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Bayesian Models of Episodic Evolution Support a Late Precambrian Explosive Diversification of the Metazoa

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Abstract

Multicellular animals, or Metazoa, appear in the fossil records between 575 and 509 million years ago (MYA). At odds with paleontological evidence, molecular estimates of basal metazoan divergences have been consistently older than 700 MYA. However, those date estimates were based on the molecular clock hypothesis, which is almost always violated. To relax this hypothesis, we have implemented a Bayesian approach to describe the change of evolutionary rate over time. Analysis of twenty-two genes from the nuclear and the mitochondrial genomes under the molecular clock assumption produced old date estimates, similar to those from previous studies. However, by allowing rates to vary in time and by taking small species sampling fractions into account, we obtained much younger estimates, broadly consistent with the fossil records. In particular, the date of protostome–deuterostome divergence was on average 582 ± 112 MYA. These results were found to be robust to specification of the model of rate change. The clock assumption thus had a dramatic effect on date estimation. However, our results appeared sensitive to the prior model of cladogenesis, although the oldest estimates (791 ± 246 MYA) were obtained under a sub-optimal model. Bayes posterior estimates of evolutionary rates indicated at least one major burst of molecular evolution at the end of the Precambrian when protostomes and deuterostomes diverged. We stress the importance of assumptions about rates on date estimation and suggest that the large discrepancies between the molecular and fossil dates of Metazoan divergences might partly be due to biases in molecular date estimation.

Introduction

The sudden appearance of numerous metazoan phyla in the early Cambrian (Knoll and Carroll 1999) suggests an explosive morphological diversification, but the timing of the divergence of the Metazoa is controversial (Valentine, Jablonski, and Erwin 1999). Paleontological evidence indicates this divergence occurred ~600 MYA (Valentine, Jablonski, and Erwin 1999; Conway Morris 2000), while molecular studies support much earlier dates (Wray, Levinton, and Shapiro 1996; Feng, Cho, and Doolittle 1997; Bromham et al. 1998; Wang, Kumar, and Hedges 1999). One hypothesis is that animals were small and unlikely to fossilize before the Cambrian explosion (Cooper and Fortey 1998), which is supported by the recent find of a crustacean in early Cambrian strata (511 MYA) (Siveter, Williams, and Waloszek 2001). However, in the absence of uncontroversial animal fossils older than the Ediacaran biota (Brasier 1998; Budd and Jensen 2000; Rasmussen et al. 2002), deep divergence dates may only be reasonably approached by indirect comparative studies.

Molecular date estimates vary widely among studies (Knoll and Carroll 1999), which indicates a possible problem when averaging results over genes evolving at different rates. However, all studies to date suggest that the basal divergence between protostomes and deuterostomes occurred more than 700 MYA (Bromham et al. 1998). This early-origin hypothesis is supported by the analysis of a large number of genes (Wray, Levinton, and Shapiro 1996; Feng, Cho, and Doolittle 1997; Gu 1998; Wang, Kumar, and Hedges 1999). However, these studies are all based on the molecular clock hypothesis (Zuckerkandl and Pauling 1965), with lineages violating the clock removed through sequential relative rate tests (Wu and Li 1985). The power of such tests was questioned (Bromham et al. 2000), and it has been suggested that violation of the clock could have drastic effects on date estimation (Ayala, Rzhetsky, and Ayala 1998; Bromham and Hendy 2000; Yoder and Yang 2000).

In this paper, we take a Bayes approach (Thorne, Kishino, and Painter 1998) to examine the effect of models of rate evolution on estimates of metazoan divergence dates. By analyzing eleven nuclear genes and eleven mitochondrial genes, we show that the Bayes analysis under the molecular clock gives date estimates comparable to those reported in previous molecular studies (Wray, Levinton, and Shapiro 1996; Feng, Cho, and Doolittle 1997; Gu 1998; Wang, Kumar, and Hedges 1999). However, when the clock assumption was relaxed, we obtained younger date estimates, largely consistent with the fossil records.

Materials and Methods

The Bayesian Framework

Divergence times (T) and evolutionary rates (R) are assigned prior distributions. Their joint posterior probability given data X is given by $p(R,T \mid X) = p(X \mid R,T)\, p(R \mid T)\, p(T)/p(X)$ (Thorne, Kishino, and Painter 1998). The tree topology is assumed known and fixed (Nielsen 1995; see supplementary information). The likelihood $p(X \mid R,T)$ was computed assuming the HKY85 + Γ nucleotide substitution model with eight rate classes (Hasegawa, Kishino, and Yano 1985; Yang 1994). The transition–transversion rate ratio κ and the among-site rate variation parameter α were integrated out over uniform priors U(0, 1000) and U(0, 100), respectively. Base frequencies were estimated using their empirical frequencies in the data and fixed in the analysis.

Prior models of rate change

Rate change is accommodated by a stochastic process in which the rate of a descendent branch follows a statistical distribution centered on the rate of the ancestral branch (Thorne, Kishino, and Painter 1998). The variance of the distribution is given by $s^2 = \sigma^2\Delta t$, where Δt is the time duration of the branch. Thus rates evolve in an autocorrelated manner from ancestor to descendent and branches far apart on the phylogeny are likely to have different rates. Parameter σ^2 determines how variable the rates are: a small σ^2 means a clock-like tree while a large σ^2 means highly variable rates (fig. 1). Here we explore two statistical distributions to model rate change over branches: the exponential distribution (EXP) and the Ornstein-Uhlenbeck process (OUP), which is a stationary Gaussian process (Aris-Brosou and Yang 2002). Because of the stationarity property, no systematic trend in the rate, either upward or downward, is assumed. EXP has no hyperparameters since the mean of the distribution is fixed at the rate of the ancestral branch. OUP has two hyperparameters: σ^2 that controls rate variation among branches and β that is a friction term. Both σ^2 and β were integrated out assuming vague prior distributions: a gamma distribution with mean 15 and variance 25 for σ^2 and a lognormal distribution with mean log(.5) and variance .75 for β. These values were chosen according to results from an Empirical Bayes study of the 18S rRNA gene (Aris-Brosou and Yang 2002), although optimal values may differ among genes.

We used the likelihood ratio test (LRT) and the posterior Bayes factor (PBF) to test the molecular clock assumption. In the LRT, twice the log likelihood difference between the clock and non-clock models is compared with a χ^2 distribution with $n - 2$ degrees of freedom for a tree of n species. PBF (Aitkin 1991) is the ratio of the likelihood L averaged over the posterior distribution under each model: $\mathrm{PBF}_{1,2} = E_{\theta \mid X}[L_1] / E_{\theta \mid X}[L_2]$. PBF was also used to compare models of rate change. This measures the weight of evidence in the sample in favor of model 1 against model 2, with values greater than 20, 100 and 1,000 meaning strong, very strong and overwhelming evidence in favor of model 1 (Aitkin 1991). The logarithm of PBF is used in the rest of the text. We note that PBF is highly controversial but used it because it is easy to calculate from the Markov chain Monte Carlo (MCMC) runs. For the data sets analyzed in this study, the LRT of the clock and PBF led to the same conclusions.

Prior model of speciation

The Bayes approach also requires modeling speciation events to specify the prior distribution of divergence times. This distribution was specified by a generalized birth-death process allowing for species sampling (Yang and Rannala 1997). The birth-death process tends to generate trees with long internal branches. Taking incomplete species sampling into account generates more realistic trees (Yang and Rannala 1997). To avoid over-parameterization, the time for the root is fixed at 1 (Rannala 2002; Aris-Brosou and Yang 2002), so that divergence times at other internal nodes are relative to that at the root. Hyperparameters of the model of speciation were integrated out: birth and death rates follow uniform prior distributions on (0, 15) and (0, 5), respectively. The sampling fraction ρ follows a uniform prior distribution on (0, .001) unless otherwise stated. After running the MCMC, a calibration date was used to rescale the relative divergence times into absolute divergence times measured in MYA.

Approximation of the posterior distribution

For each gene, the marginal posterior distributions $p(T \mid X)$ and $p(R \mid X)$ were approximated using an MCMC algorithm based on the Metropolis–Hastings sampler. The algorithm was described in Aris-Brosou and Yang (2002) and is similar to that of Thorne, Kishino, and Painter (1998). Let θ be the parameters of the model (divergence times, branch-specific rates, all the hyperparameters of the prior distributions for rates and times, κ and α). At each step of the Markov chain, a new state θ^* is proposed, differing from the current state θ by the update of one single parameter. We used normal proposal densities centered on the current parameter values. The proposed state is then accepted with probability $\min\{1, p(\theta^* \mid X) / p(\theta \mid X)\}$. For the data sets analyzed in table 1, the 50,000 first steps of the chain were discarded (burn-in) and each chain was then sampled every 500 steps until 10,000 samples were collected. Convergence was checked by running four additional shorter chains.

For the five runs, we analyzed time-series outputs for each parameter and checked consistency of the estimates across the different runs. Inferences were based on the median of the marginal distributions of parameters drawn from the longest run. The computation involved was intensive and took several months of time on a 30-processor Pentium III Beowulf cluster. The computer program implementing the models described is available at http://statgen.ncsu.edu/stephane/. A simlation study is conducted to examine the performance of the method; see Results section below.

DNA sequences

We analyzed eleven nuclear genes and eleven mitochondrial genes, with an average number of 26 taxa and 1,388 nucleotides per gene (table 1). Nucleotide sequences were obtained from Genbank for eleven nuclear genes (18S rRNA, actin, α-tubulin, β-tubulin, calreticulin, catalase, elongation factor 1 [EF-1], histone H1, heat shock protein 70 [Hsp70], protein kinase *c* [Pkc], troponin *c*) and eleven mitochondrial genes (cytochrome c oxidase [Cox] subunits I, II and III, cytochrome *b* [Cyt *b*], nicotinamide adenine dinucleotide dehydrogenase [ND] subunits 1 to 6 and 4L). These genes were chosen because (i) they are represented extensively across the Metazoa, (ii) they cover both protostome–deuterostome and echinoderm–chordate splits and (iii) at least one fossil calibration point at an age at least 300 MYA is available. Alignments were performed with ClustalW 1.8 (Thompson, Higgins, and Gibson 1994) and adjusted manually. For protein coding genes, all three codon positions were included in the analyses, with among-site rate variation accounted for using a discrete gamma model with eight rate classes (Yang 1994).

Calibration points

The phylogenetic tree for each gene is rooted using either a land plant (Arabidopsis), a fern (Polypodium for the 18S rRNA gene) or a fungus (Schizosaccharomyces for the troponin *c* gene). In order to reflect the most basal split (Parazoa–Eumetazoa), a diploblastic animal (Cnidaria) is included in the analysis whenever possible. To reduce errors associated with calibration points, only fossil-based dates were used, as in Bromham et al. (1998) (in MYA): Collembola–Pterygota, 390; Aranaea–Scorpionida, 405; Coelacanth–Dipnoi/Tetrapoda, 418; Osteichthyes–Dipnoi/Tetrapoda, 428; Asteroidea–Echinoidea, 500; Agnata–Gnathostoma, 510; Arachnida–Merostomata, 520; Cephalochordata–Chordata, 530. Each gene has between one and eight calibration points (table 1). When several calibration points were used, the median of the estimated divergence times was used as the final estimate.

We focused on two key evolutionary transitions: the protostome–deuterostome (PD) divergence, which marks the appearance of “higher Metazoa” (Eumetazoa), and the echinoderm–chordate (EC) divergence, as it predates the origin of the vertebrates.

Results and Discussion

Computer Simulation to Examine the Performance of the Bayes Algorithm

We conducted computer simulations to examine the performance of the Bayes MCMC algorithm (fig. 2). One hundred replicate datasets were generated for three trees (fig. 2A–C), each of which includes eight ingroup taxa and one outgroup taxon. Sequences, each of 1,000 nucleotides, were generated under the JC69 substitution model (Jukes and Cantor 1969) using the program EVOLVER from the PAML package (Yang 1997). Data sets were then analyzed under the same substitution model, assuming either a Bayesian molecular clock (with all branches having the same rate), or the exponential model of rate change. For each MCMC analysis, the first 20,000 samples of the chain were discarded as burn-in, after which 500 samples were collected for inference, sampling once every 1,000 iterations. Convergence was checked by time series plots for divergence times sampled from the posterior distribution. The results are presented in figure 2.

Topology 1 conforms to a perfect molecular clock, and both the Bayesian clock model and the EXP model of rate change performed well. Topology 2 also conforms to the clock but the shape of the tree reflected in the relative divergence times is very different from that expected under the birth-death process model with species sampling. For this tree, the Bayes analysis assuming the clock performed well, but the exponential model of rate change tended to overestimate divergence times and underestimate substitution rates for terminal branches (fig. 2H). In topology 3, the molecular clock is violated, with a terminal branch having a rate twice as high as all other branches. When the clock was incorrectly assumed (fig. 2F), divergence dates t_{13} and t_{15} are seriously overestimated. Note that in the true tree topology, there are only two distinct divergence dates (that is, $t_{14} = t_{15}$, and the four most recent nodes have the same age). In contrast, the exponential model of rate change performed much better. The four recent nodes had dates all close to the truth, while t_{14} and t_{15} were similar as well although slighted biased upwards (fig. 2I). Note that posterior distributions under the EXP model are wider than under the clock model for all three trees, because the rate-change model involves more parameters and incorporates more variability in the data.

Divergence dates under the Bayesian molecular clock

The molecular clock hypothesis is tested using two approaches: the LRT and the posterior Bayes factor (PBF). For every gene studied, the clock hypothesis was strongly rejected by the LRT ($P < 0.001$ for all the genes) and the PBF (table 1). Date estimates of the PD divergence under the clock are above 700 MYA for most genes (table 1), with a median (1st–3rd quartiles) of 1090 MYA (1411–841), i.e. before the Vendian (fig. 3A). These results are in agreement with previous molecular studies (Wray, Levinton, and Shapiro 1996; Feng, Cho, and Doolittle 1997; Gu 1998; Wang, Kumar, and Hedges 1999). Different genes produced substantially different estimates for the PD divergence, ranging from ~500 MYA for calreticulin to ~1,990 MYA for Cox1 (fig. 3A).

Divergence dates under models of rate change

The molecular clock assumption was relaxed by modeling rate change over time either by the exponential model (EXP) or by the Ornstein-Uhlenbeck process (OUP). Both processes are stationary, and neither assumes a trend in the evolutionary rate. In particular, unlike the model of Bromham and Hendy (2000), they do not posit fast early rates. Table 1 shows that EXP and OUP gave similar estimates, and the PD divergence is dated at 579 MYA (607–554) and 582 MYA (617–556), respectively (see also fig. 3B and C). Those estimates are broadly consistent with paleontological data (Valentine, Jablonski, and Erwin 1999). The estimates for the PD divergence are close to the minimum estimate of 588 MYA obtained by Bromham and Hendy (2000) assuming elevated rates around the root. Note that the only difference between the clock and non-clock analyses in table 1 lies in the assumption about substitution rates while all other assumptions are the same. The effect of relaxing the molecular clock is thus dramatic.

Effect of the prior assumptions on date estimates

Before drawing a conclusion, we examine the effects of several factors. First, we assess the influence of the prior by running the MCMC with no data, that is, by fixing $f(X|T, R) = 1$ in the MCMC, so that the Markov chain converges to the prior distribution. For instance, with 36 species and the four calibration points used for the mitochondrial genes, prior divergence times under the clock and under models of rate change were very similar, with t_{clock}(PD) = 526 (95% credible set (CS): 482–611) and t_{OUP}(PD) = 526 (95% CS: 482–609). While these dates are much younger than the dates estimated under the clock, they are close to the estimates under models of rate change. The results suggest that the prior for divergence times may have some influence on date estimates under models of rate change. This appears to be the same pattern as seen in the simulation study (fig. 2H).

Second, the exponential model used is crude and unrealistic, while the Ornstein-Uhlenbeck process is more complex. Table 1 shows that the latter model fitted the data better than the former for most genes (median log{PBF_{OE}} = 16). Both models clearly outperformed the clock model (e.g., log{PBF_{OC}} >> 10, table 1), and more importantly, gave consistent estimates of divergence dates for the PD and the EC splits (table 1; fig. 3, B and C). Therefore, our date estimates for these two splits appear robust to the specification of the model of rate change, and may not be improved by more complex models.

Third, the sampling fraction in the birth-death process with species sampling is known to affect the shape of the tree and thus may influence divergence date estimation. Our analysis (table 1, fig. 3) assumes that the sampling fraction has a uniform prior distribution $U(0, \rho_{\mathrm{up}})$ with the upper bound $\rho_{\mathrm{up}} = .001$. Larger ρ_{up} values did not greatly affect estimates under the clock, but gave older estimates under models of rate change: if we assume that $\rho_{\mathrm{up}} = .5$, the PD split was estimated at ~791 ± 246 MYA. However, such a large ρ_{up} was strongly rejected by the posterior Bayes factor (log $\mathrm{PBF}_{.001/.5} = 24.11$). On the other hand, assuming a ρ_{up} smaller than that used in table 1 (.0001) gave an estimate of ~561 ± 144 MYA for the PD split, which was favored by the Bayes factor (log $\mathrm{PBF}_{.001/.0001} = -6.27$). We note that this latter estimate is very close to the one found for a sampling fraction an order of magnitude larger ($\rho_{\mathrm{up}} = .001$). Thus the date estimates are stable over a reasonable range of ρ_{up} values.

Lastly, we did not account for uncertainty in the tree topology, while controversy exists concerning the metazoan phylogeny (Knoll and Carroll 1999). We note however that plausible topologies gave similar speciation date estimates in previous studies (Yoder and Yang 2000).

