Are Turtles Diapsid Reptiles?

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BIOCOMP 2013

Abstract

It has been argued that, based on a neighbor-joining analysis of a broad set of fossil reptile morphological data that turtles are diapsid reptiles. A Bayesian phylogenetic analysis does not sustain this view.

Keywords: Turtles, diapsid, neighbor-joining, Bayesian phylogenetic

1.0 Introduction

The traditional classification of reptiles is based on a single key character, the presence and style of fenestration in the temporal region of the skull. Snakes, lizards, crocodiles, dinosaurs and others are 'diapsids': they have (at least in a rudimentary form) two holes in the temporal region. Reptiles in which the skull is completely roofed, with no temporal fenestration, are called 'anapsids'. These include many Palaeozoic forms such as captorhinomorphs, procolophonids and pareiasaurs, but also include Testudines (turtles and tortoises). Consistent with this assumption, recent analyses of the affinities of Testudines have included Palaeozoic taxa only, placing them as akin to captorhinomorphs or procolophonids or nested within pareiasaurs. [4], in contrast, maintains turtles are diapsid reptiles, based on a neighbor-joining (NJ) assessment ([2]) of fossil reptile morphological data.

2.0 Method

The taxon descriptors in [5] were reformatted under Microsoft *Notebook* to be compatible with the variable coding requirements of [1]. The resulting descriptors were then analyzed under a Bayesian phylogenetic ([2]) software package (*MRBAYES*, [1]; see Figure 1). The software was run on a Dell Inspiron 545 with an Intel Core2 Quad CPU Q8200 clocked at 2.33 GHz, with 8.00 GB RAM, under *Windows Vista Home Premium/SP2.*

```
begin data;
     dimensions ntax=33 nchar=168;
     format datatype=Standard gap=- missing=? interleave=Yes;

     matrix

Seymouriadae       0000000000000000000001000000000000000000000000000000000000
Diadectomorpha     0000000000000000000001{01}00{01}00000{01}0000000000000000000101{01}000
Caseidae           0000000000100000000000010000?00100001000000000002010010001
Ophiacodontidae    00000000010000000010000{01}1000?00101001000000100001021110001
Edaphosauridae     0000?0000000010000{01}?00010000?10{01}11001000020100001021111001
Sphenacodontidae   00001000011001001010001{01}0000?10101001000020100001021111001
Gorganopsia        00001001011001001?1?00111000?20001001200120100001021211001
Cynodontia         0000110201100100210001000000?20001001200120?01212021211001
Captorhinidae      0000000000000100000000000000?1000001100010000000{02}001112010
Paleothyris        00000000000000000?0000000000?10000011000100000000000110010
Millerettidae      ?0000000000010000?010000{01}000?100000010{01}00000000000{01}0010010
Acleistorhinus     00000100001010011?0?00110000?01100001000110000002011010010
Lanthanosuchidae   000001000011100{01}1?1?00110100?00100001000011???00{02}011010010
Macroleter         00000100001010011?0?00001001?00000001100000???002010012010
Bradysaurus        0000010100101101000101000100?00000001000001001002001012110
Scutosaurus        0000010100111101000101000100?00000001000001001002001012110
Anthodon           0000010100101101000101000100?00000001000001001002001012110
Procolophon        0000010000101100100110001001?0000010110001{01}011002002012110
Owenetta           00000100001011001?0?10001001?00000111100010???00200012110
