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Title:

# What do ossification sequences tell us about the origin of extant amphibians?

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ABSTRACT—The controversial origin of extant amphibians has been studied using several sources of data and methods, including phylogenetic analyses of morphological data, molecular dating, stratigraphic data, and comparisons of ossification sequences. A consensus has failed to emerge, perhaps because the diversity of methods used hampers comparisons. We have compiled a dataset of ossification sequences of 102 terminal taxa and seven cranial bones to assess the relative support for six competing hypotheses about the origin of extant amphibians: a monophyletic origin among temnospondyls, a monophyletic origin among lepospondyls, a diphyletic origin among both temnospondyls and lepospondyls, a diphyletic origin among temnospondyls alone, and two variants of a triphyletic origin, in which anurans and urodeles come from different temnospondyl taxa while caecilians come from lepospondyls and are either closer to anurans and urodeles or to amniotes. The data are analyzed through maximum likelihood, and the AICc (corrected Akaike Information Criterion) weights of the six hypotheses allow us to assess their relative support. By an unexpectedly large margin, our analyses support a monophyletic origin among lepospondyls; a monophyletic origin among temnospondyls, the current near-consensus, is a distant second. All other hypotheses are exceedingly unlikely according to our data.

**Keywords:** macroevolution; paleontology; evo-devo; ossification sequences; Lissamphibia; Tetrapoda; phylogeny

## Introduction

Paleontologists have been studying the origin of the extant amphibian clades for more than a century. Early studies generally proposed an origin of at least some extant amphibians from temnospondyls. Cope (1888) initially suggested that batrachians (anurans and urodeles) derived from temnospondyls (a large clade of limbed vertebrates known from the Early Carboniferous to the Early Cretaceous) because he believed that the batrachian vertebral centrum was an intercentrum, the dominant central element of temnospondyls. Later, Watson (1940) argued that anurans were derived from temnospondyls because of similarities (mostly in the palate) between the temnospondyl "*Miobatrachus*" (now considered a junior synonym of *Amphibamus*) and anurans. Monophyly of extant amphibians (Lissamphibia) was proposed by Parsons and Williams (1962, 1963), an idea that was accepted more quickly by herpetologists than by paleontologists. Lissamphibian monophyly was supported by (among a few other character states) the widespread occurrence of pedicellate, bicuspid teeth. The subsequent discovery of such teeth in the amphibamid temnospondyl *Doleserpeton* (Bolt 1969) reinforced the widespread (though not universal) acceptance of an origin of Lissamphibia from within temnospondyls (e.g., Schoch and Milner 2004). Recently, this hypothesis, referred to as the temnospondyl hypothesis or TH for short (Fig. 1c), has been supported by several phylogenetic analyses based on phenotypic data matrices (e.g. Ruta and Coates 2007; Sigurdsen and Green 2011; Maddin et al. 2012; Pardo et al. 2017a, b: fig. S6).

Dissenting opinions about the origin of extant amphibians have been expressed for several decades (see Schoch and Milner 2004 for a historical review). These were initially formulated especially for the urodeles and caecilians, which are less similar to temnospondyls and lack a tympanic middle ear (which is present in most anurans and often inferred for at least some temnospondyls but absent in lepospondyls). Thus, Steen (1938) highlighted

similarities in the palate (broad cultriform process of the parasphenoid) and cheek (loss of several bones) between lysorophian lepospondyls and urodeles. Carroll and Currie (1975) and Carroll and Holmes (1980) argued that the exant amphibians had three distinct origins among early stegocephalians; while they accepted an origin of anurans among temnospondyls, they suggested that urodeles and caecilians originated from two distinct groups of lepospondyls (*Rhynchonkos* for caecilians, Hapsidopareiidae for urodeles). Later, based mostly on developmental similarities between the temnospondyl *Apateon* and urodeles, Carroll (2001, 2007) and Fröbisch et al. (2007) even proposed a second hypothesis involving a triphyletic origin of lissamphibians, with an origin of anurans and urodeles from two distinct temnospondyl groups, while the caecilians would remain in the lepospondyl clade. This is what we call the polyphyly hypothesis (PH). We have tested two versions: the one (called PH1; Fig. 1e) cautiously suggested by Fröbisch et al. (2007), which agrees with the paleontological consensus in placing all or most lepospondyls closer to Amniota than to Temnospondyli (Fig. 1b; Sigurdsen and Green 2011; Pardo et al. 2017a, b: fig. S6; Marjanović and Laurin 2018), and a version modified (PH2; Fig. 1f) to make Lissamphibia monophyletic with respect to Amniota, a fact we consider demonstrated beyond reasonable doubt by multiple molecular phylogenetic analyses (Fig. 1a; Pyron 2014; Irisarri et al. 2017; Feng et al. 2017; and references cited therein). Anderson (2007) and Anderson et al. (2008) found lissamphibian diphyly, specifically a monophyletic Batrachia among the temnospondyls while keeping the caecilians among the lepospondyls (DH1; Fig. 1g). Pardo et al. (2017b: fig. 2, S7) presented a similar hypothesis, with batrachians and caecilians having separate origins within the temnospondyls (DH2; Fig. 1h). Further, a monophyletic origin of all extant amphibians among lepospondyls has also been proposed (Laurin 1998; Pawley 2006: appendix 16; Marjanović and Laurin 2009, 2013, 2018). This will be referred to below as the lepospondyl hypothesis (LH; Fig. 1d).

Phylogenetic analyses of molecular data cannot distinguish the TH, the PH2, the DH2 or the LH from each other by topology (Fig. 1). Several other types of data and methods have, however, been used to try to discriminate between the various hypotheses on the origin of extant amphibians. In addition to classical phylogenetic analyses of morphological data matrices, these include the use of molecular (Zhang et al. 2005; Marjanović and Laurin 2007; Pardo et al. 2017b) and stratigraphic data (Marjanović and Laurin 2008) to compare the inferred divergence dates between the three main extant amphibian clades with predictions based on the TH and the LH on the one side and the PH and the DH on the other. However, developmental data, in the form of ossification sequences, have been the second-most frequently used (after classical morphological data) to argue for particular phylogenetic hypotheses. These data include mainly cranial (e.g. Schoch 2002, 2006; Schoch and Carroll 2003; Schoch and Milner 2004; Anderson 2007; Carroll 2007; Germain and Laurin 2009) and autopodial ossification sequences (e.g. Fröbisch et al. 2007, 2015). Ossification sequences of other parts of the skeleton, like the vertebrae, shoulder girdle and scales, are also documented in a few Paleozoic stegocephalians (e.g. Carroll et al. 1999; Witzmann and Schoch 2006; Anderson 2007; Carroll 2007; Olori 2013), but these have played a minor role in the controversy about the origin of extant amphibians. This study will emphasize cranial ossification sequences because these are available for the greatest number of extant taxa.

## METHODS

### *Ossification sequence data*

From all the literature we could get access to, we compiled the most extensive database on ossification sequences for osteichthyans that exists to date. The most useful sources for extant taxa included compilations: Harrington et al. (2013) for amphibians, Hugi et al. (2012) for squamates, Maxwell et al. (2010) for birds, and Weisbecker (2011) for

mammals. For the temnospondyl *Apateon* (*A. caducus* and *A. pedestris*), data come from Schoch (2004); the partial cranial sequence for the temnospondyl *Sclerocephalus* (*S. haeuseri*) comes from Werneburg (2018). The other sources of sequence data can be found in SM (Supplementary Material) 1. The data themselves and the phylogenetic trees corresponding to the tested hypotheses are included in SM 2.

The software we used to compute AICc weights (see below) cannot handle missing data. This unfortunately meant we had to discard much information. In order to keep as many taxa as possible in the analysis, we first compiled a matrix (not shown) of 153 taxa and 216 characters. Of these, we first kept characters that were scored in at least 75% of the taxa in our initial database, and taxa that were scored for at least six characters out of the seven that are scored in more than 90% of the taxa. All seven of these characters are cranial; postcranial characters are systematically scored in fewer than 25% of the taxa, so we could not use them here. In the end, we were left with 102 taxa and only seven characters (all cranial: nasal, parietal, squamosal, premaxilla, maxilla, pterygoid, and exoccipital). This data loss is not as severe as it may first seem because many excluded characters (most of them postcranial) had less than 10% scored cells (sometimes less than 1%), and most of them could not be scored for the temnospondyls *Apateon* and *Sclerocephalus*, so they could not have helped resolve the main question examined in this study. This reduced dataset used in our analyses is included in SM 2.