Episodic Molecular Evolution

It is of interest to examine whether the Cambrian explosion, as recorded by the fossils, has been preceded by a burst of molecular evolution, as suggested recently (Bromham and Hendy 2000). Figure 4 summarizes the estimates of relative rates against time from the exponential model of rate change. We define elevated relative rates as those greater than the 95th percentile of the distribution of relative rates over branches and over the sampled genes (rightmost panel of fig. 4). High relative rates occur mainly between ~640 MYA (late Riphean) and ~420 MYA (Silurian). The average relative rate is almost twice as large during this period (1.37) than either before (0.71) or after (0.62) it. Elevated rates are mainly for branches prior to the PD, the EC and the Agnatha–Gnathostoma (jawless and jawed vertebrates) divergences. The last two are contiguous and may belong to a single long period of elevated rates. Because of our limited sampling around the Parazoa–Eumetazoa split, it is difficult to detect any such burst at that time. It is remarkable that these bursts of evolution have concerned most of the twenty-two genes analyzed. We consider mutation rate variation to be an unlikely explanation for such evolutionary rate acceleration at the base of the metazoan, and a more sensible alternative is changed selective pressure. The large-scale rate acceleration might correspond to major duplication events, leading to the relaxation of selective constraints and higher evolutionary rates (Pollard and Holland 2000; Miyata and Suga 2001). Other lineages with high estimated rates (fig. 4) belong to the invertebrates, but here high rates are more gene-dependent. Subsequent “bursts” of evolution (< 400 MYA) are smaller in magnitude and mainly concern, at least in our restricted species sampling, parasites. Our date estimates suggest that divergences have been explosive, and happened in a relatively short period of time, as the PD and EC splits were found to be separated by an average of 63 MY. The environmental elements that could have triggered the Cambrian explosion remain unclear (Knoll and Carroll 1999), but our molecular date estimates are compatible with an origination of the Metazoa at about the Varanger ice age (~620–580 MYA), hereby renewing interest into possible refugia during a snowball Earth (Hyde et al. 2000).

Consistently with other studies (Ayala, Rzhetsky, and Ayala 1998; Bromham and Hendy 2000; Yoder and Yang 2000), our results highlight the importance of assumptions about evolutionary rates in divergence date estimation. By relaxing the unrealistic molecular clock assumption, we obtained date estimates for Metazoan divergences much closer to fossil data than previous molecular estimates, most of which are based on the clock assumption. A very similar pattern has been reported recently by Adkins, Walton, and Honeycutt (2003), who estimated divergence dates among rodents using a large data set of more than 4600 aligned nucleotide sequences. Those authors found that divergence dates among rodents estimated under a global molecular clock were compatible with previous molecule-based dates estimated under similar conditions, exceeding fossil-based dates. However, when a relaxed molecular clock was applied, estimated divergence dates were highly compatible with the fossil record.

We note that estimation of ancient divergence dates when the molecular clock is violated is problematic and our date estimates should be confirmed by analyzing more genes. In particular, our approach of averaging over calibration points and over multiple genes is inferior to a simultaneous analysis of multiple genes under the constraints of multiple fossil calibrations (Thorne, Kishino, and Painter 1998; Thorne and Kishino 2002). We emphasize the sensitivity of date estimation to assumptions about rates, and suggest that development of powerful estimation methodologies and accumulation of more gene sequences will eventually resolve the issue of Metazoan divergences.

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Table 1

Statistics of the Genes Analyzed and Divergence Dates of Two Major Clades (MYA)

					proto–deuterostome			echinoderm–chordate				
	n	*s*	*c*	$2\delta\ell$	CLOCK	EXP	OUP	CLOCK	EXP	OUP	log PBF_{OC}	log PBF_{OE}
Mitochondrial												
Cox1	35	1681	4	2341.22	1988	538	624	764	506	579	1005.15	-157.10
Cox2	35	804	4	740.33	1278	577	602	799	515	548	366.43	16.37
Cox3	35	813	4	1173.48	1561	540	595	786	515	557	568.48	15.02
Cyt *b*	35	1285	4	1847.06	1724	572	583	714	510	544	850.40	28.87
ND1	35	1087	4	1336.89	1358	552	574	1331	502	542	662.85	78.21
ND2	35	2589	4	747.74	995	562	558	950	503	523	371.89	20.87
ND3	35	403	4	312.29	890	582	581	636	501	531	179.28	19.49

ND4	35	1563	4	1110.97	1429	574	670	736	501	537	541.87	81.20
ND4L	35	319	4	233.19	1142	607	530	759	525	492	117.22	49.02
ND5	34	2073	4	964.33	1596	635	623	831	521	548	482.33	35.60
ND6	35	655	4	348.79	1117	806	775	658	533	543	167.91	36.88
Nuclear												
18S rRNA	40	1032	8	867.50	2048	642	639	1427	567	564	441.75	16.32
actin	14	1135	1	73.36	896	495	551	594	463	496	30.87	-2.69
α tubulin	21	1365	1	460.26	796	494	488	684	459	455	196.91	-3.00
β tubulin	18	1389	1	219.65	1197	586	593	707	527	531	102.68	-2.30
calreticulin	12	1424	1	111.17	506	490	506	482	477	473	52.09	-1.68
catalase	11	2379	1	776.12	1064	606	572	1059	594	534	260.94	-1.16

EF-1	30	2562	2	4840.38	858	601	526	854	595	523	2302.58	141.62
histone H1	13	743	1	380.29	452	558	559	445	545	524	187.01	4.01
Hsp70	12	2026	1	163.99	744	600	585	735	585	540	28.24	-1.32
Pkc	11	2633	1	724.87	519	644	555	515	636	533	237.59	7.12
troponin *c*	13	573	1	163.38	835	858	638	543	463	455	220.65	37.15

n: number of sequences; *s*: sequence length; *c*: number of calibration points; $2\delta\ell$: LRT statistic (twice the log-likelihood difference between the clock and no-clock models); d.f. = $n - 2$ for the χ^2 approximation of LRT. Date estimates are given for three models of rate change: Bayesian molecular clock (CLOCK), exponential distribution (EXP) and Ornstein-Uhlenbeck process (OUP). Posterior Bayes factors are given on a log scale: PBF_{OC} is the posterior Bayes factor $\text{PBF}_{OUP,CLOCK}$, and PBF_{OE} is $\text{PBF}_{OUP,EXP}$. Dates are averaged over independent fossil-based calibration points.

Figure legends

FIG. 1.— Effect of the variance parameter σ^2 in the model of rate change on the distribution of rates for branches. The rate r_i of a branch is drawn from a distribution that is centered around the rate r_{A} for the ancestral branch. When σ^2 is small, r_i is close to r_{A}, so that the rates of the different branches are very similar and the tree is clock-like (A and B). Conversely, a large σ^2 allows more variation in the branch-specific rates (C and D).

FIG. 2.— Performance of the Bayesian molecular clock model and the exponential model of rate change in divergence date estimation. Data sets were simulated using topologies 1 to 3 with branch lengths indicated by the scale bars (A–C), and were analyzed under the Bayesian molecular clock (D–F) model and under the exponential model of rate change (G–I). The "true" divergence times are indicated by vertical gray broken lines. The clock holds in topologies 1 and 2, while in topology 3, a terminal branch shows a two-fold rate acceleration. The density plots (D–I) represent the posterior distributions of the node times on a relative scale (0: present; 1: root time).

FIG. 3.— Posterior distributions of the divergence time between protostomes and deuterostomes. Eleven nuclear genes (magenta) and eleven mitochondrial genes (blue) were analyzed under three models of rate change: (A) the Bayesian molecular clock, (B) the exponential distribution, (C) the Ornstein-Uhlenbeck process.

FIG. 4.— Relative rates of evolution plotted against the estimated divergence dates for the twenty-two genes studied. For each gene the relative rate is calculated by the estimated rate for the branch divided by the average rate for the gene. The x-axis indicates the age of the descendent node of the branch. Horizontal lines correspond to the median (blue) and to the 95th percentile (magenta) of the distribution of relative rates over branches and genes. Four branches leading to the following divergences are indicated as: Parazoa–Eumetazoa split (✕); PD split (■); EC split (+); split basal to vertebrates (●). A schematic geological scale is also given (topmost).

Fig. 1

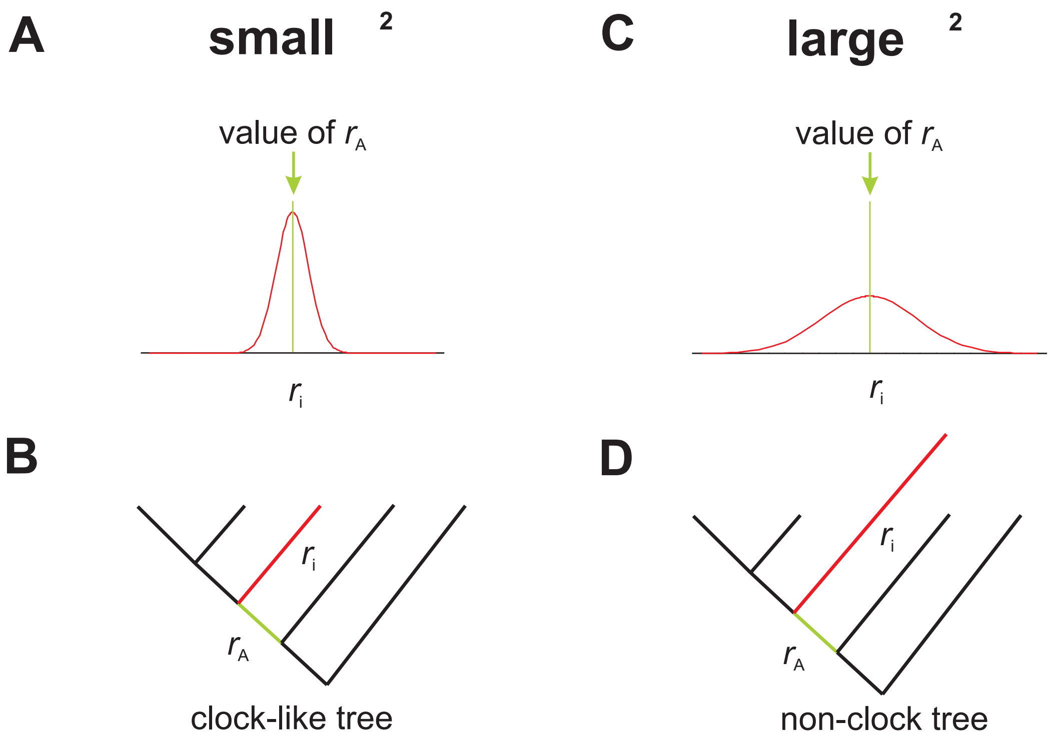
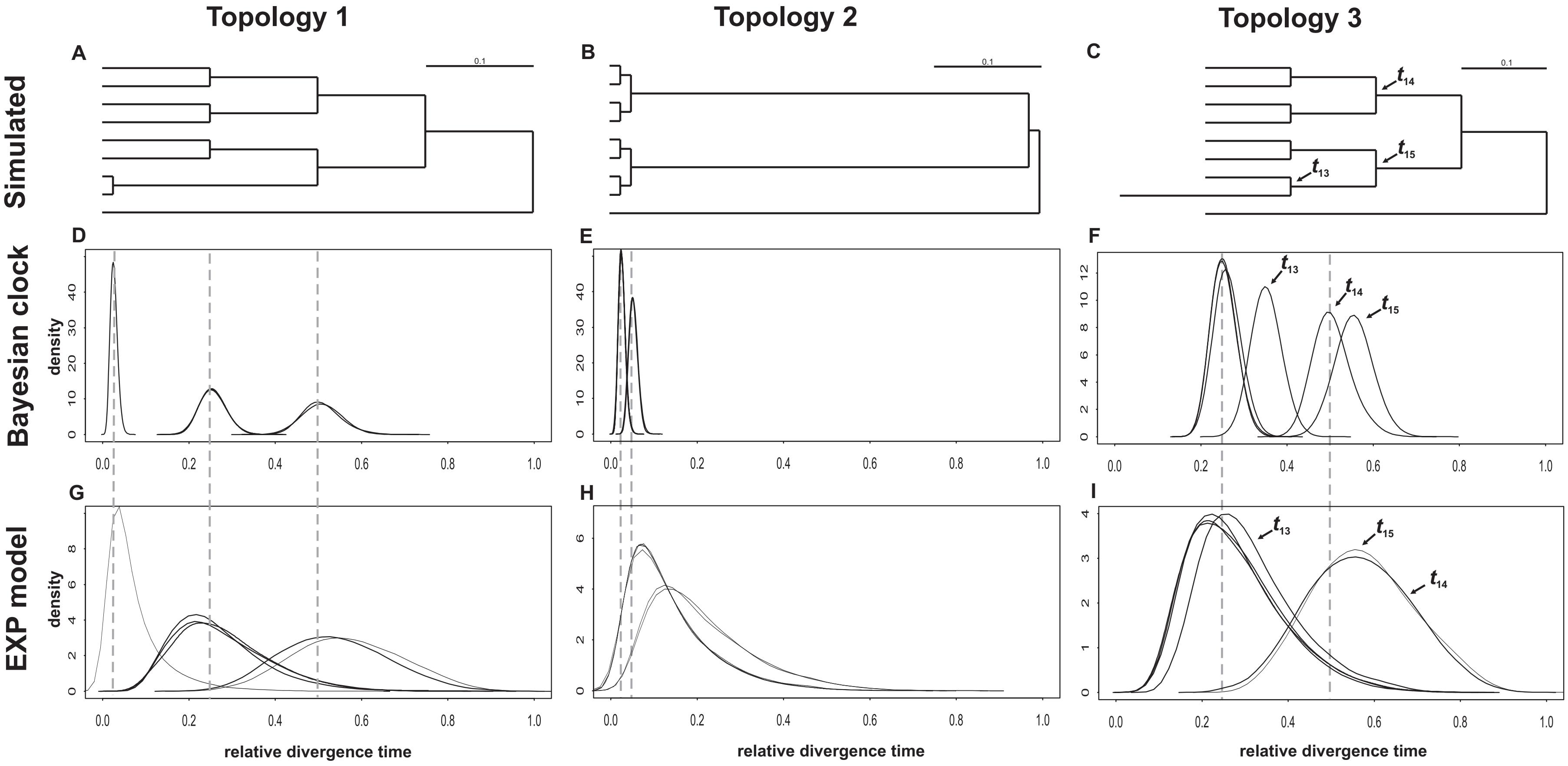

A
small 2
value of r_A
r_i
C
large 2
value of r_A
r_i
B
r_i
r_A
clock-like tree
D
r_i
r_A
non-clock tree

Fig. 2



Topology 1
Topology 2
Topology 3
Simulated
Bayesian clock
EXP model
A
B
C
D
E
F
G
H
I
0.1
t_{14}
t_{15}
t_{13}
density
0
10
20
30
40
2
4
6
8
12
0.0
0.2
0.4
0.6
0.8
1.0
relative divergence time