Araeoscelidia      00000000000001000?1000001000010000011000100000000110110010
Claudiosaurus      0000000000?001101?1?00?010000111000110001200??01013?112010
Younginiformes     000000000000000010100000100011110001111010001101011011{02}010
Kuehneosauridae    000?0110001001101?0?00001010??01002??1111300??012132212010
Testudines
001{01}0101001000{01}01{01}{02}10{01}0001{01}0?10000111110010011003002{02}12110
Rhynchocephalia
0000000000100010210100{01}0{01}01011111001111{01}120011{01}1{02}1{23}2{12}12110
Squamata
000{01}0000{012}0{01}000{01}021{012}10{01}{01}0001011{01}100210111130011{01}1{023}132{12}
12{01}10
Choristodera       1001011?11000100112?000000000?01000??110?{23}0011113112212?10
Rhynchosauria      11110111001000{01}01?01000000{01}0011110110110120011213112212110
Prolacertiformes
1001010001{01}00{01}{01}01?100010{01}0100110{01}02101101{23}001101{23}132{12}12010
Trilophosaurus     1?11000?00?00?????1?011000100?1??0???110?000?1213102212110
Archisauriformes
100{01}010001100{01}{01}01{01}{01}00000{01}01001110110110120011{01}1{03}112{12}12010
Placodus           1000000110100100212101001100111000111110?300??012132212010
Eosauropterygia
100{01}000{01}0010?0{01}021{01}?0000{01}0{01}0111100010{01}10?{23}00??{01}1{12}132212010

Seymouriadae       0000000000000000000000000000010000000000000000000000000000
Diadectomorpha     0000000010000000000000000{01}00000000{01}00000000000000000000000
Caseidae           1000000000000000000100000000000100100000000000010000000000
Ophiacodontidae    1000?000??000000000100000000000101100100000000010000000000
Edaphosauridae     100110210?000000?10110100000000111101000000010010010000000
Sphenacodontidae   1001102110000000010100000000000101100100000010010010000000
Gorganopsia        10111121?1012110120200002100000101100100100010011011100011
Cynodontia         101111211101211012020002210102210110010{01}101011011011101111
Captorhinidae      20000000000000010001002001000000001000000000000000000{01}000
Paleothyris        2001?000??0??00?0101000001?0000000100000000010010000000000
Millerettidae      2000?000?00??0010001000201000000001100000000?0001000?0?000?
Acleistorhinus     ?201?000??0??10000010002?1?10?00101000????????????????????
Lanthanosuchidae   12000000??0??1000101000{01}11??01?0?11010??000????200?????01?
Macroleter         20?0?00???0??00?0?01000211?10?001110000000000002001?0?0010
Bradysaurus        2010100101000112111111020111000011101010000?00020011110010
Scutosaurus        2010110101000112111111020111000011101011000?00020011111010
Anthodon           201011010100011211111102011100001110101?000?00020011111010
Procolophon        200{01}00110010001111120002111111001110100{01}000010020011001010
```

```
Owenetta          2000?010??1??0011112000211111001110{01}00?000?0002001?0??01?
Araeoscelidia     2001?000??0??0010011000001000100001000000000100100000000001
Claudiosaurus     200??100??0??0010011100001?101000010100 0?001101100001 0111?
Younginiformes    2001?000??00?0010001100011??0?00?110100000011001 0001?0{01}1{01}1
Kuehneosauridae   20?1?01???0??001???0?0?001?0??10?1101000100?11?1110?10????
Testudines
201011111101{01}{01}0211{01}2113{02}011111101{01}1??11{01}{02}011{01}0?1001111010
Rhynchocephalia   2{01}01?0111{01}0100011102100011010110111110 00{01}01?1001{01}001101121
Squamata
200{01}0{01}111101{01}0011{01}12010{012}11{01}{01}1110{01}11110001{02}{01}1100110011{01}1
{01}21
Choristodera      210110011?01??0111010101010?01?011101000110110111001 10111?