Of these seven characters, *Sclerocephalus* cannot presently be scored for the squamosal. Because of the potential importance of *Sclerocephalus* as a stem-caecilian according to the DH2 (Fig. 1h) and as one of only three sampled extinct taxa, we ran all analyses twice: once with *Sclerocephalus* and six characters, once without *Sclerocephalus* and with seven characters. The first set of analyses is presented in Tables 1 and 3; the second set gave very similar results and is presented in Tables 2 and 4.

Due to the scarcity of clear homologies between the skull bones of tetrapods and actinopterygians, we had to omit all actinopterygians from our analyses. As cranial ossification sequences remain unknown for finned sarcopterygians (except perhaps lungfish, whose skull bones seem mostly impossible to homologize), our analyses are restricted to limbed vertebrates.

Unfortunately, no cranial ossification sequence is available for any lepospondyl or possible lepospondyl except the highly apomorphic aïstopod *Phlegethontia*, for which three cranial ossification stages are known (Anderson et al. 2003; Anderson 2007). A recent anatomical restudy and phylogenetic analysis suggests that aïstopods are not lepospondyls (Pardo et al., 2017a); the resulting uncertainty about the position of the aïstopods (among lepospondyls or on the tetrapod stem) prevents us from using the developmental sequence of *Phlegethontia* here. The low taxon sample is more limiting for this analysis than the low character sample. However, as explained below, the absence of lepospondyl sequences in our dataset does not preclude testing the six hypotheses (TH, PH1, PH2, DH1, DH2, LH).

Given that various taxa differ in the numbers of bones and that the resolution of the sequences is also variable between taxa, these data needed to be standardized to make comparisons and computations meaningful, as suggested by Germain and Laurin (2009). For this, we used the position in the sequence (from first to last) and standardized this relative sequence position between 0 and 1 using the simple formula given by Germain and Laurin (2009). The standardized sequence position ($X_s$) is:

$$X_s = (X_i - X_{min})/(X_{max} - X_{min}),$$

where:

$X_i$ is the position of a given bone in the sequence

154 $X_{min}$ is the lowest position in the sequence (generally denoted 0 or 1)

155 $X_{max}$ is the highest position in the sequence (for instance, if there are 20 bones, $X_{min}$ is 1 and
156 the sequence is completely resolved, $X_{max} = 20$).

157 We also experimented with using size (skull length) or developmental stage as standards, but
158 this led to lower resolution (results not shown), so we worked only with sequences
159 standardized using sequence position.

160 Our ossification sequence data of extant and extinct taxa and the phylogenetic trees we
161 used are available online in the supplements to this paper on the bioRxiv web site.

162 *Analytical methods*

163 To discriminate between the six hypotheses about the origin of extant amphibians, two
164 methods are available: direct phylogenetic analysis of the sequence data, and comparisons of
165 the tree length (number of steps in regular parsimony, squared length in squared-change
166 parsimony, or similar measures) of various trees selected a priori to represent these
167 hypotheses. We selected the latter approach because relatively few data are available, and
168 thus, phylogenetic analysis of such data is unlikely to provide a well-resolved tree.

169 We used the CoMET module (Lee et al. 2006) for Mesquite 3.2 (Maddison and
170 Maddison 2017) to test the relative fit of the data on trees representing the six hypotheses.
171 CoMET calculates the likelihood and the AIC (Akaike Information Criterion) of nine
172 evolutionary models given continuous data and a tree. The nine models are obtained by
173 modifying the branch lengths of the reference tree. Thus, branches can be set to 0 (for internal
174 branches only, to yield a non-phylogenetic model), to 1 (equal or speciational model), left
175 unchanged from their original length (gradual evolution), or set free and evaluated from the
176 data (free model). This can be applied to internal and/or external branches, and various

combinations of these yield nine models (Lee et al. 2006: fig. 1). Among these nine models two have been frequently discussed in the literature and are especially relevant: gradual evolution, in which branch lengths (here representing evolutionary time) have not been changed, and a speciational model, in which all branches are set to the same length, and which has some similarities with Gould and Eldredge's (1972) punctuated equilibria model (though a model with one internal branch stemming from each note set to 0 and the other set to 1 would be even closer to the original formulation of that model). In this study, we assessed the fit of six of the nine models covered by CoMET; the other three (the punctuated version of distance, equal and free) could not be assessed due to problems in the current version of CoMET and possibly the size of our dataset (the corresponding author T. Oakley, pers. comm., has not yet been able to look into them).

Provided that the same evolutionary model is optimal for all compared hypotheses (this condition is met, as shown below), the AIC weights of the various trees under the same model can be used to assess the support for each tree. This can show not only which tree is best supported, but how many times more probable the best tree is compared to the alternatives – another reason to prefer this approach over a phylogenetic analysis. Given the small sample size (which here is the number of characters), we computed the corrected AIC (AICc) and the AICc weights using the formulae given by Anderson and Burnham (2002) and Wagenmakers and Farrell (2004).

Our tests make sense only in the presence of a phylogenetic signal in the data. In addition to the test of evolutionary model in CoMET evoked above (which tests non-phylogenetic as well as phylogenetic models), we performed a test based on squared-change parsimony (Maddison 1991) and random taxon reshuffling (Laurin 2004). For this test, we compared the length of the LH reference tree (with and without *Sclerocephalus*) to a population of 10,000 random trees produced by taxon reshuffling.

*Reference phylogenies*

We built a reference timetree that attempts to capture the most consensual relationships (Fig. 2). The tree was compiled in Mesquite (Maddison and Maddison 2017) and time-calibrated using the Stratigraphic Tools (Josse et al. 2006). For consistency and to avoid the effects of gaps in the fossil record, we used molecular divergence dates whenever possible. The main sources we used for topology and divergence times are as follows:

The phylogeny of lissamphibians follows the work of Pyron (2014). However, several data bases have been used for the temporal calibration of the tree: Germain and Laurin (2009) was used for the urodeles, whereas Feng et al. (2017), supplemented by Bossuyt and Roelants (2009) and Pyron (2014), was used for the anurans as well as more rootward nodes (Batrachia, Lissamphibia, Tetrapoda; also Amniota). Marjanović and Laurin (2014) was used for the Ranidae, Ceratophryidae, and Hylidae.

The sediments that have preserved the temnospondyls *Apateon* and *Sclerocephalus* are not easy to correlate with each other or with the global chronostratigraphic scale. Combining stratigraphic information from Schoch (2014a), Schneider et al. (2015) and Werneburg (2018), we have placed all three sampled species (*A. pedestris*, *A. caducus*, *S. haeuseri*) at the Sakmarian/Artinskian stage boundary (Permian; 290.1 Ma ago); combining stratigraphic information from Schneider et al. (2015) with the phylogeny in Schoch (2014a), we have tentatively placed the divergence between the two *Apateon* species (which are not sister-groups: Schoch 2014a) at the Kasimovian/Gzhelian stage boundary (Carboniferous; 303.7 Ma ago). The age of the last common ancestor of *Apateon* and *Sclerocephalus* depends strongly on temnospondyl phylogeny, which remains unresolved (Pardo et al. 2017b; Marjanović and Laurin 2018; and numerous references in both); as a compromise between the different

225 options, we have provisionally placed it at the boundary between the Early and the Late
226 Carboniferous (Serpukhovian/Bashkirian, 323.2 Ma ago) where applicable.

227 For the birds, Pons et al. (2005) was used for the Laridae, Wang et al. (2013) for the
228 Phasianidae and Gonzales et al. (2009) for the Anatidae. The temporal calibration was taken
229 from Prum et al. (2015) as recommended by Berv and Field (2017); gaps were filled in using
230 the database http://www.birdtree.org.