Fig. 3

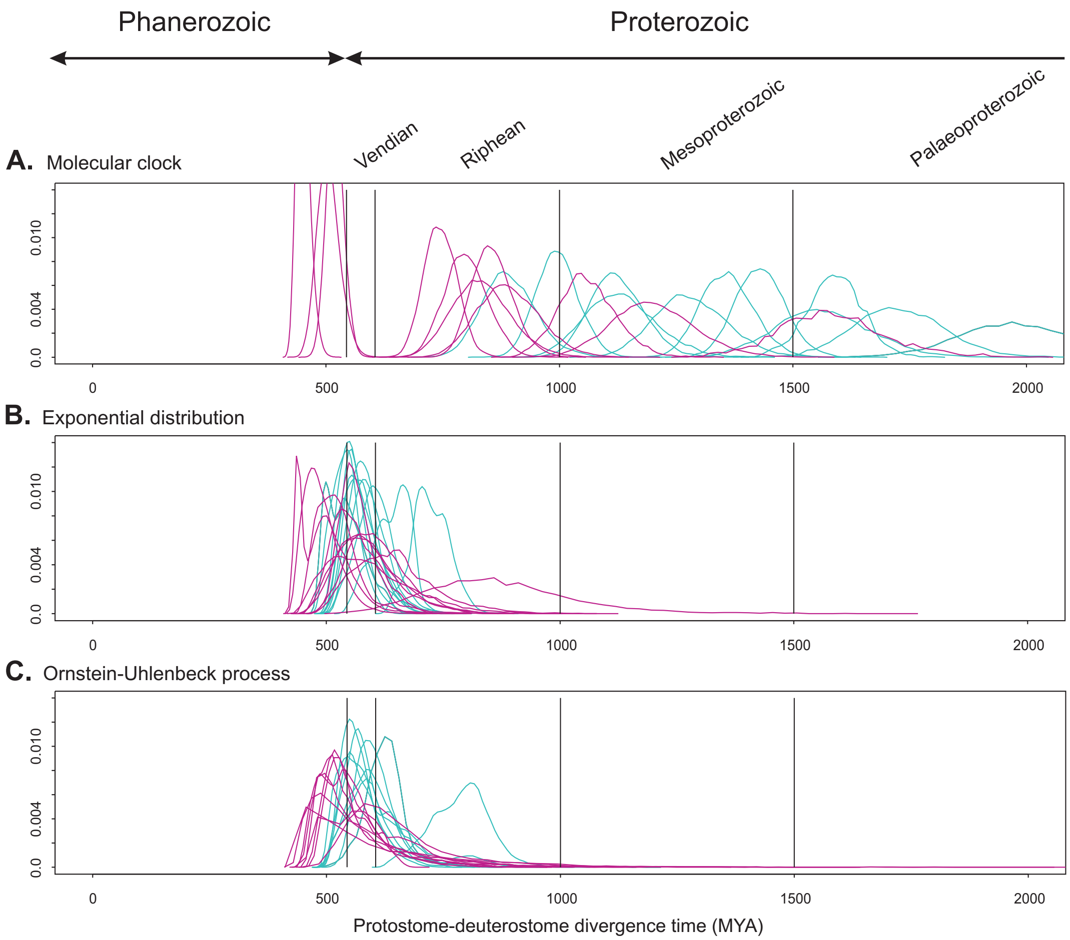
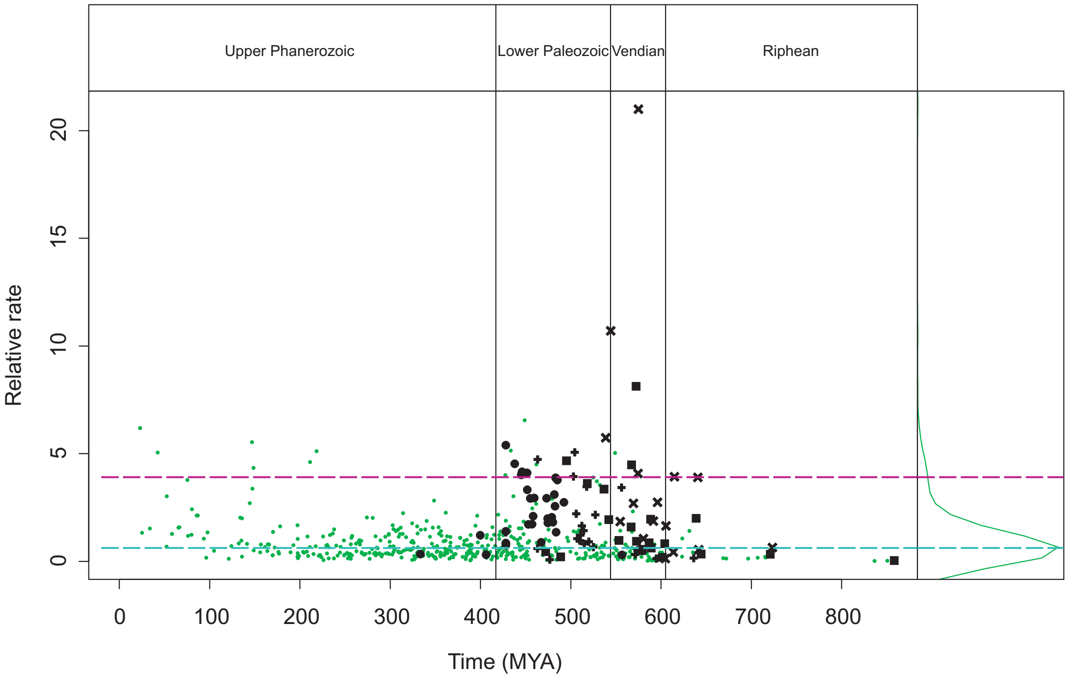

Phanerozoic
Proterozoic
Vendian
Riphean
Mesoproterozoic
Palaeoproterozoic
A. Molecular clock
0.0
0.004
0.010
0
500
1000
1500
2000
B. Exponential distribution
0.0
0.004
0.010
0
500
1000
1500
2000
C. Ornstein-Uhlenbeck process
0.0
0.004
0.010
0
500
1000
1500
2000
Protostome-deuterostome divergence time (MYA)

Fig. 4


Upper Phanerozoic
Lower Paleozoic
Vendian
Riphean
Relative rate
0
5
10
15
20
Time (MYA)
0
100
200
300
400
500
600
700
800

MBE MS# 3091
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Bayesian Models of Episodic Evolution Support a Late Precambrian Explosive Diversification of the Metazoa

Supplementary information

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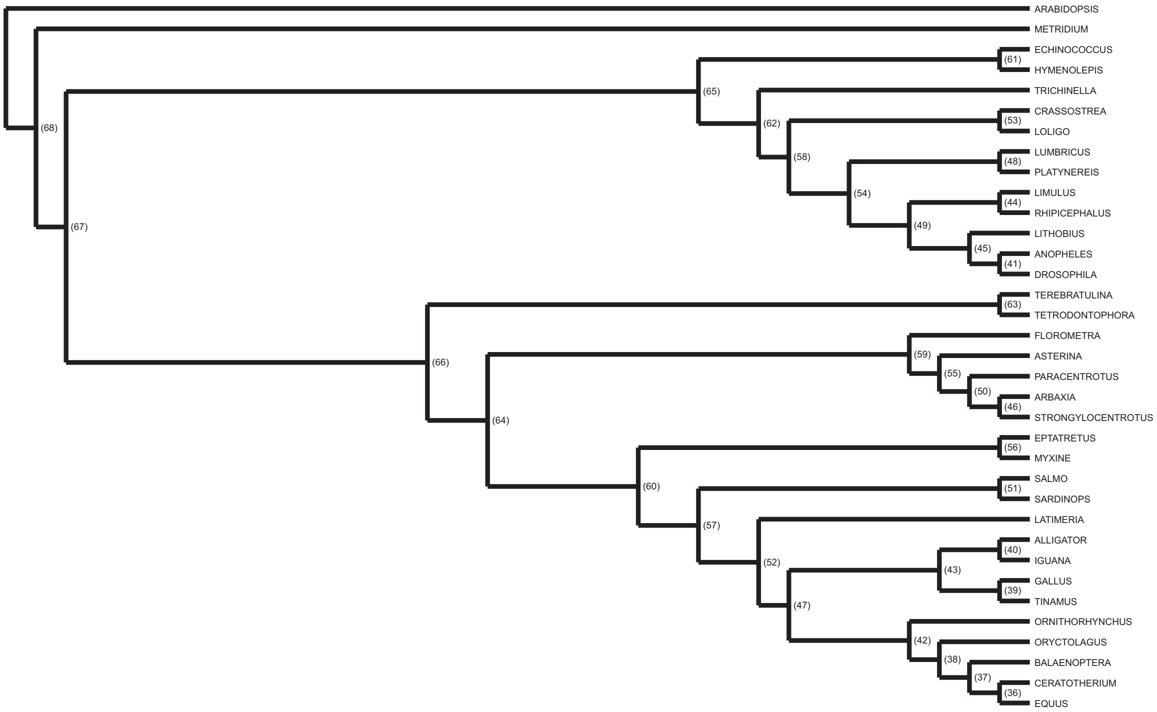
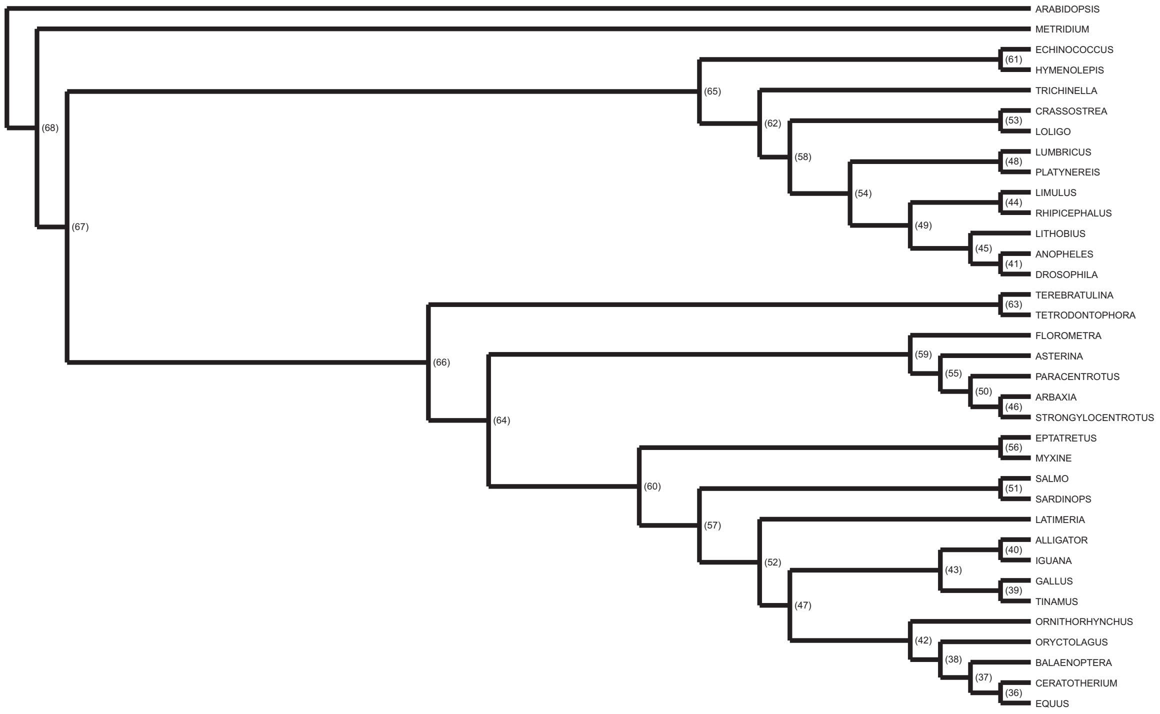
This document contains the posterior estimates (median of the distributions) of the divergence times under the Bayesian molecular clock (CLOCK), the exponential (EXP) and the Ornstein-Uhlenbeck (OUP) models of rate change, as well as the tree topology used for the individual genes. Estimates are given as estimated by the algorithm used (“relative dates”), on a (0,1) scale, and their conversion on an absolute scale in MYA. Highlighted (in bold) are the time estimates for the protostome–deuterostome and the echinoderm–chordate divergences.

Cox1



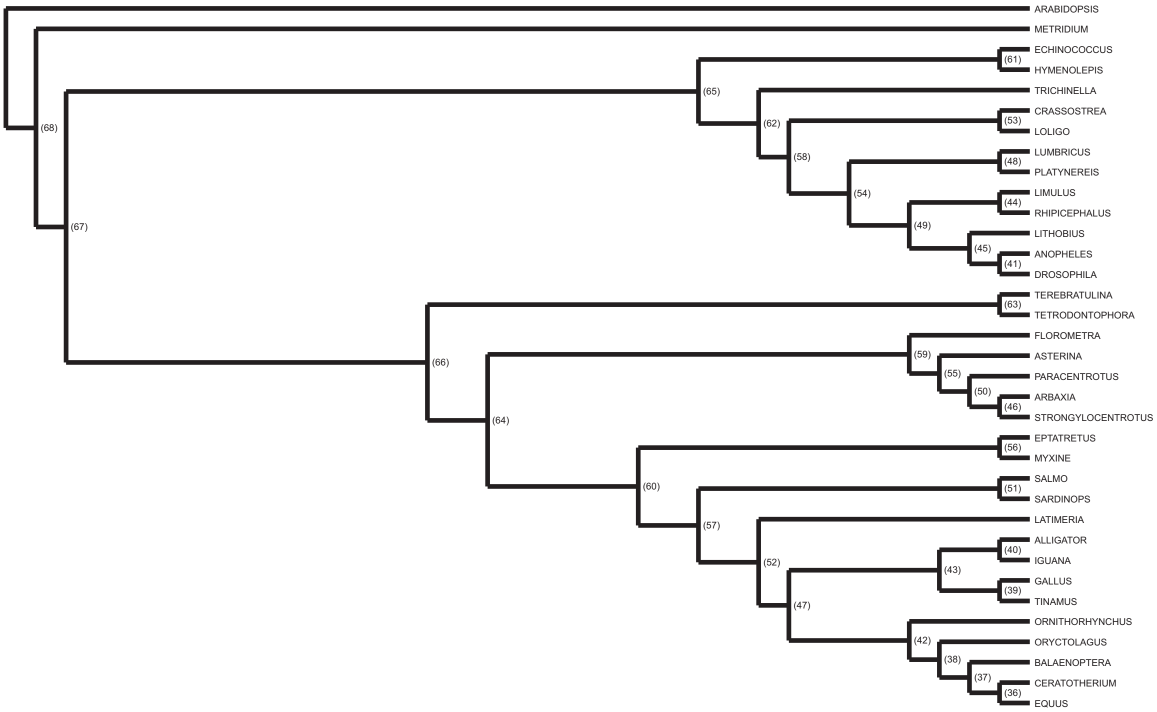
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0217	135.43	0.2916	158.3	0.0328	20.636
t[37]	0.0328	205.02	0.4225	229.37	0.4224	265.66
t[38]	0.0418	260.84	0.5809	315.34	0.4958	311.8
t[39]	0.0304	190.08	0.4585	248.89	0.5159	324.43
t[40]	0.0461	287.96	0.62	336.59	0.496	311.92
t[41]	0.0308	192.46	0.3297	179	0.2419	152.14
t[42]	0.0457	285.25	0.6233	338.39	0.509	320.07
t[43]	0.05	312.47	0.6822	370.34	0.5793	364.32
t[44]	0.0681	425.56	0.6291	341.55	0.3587	225.55
t[45]	0.0692	432.13	0.673	365.37	0.4822	303.27
t[46]	0.0402	250.8	0.519	281.74	0.3682	231.54
t[47]	0.0587	366.79	0.7507	407.57	0.6121	384.94
t[48]	0.0735	459.26	0.467	253.51	0.5766	362.58
t[49]	0.073	455.91	0.7223	392.15	0.6382	401.32
t[50]	0.0422	263.84	0.6219	337.64	0.4274	268.8
t[51]	0.0378	236.26	0.6502	352.96	0.4004	251.82
t[52]	0.0611	381.38	0.791	429.42	0.6611	415.73
t[53]	0.1388	867.15	0.8946	485.67	0.7216	453.77
t[54]	0.1222	763.38	0.9356	507.92	0.8203	515.85
t[55]	0.0547	341.6	0.6975	378.66	0.585	367.9
t[56]	0.0308	192.37	0.5759	312.63	0.6622	416.41
t[57]	0.0624	389.54	0.8037	436.32	0.7235	455.01
t[58]	0.1406	878.25	0.9529	517.31	0.9044	568.73
t[59]	0.0798	498.17	0.7745	420.47	0.6888	433.16
t[60]	0.0808	504.72	0.8393	455.62	0.7957	500.41
t[61]	0.0411	256.93	0.2461	133.58	0.188	118.25
t[62]	0.3174	1982.4	0.9748	529.18	0.9852	619.54
t[63]	0.1059	661.64	0.9232	501.17	0.8588	540.1
t[64]	**0.1223**	**764.16**	**0.9323**	**506.13**	**0.9212**	**579.3**
t[65]	0.3178	1985.2	0.9835	533.92	0.9893	622.15
t[66]	0.1268	792.24	0.9819	533.07	0.985	619.45
t[67]	**0.3183**	**1988.1**	**0.9917**	**538.37**	**0.9929**	**624.42**
t[68]	0.3194	1995	0.9963	540.89	0.9972	627.1
t[69]	1	6246.3	1	542.89	1	628.87

Cox2



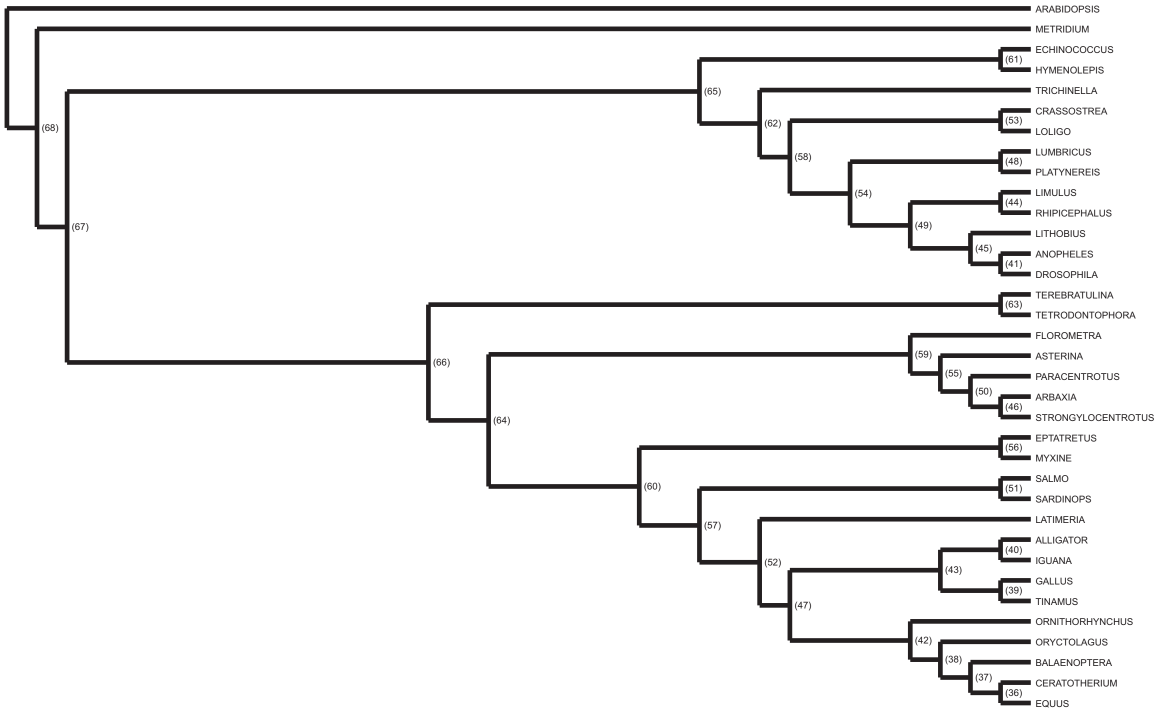
	CLOCK		EXP		OUP	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0344	104.06	0.3225	187.68	0.3185	194.71
t[37]	0.0471	142.74	0.4328	251.85	0.4066	248.57
t[38]	0.0651	197.24	0.5027	292.52	0.4836	295.67
t[39]	0.045	136.21	0.2683	156.16	0.4071	248.9
t[40]	0.1049	317.64	0.5864	341.29	0.4453	272.25
t[41]	0.0607	183.84	0.2961	172.34	0.2004	122.53
t[42]	0.0812	246.01	0.5702	331.83	0.5492	335.74
t[43]	0.1094	331.2	0.6314	367.46	0.5706	348.86
t[44]	0.1855	561.73	0.6006	349.55	0.5494	335.87
t[45]	0.1639	496.17	0.6251	363.77	0.5074	310.2
t[46]	0.0634	191.97	0.4271	248.58	0.3851	235.44
t[47]	0.1122	339.67	0.6625	385.53	0.6339	387.55
t[48]	0.1659	502.19	0.4722	274.83	0.6864	419.65
t[49]	0.1991	602.82	0.7108	413.66	0.6775	414.21
t[50]	0.0706	213.62	0.5325	309.88	0.5399	330.06
t[51]	0.0493	149.29	0.5987	348.42	0.4737	289.61
t[52]	0.1263	382.55	0.7509	437.01	0.7091	433.5
t[53]	0.3219	974.62	0.8769	510.3	0.8101	495.27
t[54]	0.2995	907	0.9012	524.46	0.8635	527.87
t[55]	0.1028	311.19	0.6664	387.84	0.7014	428.82
t[56]	0.0525	159.04	0.3294	191.72	0.1985	121.34
t[57]	0.1293	391.61	0.7734	450.11	0.7509	459.09
t[58]	0.3312	1002.9	0.9244	537.96	0.9261	566.2
t[59]	0.1428	432.36	0.7278	423.54	0.7469	456.6
t[60]	0.213	644.91	0.822	478.4	0.8354	510.7
t[61]	0.0967	292.77	0.1172	68.201	0.141	86.179
t[62]	0.4154	1257.7	0.9563	556.55	0.9561	584.53
t[63]	0.2469	747.6	0.7653	445.36	0.7306	446.66
t[64]	**0.2638**	**798.66**	**0.8847**	**514.86**	**0.8963**	**547.97**
t[65]	0.4188	1268	0.9724	565.87	0.9693	592.58
t[66]	0.3237	980.24	0.9805	570.63	0.9651	590
t[67]	**0.422**	**1277.8**	**0.9919**	**577.23**	**0.9845**	**601.88**
t[68]	0.427	1292.8	0.9966	579.99	0.9933	607.23
t[69]	1	3027.9	1	581.96	1	611.35

