A supertree of Temnospondyli: cladogenetic patterns in the most species-rich group of early tetrapods

Marcello Ruta1,*, Davide Pisani2, Graeme T. Lloyd1 and Michael J. Benton1

1Department of Earth Sciences, University of Bristol, Wills Memorial Building, Queen’s Road, Bristol BS8 1RJ, UK
2Laboratory of Evolutionary Biology, The National University of Ireland, Maynooth, Kildare, Ireland

As the most diverse group of early tetrapods, temnospondyls provide a unique opportunity to investigate cladogenetic patterns among basal limbed vertebrates. We present five species-level supertrees for temnospondyls, built using a variety of methods. The standard MRP majority rule consensus including minority components shows slightly greater resolution than other supertrees, and its shape matches well several currently accepted hypotheses of higher-level phylogeny for temnospondyls as a whole. Also, its node support is higher than those of other supertrees (except the combined standard plus Purvis MRP supertree). We explore the distribution of significant as well as informative changes (shifts) in branch splitting employing the standard MRP supertree as a reference, and discuss the temporal distribution of changes in time-sliced, pruned trees derived from this supertree. Also, we analyse those shifts that are most relevant to the end-Permain mass extinction. For the Palaeozoic, shifts occur almost invariably along branches that connect major Palaeozoic groups. By contrast, shifts in the Mesozoic occur predominantly within major groups. Numerous shifts bracket narrowly the end-Permian extinction, indicating not only rapid recovery and extensive diversification of temnospondyls over a short time period after the extinction event (possibly less than half a million years), but also the role of intense cladogenesis in the late part of the Permian (although this was counteracted by numerous ‘background’ extinctions).

Keywords: Temnospondyli; supertree; diversification; Stereospondyli; Permian; Triassic

1. INTRODUCTION

The origin and early radiation of limbed vertebrates (or tetrapods) are the focus of much renewed interest and interdisciplinary research at the interface between evolutionary and developmental biology. Outstanding palaeontological discoveries continue to refine the complex picture of character acquisition and transformation that accompanied vertebrate terrestrialization. At the same time, phylogeny-based macroevolutionary studies are beginning to decipher quantitative and qualitative aspects of early tetrapod diversification (e.g. Laurin 2004; Ruta et al. 2006; Wagner et al. 2006; Marjanovic & Laurin 2007). In the present work, we examine for the first time models of cladogenesis (i.e. branch subdivision) in Temnospondyli, the largest group of primitive tetrapods. Our focus is the identification of portions of the temnospondyl phylogeny that underwent significant changes in lineage splitting. This is achieved through analyses of the degree of asymmetry (i.e. imbalance), count of taxa and overall shape of the two branches that descend from each internal node in the phylogeny. Throughout, we employ the term ‘branching’ in the sense of multiplication of lineages. In this context, ‘branching’ is more appropriate than ‘diversification’, as our analysis revolves exclusively around tree topology and is not concerned with measures of morphological character change or with the tempo and mode of evolutionary transformations (e.g. Simpson 1944).

Comprehensive temnospondyl phylogenies are still unavailable. Compilation of an exhaustive data matrix for all described species is unrealistic at present. However, several species-level trees have been published for nearly all families or superfamilies of temnospondyls. Thus, given a set of partially overlapping input trees, supertree techniques can be used to combine these trees and generate a synthetic ‘consensus’ phylogeny (e.g. Pisani & Wilkinson 2002; Wilkinson et al. 2005a; Pisani et al. 2007). Here, we present various supertrees for temnospondyls built using different methods and employ them for an analysis of cladogenetic patterns. Temnospondyls are particularly amenable to this kind of analysis and to macroevolutionary studies in general, given their sheer diversity and their broad stratigraphical and geographical distributions, with records from all continents and spanning approximately 210 Myr (Early Carboniferous–late Early Cretaceous; Milner 1990).

Temnospondyls are of great zoological significance because they are implicated in the current debate on amphibian origins (e.g. Ruta & Coates 2007), although this is disputed by a number of researchers (e.g. Vallin & Laurin 2004). Regardless of their systematic affinities, temnospondyls present an interesting case study for elucidating macroevolutionary patterns in species-rich fossil groups.