231 Several papers, mainly Tarver et al. (2016), were used for the phylogeny and
232 divergence times of mammals. For the Muridae, two references were used: Lecompte et al.
233 (2008) and the website "Mammals species of the World" (http://vertebrates.si.edu) for the
234 position of two taxa: *Mesocricetus auratus* and *Peromyscus melanophrys*. Other species were
235 placed following the work of Meredith et al. (2011), which also gives divergence times. We
236 caution, however, that all available molecular dates for Paleogene and earlier mammal nodes
237 may be overestimates (Berv and Field 2017).

238 Three references were also used to integrate squamates in the phylogenetic tree and for
239 the calibration of divergence times: Brandley et al. (2005), Rabosky et al. (2014), Reeder
240 (2003). Sterli et al. (2013) was used for turtles.

241 For turtles, there is now a near-consensus that they are diapsids, a hypothesis that is
242 not necessarily incompatible with an origin among "parareptiles" (Laurin and Piñeiro 2017).
243 Thus, following most recent molecular phylogenies (e.g., Hugall et al. 2007; Irisarri et al.
244 2017), we have inserted them as the sister-group of Archosauria.

245 Given that we disagree with several of the calibration dates in Irisarri et al. (2017), we
246 have not used divergence dates from that source.

To discriminate between the hypotheses on lissamphibian origins, we inserted the temnospondyl *Apateon* in the tree where each predicts that it should be (Fig. 1c–h). Thus, according to the TH (Fig. 1c), *Apateon* lies on the lissamphibian stem (Ruta and Coates 2007; Anderson 2007). Under the LH (Fig. 1d), *Apateon* lies on the tetrapod stem (Laurin 1998). Under both versions of the DH (Fig. 1g, h), *Apateon* lies on the batrachian stem (Pardo et al. 2017b: fig. 2, S7). Under both versions of the PH (Fig. 1e, f), *Apateon* lies on the caudate stem (Carroll 2007; Fröbisch et al. 2007). Within the DH and the PH, both versions of each differ in the position of Gymnophiona. Thus, despite the absence of any lepospondyl in our ossification sequence dataset, our taxonomic sample allows us to test all competing hypotheses.

*Sclerocephalus* is the sister-group of *Apateon* under the LH (Fig. 1d), immediately rootward of it (on the lissamphibian stem) under the TH (Fig. 1c) and likewise (but on the batrachian stem) under the DH1 (Fig. 1g), on the caecilian stem under the DH2 (Fig. 1h) and the sister-group of Batrachia (including *Apateon*) under both versions of the PH (Fig. 1e, f).

To ensure that our analyses were not biased in favor of a given hypothesis, in case that a continuous evolutionary model were favored, we adjusted the branch lengths such that the sum of branch lengths was equal between the compared topologies.

## RESULTS

The phylogenetic speciational model (in which all branch lengths are equal) had overwhelming support whether or not *Sclerocephalus* (Table 1) or the squamosal (Table 2) were included; the five other examined models had AICc weights $< 10^{-11}$. Thus, from here on, we compare support of the six competing topologies using only this model. This simplifies the discussion, because it means that the original branch lengths are irrelevant (under that model, all branch lengths are equal). Thus, the only variable is the topology. This, along with

271 the test based on squared-change parsimony and random taxon reshuffling indicates that the
272 phylogenetic signal in these data is strong; the latter test (Laurin, 2004) indicates that the
273 probability that the observed covariation between the data and the tree reflects a random
274 distribution is less than 0.0001 (none of the 10,000 random trees generated were as short as
275 the reference tree).

276 Using the speciational model, the AICc weights of the six compared topologies
277 indicate that there is strong support for the LH, with an AICc weight of 0.987 when
278 *Sclerocephalus* is included (Table 3) and 0.895 when the squamosal is included instead (Table
279 4). Of the other topologies, the TH was by far the best supported, with an AICc weight of
280 0.013 (with *Sclerocephalus*) or 0.094 (with the squamosal), which is 75.11 or 9.54 times less
281 than for the LH. Both versions of the DH and of the PH have negligible support (AICc
282 weights < 0.01 when the squamosal is included, < 0.0001 when *Sclerocephalus* is included).
283 The least support is found for the PH2 when *Sclerocephalus* is included, and for the DH1
284 when the squamosal is included. In both cases, the recently proposed DH2 (Pardo et al.
285 2017b) fares second-worst by a small margin. Notably, the DH1 contradicts the modern
286 consensus on lissamphibian monophyly (Fig. 1g), while the PH2 and the DH2 fulfill this
287 constraint from the molecular but not the paleontological point of view, having lissamphibian
288 monophyly with respect to amniotes but not with respect to temnospondyls (Fig. 1f, h).

289 DISCUSSION

290 *Phylogenetic signal*

291 In his discussion of previous attempts to draw phylogenetic conclusions from ossification
292 sequences (e.g. Schoch and Carroll 2003), Anderson (2007) noted that ossification sequences
293 seemed to abound in symplesiomorphies and in autapomorphies of terminal taxa, while
294 potential synapomorphies were scarce. This pessimism was seemingly confirmed by Schoch

295 (2006) in a paper that was published after Anderson's (2007) book chapter had gone to press:
296 not only were many similarities in the cranial ossification sequences across Osteichthyes
297 found to be symplesiomorphies, but a phylogenetic analysis of cranial ossification sequences
298 did not recover Mammalia, Sauropsida, Amniota or Lissamphibia as monophyletic. Along
299 with these untenable results, Schoch (2006) dismissed another: the position of the
300 temnospondyl *Apateon caducus* (the only included extinct taxon) outside the tetrapod crown-
301 group, i.e. the lepospondyl hypothesis on lissamphibian origins (LH).

302 While ossification sequences alone may not provide enough data for a phylogenetic
303 analysis, our datasets (with much larger taxon samples than in Schoch 2006) fit some tree
304 topologies much better than others. Both the tests using CoMET and squared-change
305 parsimony with random taxon reshuffling overwhelmingly support the presence of a
306 phylogenetic signal in these data; the null hypothesis of the absence of a phylogenetic signal
307 can be rejected in both cases, given that it has a probability of $< 10^{-97}$ in the first case, and <
308 $10^{-4}$ in the second case. We conclude that it contains a strong phylogenetic signal, and are
309 therefore cautiously optimistic about future contributions of ossification sequences to
310 phylogenetics.

311 *Suggestions for future extensions and uses of our dataset*

312 The postcranial ossification sequences of the lepospondyls *Microbrachis* and *Hyloplesion*
313 were described by Olori (2013). While they feature lower resolution than most sequences
314 used here, a study comparable to ours but focusing on postcranial elements could be carried
315 out in the future, if only with fewer taxa (as long as missing data remain a problem). This
316 would be worth doing because postcranial ossification sequences, like cranial ones, have been
317 used as arguments in the discussion on lissamphibian origins.

Specifically, Carroll et al. (1999) stated that the neural arches ossify before the centra in frogs and temnospondyls, but not in salamanders, caecilians or lepospondyls. When it was found that the centra do ossify first in a few cryptobranchoid salamanders, Carroll (2007: 30) took this as "strong evidence that the most primitive crown-group salamanders had a sequence of vertebral development that is common to frogs and labyrinthodonts (but distinct from that of lepospondyls)". In fact, apart from tail regeneration in *Hyloplesion* and *Microbrachis* (where the centra ossify before the neural arches: Olori 2015; Fröbisch et al. 2015; van der Vos et al. 2017), only one incompletely ossified vertebral column (referred to *Utaherpeton*) is known of any putative lepospondyl. "In this specimen, […] five neural arches […] have ossified behind the most posterior centrum." (Carroll and Chorn 1995: 40–41) Carroll's (2007: 85) claim that "the centra always ossified prior to the arches" in lepospondyls is therefore rather puzzling.