Cox3



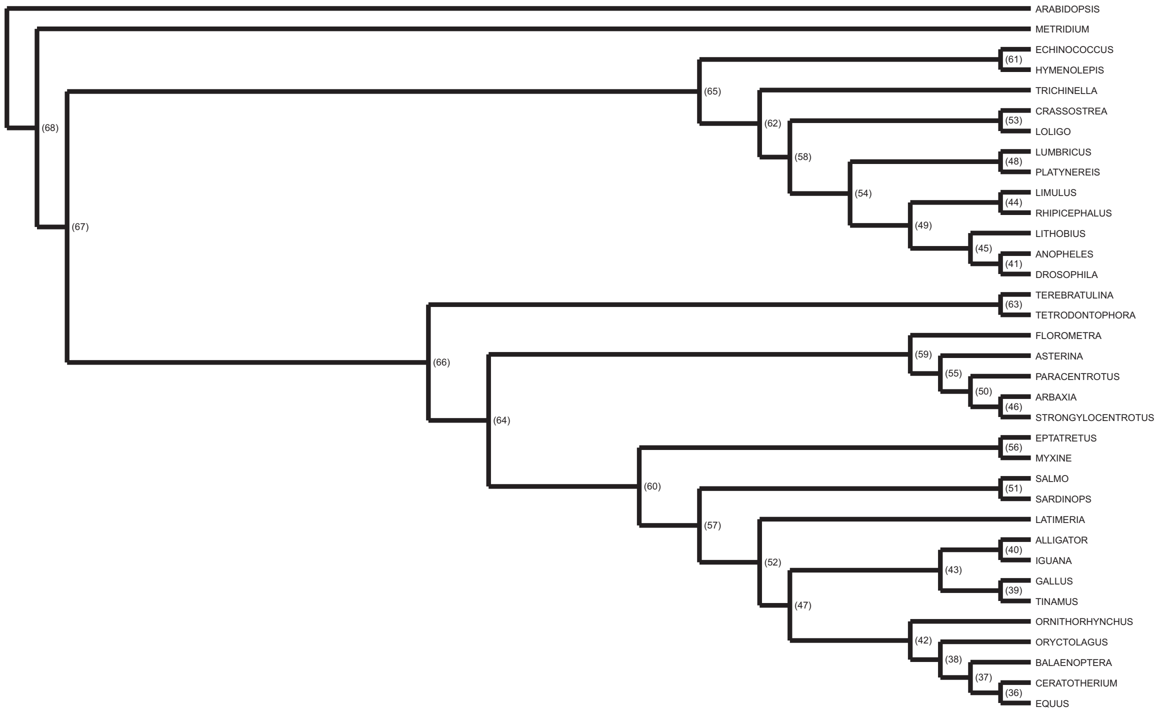
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0415	152.34	0.3729	202.8	0.4806	290.56
t[37]	0.0546	200.44	0.4652	253.02	0.536	324.04
t[38]	0.0668	244.93	0.5431	295.37	0.5647	341.41
t[39]	0.058	212.86	0.3698	201.14	0.3737	225.91
t[40]	0.0906	332.37	0.6721	365.53	0.556	336.14
t[41]	0.0503	184.69	0.2832	154.01	0.3112	188.15
t[42]	0.081	297.26	0.6234	339.03	0.6198	374.72
t[43]	0.0981	359.92	0.7436	404.41	0.6561	396.66
t[44]	0.1736	636.91	0.6456	351.15	0.5934	358.75
t[45]	0.1539	564.57	0.5912	321.53	0.6394	386.54
t[46]	0.0794	291.29	0.4852	263.9	0.5005	302.6
t[47]	0.102	374.36	0.7752	421.63	0.709	428.64
t[48]	0.1433	525.78	0.5474	297.7	0.6134	370.83
t[49]	0.1815	665.96	0.7354	399.97	0.7308	441.79
t[50]	0.0888	325.72	0.5883	319.95	0.6271	379.1
t[51]	0.0645	236.47	0.6172	335.67	0.6163	372.62
t[52]	0.106	389.04	0.8126	441.94	0.7444	450.06
t[53]	0.309	1133.5	0.938	510.14	0.8629	521.68
t[54]	0.2487	912.32	0.9431	512.96	0.8773	530.41
t[55]	0.1286	471.97	0.76	413.34	0.7638	461.77
t[56]	0.0582	213.54	0.5738	312.08	0.4988	301.57
t[57]	0.1082	396.98	0.8246	448.48	0.7676	464.07
t[58]	0.3144	1153.4	0.9664	525.6	0.9379	567.01
t[59]	0.1677	615.37	0.8126	441.95	0.8595	519.62
t[60]	0.1445	530.14	0.8557	465.39	0.8266	499.71
t[61]	0.1078	395.53	0.0867	47.155	0.1217	73.593
t[62]	0.4212	1545.4	0.9789	532.41	0.9704	586.65
t[63]	0.2107	773	0.8435	458.78	0.7683	464.5
t[64]	**0.2142**	**786.01**	**0.9474**	**515.28**	**0.9205**	**556.53**
t[65]	0.4238	1554.7	0.987	536.79	0.9782	591.37
t[66]	0.2375	871.46	0.9843	535.35	0.963	582.17
t[67]	**0.4254**	**1560.9**	**0.9937**	**540.43**	**0.9844**	**595.14**
t[68]	0.4299	1577.2	0.9974	542.46	0.9916	599.46
t[69]	1	3668.9	1	543.88	1	604.57

CytB



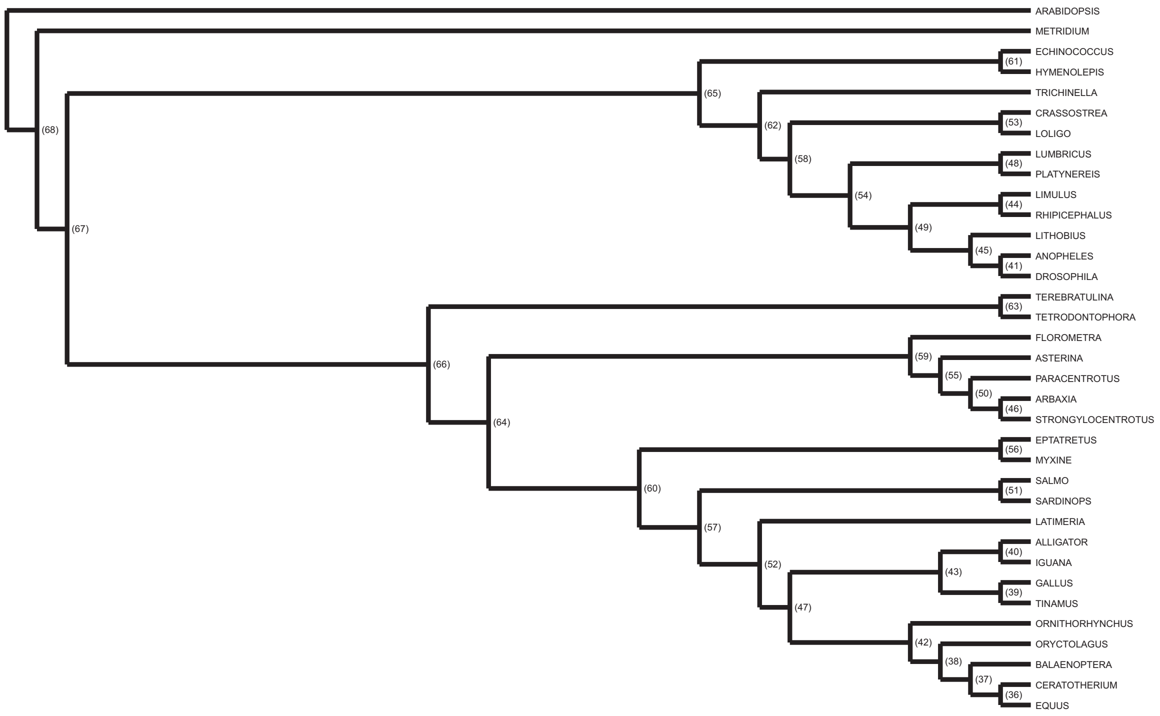
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0308	120.18	0.3245	186.66	0.1698	100.06
t[37]	0.0455	177.21	0.4719	271.45	0.3646	214.87
t[38]	0.0548	213.47	0.5468	314.56	0.485	285.83
t[39]	0.0409	159.19	0.4041	232.46	0.0859	50.637
t[40]	0.0785	305.82	0.5966	343.23	0.4401	259.36
t[41]	0.0555	216.35	0.383	220.31	0.0983	57.909
t[42]	0.0737	287.03	0.6238	358.86	0.5989	352.9
t[43]	0.0824	321.18	0.6551	376.84	0.594	350.04
t[44]	0.1257	489.62	0.5269	303.13	0.7122	419.67
t[45]	0.1257	489.78	0.6755	388.59	0.777	457.87
t[46]	0.056	218.14	0.4602	264.74	0.1881	110.82
t[47]	0.0888	346.1	0.7131	410.22	0.7366	434.06
t[48]	0.1228	478.52	0.6675	384.01	0.6795	400.43
t[49]	0.1399	545.31	0.7403	425.85	0.8489	500.28
t[50]	0.0594	231.28	0.5115	294.24	0.4309	253.93
t[51]	0.0501	195.26	0.4825	277.55	0.4966	292.64
t[52]	0.0934	363.75	0.7589	436.59	0.7457	439.46
t[53]	0.4286	1670.2	0.9719	559.12	0.9066	534.24
t[54]	0.1707	665.11	0.9606	552.62	0.9192	541.69
t[55]	0.1176	458.25	0.741	426.27	0.7368	434.17
t[56]	0.0677	263.69	0.5145	295.95	0.5333	314.3
t[57]	0.1024	399.1	0.8043	462.71	0.8072	475.7
t[58]	0.4378	1706	0.9813	564.52	0.9781	576.37
t[59]	0.1343	523.15	0.7926	455.98	0.8181	482.1
t[60]	0.1564	609.56	0.8285	476.62	0.8886	523.66
t[61]	0.0803	313.08	0.1122	64.558	0.1969	116.05
t[62]	0.44	1714.3	0.9869	567.76	0.981	578.09
t[63]	0.1569	611.45	0.9036	519.82	0.7977	470.06
t[64]	**0.1832**	**713.97**	**0.8862**	**509.83**	**0.9236**	**544.25**
t[65]	0.4413	1719.5	0.9913	570.25	0.9856	580.8
t[66]	0.199	775.5	0.9819	564.84	0.9774	575.97
t[67]	**0.4424**	**1723.7**	**0.9949**	**572.33**	**0.9897**	**583.23**
t[68]	0.4449	1733.6	0.9979	574.06	0.9959	586.88
t[69]	1	3896.6	1	575.27	1	589.29

ND1



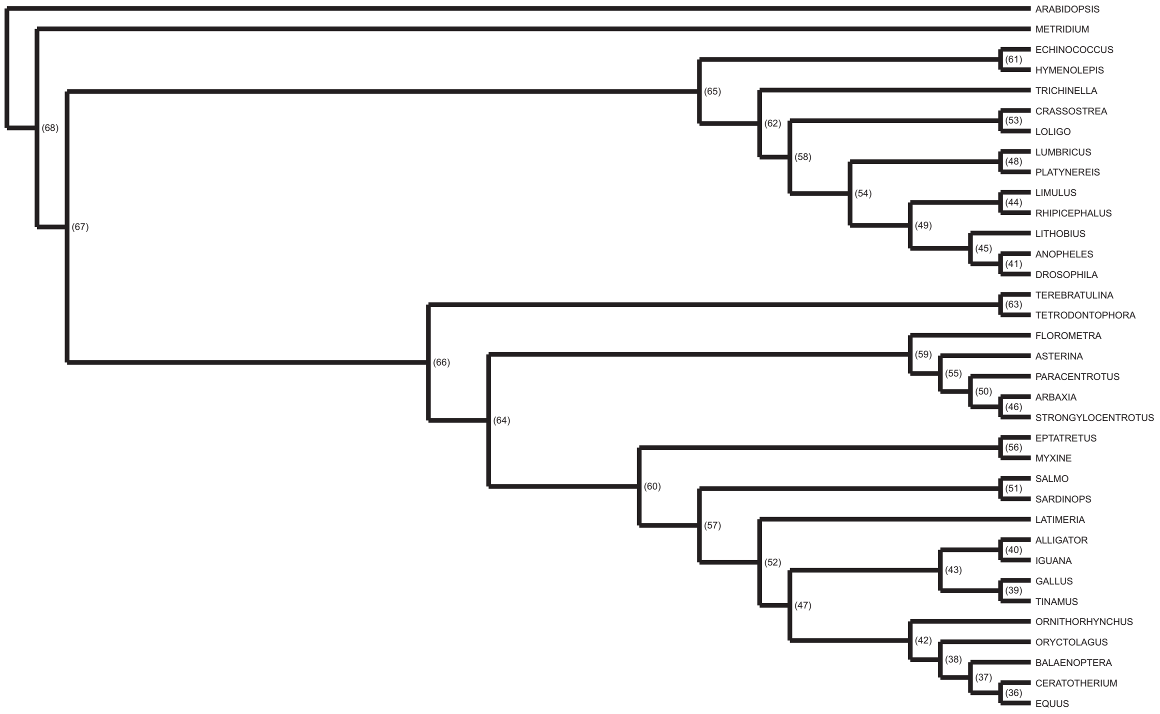
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0579	109.44	0.3237	179.52	0.1652	99.849
t[37]	0.0806	152.5	0.4331	240.18	0.3278	198.11
t[38]	0.1092	206.49	0.5177	287.07	0.4703	284.23
t[39]	0.0942	178.1	0.4267	236.65	0.4939	298.53
t[40]	0.1202	227.31	0.4901	271.78	0.4701	284.12
t[41]	0.0768	145.21	0.1283	71.133	0.0857	51.825
t[42]	0.1356	256.48	0.5782	320.63	0.5552	335.57
t[43]	0.1479	279.7	0.5818	322.63	0.5621	339.73
t[44]	0.2222	420.17	0.2666	147.83	0.2049	123.83
t[45]	0.1909	361.01	0.2461	136.5	0.1636	98.858
t[46]	0.129	244.05	0.5313	294.62	0.1983	119.84
t[47]	0.172	325.21	0.6601	366.04	0.644	389.2
t[48]	0.2779	525.6	0.6248	346.48	0.8035	485.61
t[49]	0.2249	425.3	0.271	150.29	0.2228	134.66
t[50]	0.1351	255.57	0.5588	309.88	0.4792	289.65
t[51]	0.1331	251.74	0.6355	352.42	0.6697	404.77
t[52]	0.1978	374.14	0.741	410.92	0.7092	428.64
t[53]	0.6971	1318.4	0.9698	537.77	0.8467	511.74
t[54]	0.7017	1327.2	0.9722	539.13	0.8757	529.24
t[55]	0.6929	1310.5	0.8639	479.05	0.8507	514.13
t[56]	0.0892	168.77	0.4851	269.01	0.5052	305.34
t[57]	0.2027	383.37	0.7676	425.67	0.7464	451.14
t[58]	0.7057	1334.7	0.9823	544.73	0.9034	545.99
t[59]	0.6979	1319.9	0.887	491.89	0.8756	529.18
t[60]	0.2588	489.42	0.8369	464.1	0.8229	497.34
t[61]	0.1176	222.36	0.1267	70.278	0.4192	253.34
t[62]	0.7124	1347.3	0.9883	548.03	0.9197	555.88
t[63]	0.7038	1331.1	0.9641	534.63	0.9194	555.68
t[64]	**0.704**	**1331.4**	**0.9058**	**502.28**	**0.8972**	**542.26**
t[65]	0.7153	1352.9	0.9924	550.35	0.9295	561.81
t[66]	0.7106	1344	0.9877	547.73	0.9426	569.68
t[67]	**0.7179**	**1357.7**	**0.9962**	**552.44**	**0.9503**	**574.32**
t[68]	0.7234	1368.1	0.9984	553.68	0.9892	597.88
t[69]	1	1891.3	1	554.54	1	604.39