We address two main questions: (i) which portions of temnospondyl phylogeny underwent a significant branching shift (i.e. a significant change in lineage
multiplication)? and (ii) what was the distribution of shifts immediately before and after the end-Permian extinction? The end-Permian extinction (Benton 2003; Benton et al. 2004; Erwin 2006) had by far the most dramatic impact on terrestrial and marine life of all known mass extinctions and continues to play an unrivalled role in our understanding of the consequences that large-scale biological crises have for faunal turnovers, ecological remodelling and adaptive radiations.

2. MATERIAL AND METHODS

(a) Input trees
Temnospondyl phylogenies (see appendix 1a in the electronic supplementary material) were chosen according to several criteria (Ruta et al. 2003; Bininda-Emonds et al. 2004). First, current knowledge of the large-scale interrelationships of temnospondyls relies extensively on Milner’s (1990) over-archi family-level study, which was therefore selected as the starting point for our survey. Second, we included trees with four or more temnospondyl taxa (either species or supraspecific units). Third, if an original tree included different tetrapod groups, then only its temnospondyl portion was used as an input tree. Fourth, if several trees were obtained from an original analysis, we used either their strict consensus (if presented) or the tree selected by the original author(s) (in the absence of a consensus). Last manually generated trees were considered only if accompanied by apomorphy lists. Original data were not reanalysed (for supraspecific taxon replacement, see ‘a’ in the electronic supplementary material).

(b) Supertree analyses
Input trees were built using MacClade 4 (Maddison & Maddison 2005), Baum & Ragan’s (1993) and Purvis’ (1995) codings were employed to generate matrix representations of these trees and to build standard and Purvis MRP supertrees, respectively. In addition, we used the Baum and Ragan’s matrix to generate a split-fit supertree (SFT; Wilkinson et al. 2005b). Finally, we built a most similar supertree (MSS; Creevey et al. 2004) and a combined consensus of the standard plus Purvis MRP supertrees (for details of methods, see ‘b’ in the electronic supplementary material). All supertrees represent Majority Rule consensus topologies including minority components (see appendix 1b–f in the electronic supplementary material).

For the MRP analyses, we carried out two independent searches, using Quicke et al.’s (2001) strategy for estimating large phylogenies and Nixon’s (1999) parsimony ratchet (implemented in PERL-RAT-PL v. 1.0.9a; Bininda-Emonds 2004). Both of these methods produced trees of identical minimal length. A maximum of 100 000 trees were retained owing to computer memory limitations. A standard plus Purvis MRP supertree was built from 200 000 trees (i.e. using 100 000 trees from each of the standard and Purvis MRP analyses).

Methodological artefacts are common in phylogenetic reconstruction (e.g. Delsuc et al. 2005). As the standard and Purvis MRP methods show different properties and opposite shape-related biases (e.g. Wilkinson et al. 2005b), we conjecture that nodes present in the consensus of the combined standard plus Purvis MRP are less likely to be artefactual. Therefore, we regard this consensus (see appendix 1f and g in the electronic supplementary material) as the most conservative estimate of temnospondyl phylogeny, given available data, although it is obviously less resolved than both the standard and the Purvis MRP supertrees.

PAUP* v. 4.0b10 (Swofford 1998) was used to run all the parsimony analyses and to calculate consensus topologies. We employed the following programs: MIX (Felsenstein 2000) for the SPTT analysis (1000 random additions; threshold parsimony set to 1.8); CLANN (Creevey & McInerney 2005) for the MSS analysis (default settings); and MsA (http://www.agapow.net) to calculate Colless’ imbalance index (Colless 1995) for the standard MRP supertree.

Node support, shown here only for standard MRP (figure 1) and for the consensus of standard plus Purvis MRP (see appendix 1g in the electronic supplementary material), is expressed using Wilkinson et al.’s (2005a) V1 index. Values of this index range from +1 to −1 and show different degrees of support between supertree and input trees, i.e. from complete support to total conflict. Our dataset is not amenable to input tree bootstrapping (Creevey et al. 2004; see also Burleigh et al. 2006) due to sparse taxon sampling (Moore et al. 2006).