Fröbisch et al. (2007, 2015) pointed out that the first two digital rays (digits, metapodials and distal carpals/tarsals) ossify before the others ("preaxial polarity") in salamanders and the temnospondyls *Apateon*, *Micromelerpeton* and *Sclerocephalus*, while the fourth ossifies first ("postaxial polarity") in amniotes, frogs and "probably" (Fröbisch et al. 2015: 233, 234) the lepospondyls *Microbrachis* and *Hyloplesion*. This latter inference, however, is based only on a delay in the ossification of the fifth ray that is shared specifically with sauropsid amniotes (Olori 2015). Ossification sequences (however partial) of the other four rays in any lepospondyl are currently limited to the tarsus of *Batropetes*, which clearly shows preaxial polarity (Glienke 2015: fig. 6O–S; Marjanović and Laurin 2018), and that of the putative lepospondyl *Sauropleura*, in which likewise the second distal tarsal ossified before all others (Marjanović and Laurin 2018). Outside of temno- and lepospondyls, Marjanović and Laurin (2013, 2018) presented evidence that preaxial polarity is plesiomorphic, widespread and dependent on the use of the still developing limbs for

343 locomotion, which would explain why it was independently lost in amniotes and frogs and
344 reduced (the third ray ossifies first) in direct-developing salamanders.

345 Given the phylogenetic signal we have found in cranial ossification sequences, we
346 think that ossification sequence data should eventually be added to phenotypic datasets for
347 analyses of tetrapod phylogeny. Indeed, an analysis of amniote phylogeny using data from
348 organogenesis sequences (coded using event-pairing in Parsimov) already exists (Werneburg
349 and Sánchez-Villagra 2009). The usefulness of such data for phylogenetic inference was
350 further tested, with encouraging results, by Laurin and Germain (2011), and the present
351 analysis adds additional support for it.

352 *On the origin of lissamphibians, or the preservation of favoured lepospondyls in the struggle*
353 *for life*

354 The strong support for the lepospondyl hypothesis that we have found is surprising because
355 cranial ossification sequence data, especially those of the Permo-Carboniferous temnospondyl
356 *Apateon*, have often been claimed to contradict the LH; especially, similarities between
357 *Apateon* and extant urodeles, in particular the supposedly "primitive" hynobiid *Ranodon*, have
358 often been emphasized (Schoch and Carroll 2003; Schoch and Milner 2004; Carroll 2007;
359 Schoch 2014b). However, other studies have already raised doubts about some of these claims
360 (e.g. Schoch 2006; Anderson 2007; Germain and Laurin 2009). Schoch (2006) and Anderson
361 (2007) concluded that most characters shared between *Apateon* and urodeles were
362 plesiomorphies. Germain and Laurin (2009) also demonstrated that, far from being very
363 similar to the ancestral urodele morphotype (contra Schoch and Carroll 2003 or Carroll 2007),
364 the cranial ossification sequence of *Apateon* was statistically significantly different from that
365 of the hypothetical last common ancestor of all urodeles (as suspected by Anderson 2007).
366 However, these earlier studies did not clearly show which of the various hypotheses on

lissamphibian origins the ossification sequences of *Apateon* – or the newly available partial sequence (Werneburg 2018) of the phylogenetically distant temnospondyl *Sclerocephalus* – supported most. This is what we have attempted to do here.

We conclude that cranial ossification sequences of amniotes, lissamphibians and temnospondyls provide support for the LH that is independent of the phylogenetic analyses of Laurin (1998), Pawley (2006: appendix 16) or Marjanović and Laurin (2009, 2018).

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## References

Anderson J.S. 2007. Incorporating ontogeny into the matrix: a phylogenetic evaluation of developmental evidence for the origin of modern amphibians. Pages 182–227 in Anderson J.S., Sues H.-D., editors. Major transitions in vertebrate evolution. Bloomington: Indiana University Press.

Anderson J.S., Carroll R.L., Rowe T.B. 2003. New information on *Lethiscus stocki* (Tetrapoda: Lepospondyli: Aistopoda) from high-resolution computed tomography and a phylogenetic analysis of Aistopoda. Can. J. Earth Sci. 40:1071–1083.

Anderson D.R., Burnham K.P. 2002. Avoiding pitfalls when using information-theoretic methods. J. Wildl. Manag. 66:912–918.

Anderson J.S., Reisz R.R., Scott D., Fröbisch N.B., Sumida S.S. 2008. A stem batrachian from the Early Permian of Texas and the origin of frogs and salamanders. Nature 453:515–518.

Berv J.S., Field D.J. 2017 (printed 2018). Genomic signature of an avian Lilliput Effect across the K-Pg Extinction. Syst. Biol. 67:1–13.

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

Bossuyt F., Roelants K. 2009. Frogs and toads (Anura). Pages 357–364 in Hedges S.B., Kumar S., editors. The Timetree of Life. New York: Oxford University Press.

Brandley M.C., Schmitz A., Reeder T.W. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54:373-390.

Carroll R.L. 2001. The origin and early radiation of terrestrial vertebrates. J. Paleontol. 75:1202–1213.

Carroll R.L. 2007. The Palaeozoic ancestry of salamanders, frogs and caecilians. Zool. J. Linn. Soc. 150 (suppl. 1):1–140.

Carroll R.L., Chorn J. 1995. Vertebral development in the oldest microsaur and the problem of “lepospondyl” relationships. J. Vert. Paleont. 15:37–56.

Carroll R.L., Currie P.J. 1975. Microsaurs as possible apodan ancestors. Zool. J. Linn. Soc. 57:229–247.

Carroll R.L., Holmes R. 1980. The skull and jaw musculature as guides to the ancestry of salamanders. Zool. J. Linn. Soc. 68:1–40.

Carroll R.L., Kuntz A., Albright K. 1999. Vertebral development and amphibian evolution. Evol. Dev. 1:36–48.

Cope E.D. 1888. On the intercentrum of the terrestrial Vertebrata. Trans. Am. Phil. Soc. 16:243–253.

Eldredge N., Gould S.J. 1972. Punctuated equilibria: an alternative to phyletic gradualism. Pages 82–115 in Schopf T.J.M., editor. Models in Paleobiology. San Francisco: Freeman, Cooper & Company.

Feng Y.-J., Blackburn D.C., Liang D., Hillis D.M., Wake D.B., Cannatella D.C., Zhang P. 2017. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. Proc. Natl. Acad. Sci. USA E5864–E5870.

Fröbisch N.B., Carroll R.L., Schoch R.R. 2007. Limb ossification in the Paleozoic branchiosaurid *Apateon* (Temnospondyli) and the early evolution of preaxial dominance in tetrapod limb development. Evol. Dev. 9:69–75.

Fröbisch N.B., Bickelmann C., Olori J.C., Witzmann F. 2015. Deep-time evolution of regeneration and preaxial polarity in tetrapod limb development. Nature 527:231–234.

Germain D., Laurin M. 2009. Evolution of ossification sequences in salamanders and urodele origins assessed through event-pairing and new methods. Evol. Dev. 11:170–190.

Glienke S. 2015. Two new species of the genus *Batropetes* (Tetrapoda, Lepospondyli) from the Central European Rotliegend (basal Permian) in Germany. J. Vert. Paleont. 35:e918041.

Gonzalez J., Düttmann H., Wink M. 2009. Phylogenetic relationships based on two mitochondrial genes and hybridization patterns in Anatidae. J. Zool. 279:310–318.

Harrington S.M., Harrison L.B., Sheil C.A. 2013. Ossification sequence heterochrony among amphibians. Evol. Dev. 15:344–364.

Hugall A.F., Foster R., Lee M.S.Y. 2007. Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. Syst. Biol. 56:543–563.

Hugi J., Hutchinson M.N., Koyabu D., Sánchez-Villagra M.R. 2012. Heterochronic shifts in the ossification sequences of surface- and subsurface-dwelling skinks are correlated with the degree of limb reduction. Zoology 115:188–198.

Irisarri I., Baurain D., Brinkmann H., Delsuc F., Sire J.-Y., Kupfer A., Petersen J., Jarek M., Meyer A., Vences M., Philippe H. 2017. Phylotranscriptomic consolidation of the jawed vertebrate timetree. Nature Ecol. Evol. 1:1370–1378.