ND2



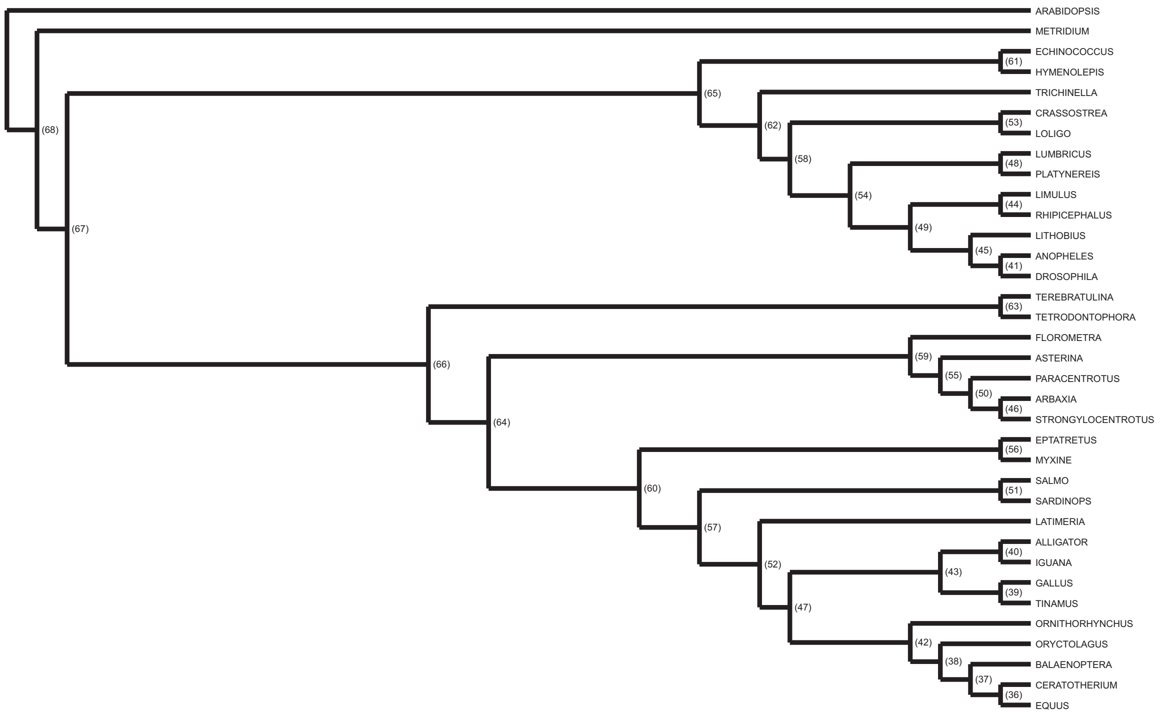
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0598	73.106	0.2657	150.38	0.1581	89.771
t[37]	0.0879	107.55	0.3425	193.82	0.3942	223.79
t[38]	0.1235	151.06	0.4133	233.92	0.5258	298.51
t[39]	0.1088	133.11	0.4119	233.09	0.4682	265.81
t[40]	0.2258	276.22	0.6067	343.34	0.5533	314.11
t[41]	0.1352	165.35	0.3743	211.84	0.2601	147.65
t[42]	0.1782	218.06	0.5345	302.49	0.5986	339.82
t[43]	0.2327	284.71	0.6458	365.46	0.6264	355.62
t[44]	0.3779	462.39	0.6372	360.59	0.6454	366.41
t[45]	0.2971	363.45	0.5954	336.93	0.5837	331.39
t[46]	0.1484	181.5	0.4339	245.54	0.4436	251.87
t[47]	0.2567	314.01	0.7073	400.26	0.7019	398.49
t[48]	0.3715	454.5	0.552	312.38	0.5761	327.05
t[49]	0.3891	476.08	0.6746	381.77	0.6566	372.78
t[50]	0.1521	186.04	0.5194	293.93	0.5032	285.71
t[51]	0.1516	185.53	0.5729	324.21	0.5675	322.19
t[52]	0.2631	321.94	0.7422	420.05	0.7625	432.89
t[53]	0.5708	698.31	0.7804	441.63	0.6541	371.38
t[54]	0.4929	603.04	0.821	464.61	0.7666	435.23
t[55]	0.7255	887.63	0.7679	434.58	0.8469	480.82
t[56]	0.1022	125.04	0.3188	180.4	0.5271	299.27
t[57]	0.2691	329.22	0.7526	425.92	0.8005	454.46
t[58]	0.5898	721.63	0.8531	482.81	0.8146	462.45
t[59]	0.7342	898.24	0.8151	461.28	0.886	503.02
t[60]	0.4116	503.57	0.8384	474.46	0.8839	501.83
t[61]	0.1293	158.2	0.115	65.056	0.0472	26.8
t[62]	0.7968	974.88	0.9593	542.88	0.9102	516.72
t[63]	0.3171	387.99	0.4845	274.19	0.6794	385.71
t[64]	**0.7767**	**950.29**	**0.888**	**502.55**	**0.9219**	**523.37**
t[65]	0.8075	987.96	0.9829	556.28	0.9523	540.64
t[66]	0.7886	964.82	0.9795	554.34	0.9693	550.29
t[67]	**0.8131**	**994.8**	**0.9932**	**562.1**	**0.9835**	**558.38**
t[68]	0.8851	1082.8	0.9972	564.35	0.9925	563.5
t[69]	1	1223.4	1	565.93	1	567.73

ND3



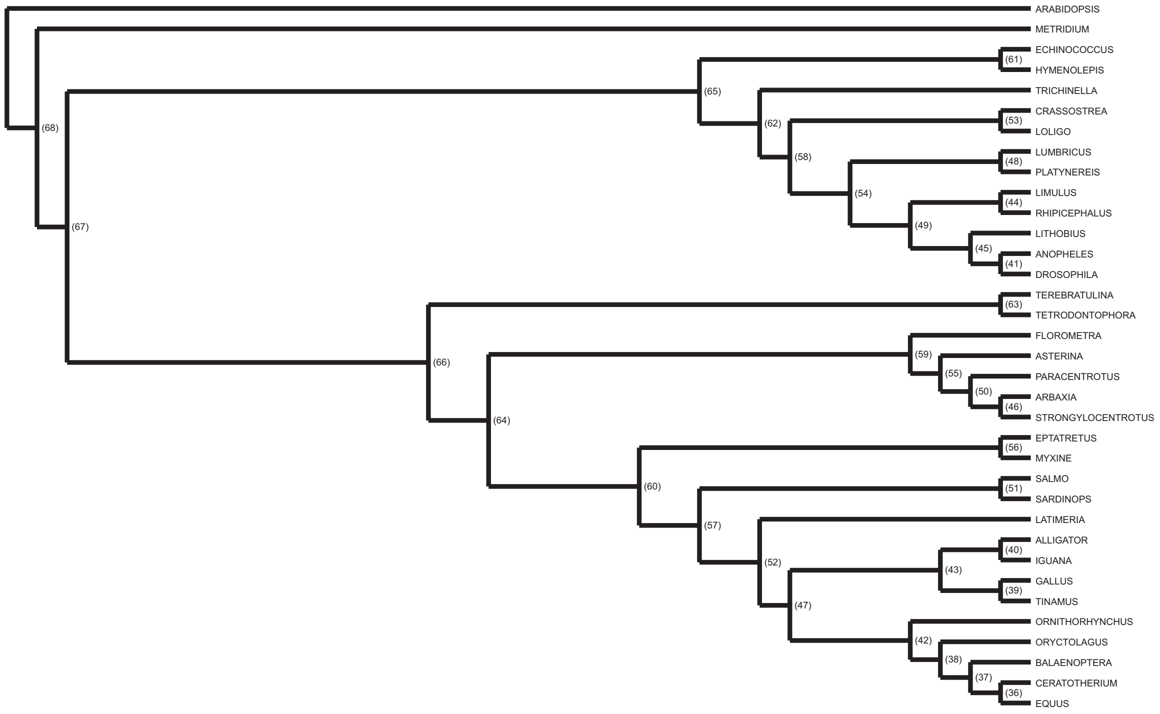
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0681	83.909	0.245	143.46	0.218	137.38
t[37]	0.0954	117.49	0.3528	206.59	0.3471	218.67
t[38]	0.1394	171.71	0.4718	276.24	0.4558	287.17
t[39]	0.1244	153.24	0.3951	231.35	0.4296	270.66
t[40]	0.1725	212.36	0.482	282.25	0.4644	292.61
t[41]	0.234	288.16	0.5446	318.89	0.467	294.25
t[42]	0.1777	218.77	0.5209	305.02	0.5129	323.13
t[43]	0.2112	260.08	0.6258	366.42	0.5815	366.36
t[44]	0.3544	436.38	0.6959	407.47	0.5596	352.54
t[45]	0.3735	459.97	0.7694	450.51	0.6181	389.41
t[46]	0.2197	270.5	0.573	335.52	0.542	341.45
t[47]	0.2664	328.02	0.7483	438.17	0.651	410.16
t[48]	0.3434	422.89	0.5489	321.43	0.5624	354.32
t[49]	0.3928	483.67	0.8048	471.25	0.6694	421.74
t[50]	0.4543	559.47	0.688	402.84	0.6975	439.42
t[51]	0.1786	219.91	0.626	366.54	0.5814	366.31
t[52]	0.2706	333.15	0.7623	446.38	0.6738	424.5
t[53]	0.5593	688.76	0.9051	529.99	0.7038	443.44
t[54]	0.4783	588.99	0.8552	500.74	0.7361	463.74
t[55]	0.4664	574.28	0.7411	433.94	0.7816	492.45
t[56]	0.1047	128.88	0.4331	253.63	0.6815	429.36
t[57]	0.2775	341.66	0.775	453.82	0.7009	441.57
t[58]	0.6013	740.43	0.9599	562.1	0.8441	531.83
t[59]	0.4951	609.62	0.8054	471.64	0.8137	512.67
t[60]	0.3926	483.44	0.8189	479.51	0.7786	490.54
t[61]	0.1933	238.05	0.195	114.19	0.3956	249.25
t[62]	0.7001	862.09	0.9758	571.38	0.8827	556.15
t[63]	0.5392	663.94	0.8935	523.23	0.7578	477.43
t[64]	**0.5164**	**635.87**	**0.8558**	**501.11**	**0.8424**	**530.76**
t[65]	0.7154	880.96	0.9858	577.23	0.9065	571.12
t[66]	0.6798	837.07	0.9844	576.43	0.9141	575.91
t[67]	**0.7231**	**890.38**	**0.9931**	**581.54**	**0.9224**	**581.12**
t[68]	0.751	924.76	0.9971	583.87	0.9756	614.69
t[69]	1	1231.4	1	585.56	1	630.03

ND4



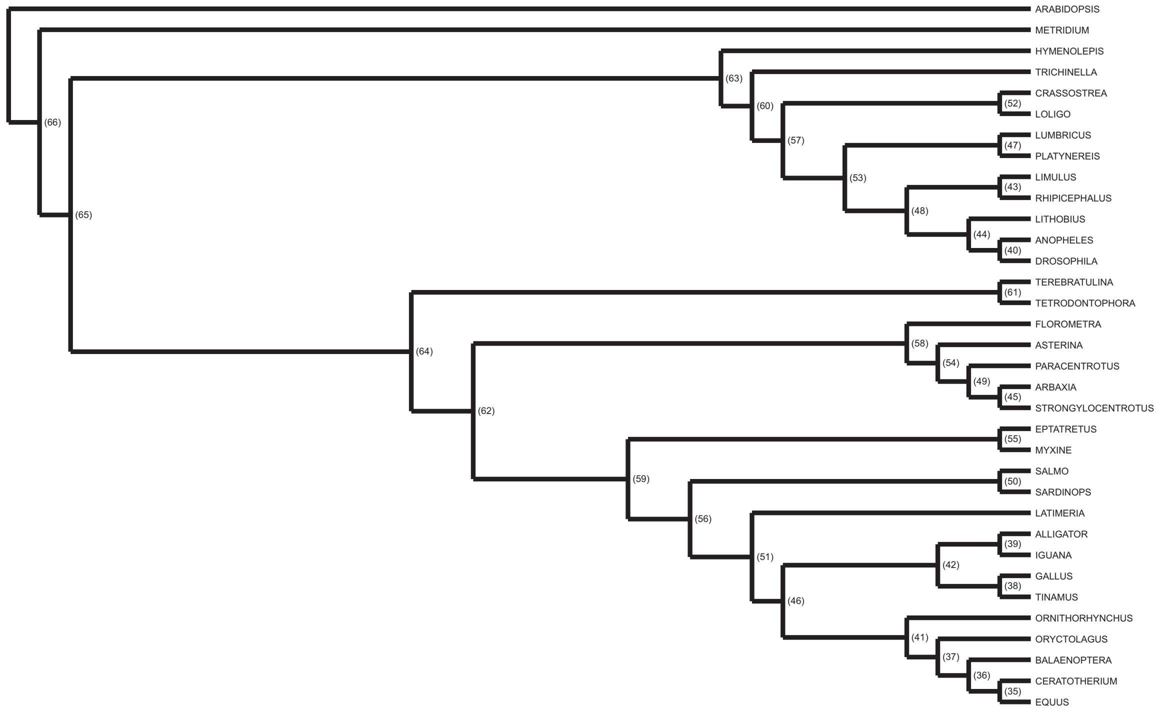
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0554	99.138	0.2951	170.27	0.1248	83.892
t[37]	0.0774	138.59	0.404	233.13	0.1739	116.94
t[38]	0.1061	189.82	0.499	287.96	0.2267	152.43
t[39]	0.0946	169.27	0.4493	259.25	0.3296	221.65
t[40]	0.1555	278.38	0.5785	333.8	0.396	266.29
t[41]	0.0722	129.27	0.1132	65.299	0.1393	93.684
t[42]	0.1506	269.56	0.6136	354.1	0.312	209.81
t[43]	0.1644	294.24	0.6358	366.92	0.4228	284.32
t[44]	0.2141	383.21	0.2548	147.05	0.3432	230.76
t[45]	0.2177	389.64	0.2509	144.8	0.3428	230.48
t[46]	0.1358	243	0.4893	282.33	0.308	207.1
t[47]	0.1996	357.29	0.7148	412.51	0.4653	312.86
t[48]	0.2298	411.35	0.4975	287.09	0.4539	305.2
t[49]	0.231	413.46	0.2854	164.67	0.3923	263.78
t[50]	0.1399	250.41	0.5354	308.94	0.3807	255.95
t[51]	0.1107	198.22	0.6115	352.89	0.3125	210.15
t[52]	0.2114	378.4	0.7668	442.5	0.6371	428.36
t[53]	0.7708	1379.8	0.9646	556.66	0.9581	644.26
t[54]	0.7749	1387	0.968	558.59	0.9624	647.12
t[55]	0.2394	428.57	0.6982	402.9	0.7062	474.84
t[56]	0.0831	148.78	0.5832	336.55	0.3066	206.17
t[57]	0.2141	383.26	0.7766	448.14	0.6566	441.48
t[58]	0.7804	1396.9	0.9786	564.7	0.9713	653.11
t[59]	0.3293	589.35	0.7652	441.6	0.7178	482.65
t[60]	0.3182	569.6	0.8374	483.25	0.7516	505.38
t[61]	0.1294	231.58	0.2471	142.61	0.1869	125.65
t[62]	0.7872	1409.1	0.9844	568.08	0.9843	661.87
t[63]	0.7768	1390.5	0.9564	551.92	0.966	649.52
t[64]	**0.4111**	**735.81**	**0.8685**	**501.16**	**0.7988**	**537.09**
t[65]	0.7941	1421.5	0.9894	570.97	0.99	665.69
t[66]	0.7843	1403.9	0.9844	568.07	0.9908	666.25
t[67]	**0.7985**	**1429.2**	**0.9949**	**574.11**	**0.9957**	**669.53**
t[68]	0.8076	1445.5	0.9979	575.84	0.9981	671.14
t[69]	1	1790	1	577.06	1	672.41