Wilkinson et al. (2005b) have shown that biases occur in most supertree methods, and within the limits of feasibility, method dependence of results should be assessed. No approach exists for ranking a set of supertrees based upon their fit to the input trees (as in the standard Shimodaira’s (2002) approximately unbiased test). Therefore, we employed a tree-based randomization test (see Rodrigo et al. 1993) to evaluate the degree of similarity of our supertrees (see appendix 1b–f in the electronic supplementary material; figure 1) relative to a set of random trees of identical size (i.e. having the same number of taxa). In PAUP*, we calculated pairwise tree-to-tree distances among our supertrees using Robinson & Foulds’ (1981) symmetric difference. For two trees, the symmetric difference is the number of splits present on one tree but not on the other (a split is the partition of a set of taxa into two non-empty subsets; Thorley & Page 2000). We thus generated 1000 random trees of the same size as our supertrees (173 taxa) and calculated tree-to-tree distances for each pair of random trees. Finally, we plotted the distribution of these distances and tested whether our supertrees are more similar to one another than are 99% of the random trees. In addition, we used the Templeton (1983) test to assess whether topological differences among our supertrees are significant when evaluated against the standard MRP matrix. The application of this test is not dissimilar from that in character-based analyses. As used here, the test evaluates statistically significant differences in supertree topologies against a matrix in which ‘characters’ represent nodes in the input trees rather than biological traits.

(c) Diversification analyses
Chan & Moore (2002, 2005) and Moore et al. (2004) devised a tree topology-dependent method for detecting diversification rate shifts (i.e. significant changes in lineage branching, based upon differences in the number of taxa and degree of imbalance on the left and right branches subtended by tree nodes) and for calculating whole-tree tests of rate variations. This method is implemented in SYMMETREE v. 1 (http://www.phylodiversity.net/bmoore/software_symmetree.html). Briefly, in the triplet (A(BC)), the probability that a shift occurred along the internal branch that subtends BC is calculated through a statistic termed $D_1$. This is expressed as
Figure 1. Standard MRP majority rule consensus supertree with minority components; V1 support values (Wilkinson et al. 2005a) are reported for each node.
Figure 2. (a) Hypothetical tree consisting of taxa a–l plotted on a time scale with stages 1 and 2, illustrating the time-slicing approach (see text). (b,c) Pruned trees related to stages 1 and 2. The internal branches and most inclusive (more basal) nodes of the tree have been drawn mostly outside the two stages in which taxa a–l occur to avoid line crowding. In fact, the age of node 'x' subtending taxa a–l should be placed correctly in stage 1; as additional examples, the node subtending taxa c–h belongs to stage 1 while the node subtending taxa i–l belongs to stage 2.

...a function of two likelihood ratios that compare the probabilities of observed differences in diversity at the internal and basal node of the triplet under a homogeneous (equal rate model, ERM) and a heterogeneous (unequal branching rates) diversification (for SYMMETREE search settings, see appendix 1 in the electronic supplementary material). A rate shift (if present) refers to the more recent taxa (or groups of taxa) to have a ghost lineage extension. The age of the internal nodes in the stage-based pruned topologies. We note that in...
We calculated the mean of all the absolute $D_1$ values (output in SYMMETREE) associated with internal nodes of the same age in a pruned topology; significant differences (if any) in $|D_1|$ means (one value of $|D_1|$ mean for each internal node age in a pruned topology) were assessed through a one-way ANOVA. This method is similar to that used by Jones et al. (2005) for supertrees that include extant terminals, and in which internal nodes are dated using either fossils or molecular clocks. We confine our discussion to pruned topologies that have direct relevance to the Permian extinction and pattern of post-extinction recovery (figure 4b–f).

3. RESULTS

(a) Supertree shape and balance
The standard MRP supertree (figure 1), used only as a ‘guide tree’ for comparing distributions of branching shifts in various time intervals, is 620 steps long with 171 internal nodes and a Colless’ imbalance index of 0.168369. A Templeton test shows that the various supertrees (see appendix 1b–g in the electronic supplementary material) differ significantly from each other ($p<0.0001$) but are more similar to one another than a set of randomly generated trees of identical size ($p<0.01$). Note that the length of the supertree has the same meaning as in character-based analyses, i.e. the total number of character-state changes, where a change refers to a transition between node absence and node presence in the input trees. Node support for the standard MRP supertree is higher than that for other supertrees, except the combined standard plus Purvis MRP supertree.