Josse S., Moreau T., Laurin M. 2006. Stratigraphic tools for Mesquite, version 1.0. http://mesquiteproject.org/packages/stratigraphicTools/

Laurin M. 1998. The importance of global parsimony and historical bias in understanding tetrapod evolution. Part I. Systematics, middle ear evolution, and jaw suspension. Ann. Sci. Nat., Zool., 13$^{e}$ Sér. 19:1–42.

Laurin M. 2004. The evolution of body size, Cope's rule and the origin of amniotes. Syst. Biol. 53:594-622.

Laurin M., Germain D. 2011. Developmental characters in phylogenetic inference and their absolute timing information. Syst. Biol. 60:630–644.

Laurin M., Piñeiro G. 2017. A reassessment of the taxonomic position of mesosaurs, and a surprising phylogeny of early amniotes. Front. Earth Sci. 5:88.

Lecompte E., Aplin K., Denys C., Catzeflis F., Chades M., Chevret P. 2008. Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences, with a new tribal classification of the subfamily. BMC Evol. Biol. 8:199.

Lee C., Blay S., Mooers A.Ø., Singh A., Oakley T.H. 2006. CoMET: A Mesquite package for comparing models of continuous character evolution on phylogenies. Evol. Bioinformatics Online 2:193–196.

Maddin H.C., Jenkins F.A. Jr., Anderson J.S. 2012. The braincase of *Eocaecilia micropodia* (Lissamphibia, Gymnophiona) and the origin of caecilians. PLOS ONE 7:e50743.

Maddison W.P. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. Syst. Zool. 40:304–314.

Maddison W.P., Maddison D.R. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2. http://mesquite.wikispaces.com

Marjanović D., Laurin M. 2008. Assessing confidence intervals for stratigraphic ranges of higher taxa: The case of Lissamphibia. Acta Palaeont. Pol. 53:413–432.

Marjanović D., Laurin M. 2009. The origin(s) of modern amphibians: a commentary. Evol. Biol. 36:336–338.

Marjanović D., Laurin M. 2013. The origin(s) of extant amphibians: a review with emphasis on the “lepospondyl hypothesis”. Geodiversitas 35:207–272.

Marjanović D., Laurin M. 2014. An updated paleontological timetree of lissamphibians, with comments on the anatomy of Jurassic crown-group salamanders (Urodela). Hist. Biol. 26:535–550.

Marjanović D., Laurin M. 2018. Reproducibility in phylogenetics: reevaluation of the largest published morphological data matrix for phylogenetic analysis of Paleozoic limbed vertebrates. PeerJ Preprints 6:e1596v3.

Maxwell E.E., Harrison L.B., Larsson H.C.E. 2010. Assessing the phylogenetic utility of sequence heterochrony: evolution of avian ossification sequences as a case study. Zoology 113:57–66.

Meredith R.W., Janečka J.E., Gatesy J., Ryder O.A., Fisher C.A., Teeling E.C., Goodbla A., Eizirik E., Simão T.L.L., Stadler T., Rabosky D.L., Honeycutt R.L., Flynn J.J., Ingram C.M., Steiner C., Williams T.L., Robinson T.J., Burk-Herrick A., Westerman M., Ayoub N.A., Springer M.S., Murphy W.J. 2011. Impacts of the Cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science 334:521–524.

Olori J.C. 2013. Ontogenetic sequence reconstruction and sequence polymorphism in extinct taxa: an example using early tetrapods (Tetrapoda: Lepospondyli). Paleobiology 39:400–428.

Olori J.C. 2015. Skeletal morphogenesis of *Microbrachis* and *Hyloplesion* (Tetrapoda: Lepospondyli), and implications for the developmental patterns of extinct, early tetrapods. PLOS ONE 10:e0128333.

Pardo J.D., Szostakiwskyj M., Ahlberg P.E., Anderson J.S. 2017a. Hidden morphological diversity among early tetrapods. Nature 546:642–645.

Pardo J.D., Small B.J., Huttenlocker A.K. 2017b. Stem caecilian from the Triassic of Colorado sheds light on the origins of Lissamphibia. Proc. Natl. Acad. Sci. U.S.A. 114:E5389–E5395.

Parsons T.S., Williams E.E. 1962. The teeth of Amphibia and their relation to amphibian phylogeny. J. Morph. 110:375–389.

Parsons T.S., Williams E.E. 1963. The relationships of the modern Amphibia: A re-examination. Q. Rev. Biol. 38:26–53.

Pawley K. 2006. The postcranial skeleton of temnospondyls (Tetrapoda: Temnospondyli) [doctoral thesis]. Melbourne: La Trobe University. http://hdl.handle.net/1959.9/405644

Pons J.-M., Hassanin A., Crochet P.-A. 2005. Phylogenetic relationships within the Laridae (Charadriiformes: Aves) inferred from mitochondrial markers. Mol. Phyl. Evol. 37:686–699.

Prum R.O., Berv J.S., Dornburg A., Field D.J., Townsend J.P., Lemmon A.M., Lemmon A.R. 2015. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. Nature 526:569–573.

Pyron R.A. 2014. Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. Syst. Biol. 63:779–797.

Rabosky D.L., Donnellan S.C., Grundler M., Lovette I.J. 2014. Analysis and visualization of complex macroevolutionary dynamics: an example from Australian scincid lizards. Syst. Biol. 63:610-627.

Reeder T.W. 2003. A phylogeny of the Australian *Sphenomorphus* group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Tribolonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. Mol. Phyl. Evol. 27:384-397.

Ruta M., Coates M.I. 2007. Dates, nodes and character conflict: addressing the lissamphibian origin problem. J. Syst. Palaeontol. 5:69–122.

Schneider J.W., Werneburg R., Rößler R., Voigt S., Scholze F. 2015. Example for the description of basins in the CPT Nonmarine-Marine Correlation Chart – Thuringian Forest Basin, East Germany. Permophiles 61:29–35.

Schoch R.R. 2002. The formation of the skull in Paleozoic and extant amphibians.

Paleobiology 28:378–396.

Schoch R.R. 2004. Skeleton formation in the Branchiosauridae: a case study in comparing ontogenetic trajectories. J. Vert. Paleont. 24:309–319.

Schoch R.R. 2006. Skull ontogeny: developmental patterns of fishes conserved across major tetrapod clades. Evol. Dev. 8:524–536.

Schoch R.R. 2014a. First evidence of the branchiosaurid temnospondyl *Leptorophus* in the Early Permian of the Saar-Nahe Basin (SW Germany). N. Jb. Geol. Paläont. Abh. 272:225–236.

Schoch R.R. 2014b. Amphibian skull evolution: the developmental and functional context of simplification, bone loss and heterotopy. J. Exp. Zool. (Mol. Dev. Evol.) 322B:619–630.

Schoch R.R., Carroll R.L. 2003. Ontogenetic evidence for the Paleozoic ancestry of salamanders. Evol. Dev. 5:314–324.

Schoch R.R., Milner A.R. 2004. Structure and implications of theories on the origin of lissamphibians. Pages 345–377 in Arratia G., Wilson M.V.H., Cloutier R., editors. Recent Advances in the Origin and Early Radiation of Vertebrates. Munich: Dr. Friedrich Pfeil.

Sigurdsen T., Green D.M. 2011. The origin of modern amphibians: a re-evaluation. Zool. J. Linn. Soc. 162:457–469.

Steen M.C. 1938. On the fossil Amphibia from the Gas Coal of Nýřany and other deposits in Czechoslovakia. Proc. Zool. Soc. Lond. 108:205–283.

Sterli J., Pol D., Laurin M. 2013. Incorporating phylogenetic uncertainty on phylogeny-based paleontological dating and the timing of turtle diversification. Cladistics 29:233–246.

Tarver J.E., dos Reis M., Mirarab S., Moran R.J., Parker S., O'Reilly J.E., King B.L., O'Connell M.J., Asher R.J., Warnow T., Peterson K.J., Donoghue P.C.J., Pisani D. 2016. The interrelationships of placental mammals and the limits of phylogenetic

551 inference. Genome Biol. Evol. 8:330–344.