ND4L



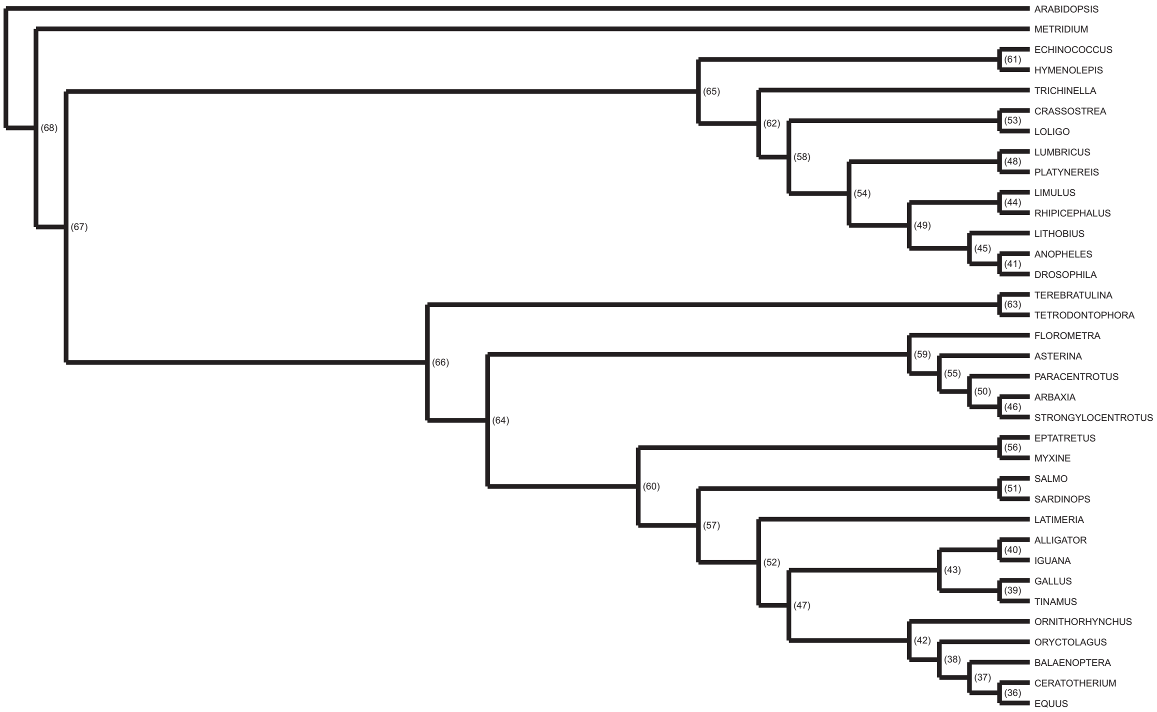
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0795	94.308	0.2749	167.78	0.1242	67.513
t[37]	0.1101	130.67	0.3513	214.43	0.3881	210.96
t[38]	0.1411	167.4	0.4276	261	0.5065	275.35
t[39]	0.1328	157.57	0.3821	233.25	0.4857	264.02
t[40]	0.2611	309.81	0.5619	342.93	0.4989	271.18
t[41]	0.1226	145.5	0.1683	102.73	0.2308	125.46
t[42]	0.2129	252.68	0.5214	318.22	0.6055	329.16
t[43]	0.2845	337.56	0.6331	386.4	0.682	370.74
t[44]	0.2996	355.46	0.3094	188.82	0.3503	190.44
t[45]	0.3011	357.34	0.273	166.62	0.3372	183.29
t[46]	0.1455	172.6	0.4426	270.12	0.2518	136.88
t[47]	0.3196	379.26	0.6936	423.34	0.7874	428.02
t[48]	0.4508	534.88	0.7282	444.46	0.7766	422.16
t[49]	0.3921	465.31	0.3798	231.8	0.4518	245.6
t[50]	0.1513	179.58	0.484	295.42	0.3981	216.42
t[51]	0.1669	198.01	0.6472	395.01	0.5324	289.44
t[52]	0.326	386.8	0.7246	442.26	0.8327	452.67
t[53]	0.8611	1021.8	0.9339	569.99	0.7892	429.02
t[54]	0.8804	1044.7	0.9415	574.66	0.8392	456.21
t[55]	0.2812	333.7	0.61	372.31	0.6466	351.48
t[56]	0.1408	167.12	0.5148	314.19	0.6159	334.79
t[57]	0.3318	393.71	0.7367	449.67	0.844	458.82
t[58]	0.902	1070.3	0.9637	588.21	0.8882	482.82
t[59]	0.5818	690.34	0.7841	478.61	0.8168	444
t[60]	0.4675	554.76	0.7921	483.48	0.8714	473.72
t[61]	0.1622	192.49	0.1507	92.001	0.2767	150.41
t[62]	0.9236	1096	0.9757	595.54	0.9195	499.84
t[63]	0.8843	1049.3	0.9445	576.5	0.7696	418.35
t[64]	**0.6396**	**758.96**	**0.8608**	**525.43**	**0.9047**	**491.78**
t[65]	0.9472	1124	0.9856	601.57	0.9474	514.99
t[66]	0.9306	1104.3	0.9851	601.26	0.9623	523.09
t[67]	**0.9625**	**1142.1**	**0.9938**	**606.58**	**0.9745**	**529.72**
t[68]	0.9787	1161.3	0.9975	608.81	0.9866	536.34
t[69]	1	1186.6	1	610.36	1	543.6

ND5



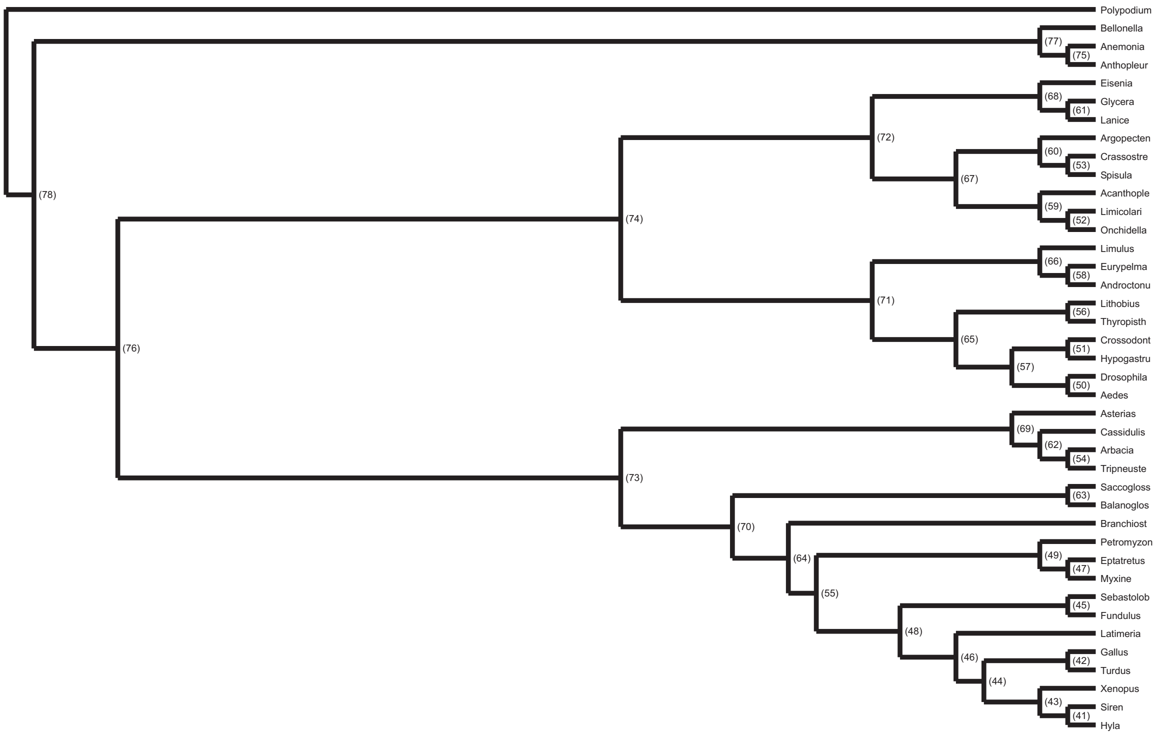
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[35]	0.0381	94.569	0.2028	128.95	0.1367	85.49
t[36]	0.0645	160.11	0.3434	218.32	0.2552	159.57
t[37]	0.0884	219.63	0.4336	275.65	0.4433	277.21
t[38]	0.0709	176.02	0.3682	234.13	0.3205	200.46
t[39]	0.122	302.87	0.4699	298.77	0.5299	331.36
t[40]	0.0623	154.73	0.0849	54.004	0.0951	59.478
t[41]	0.1102	273.72	0.5116	325.28	0.6119	382.63
t[42]	0.1312	325.82	0.5432	345.34	0.5939	371.38
t[43]	0.1841	457.33	0.2573	163.59	0.2378	148.73
t[44]	0.1839	456.73	0.2794	177.64	0.2409	150.63
t[45]	0.1084	269.18	0.5716	363.45	0.3879	242.58
t[46]	0.1481	367.87	0.6232	396.26	0.6588	411.98
t[47]	0.1835	455.65	0.3851	244.81	0.5669	354.51
t[48]	0.1908	473.75	0.293	186.3	0.2616	163.58
t[49]	0.11	273.15	0.5874	373.44	0.5169	323.25
t[50]	0.0834	207.16	0.6697	425.77	0.5643	352.86
t[51]	0.1521	377.65	0.7007	445.52	0.6878	430.1
t[52]	0.6175	1533.6	0.9425	599.25	0.8869	554.64
t[53]	0.6186	1536.3	0.9479	602.67	0.8902	556.71
t[54]	0.1859	461.78	0.7139	453.9	0.7172	448.48
t[55]	0.0666	165.46	0.5396	343.07	0.3474	217.25
t[56]	0.1531	380.14	0.7152	454.72	0.7032	439.74
t[57]	0.6229	1546.9	0.9628	612.13	0.9215	576.23
t[58]	0.2431	603.78	0.7586	482.32	0.78	487.8
t[59]	0.2239	556.02	0.7555	480.33	0.7932	496.05
t[60]	0.6316	1568.5	0.9792	622.57	0.9562	597.97
t[61]	0.6279	1559.4	0.9752	620.05	0.9606	600.72
t[62]	**0.3346**	**830.92**	**0.82**	**521.34**	**0.8767**	**548.27**
t[63]	0.6353	1577.7	0.9878	628.05	0.9737	608.93
t[64]	0.6336	1573.6	0.991	630.08	0.9843	615.51
t[65]	0.6376	1583.5	0.9957	633.08	0.9886	618.23
t[66]	**0.6425**	**1595.8**	**0.9982**	**634.68**	**0.9955**	**622.51**
t[67]	1	2483.5	1	635.8	1	625.35

ND6



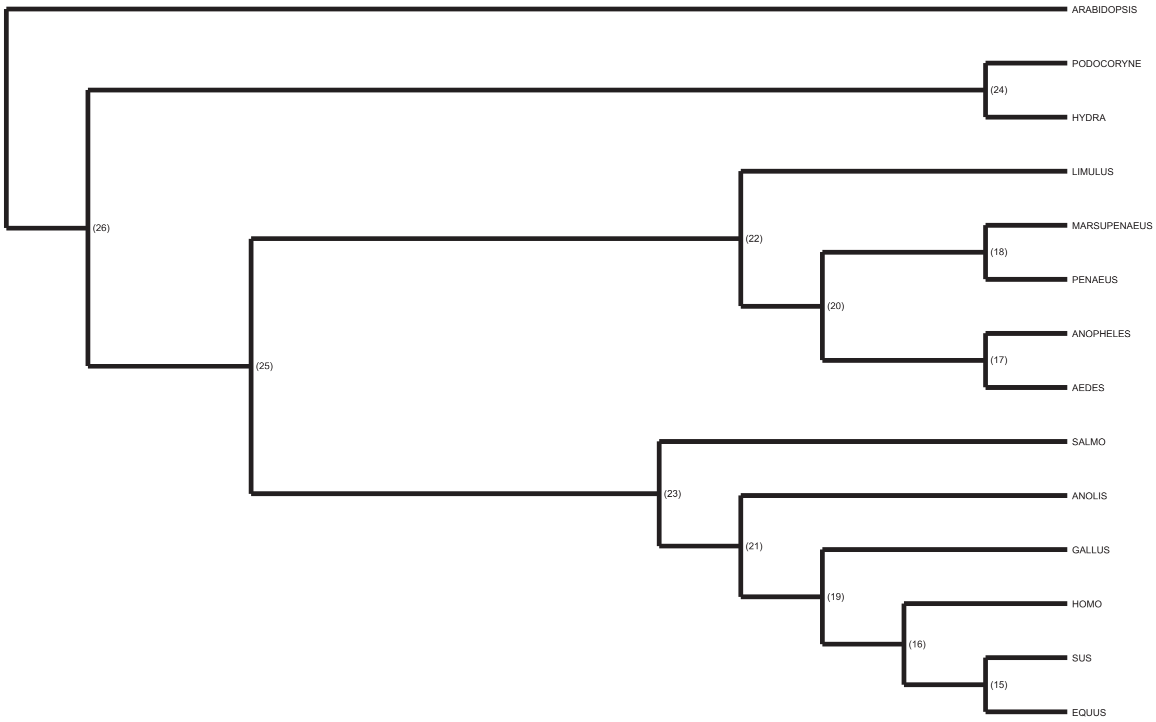
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[36]	0.0674	79.338	0.1334	108.23	0.0677	53.875
t[37]	0.1033	121.56	0.1964	159.36	0.1615	128.53
t[38]	0.1504	177.02	0.2726	221.25	0.3024	240.6
t[39]	0.1277	150.23	0.2207	179.11	0.2603	207.08
t[40]	0.2667	313.87	0.4276	346.98	0.398	316.7
t[41]	0.1536	180.71	0.3207	260.24	0.1777	141.38
t[42]	0.2057	242.03	0.3383	274.49	0.376	299.13
t[43]	0.2795	328.85	0.4609	373.98	0.4292	341.53
t[44]	0.3665	431.27	0.6667	541	0.5976	475.47
t[45]	0.3603	423.95	0.7643	620.21	0.61	485.33
t[46]	0.1581	186.04	0.3559	288.81	0.2232	177.59
t[47]	0.3198	376.26	0.5229	424.29	0.499	397.05
t[48]	0.4326	509.05	0.5156	418.39	0.6259	497.99
t[49]	0.4013	472.26	0.8153	661.59	0.694	552.18
t[50]	0.1679	197.61	0.3765	305.5	0.2733	217.49
t[51]	0.2488	292.8	0.4928	399.86	0.4725	375.92
t[52]	0.3229	379.93	0.5368	435.57	0.5243	417.13
t[53]	0.7807	918.64	0.9324	756.6	0.6507	517.74
t[54]	0.5723	673.42	0.8753	710.32	0.7686	611.55
t[55]	0.3296	387.9	0.5278	428.28	0.4393	349.56
t[56]	0.1424	167.57	0.4524	367.15	0.4191	333.46
t[57]	0.3309	389.41	0.544	441.47	0.5469	435.13
t[58]	0.8211	966.21	0.9592	778.41	0.8232	654.98
t[59]	0.3563	419.29	0.5712	463.54	0.5514	438.71
t[60]	0.4817	566.79	0.6041	490.23	0.6423	511.07
t[61]	0.1736	204.22	0.2794	226.7	0.3463	275.57
t[62]	0.8492	999.23	0.973	789.6	0.8773	698.02
t[63]	0.6881	809.67	0.9263	751.69	0.8215	653.61
t[64]	**0.5588**	**657.54**	**0.6569**	**533.08**	**0.6826**	**543.11**
t[65]	0.8746	1029.2	0.985	799.35	0.9288	739.01
t[66]	0.9347	1099.9	0.9858	799.94	0.9548	759.72
t[67]	**0.9489**	**1116.5**	**0.9938**	**806.48**	**0.9736**	**774.63**
t[68]	0.9813	1154.7	0.9975	809.46	0.9874	785.66
t[69]	1	1176.7	1	811.48	1	795.67