Appendix 1k in the electronic supplementary material shows nodes common to all supertrees. Using the standard MRP as a reference, we identified each of its nodes with a pair of taxa bracketing the group subtended by the node of interest. Nodes that consist of the same taxa with the same mutual relationships as in the standard MRP are marked with tick marks. The topology of the standard MRP conforms largely to Milner’s (1990) arrangement of major temnospondyl groups but contradicts Yates & Warren’s (2000) alternative hypothesis of large-scale interrelationships. Three monophyletic groups of mostly Carboniferous and/or Permian species occur in order of increasing distance from the supertree root: Edopoidea, Dvinosauria and an assemblage of Parioxys, Eryops and Dissorophoidea. Two remaining groups complete the picture of temnospondyl diversity, namely the basal Archegosauriformes and the Stereospondyli. The latter are the most species rich of all temnospondyls and represent a predominantly Mesozoic (especially Lower and Middle Triassic) radiation. The stereospondyls consist of Rhinesuchidae, Rhytidostea, Capitosauroidae and Trematosauroidae (figure 1). For an overview of temnospondyls, the reader is referred to Milner (1990) and Schoch & Milner (2000).
Figure 4. Pruned, stage-related topologies deriving from the standard MRP supertree: (a) Sakmarian, (b) Kazanian, (c) Tatarian, (d) Induan, (e) Olenekian and (f) Anisian. Stage names refer to the age of the terminal units plus ghost lineage extensions of younger taxa, such as are inferred from the supertree. Filled and open circles indicate significant and informative shifts, respectively. Asterisks mark branches along which a significant or informative change in lineage splitting takes place.
(b) Temporal distribution of branching shifts

The temporal distribution of branching shifts is discussed with reference to stage-related pruned topologies derived from the complete standard MRP supertree (figures 3 and 4; see also appendix 1 in the electronic supplementary material). However, it is also useful to consider the age of the branch along which a particular shift takes place, as that branch may be older than the stage being examined. This is important for an assessment of differences in the temporal distribution of $|A_1|$ values (see above). It is likewise useful to remember that if a node has undergone a rate shift (i.e. a significant change in lineage splitting), the shift itself is related to the more diverse of the two branches subtended by that node (Chan & Moore 2002, 2005). For instance, in figure 4d, the filled circle identifies a node with a significant shift, subtending all taxa comprised between Broomistega and Batrachosuchus browni. The shift proper has thus occurred along the branch (marked with an asterisk) including all taxa comprised between Odenwaldia and B. browni. The branch in question (or, more conveniently, the node at the end of the branch that is placed further away from the root of the tree) is Kazanian in age, as is the node marked by the open circle. In this example, terminal taxa (including range extensions for younger taxa) are Induan in age.

In this section, all branching shifts in each pruned topology are presented with reference to the stratigraphical stages in which terminal taxa occur in that topology (figures 3 and 4). For instance, the Induan pruned tree (figure 4d) shows Induan taxa as well as more recent taxa with a range extended back into that stage. In §3c, shifts are discussed with reference to the age of the branches on which they take place. Asterisks (figures 3 and 4) mark such branches.