Rhynchosauria     211010010?01?00210{01}2100011111010111210001001100100011 0111?
Prolacertiformes
21011{01}10??01?001000{12}1000{01}1?10100111010001{02}0?1{01}11{01}001101101
Trilophosaurus    211?10111101?00110121102111 00??01112100?1{12}011011000?10111?
Archisauriformes
2{01}1111?111011{01}01110{12}100{012}{01}1{01}10100?11010001{012}0?1{01}11{01}101101111
Placodus          2001?1101101?120120201011111001011101000000?1111111?1?1110
Eosauropterygia
2{01}01???11101?1201{01}?2110{01}{01}10?0110?110{01}0?01{01}1?11111010101{01}{01}0

Seymouriadae      0000000000000000000000000000000000000000000000000?00
Diadectomorpha    0000000000000000000000000000000000000000000000000?00
Caseidae          1000000000000000000000000000000100000000000000000?00
Ophiacodontidae   {01}000000000000000000000000000000100000000000000000?00
Edaphosauridae    10000000000000??0000000000000001?00????0?0???0??0?00
Sphenacodontidae  1000000000000000000000000000000100000000000000000?00
Gorganopsia       1000000001200010100011012101011122101000010020000?0?
Cynodontia        1200?01002200111101011112111011121101010011021100?01
Captorhinidae     0001000002000000000000000000000100000000010000000?00
Paleothyris       0001000101000000000000000000000100000000010001100?00
Millerettidae     00?00?01?1?00?0?0100000000??00?100001000?10?00100?00
Acleistorhinus    ????????????????????????????????????????????????0???
Lanthanosuchidae  0?????0?????????????????????????????????????????0?0?
Macroleter        10?1000001000000010001000001?00100001000010001000?0?
Bradysaurus       11?10000?2200100011011000001000110001000021020011001
Scutosaurus       11?10000?2200100011011001001000110001000021020011211
Anthodon          21?10010?2200100010011001001000110001000021020012111
Procolophon       10?100{01}00210010001001100000100011100100002011001{01}000
Owenetta          ???10000?10101??0100?0000???0?01?0?????0?????1??0?00
Araeoscelidia     000100010{12}00000000000000000001001000000000110101100?00
Claudiosaurus     0010010112100101000010 1?011100010000000001010 0100?00
Younginiformes    ?010000011{02}0{01}1010100101?010110010000010011010 1100?00
Kuehneosauridae   0010000101012?111?00101?0101???10??????0?10101?00?01
Testudines        22?01{01}1111{02}1011{01}11000101101111111110{01}11100211200{01}201?
Rhynchocephalia   00100001{01}12000111000101{01}01010111110{01}1111120101100?00
Squamata          0010001111{02}10{01}111000101{01}0101{01}11111011111{01}20101100?01
Choristodera      ?010000011{02}121110?00101001111??10?101110?10?01100?00
Rhynchosauria     0010{01}00011010110000110110101111120101110110101110?00
Prolacertiformes  001000001201{12}111{01}001101?0101100120{01}{01}11{01}0{01}10101100?00
Trilophosaurus    101000001201000000011011010110012010111011010 1100?00
Archisauriformes
{01}010{01}{01}01{01}111011100011010 3{12}{01}11011201{01}111001010 1101000
Placodus          00101111110121??1?00101001111?01000110000?01000020?0
Eosauropterygia
20101{01}1111{01}{01}{12}11?1?00101{01}0{01}11{01}1010001100002010 0000??0
;
end;
```

```
begin mrbayes;
   log start filename=turtle_vardata_log.log replace;
   set autoclose=yes;
   mcmcp nruns=2 ngen=8000000 printfreq=100
       samplefreq=100 nchains=4 savebrlens=yes
       filename=turtle_vardata;
   mcmc;
   plot filename=turtle_vardata.run1.p;
```

```
   plot filename=turtle_vardata.run2.p;
   sumt filename=turtle_vardata burnin=10000 contype=halfcompat;
   log stop;
end;
```