18S rRNA



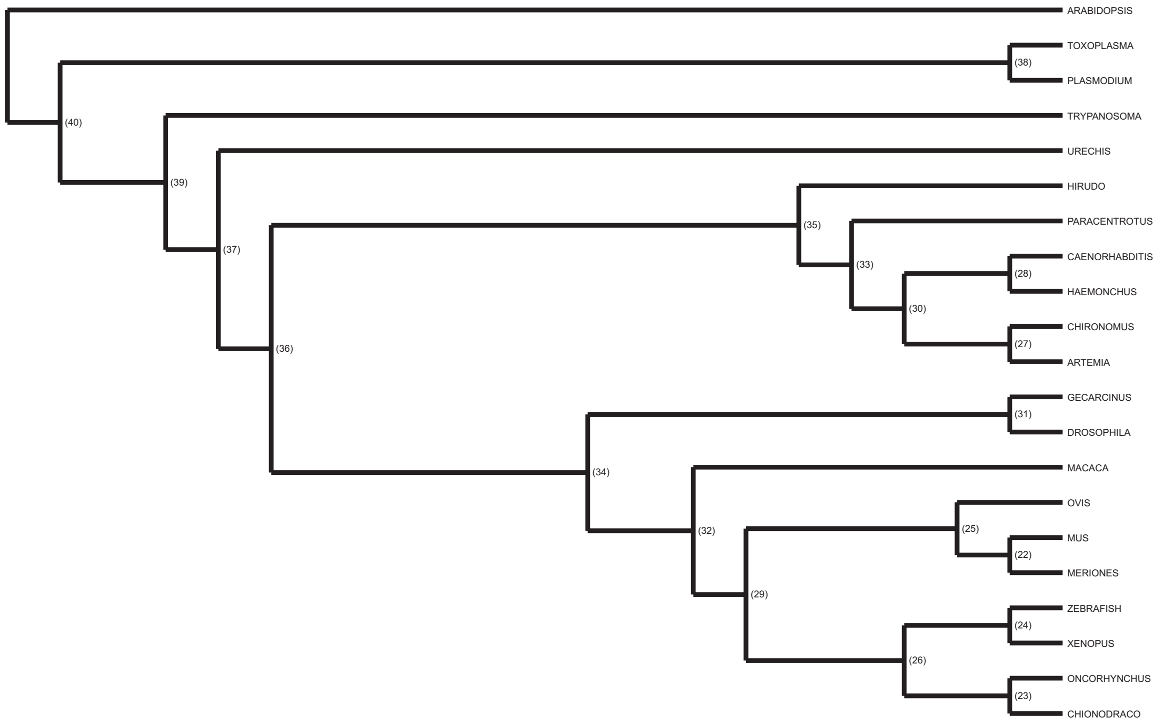
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[41]	0.04	94.03	0.39	253.19	0.41	296.55
t[42]	0.04	76.41	0.24	156.07	0.40	285.52
t[43]	0.05	111.60	0.47	302.24	0.48	347.24
t[44]	0.15	315.09	0.61	396.91	0.54	388.41
t[45]	0.17	360.63	0.64	417.59	0.43	309.75
t[46]	0.16	336.13	0.65	420.86	0.57	411.21
t[47]	0.01	17.44	0.05	33.78	0.10	73.07
t[48]	0.18	375.50	0.67	436.48	0.59	425.17
t[49]	0.36	765.39	0.58	377.65	0.49	352.92
t[50]	0.32	681.97	0.30	197.21	0.47	333.50
t[51]	0.08	173.05	0.26	172.05	0.46	330.43
t[52]	0.05	116.44	0.25	163.91	0.26	186.84
t[53]	0.25	529.86	0.55	355.92	0.46	332.22
t[54]	0.03	64.03	0.54	350.08	0.40	284.22
t[55]	0.40	841.45	0.71	459.37	0.63	452.47
t[56]	0.38	800.81	0.56	361.50	0.53	382.45
t[57]	0.55	1161.68	0.72	470.33	0.63	450.26
t[58]	0.17	367.65	0.59	384.74	0.58	413.03
t[59]	0.29	619.54	0.74	478.35	0.55	392.45
t[60]	0.28	597.21	0.68	443.85	0.54	385.20
t[61]	0.00	5.82	0.19	121.23	0.18	131.51
t[62]	0.04	77.58	0.60	390.29	0.65	462.87
t[63]	0.34	731.15	0.67	435.36	0.60	427.37
t[64]	0.51	1078.36	0.78	508.89	0.69	491.86
t[65]	0.56	1185.00	0.79	514.43	0.67	479.62
t[66]	0.24	503.87	0.73	475.93	0.66	470.12
t[67]	0.32	689.25	0.80	519.62	0.67	482.49
t[68]	0.28	604.76	0.78	506.06	0.61	436.73
t[69]	0.20	435.82	0.73	475.38	0.68	488.28
t[70]	0.55	1172.21	0.85	549.33	0.73	523.61
t[71]	0.56	1194.61	0.81	524.79	0.70	501.80
t[72]	0.33	707.50	0.82	532.29	0.72	517.36
t[73]	0.56	1192.18	0.86	556.27	0.76	544.00
t[74]	0.61	1289.44	0.84	549.04	0.76	541.95
t[75]	0.06	122.02	0.39	255.33	0.54	383.96
t[76]	**0.67**	**1426.82**	**0.87**	**567.36**	**0.79**	**564.26**
t[77]	0.18	388.06	0.63	406.52	0.62	441.08
t[78]	**0.96**	**2047.80**	**0.99**	**641.75**	**0.89**	**638.82**
t[79]	1.00	2130.05	1.00	649.82	1.00	716.24

actin



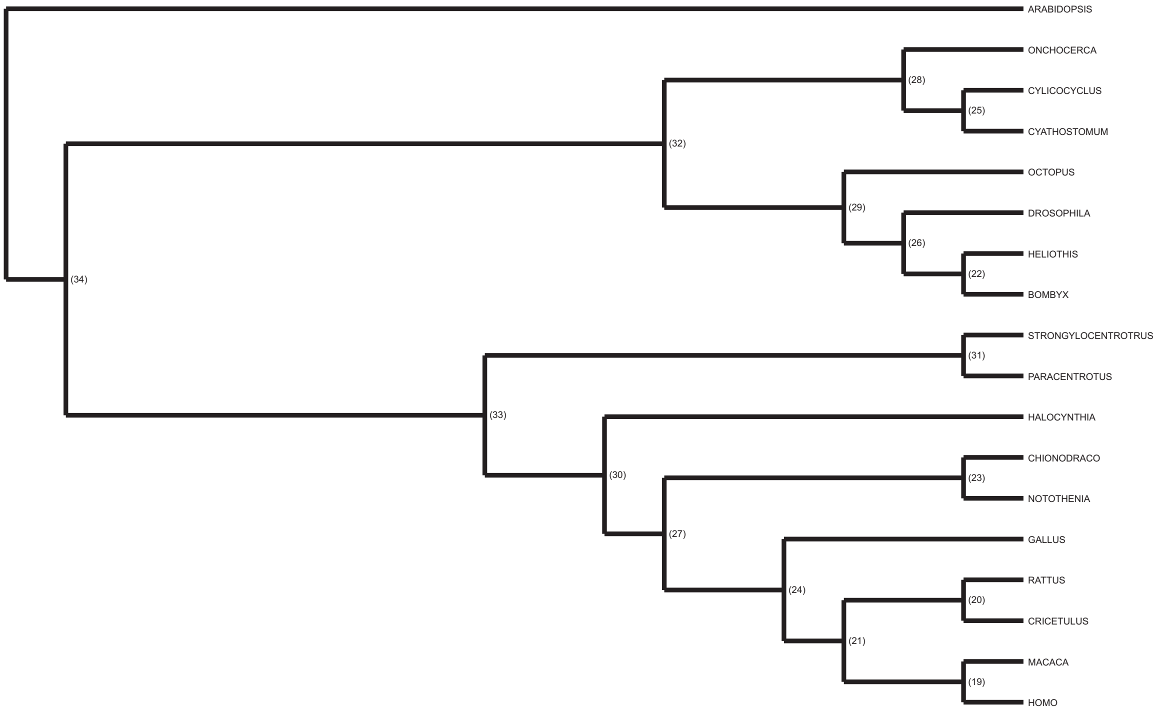
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[15]	0.3153	306.24	0.563	283.4	0.3439	207.59
t[16]	0.3578	347.45	0.7216	363.24	0.4992	301.34
t[17]	0.4005	388.92	0.6055	304.77	0.5332	321.83
t[18]	0.4966	482.31	0.7777	391.45	0.5617	339.06
t[19]	0.3726	361.87	0.7725	388.84	0.5899	356.1
t[20]	0.515	500.16	0.8269	416.23	0.6506	392.72
t[21]	0.4204	408.25	0.8208	413.17	0.6524	393.77
t[22]	0.5793	562.58	0.8985	452.27	0.7478	451.39
t[23]	0.4407	428	0.8503	428	0.7091	428
t[24]	0.402	390.39	0.6442	324.23	0.5341	322.37
t[25]	**0.6121**	**594.46**	**0.9206**	**463.4**	**0.8219**	**496.11**
t[26]	**0.9221**	**895.53**	**0.9841**	**495.34**	**0.9136**	**551.47**
t[27]	1	971.18	1	503.35	1	603.61

α tubulin



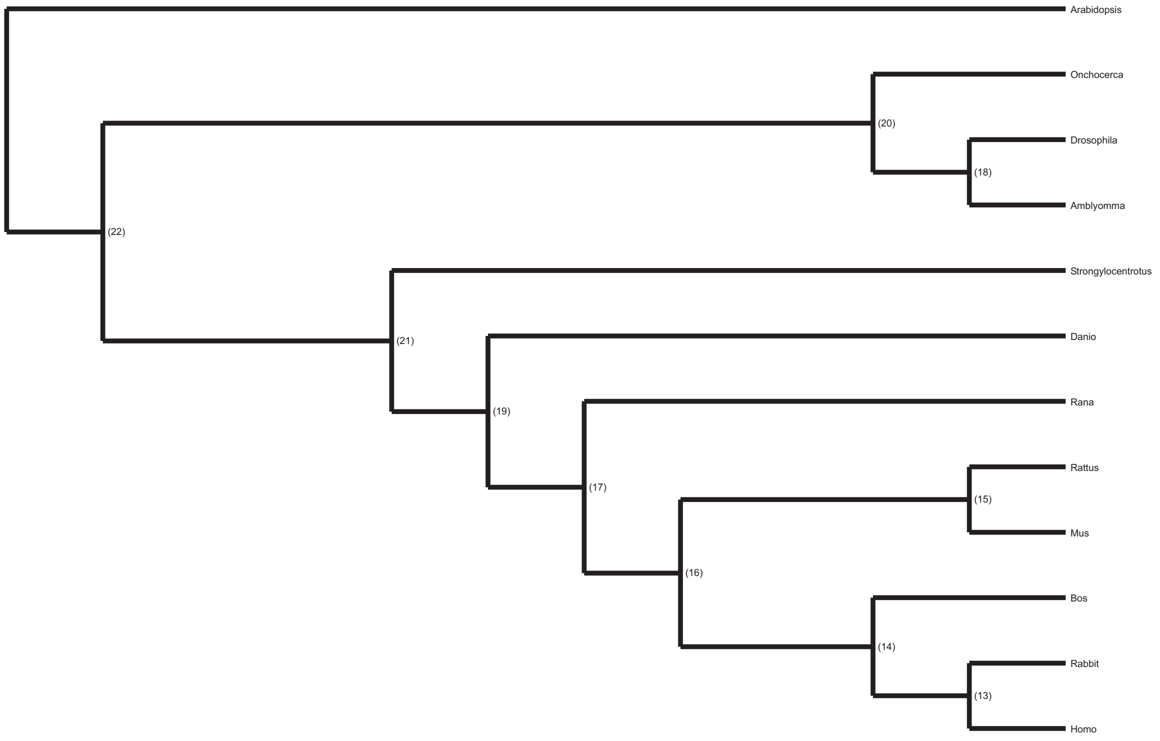
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[22]	0.0519	75.184	0.4584	280.39	0.0855	55.391
t[23]	0.2313	334.9	0.5516	337.41	0.376	243.67
t[24]	0.4611	667.79	0.4774	291.99	0.2945	190.83
t[25]	0.4763	689.72	0.5489	335.73	0.4535	293.89
t[26]	0.0855	123.84	0.5997	366.81	0.4434	287.34
t[27]	0.2448	354.52	0.4239	259.26	0.3538	229.28
t[28]	0.4885	707.37	0.4288	262.3	0.4752	307.94
t[29]	0.2405	348.2	0.6623	405.09	0.5346	346.45
t[30]	0.2673	387.03	0.586	358.45	0.5793	375.43
t[31]	0.4342	628.82	0.6191	378.68	0.5011	324.69
t[32]	0.5117	741.02	0.7048	431.12	0.6062	392.82
t[33]	0.2956	428	0.6997	428	0.6605	428
t[34]	0.5407	783.03	0.7708	471.49	0.6965	451.34
t[35]	**0.4722**	**683.83**	**0.7503**	**458.93**	**0.7025**	**455.23**
t[36]	**0.5493**	**795.51**	**0.8071**	**493.66**	**0.7532**	**488.07**
t[37]	0.5541	802.36	0.8272	505.98	0.7842	508.18
t[38]	0.7495	1085.4	0.6513	398.38	0.6896	446.9
t[39]	0.8855	1282.3	0.9396	574.73	0.8408	544.82
t[40]	0.9887	1431.8	0.9871	603.8	0.9087	588.88
t[41]	1	1448.1	1	611.67	1	648.02

β tubulin



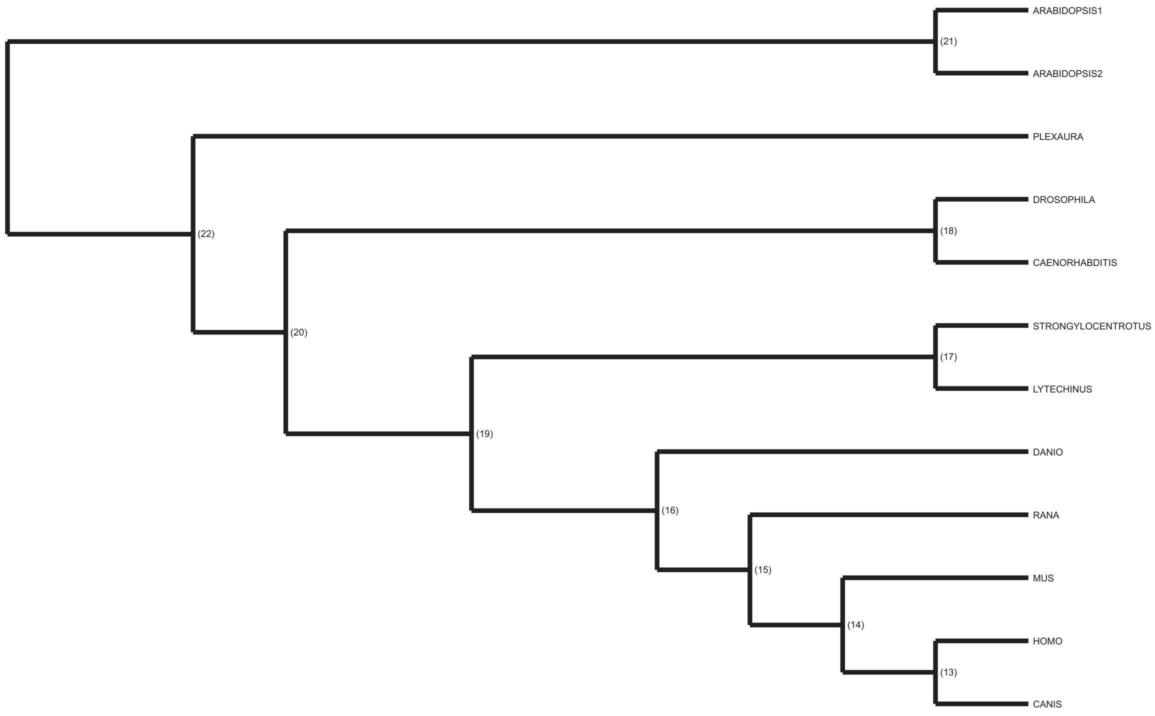
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[19]	0.0183	25.638	0.2193	116.53	0.0087	5.6641
t[20]	0.0898	125.98	0.3741	227.69	0.0258	16.847
t[21]	0.113	158.58	0.4546	279.27	0.0464	30.307
t[22]	0.6871	964.31	0.7711	455.03	0.5431	354.85
t[23]	0.2609	366.17	0.6244	367.96	0.6479	423.29
t[24]	0.274	384.57	0.6995	413.72	0.6172	403.23
t[25]	0.0453	63.604	0.1908	118.63	0.0232	15.17
t[26]	0.7044	988.57	0.8221	485.29	0.616	402.49
t[27]	0.305	428	0.7249	428	0.6551	428
t[28]	0.508	712.93	0.5212	276.79	0.5414	353.7
t[29]	0.8123	1140	0.9048	536.17	0.7254	473.95
t[30]	0.486	682.09	0.85	501.77	0.7424	485.02
t[31]	0.0612	85.829	0.5684	345.03	0.3884	253.75
t[32]	0.8407	1179.8	0.9567	567.39	0.8347	545.32
t[33]	**0.5038**	**707.11**	**0.8937**	**527.21**	**0.8133**	**531.34**
t[34]	**0.8526**	**1196.6**	**0.987**	**586.25**	**0.9073**	**592.76**
t[35]	1	1403.5	1	592.73	1	653.33

calreticulin



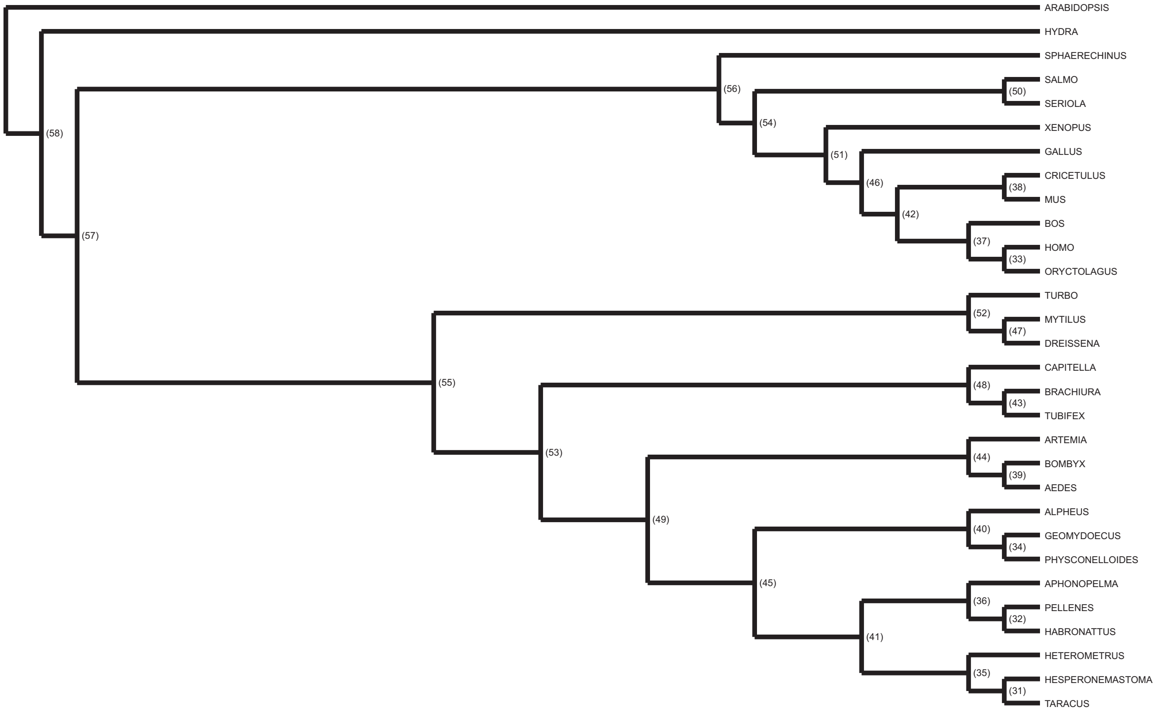
	CLOCK		EXP		OUP	
	relative	absolute	relative	absolute	relative	absolute
t[13]	0.1658	110.93	0.4645	230.85	0.2892	173.24
t[14]	0.2657	177.79	0.5603	278.46	0.4592	275
t[15]	0.0426	28.493	0.3746	186.2	0.0171	10.25
t[16]	0.2705	181	0.5931	294.79	0.5168	309.55
t[17]	0.5352	358.08	0.736	365.81	0.6365	381.23
t[18]	0.4676	312.91	0.5122	254.56	0.5071	303.72
t[19]	0.6397	428	0.8611	428	0.7146	428
t[20]	0.7433	497.35	0.9549	474.6	0.7532	451.11
t[21]	**0.7199**	**481.7**	**0.9605**	**477.4**	**0.7894**	**472.79**
t[22]	**0.7561**	**505.93**	**0.9851**	**489.63**	**0.8453**	**506.27**
t[23]	1	669.11	1	497.02	1	598.94