SYMME TREE located the following nine shifts: one in the Carboniferous (Moscovian); four in the Permian (Aossilan, Sakmarian, Kazanian and Tatarian); and four in the Triassic (two Induan, one Olenekian and one Anisian). In the Moscovian (figure 3a), a significant shift occurs along the branch subtending dissorophoids (Stegeopterus to Doleserpeton). In the Asselian (figure 3b), an informative shift marks the group bracketed by Lysipterygium and Schoenfelderpeton. A significant shift in the Sakmarian (figure 4a) subsumes basal archeosauriforms (Lysipterygium to Collidosuchus, but including also the early stereospondyl Rhinesuchoides) as well as a group consisting of cryopoids, paraxooids and dissorophoids (Paroxyx to Eimerisaurus). The Kazanian is marked by a significant shift (figure 4b) along a branch that leads to an assemblage of archeosauriforms (Konzhukovia to Prionosuchus) and of pelotobatrachids plus basal stereospondyls (Pelotobatrachus to Uranocentrodon). In the Tatarian, an informative shift (figure 4c) is observed near the base of the stereospondyls (Rhineasuchus to Trachosaurus). In the Induan (figure 4d), a significant shift marks the radiation of stereospondyls other than rhinesuchids (Odenwaldia to Batrachosuchus browni). In this radiation, two sister groups are recognized, i.e. rhystidosaurae (Eobydshkerina to B. browni) and capitosaurians (Odenwaldia to Wassertosaurus). An informative shift within rhystidosaurae occurs along the branch that subtends all taxa between Pneumatostega and B. browni. The last two shifts to be considered, in the Olenekian (figure 4e) and in the Anisian (figure 4f), underpin two radiations within capitosaurid stereospondyls (Cherninia to Stancephalosaurus birdi and Paracyclotosaurus to Mazidosaurus, respectively).

c) Analysis of variance in the temporal distribution of shifts

The pruned topologies that have yielded shifts can now be looked at, in a more general way, as a frame of reference for investigating temporal variations in lineage splitting. For this purpose, we consider the differences in the $|A_1|$ means for each topology. We assess the overall significance of these differences through a one-way ANOVA and seek to pinpoint significant differences in pairwise comparisons of sampled $|A_1|$ values through a post hoc J. W. Tukey’s (1953, unpublished manuscript) honestly significant differences (HSD) test. Where possible, we have lumped together samples of $|A_1|$ values for different stages, especially when only one or very few nodes in a pruned tree belong to a certain stage (as is generally the case for the most inclusive tree nodes).

For the Kazanian pruned tree (figure 4b), we have 15 Kazanian, 1 Sakmarian, 2 Asselian and 4 Moscovian nodes. Differences among the means of $|A_1|$ values, related to Kazanian, Sakmarian plus Asselian, and Moscovian, are marginally non-significant ($F = 3.4209; p = 0.051758$).

For the Tatarian pruned tree (figure 4c), there are 9 Tatarian, 6 Kazanian, 1 Asselian and 5 Moscovian nodes. Differences among means of $|A_1|$ values for Tatarian, Kazanian and Asselian plus earlier stages are significant ($F = 3.9422; p = 0.038029$), although a post hoc HSD Tukey’s test does not highlight significant differences in any of the pairwise comparisons for the selected time intervals.

For the Induan pruned tree (figure 4d), ages of nodes are distributed as follows: 47 Induan, 6 Tatarian, 4 Kazanian, 1 Moscovian and 1 Viséan. Differences among means of $|A_1|$ values relate to Induan, Tatarian and Kazanian plus earlier stages and appear to be significant ($F = 17.1205; p < 0.0001$). A post hoc HSD Tukey’s test shows that significant differences between means of $|A_1|$ values concern comparisons between Induan and Tatarian and between Induan and Kazanian plus earlier stages ($p < 0.01$). More specifically, the mean $|A_1|$ value in the Induan is significantly lower than those associated with the other time intervals.

For the Olenekian pruned tree (figure 4e), node ages are distributed as follows: 18 Olenekian, 19 Induan, 2 Tatarian and 1 Kazanian. There are significant differences among the means of $|A_1|$ values for Olenekian, Induan and Tatarian plus earlier stages ($F = 3.9976; p = 0.026567$), and a post hoc test shows that the significant difference relates to the comparison between Olenekian and Tatarian plus earlier stages ($p < 0.05$). In the Olenekian, the mean $|A_1|$ value is significantly lower than in the Tatarian plus earlier stages.

For the Anisian (figure 4f), node ages are distributed as follows: 13 Anisian, 2 Olenekian, 8 Induan and 3 Kazanian. Differences among means of $|A_1|$ values for Anisian, Olenekian and Induan plus earlier stages are not significant ($F = 3.0898; p = 0.064783$).