552 van der Vos W., Witzmann F., Fröbisch N.B. 2017. Tail regeneration in the Paleozoic
553 tetrapod *Microbrachis pelikani* and comparison with extant salamanders and squamates.
554 J. Zool. 304:34–44

555 Wagenmakers E.-J., Farrell S. 2004. AIC model selection using Akaike weights. Psychon.
556 Bull. Rev. 11:192–196.

557 Wang N., Kimball R.T., Braun E.L., Liang B., Zhang Z. 2013. Assessing phylogenetic
558 relationships among Galliformes: a multigene phylogeny with expanded taxon sampling
559 in Phasianidae. PLOS ONE 8:1–12.

560 Watson D.M.S. 1940. The origin of frogs. Trans. R. Soc. Edinburgh 60:195–231.

561 Weisbecker V. 2011. Monotreme ossification sequences and the riddle of mammalian skeletal
562 development. Evolution 65:1323–1335.

563 Werneburg R. 2018 (for 2017). Earliest 'nursery ground' of temnospondyl amphibians in the
564 Permian. Semana 32:3–42.

565 Werneburg I., Sánchez-Villagra M.R. 2009. Timing of organogenesis support basal position
566 of turtles in the amniote tree of life. BMC Evol. Biol. 9:82.

567 Witzmann F., Schoch R.R. 2006. Skeletal development of the temnospondyl
568 *Acanthostomatops vorax* from the Lower Permian Döhlen Basin of Saxony. Trans. R.
569 Soc. Edinburgh: Earth Sci. 96:365–385.

570 Zhang P., Zhou H., Chen Y.-Q., Liu Y.-F., Qu L.-H. 2005. Mitogenomic perspectives on the
571 origin and phylogeny of living amphibians. Syst. Biol. 54:391–400.

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575 FIGURE LEGENDS

576 FIGURE 1. Hypotheses on the relationships of the extant amphibian clades since the mid-20th
577 century. The names of terminal taxa sampled here are in boldface; the names of larger clades
578 are placed toward the right end of a branch if they have minimum-clade (node-based)
579 definitions, to the left if they have maximum-clade (branch-based) definitions. The first two
580 trees (a, b) show the current consensus; the other trees (c–h) show the various tested
581 paleontological hypotheses. a) Consensus of the latest and largest phylogenetic analyses of
582 molecular data (Pyron 2014; Irisarri et al. 2017; Feng et al. 2017); all named clades are
583 therefore extant. Note the monophyly of the extant amphibians (Lissamphibia, marked with a
584 light gray dot) with respect to Amniota. b) Consensus of all analyses of Paleozoic limbed
585 vertebrates (latest and largest: Pawley 2006; Sigurdsen and Green 2011; Pardo et al. 2017a, b:
586 fig. S6; Marjanović and Laurin 2018), omitting the extant amphibian clades. Note the
587 monophyly of "lepospondyls" + amniotes (marked with a dark gray dot). c) TH: Lissamphibia
588 nested among temnospondyls, close to *Apateon* (most recently found by Sigurdsen and Green
589 2011; Maddin et al. 2012; Pardo et al. 2017a, b: fig. S6; argued for by Schoch and Milner
590 2004, Schoch 2014b and others). Compatible with both a and b (light gray dots). d) LH:
591 Lissamphibia nested among "lepospondyls" (found most recently by Pawley 2006;
592 Marjanović and Laurin 2009, 2018); consequently, the temnospondyls *Apateon* and
593 *Sclerocephalus* are not crown-group tetrapods. Compatible with both a) and b) (light gray
594 dots). e) PH1: Urodela as temnospondyls close to *Apateon*, Anura as a separate clade of
595 temnospondyls, Gymnophiona as "lepospondyls" (found by Carroll 2007; argued for by
596 Carroll 2001; Schoch and Carroll 2003; very cautiously Fröbisch et al. 2007). Compatible
597 with b) (dark gray dot) but not with a) (light gray circle). f) PH2: like PH1, but with

598 monophyletic extant amphibians with respect to amniotes (a) restored (light gray dot) at the
599 expense of compatibility with the paleontological consensus concerning the position of
600 temnospondyls, lepospondyls, and amniotes (b) (dark gray circle). g) DH1: Batrachia as
601 temnospondyls close to *Apateon*, Gymnophiona as "lepospondyls" (found by Anderson 2007;
602 Anderson et al. 2008). Compatible with b) (dark gray dot) but not with a) (light gray circle).
603 h) DH2: Batrachia as temnospondyls close to *Apateon*, Gymnophiona as temnospondyls
604 distantly related to *Sclerocephalus* (found by Pardo et al. 2017b in an analysis that included
605 only temnospondyls and lissamphibians: fig. 2, S7). Compatible with both a) and b).

606 FIGURE 2. Reference phylogeny used for some of the analyses, illustrating the LH. The tree
607 was time-calibrated, but analyses showed that branch lengths are irrelevant, given that the best
608 model is speciational (Table 1).

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611 TABLE 1. AICc and AICc weights of six evolutionary models given our data (102 taxa,
612 including the temnospondyl *Sclerocephalus*; six characters) and reference tree. This was
613 performed on the tree representing the LH (lepospondyl hypothesis), but doing this on other
614 trees leads to similar results. Numbers presented with four significant digits; best values in
615 boldface. Abbreviations: k, number of estimable parameters; l, likelihood; wi, weight; $\Delta_i$,
616 difference of AICc from that of the Pure-Phylogenetic / Equal model.

| | **AIC** | **l** | **k** | **AICc** | **$\Delta_i$ AICc** | **wi(AICc)** |
|---|---|---|---|---|---|---|
| Pure-Phylogenetic / Distance | −569.0 | 285.5 | **1** | −567.5 | 780.4 | 3.461 E−170 |
| Pure-Phylogenetic / Equal | **−1173** | **587.5** | **1** | **−1171** | **0** | **1.000** |
| Pure-Phylogenetic / Free | 2.000 E10 | −1.000 E10 | 306 | 2.000 E10 | 2.000 E10 | 0 |
| Non-Phylogenetic / Distance | −439.8 | 220.9 | **1** | −438.3 | 909.6 | 3.026 E−198 |
| Non-Phylogenetic / | −899.5 | 450.7 | **1** | −898.0 | 450.0 | 1.959 E−98 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Equal | | | | | | |
| Non-Phylogenetic / Free | 2.000 E10 | −1.000 E10 | 154 | 2.000 E10 | 2.000 E10 | 0 |

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TABLE 2. As Table 1, but with 101 taxa, excluding *Sclerocephalus*, and six characters. Abbreviations and boldface as in Table 1.

| | **AIC** | **l** | **k** | **AICc** | **$\Delta_i$ AICc** | **wi(AICc)** |
|---|---|---|---|---|---|---|
| Pure-Phylogenetic / Distance | −718.3 | 360.2 | **1** | −717.1 | 630.8 | 1.046 E−137 |
| Pure-Phylogenetic / Equal | **−1350** | **676.1** | **1** | **−1349** | **0** | **1.000** |
| Pure-Phylogenetic / Free | 2.000 E10 | −1.000 E10 | 306 | 2.000 E10 | 2.000 E10 | 0 |
| Non-Phylogenetic / Distance | −549.5 | 275.7 | **1** | −548.3 | 799.6 | 2.282 E−174 |
| Non-Phylogenetic / Equal | −899.5 | 450.7 | **1** | −898.3 | 449.7 | 2.278 E−98 |
| Non-Phylogenetic / Free | −649.8 | 478.9 | 154 | −1293 | 55.11 | 1.078 E−12 |

620

621 TABLE 3. AIC and AICc weights of the six topologies, reflecting the six hypotheses about the
622 origin of extant amphibians, for the speciational model (called Pure-Phylogenetic / Equal in
623 Tables 1 and 2), given a sample with 102 taxa (including *Sclerocephalus*) and six characters.
624 Abbreviations and boldface as in Table 1, except $\Delta_i$: difference of AICc from that of the LH.
625 Hypotheses from top to bottom: TH: monophyletic origin among temnospondyls; PH1:
626 triphyletic origin (anurans and urodeles from different temnospondyls, caecilians from
627 lepospondyls); PH2: triphyletic origin from temnospondyls; DH1: diphyletic origin (anurans
628 and urodeles from temnospondyls, caecilians from lepospondyls); DH2: diphyletic origin
629 from temnospondyls; LH: monophyletic origin from lepospondyls; see text for details and
630 references.

