallest clade that includes all extant frogs (a crown-group).

Apoda: the smallest clade that includes all extant caecilians (a crown-group).

Batrachia: the smallest clade that contains Salientia and Caudata (a crown-group).

Caudata: the largest clade that includes Urodela but neither Anura nor Apoda.

Dipnomorpha: the largest clade that includes lungfish but not tetrapods.

Gymnophiona: the largest clade that includes Apoda but neither Anura nor Urodela.

Lissamphibia: the smallest clade that includes Apoda, Anura and Urodela, but not Amniota (a crown-group).

Salientia: the largest clade that includes Anura but neither Urodela nor Apoda.

Sauropsida: the largest clade that includes birds but not mammals.

Stegocephali: the smallest clade that includes all limbed vertebrates. Often called Tetrapoda in the literature.

Tetrapoda: the smallest clade that contains Lissamphibia and Amniota (a crown-group).

Tetrapodomorpha: the largest clade that includes tetrapods but not lungfish.

Theropsida: the largest clade that includes mammals but not birds. Often called Synapsida in the literature.

Urodela: the smallest clade that includes all extant salamanders (sensu lato; cryptobranchoids, sirenids, and salamandroids).

NOTE ADDED IN PROOF

Using many color photographs, Wang et al. (2005) have shown that the Daohugou Beds and the overlying and underlying strata are complexly folded, and that the Daohugou Beds overlie the 159 to 164 My old ignimbrite of the Tiaojishan Formation. Reports of the Tuchengzi Formation underlying the Daohugou Beds, however, were due to misidentifications; the position of the Tuchengzi Formation relative to the Daohugou Beds remains unknown. Therefore the age of the Daohugou Beds may be Oxfordian to Barremian. "We propose that the Daohugou fossil assemblage probably represents the earliest evolutionary stage of the Jehol Biota based on both vertebrate biostratigraphy and the sedimentological and volcanic features which suggest the Daohugou deposit belongs to the same cycle of volcanism and sedimentation as the [end-Barremian and early Aptian] Yixian Formation of the Jehol Group." (Wang et al. 2005:2369). Thus, *Pangerpeton, Jeholotriton, Liaoxitriton daohugouensis* and *Chunerpeton* could conceivably be somewhat older, but more probably younger, than indicated in Figure 3.

Wang, X., Z. Zhou, H. He, F. Jin, Y. Wang, J. Zhang, Y. Wang, X. Xu, and F. Zhang. 2005. Stratigraphy and age of the Daohugou Bed in Ningcheng, Inner Mongolia. Chin. Sci. Bull. (English edition) 50:2369–2376.