4. DISCUSSION

(a) Branching shifts and secular diversification trends in temnospondyls

In the majority of pruned trees with Palaeozoic species (figures 3b and 4a–c), shifts take place along branches that connect major groups, thus marking the separation between distinct radiations. For example, the shift in figure 3b, dating back to the Moscovian, underpins the
radiation of all post-edopoid and post-dinosaur temnospondyls. The shift in figure 4a marks approximately the same radiation although the latter shows only a partial taxonomic overlap with that in figure 3b. Taken together, these shifts testify to the rapid diversification of some of the most diverse among all groups of Permo-Carboniferous temnospondyls. The shifts in figures 4b,c are observed near the base of stereospondyls. In these two figures, either a separation of basal archegosauriforms from basal stereospondyls or a split between basal and ‘higher’ stereospondyls (sensu Schoch & Milner 2000; see also Yates & Warren 2000) is highlighted.

The pattern of ‘between-groups’ shift distribution that characterizes most Palaeozoic pruned trees contrasts with a ‘within-group’ shift distribution in one Palaeozoic (figure 3a) and all of the Triassic (figure 4d–f) pruned trees. The Palaeozoic shift occurs within a subset of dissorophoids, whereas shifts in figure 4d–f underpin lineage-splitting events within assorted stereospondyls, in particular rhizodonts and capitosaurids.

(b) Temnospondyls and the end-Permian extinction

The chronology of the four shifts in figure 4d–f (highlighted by asterisks) is interesting. Two of these shifts (figure 4e,f) took place in the Induan, one (figure 4d, open circle plus asterisk) in the Tatarian and one (figure 4d, filled circle plus asterisk) in the Kazanian. Together, the four shifts bracket narrowly the end-Permian extinction: in particular, the Induan shifts took place about half a million years after the extinction. This suggests that temnospondyls underwent a relatively rapid post-extinction recovery with the appearance of several new lineages shortly after their catastrophic decline (in terms of observed diversity values). However, the Late Permian shifts also testify to an increase in lineage splitting, which took place over a period of approximately 18 Myr before the extinction (an additional Kazanian shift is highlighted in figure 4e).

The pattern of temnospondyl diversification near the end of the Palaeozoic thus appears to be complicated by an interplay between multiple ‘background’ extinctions in the late Permian and a series of interspersed cladogenetic events. Milner (1990) noted that the Upper Permian record of temnospondyls includes late range extensions of Lower Permian groups, rather than early range extensions of Lower Triassic groups. This observation led Milner (1990) to argue in favour of an extinction-driven pattern of diversification and against a gradual decline in diversity throughout the Permian. A gradual decline would also imply a slow emergence of Triassic lineages, a model that is not supported by the explosive nature of the stereospondyl radiation and by the location of branching shifts in the Induan.

However, we note that diversification shifts occurred also on branches preceding the Permian extinction, as early as three million years before the extinction event proper. These finds may not be reconciled with Milner’s (1990, p. 335) hypothesis of a ‘… cessation of appearance of families’ in the Upper Permian, for which Milner (1990) invoked taphonomic artefacts. In this scenario, extinction was responsible for the decimation of several Upper Permian temnospondyl groups in Laurasia. However, in Gondwana, some other groups survived into the Triassic. The distribution of shifts in relation to the geographical occurrences of taxa is noteworthy in this respect. Thus, several basal members that belong to groups underpinned by shifts in figure 4c,d are found in South Africa. Furthermore, most of the taxa in the bottom half of the tree in figure 4d occur in Gondwana.

The biogeographic implications associated with the distribution of shifts are that, although a large number of temnospondyls went extinct across Laurasia before the end-Palaeozoic, a series of speciation bursts occurred in rapid succession in the late Permian, which led to the appearance of several new families in the Southern Hemisphere. Dispersal probably involved spread into the southeastern portions of Gondwana and subsequent colonization of Laurasia. Additional work is needed to correlate shifts with profiles of observed and inferred (i.e. corrected with ghost lineage extensions) diversities for the group as a whole across the Permo-Triassic boundary. Research in progress suggests (although results are still preliminary) that the ‘burst’ of diversification in the aftermath of the end-Permian extinction is significantly mitigated by inclusion of ghost lineages. Furthermore, pre- and post-extinction shifts imply the occurrence of ‘pulses’ of diversification with a distinct geographical ‘fingerprint’. It will therefore be interesting to explore the impact of ancestral area distributions and dispersal routes on profiles of diversity across the Palaeozoic–Mesozoic transition.

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