| **Hypothesis** | **AIC** | **l** | **AICc** | **$\Delta_i$ AICc** | **wi(AICc)** |
|---|---|---|---|---|---|
| TH | −1164 | 583.2 | −1163 | 8.638 | 0.01314 |
| PH1 | −1148 | 574.8 | −1146 | 25.37 | 3.062 E−06 |
| PH2 | −1147 | 574.4 | −1145 | 26.18 | 2.034 E−06 |
| DH1 | −1154 | 578.1 | −1153 | 18.65 | 8.799 E−05 |
| DH2 | −1147 | 574.5 | −1146 | 25.94 | 2.302 E−06 |
| LH | **−1173** | **587.5** | **−1171** | **0** | **0.9868** |

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632

633 TABLE 4. As Table 3, but for a sample with 101 taxa, excluding *Sclerocephalus*, and seven

634 characters. Abbreviations, boldface and hypotheses as in Table 3.

| **Hypothesis** | **AIC** | **l** | **AICc** | **$\Delta_i$ AICc** | **wi(AICc)** |
|---|---|---|---|---|---|
| TH | −1346 | 673.8 | −1345 | 4.511 | 0.09377 |
| PH1 | −1338 | 670.2 | −1337 | 11.83 | 0.002409 |
| PH2 | −1341 | 671.4 | −1340 | 9.419 | 0.008059 |
| DH1 | −1335 | 668.6 | −1334 | 14.92 | 5.157 E−04 |
| DH2 | −1336 | 669.0 | −1335 | 14.24 | 7.224 E−04 |
| LH | **−1350** | **676.1** | **−1349** | **0** | **0.8945** |

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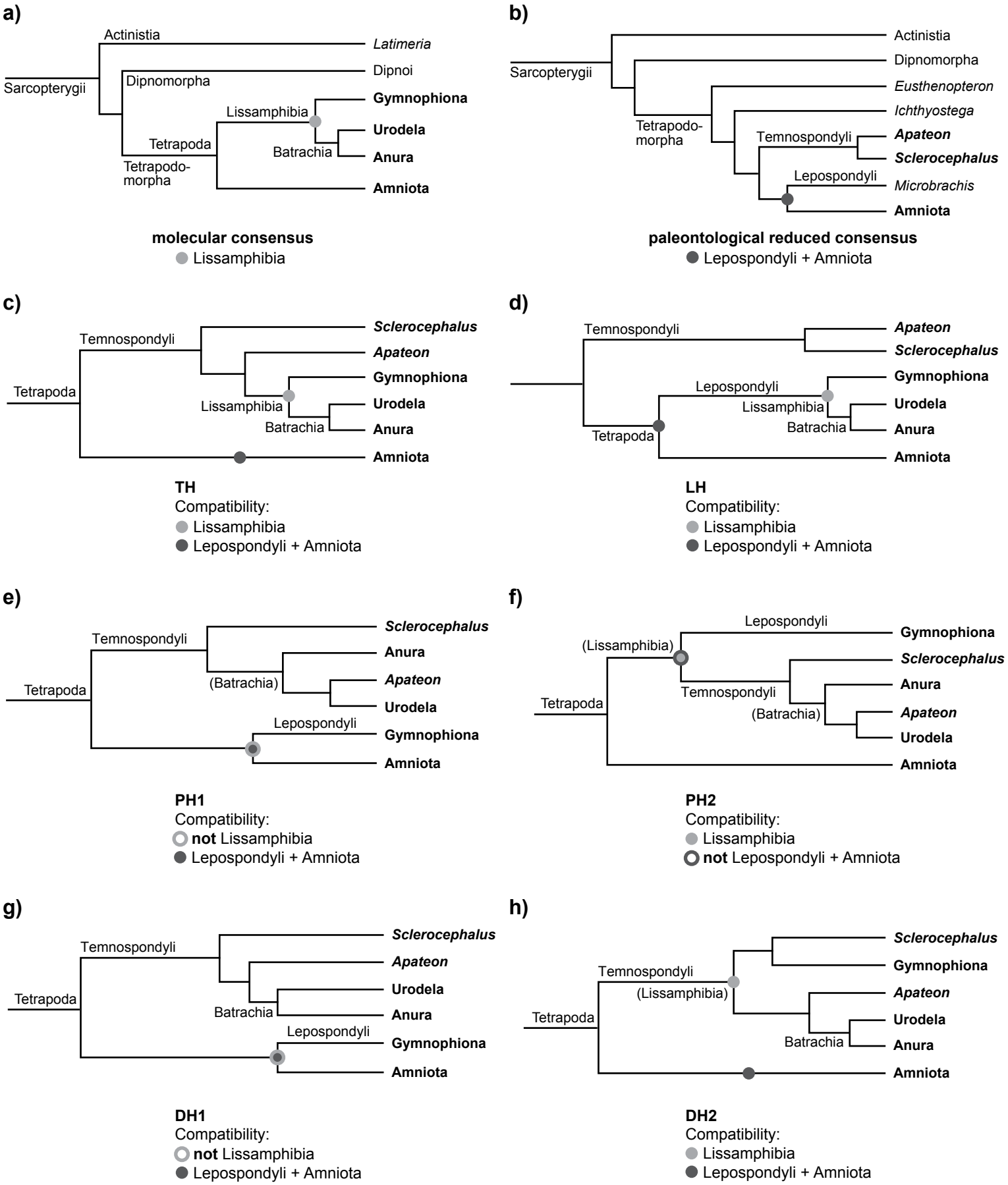
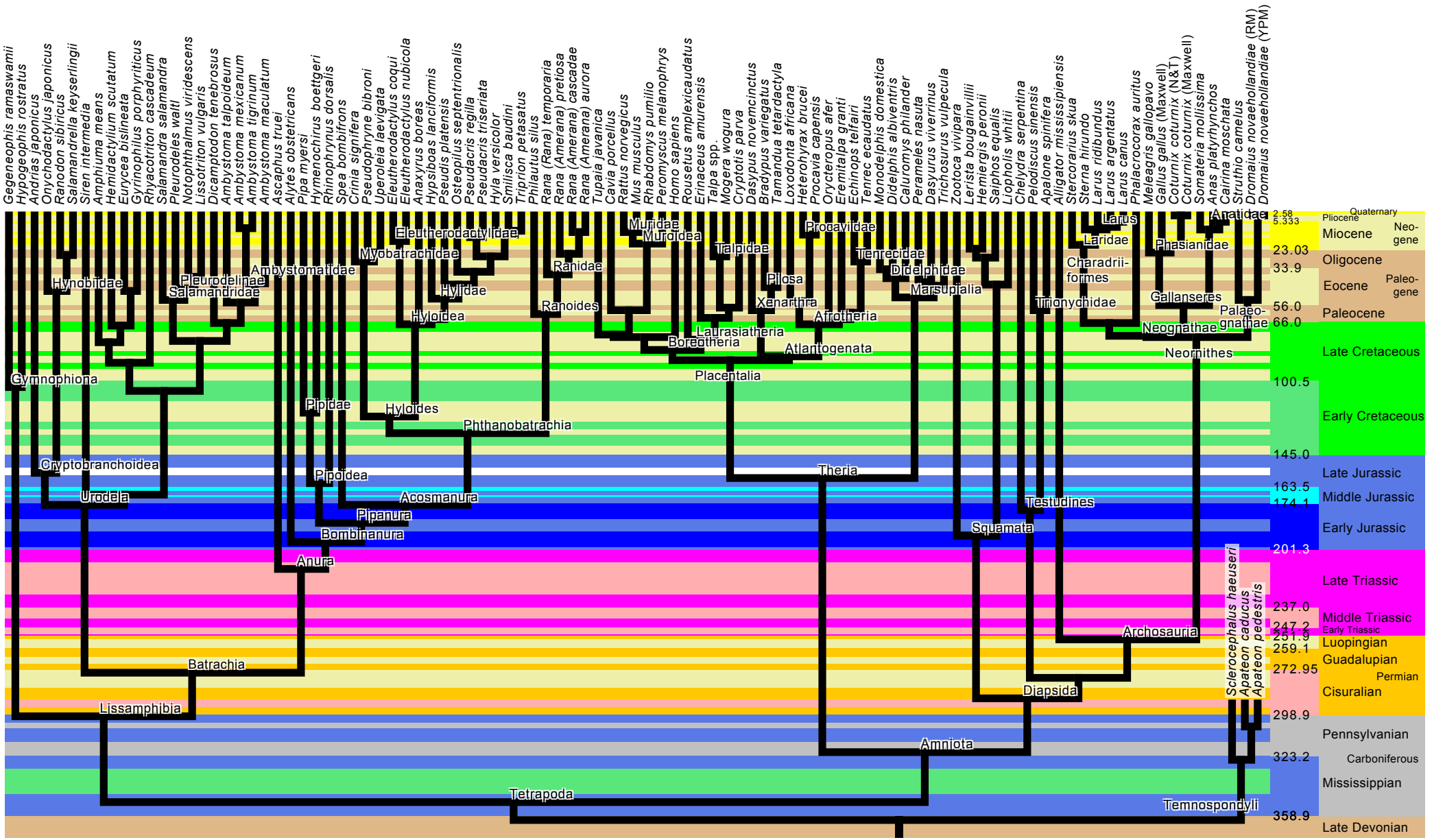
## SUPPLEMENTARY MATERIAL