Figure 1. Template of the *MRBAYES* script [1]) used in this study. The script creates 8000000 (*ngen*) Markov Chain ([6]) generations, (Monte Carlo, [7]) sampling every 100 (*samplefreq*) generations. The first 10000 (*burnin*) trees are discarded. Partial tree consensus (*contype*) is allowed. For definitions of other parameters used in this script, see [1]. A description of the character coding shown in the data matrix can be found in [5]

3.0 Results

Figure 2 is the tree generated by the script shown in Figure 1 running under [1]. The time to produce this tree was ~3 hours. Based on the system monitor, two of the four cores on the system performed 99% of the computational work. Total CPU utilization ranged from about 25% to 50%. The computation required approximately 1 GB memory.

```
/-Seymouriadae (1)
|
|- Diadectomorpha (2)
|
|    /- Caseidae (3)
|    |
| /--+   / Ophiacodontidae (4)
| |  |   |
| |  \---+   /--- Edaphosauridae (5)
| |      |   |
| |      \---+ / Sphenacodontidae (6)
+ |          | |
| |          \-+                  / Gorganopsia (7)
| |            \------------------+
| |                               \------------- Cynodontia (8)
| |
| |    /-- Captorhinidae (9)
| |    |
| |    |/- Paleothyris (10)
| |  /-+|
| |  | ||   /- Araeoscelidia (20)
\-+  | \+   |
  |  |  |   |         /------ Claudiosaurus (21)
  |  |  \---+         |
  |  |      |         |   /- Younginiformes (22)
  |  |      |         |   |
  |  |      \---------+   |              /------ Kuehneosauridae (23)
  |  |                |   |              |
  |  |                |   |              |     /-- Rhynchocephalia (25)
  |  |                |   |              |-----+
  |  |                \---+         /----+     \-- Squamata (26)
  |  |                    |         |    |
  |  |                    |         |    |        /-------- Placodus (32)
  |  |                    |         |    \--------+
  |  |                    |         |             \- Eosauropterygia (33)
  \--+                    \---------+
     |                              |  /------- Choristodera (27)
     |                              |  |
     |                              |  |         /----- Rhynchosauria (28)
     |                              |  |   /----+
     |                              \--+   |    \------- Trilophosaurus (30)
     |                                 | /-+
     |                                 | | \---- Archisauriform~ (31)
```

```
     |                                 \-+
     |                                   \---- Prolacertiform~ (29)
     |
     |  /- Millerettidae (11)
     |  |
     |  |             /--- Acleistorhinus (12)
     |  |      /------+
     \--+      |      \----- Lanthanosuchid~ (13)
        |      |
        |      |  / Macroleter (14)
        \------+  |
               |  |                                / Bradysaurus (15)
               |  |                                |
               |  |                   /------------+- Scutosaurus (16)
               \--+                   |            |
                  |      /------------+            \- Anthodon (17)
                  |      |            |
                  |      |            \------------------------- Testudines (24)
                  \------+
                         |   /--- Procolophon (18)
                         \---+
                             \-- Owenetta (19)

|---------| 0.200 expected changes per site

Calculating tree probabilities...

Credible sets of trees (14565 trees sampled):
   50 % credible set contains 128 trees
   90 % credible set contains 4171 trees
   95 % credible set contains 8565 trees
   99 % credible set contains 13365 trees
```

Figure 2. Phylogenetic tree produced by the script shown in Figure 1. Some taxon names are truncated; full names can be found in [5].

4.0 Conclusions and discussion

The results in Section 3.0 motivate several observations:

1. Figure 2 shows affinities between Testudines and several of the parareptiles (Procolophonidae, Bradysaurus, Anthodon, and Scutosaurus), as originally claimed in [1]. However, the analysis does not sustain the claim in [1] that Testudines is a sister group of the Sauropterygia (e.g., the pleisiosaurs, nothosaurs, and pachypleurosaurs).

2. The morphology of turtles derives characteristics from diverse reptilian groups that crosscut the diapsid/anapsid distinction. This is evidence that the diapsid/anapsid distinction is of limited taxonomic utility.

3. The results of this study generally agree with those of [3], which is based on Maximum Parsimony.

4. It is not unusual for different phylogenetic methods to produce somewhat different results when applied to the same data set. Except on data sets containing no more than a few tens of taxa, today's practical phylogenetic algorithms must use some approximations and heuristics in order to execute in tolerable time. The NJ algorithm used in [5] for example, restricts its combinatorial tree searches to a relatively localized region of tree space; the Bayesian algorithm used in the present study samples less than the full population of generations produced. Bayesian methods have the distinct theoretical advantage, however, that if the sample selected is large enough, the Central Limit Theorem ([8], Chap. 7) guarantees the solution based on the sample

will converge to the population distribution of trees; NJ cannot be guaranteed to satisfy this criterion.

5.0 Acknowledgements

This work benefited from discussions with Tony Pawlicki, with Town Peterson and Kris Krishtalka of the University of Kansas Biodiversity Institute, and with Joan Hunt of the University of Kansas Medical Center. For any problems that remain, I am solely responsible.

6.0 References

[1] Ronquist F and Huelsenbeck JP. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (2003), 1572-1574. Software is available at
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[2] Felsenstein J. *Inferring Phylogenies.* Sinauer Associates. 2004.

[3] Lee MSY. The origin of the turtle body plan: bridging a famous morphological gap. *Science* 261 (1993), 1716-1720.

[4] Rieppel O and deBraga M. Turtles as diapsid reptiles. *Nature*, Vol. 384 (5 December 1996), 453-455.

[5] O. Rieppel and M. deBraga. Supplementary information for [4] . This data was once available on the *Nature* web site, URL http://www.nature.com, but no longer appears to be. A copy to the morphological data, together with a description of the morphological characters (and associated references) can be obtained from me on request.

[6] Gilks WR, Richardson S, and Spiegelhalter DJ. *Markov Chain Monte Carlo in Practice.* Chapman and Hall. 1996.

[7] Liu JS. *Monte Carlo Strategies in Scientific Computing.* Springer. 2001.

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