ONLINE SUPPLEMENTARY DOCUMENT 1. Sources of the ossification sequence data.

ONLINE SUPPLEMENTARY DOCUMENT 2. Data matrix in NEXUS format for Mesquite.


a)
Actinistia
Latimeria
Dipnoi
Sarcopterygii
Dipnomorpha
Gymnophiona
Lissamphibia
Urodela
Tetrapoda
Batrachia
Anura
Tetrapodo-
morpha
Amniota
molecular consensus
Lissamphibia
b)
Actinistia
Dipnomorpha
Sarcopterygii
Eusthenopteron
Ichthyostega
Tetrapodo-
morpha
Temnospondyli
Apateon
Sclerocephalus
Lepospondyli
Microbrachis
Amniota
paleontological reduced consensus
Lepospondyli + Amniota
c)
Sclerocephalus
Temnospondyli
Apateon
Gymnophiona
Tetrapoda
Lissamphibia
Urodela
Batrachia
Anura
Amniota
TH
Compatibility:
Lissamphibia
Lepospondyli + Amniota
d)
Temnospondyli
Apateon
Sclerocephalus
Gymnophiona
Lepospondyli
Lissamphibia
Urodela
Batrachia
Anura
Tetrapoda
Amniota
LH
Compatibility:
Lissamphibia
Lepospondyli + Amniota
e)
Sclerocephalus
Temnospondyli
Anura
(Batrachia)
Apateon
Tetrapoda
Urodela
Lepospondyli
Gymnophiona
Amniota
PH1
Compatibility:
not Lissamphibia
Lepospondyli + Amniota
f)
Lepospondyli
Gymnophiona
(Lissamphibia)
Sclerocephalus
Anura
Temnospondyli
Tetrapoda
(Batrachia)
Apateon
Urodela
Amniota
PH2
Compatibility:
Lissamphibia
not Lepospondyli + Amniota
g)
Sclerocephalus
Temnospondyli
Apateon
Urodela
Tetrapoda
Batrachia
Anura
Lepospondyli
Gymnophiona
Amniota
DH1
Compatibility:
not Lissamphibia
Lepospondyli + Amniota
h)
Sclerocephalus
Gymnophiona
Temnospondyli
(Lissamphibia)
Apateon
Tetrapoda
Urodela
Batrachia
Anura
Amniota
DH2
Compatibility:
Lissamphibia
Lepospondyli + Amniota



Gegeneophis ramaswamii
Hypogeophis rostratus
Andrias japonicus
Onychodactylus japonicus
Ranodon sibiricus
Salamandrella keyserlingii
Siren intermedia
Amphiuma means
Hemidactylium scutatum
Eurycea bislineata
Gyrinophilus porphyriticus
Rhyacotriton cascadeum
Salamandra salamandra
Pleurodeles waltl
Notophthalmus viridescens
Lissotriton vulgaris
Dicamptodon tenebrosus
Ambystoma talpoideum
Ambystoma mexicanum
Ambystoma tigrinum
Ambystoma maculatum
Ascaphus truei
Alytes obstetricans
Pipa myersi
Hymenochirus boettgeri
Rhinophrynus dorsalis
Spea bombifrons
Crinia signifera
Pseudophryne bibroni
Uperoleia laevigata
Eleutherodactylus coqui
Eleutherodactylus nubicola
Anaxyrus boreas
Hypsiboas lanciformis
Pseudis platensis
Osteopilus septentrionalis
Pseudacris regilla
Pseudacris triseriata
Hyla versicolor
Smilisca baudini
Triprion petasatus
Philautus silus
Rana (Rana) temporaria
Rana (Amerana) pretiosa
Rana (Amerana) cascadae
Rana (Amerana) aurora
Tupaia javanica
Cavia porcellus
Rattus norvegicus
Mus musculus
Rhabdomys pumilio
Peromyscus melanophrys
Homo sapiens
Rousetus amplexicaudatus
Erinaceus amurensis
Talpa spp.
Mogera wogura
Cryptotis parva
Dasypus novemcinctus
Bradypus variegatus
Tamandua tetardactyla
Loxodonta africana
Heterohyrax brucei
Procavia capensis
Orycteropus afer
Eremitalpa granti
Echinops telfairi
Tenrec ecaudatus
Monodelphis domestica
Didelphis albiventris
Caluromys philander
Perameles nasuta
Dasyurus viverrinus
Trichosurus vulpecula
Zootoca vivipara
Lerista bougainvillii
Hemiergis peronii
Saiphos equalis
Liopholis whitii
Chelydra serpentina
Pelodiscus sinensis
Apalone spinifera
Alligator mississipiensis
Stercorarius skua
Sterna hirundo
Larus ridibundus
Larus argentatus
Larus canus
Phalacrocorax auritus
Meleagris gallopavo
Gallus gallus (Maxwell)
Coturnix coturnix (N&T)
Coturnix coturnix (Maxwell)
Somateria mollissima
Anas platyrhynchos
Cairina moschata
Struthio camelus
Dromaius novaehollandiae (RM)
Dromaius novaehollandiae (YPM)
Hynobiidae
Pleurodelinae
Salamandridae
Ambystomatidae
Myobatrachidae
Eleutherodactylidae
Hylidae
Hyloidea
Ranidae
Ranoides
Muridae
Muroidea
Talpidae
Procavidae
Tenrecidae
Didelphidae
Marsupialia
Pilosa
Xenarthra
Afrotheria
Laurasiatheria
Boreotheria
Atlantogenata
Placentalia
Larus
Laridae
Charadrii-formes
Trionychidae
Phasianidae
Gallanseres
Neognathae
Palaeo-gnathae
Anatidae
Neornithes
Gymnophiona
Pipidae
Hyloides
Phthanobatrachia
Cryptobranchoidea
Pipoidea
Urodela
Acosmanura
Pipanura
Bombinanura
Anura
Theria
Testudines
Squamata
Archosauria
Diapsida
Batrachia
Lissamphibia
Amniota
Tetrapoda
Temnospondyli
Sclerocephalus haeuseri
Apateon caducus
Apateon pedestris
2.58
5.333
23.03
33.9
56.0
66.0
100.5
145.0
163.5
174.1
201.3
237.0
247.2
251.9
259.1
272.95
298.9
323.2
358.9
Quaternary
Pliocene
Miocene
Neo-gene
Oligocene
Eocene
Paleo-gene
Paleocene
Late Cretaceous
Early Cretaceous
Late Jurassic
Middle Jurassic
Early Jurassic
Late Triassic
Middle Triassic
Early Triassic
Luopingian
Guadalupian
Permian
Cisuralian
Pennsylvanian
Carboniferous
Mississippian
Late Devonian