catalase



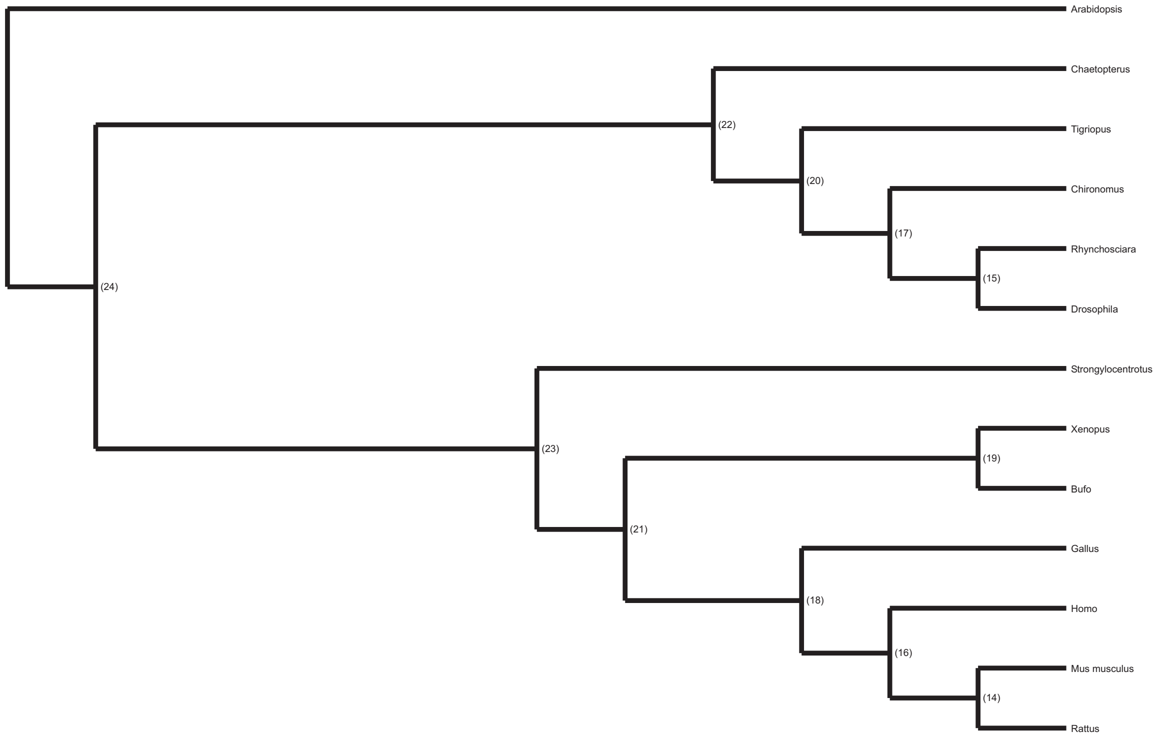
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[13]	0.0748	105.32	0.2612	167.53	0.067	49.415
t[14]	0.1178	165.92	0.3944	252.9	0.1889	139.22
t[15]	0.2505	352.84	0.5546	355.66	0.4482	330.37
t[16]	0.3039	428	0.6674	428	0.5806	428
t[17]	0.1434	201.94	0.0867	55.595	0.1098	80.971
t[18]	0.4977	700.91	0.7537	483.31	0.5663	417.46
t[19]	**0.752**	**1059.1**	**0.9262**	**593.95**	**0.7247**	**534.25**
t[20]	**0.7553**	**1063.7**	**0.9454**	**606.3**	**0.7762**	**572.18**
t[21]	0.2219	312.46	0.6765	433.85	0.2274	167.63
t[22]	0.9963	1403.1	0.9832	630.54	0.8755	645.37
t[23]	1	1408.4	1	641.29	1	737.16

EF-1



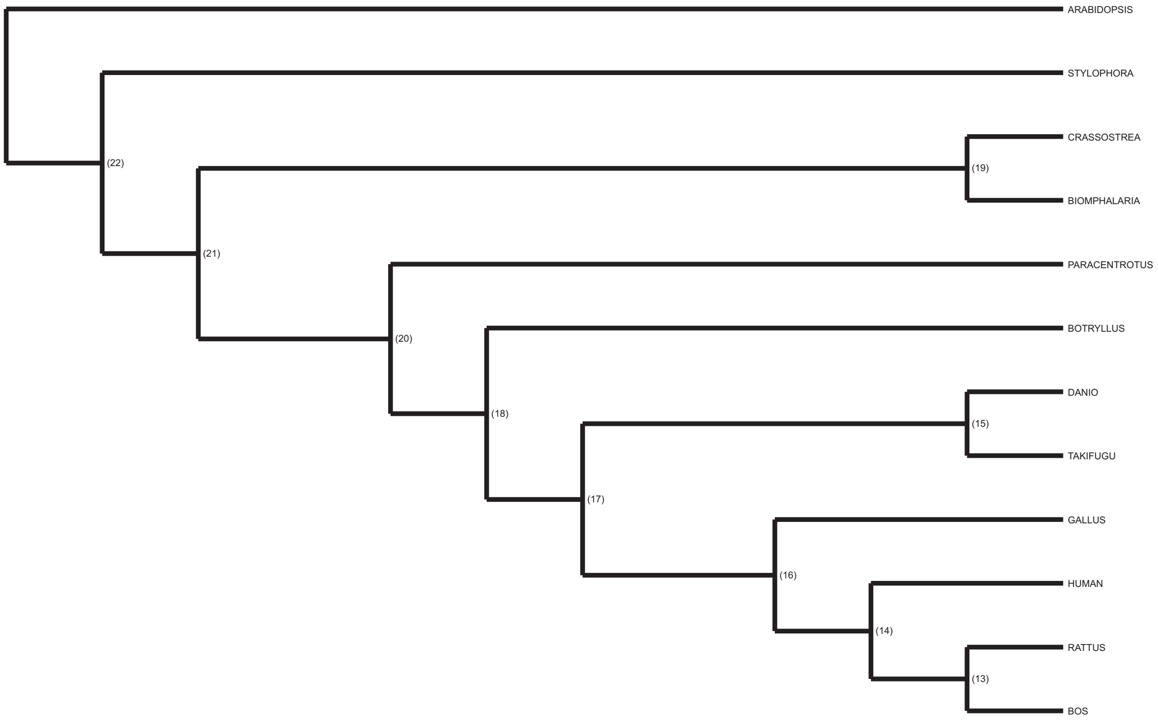
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[31]	0.195	167.85	0.3921	237.53	0.4914	264.65
t[32]	0.037	31.868	0.1589	96.244	0.0156	8.3886
t[33]	0.1992	171.5	0.6418	388.77	0.4208	226.62
t[34]	0.2431	209.29	0.3258	197.35	0.4062	218.77
t[35]	0.3144	270.65	0.543	328.95	0.6419	345.71
t[36]	0.2275	195.86	0.4686	283.9	0.5285	284.64
t[37]	0.2014	173.38	0.6805	412.25	0.572	308.07
t[38]	0.0712	61.303	0.5353	324.3	0.0611	32.88
t[39]	0.8008	689.29	0.9657	585.03	0.8464	455.85
t[40]	0.3992	343.59	0.5778	350.06	0.6299	339.26
t[41]	0.3775	324.99	0.6147	372.36	0.708	381.31
t[42]	0.2034	175.08	0.6978	422.71	0.6719	361.86
t[43]	0.2243	193.07	0.3826	231.81	0.4235	228.07
t[44]	0.8417	724.53	0.9733	589.6	0.8739	470.64
t[45]	0.5492	472.74	0.7346	445.04	0.815	438.91
t[46]	0.979	842.72	0.8623	522.4	0.9265	499
t[47]	0.3098	266.64	0.5398	327.02	0.5924	319.04
t[48]	0.4029	346.85	0.72	436.16	0.6783	365.3
t[49]	0.9904	852.52	0.979	593.09	0.951	512.17
t[50]	0.2376	204.53	0.7489	453.65	0.0467	25.166
t[51]	0.985	847.91	0.8944	541.8	0.9473	510.18
t[52]	0.4376	376.66	0.7989	483.96	0.9302	500.94
t[53]	0.992	853.87	0.9839	596.03	0.9641	519.21
t[54]	0.9874	849.91	0.919	556.7	0.9592	516.6
t[55]	0.9938	855.48	0.9884	598.77	0.9733	524.17
t[56]	**0.9918**	**853.76**	**0.982**	**594.89**	**0.972**	**523.49**
t[57]	**0.9962**	**857.51**	**0.9926**	**601.3**	**0.9772**	**526.27**
t[58]	0.9982	859.23	0.9982	604.68	0.9922	534.35
t[59]	1	860.8	1	605.8	1	538.56

histone H1



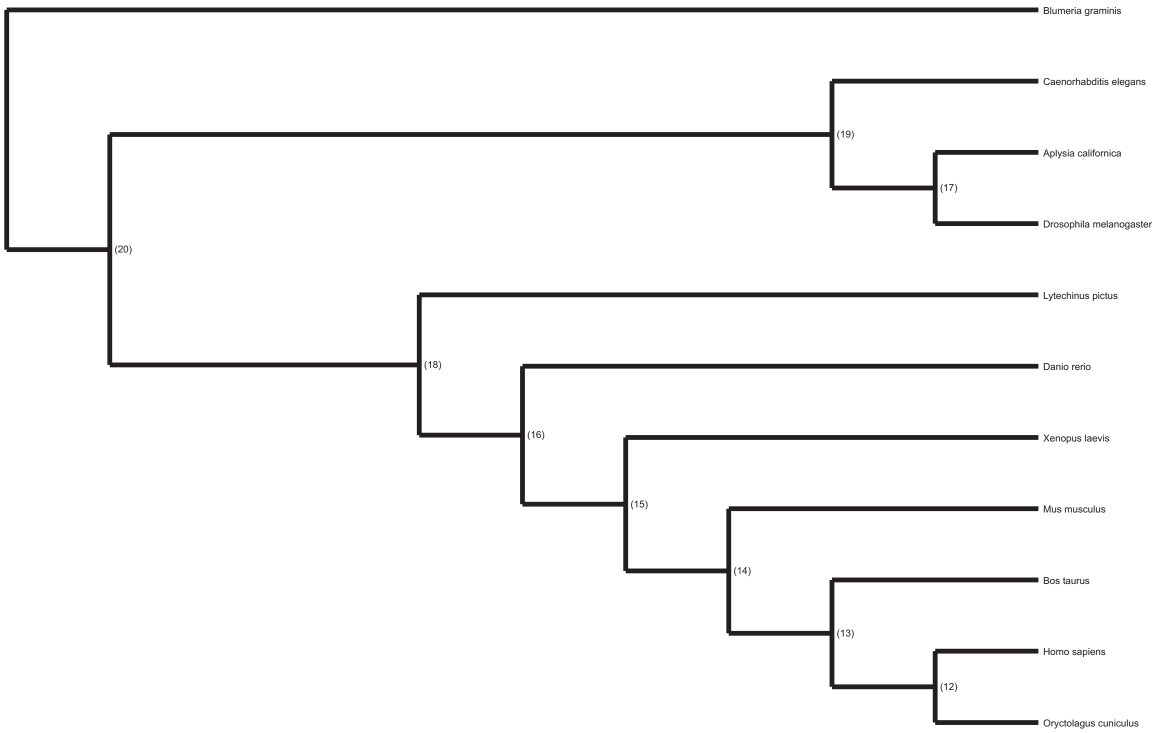
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[14]	0.6486	368.29	0.6297	355.46	0.4991	305.28
t[15]	0.3327	188.91	0.3773	212.99	0.5056	309.26
t[16]	0.6613	375.53	0.6665	376.22	0.5996	366.79
t[17]	0.4989	283.3	0.5967	336.79	0.6406	391.87
t[18]	0.6941	394.13	0.7291	411.55	0.6846	418.78
t[19]	0.7392	419.72	0.7294	411.71	0.6159	376.74
t[20]	0.5694	323.32	0.8523	481.06	0.7175	438.92
t[21]	0.7537	428	0.7583	428	0.6997	428
t[22]	0.5905	335.31	0.9195	519.02	0.8162	499.3
t[23]	**0.7839**	**445.12**	**0.966**	**545.24**	**0.8562**	**523.75**
t[24]	**0.7954**	**451.63**	**0.9881**	**557.74**	**0.9144**	**559.32**
t[25]	1	567.84	1	564.46	1	611.71

Hsp70



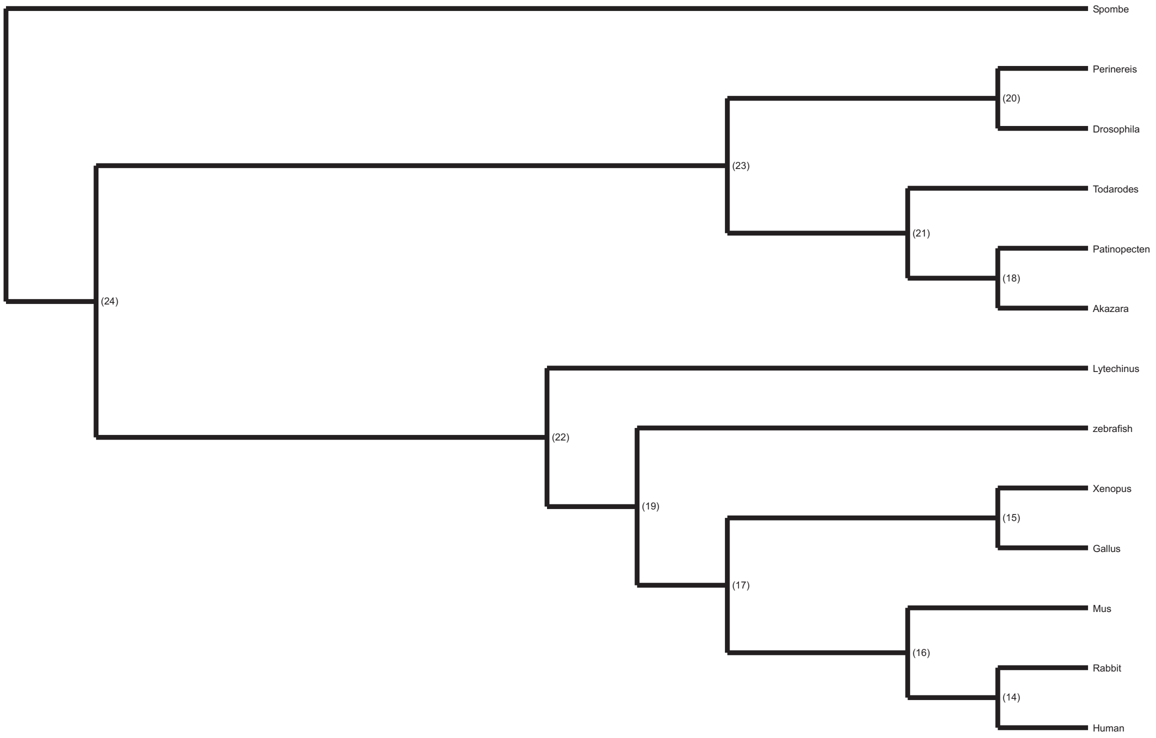
	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[13]	0.0373	82.782	0.2227	138.7	0.0965	75.16
t[14]	0.139	308.6	0.4834	301.08	0.3667	285.67
t[15]	0.1227	272.37	0.5137	319.97	0.3225	251.28
t[16]	0.1832	406.91	0.6429	400.44	0.4811	374.83
t[17]	0.1927	428	0.6871	428	0.5494	428
t[18]	0.3138	696.78	0.8658	539.29	0.6326	492.88
t[19]	0.3194	709.26	0.9312	580.02	0.6674	519.96
t[20]	**0.3311**	**735.19**	**0.9387**	**584.69**	**0.6931**	**539.95**
t[21]	**0.3348**	**743.56**	**0.9639**	**600.43**	**0.7504**	**584.6**
t[22]	0.4156	922.79	0.9815	611.4	0.8277	644.83
t[23]	1	2220.6	1	622.9	1	779.07

Pkc



	CLOCK		**EXP**		**OUP**	
	relative	absolute	relative	absolute	relative	absolute
t[12]	0.0479	24.981	0.0387	25.182	0.0382	23.185
t[13]	0.2588	134.91	0.1229	79.851	0.2961	179.54
t[14]	0.8101	422.35	0.5925	385.04	0.5448	330.35
t[15]	0.8176	426.29	0.6517	423.55	0.6819	413.47
t[16]	0.8209	428	0.6586	428	0.7059	428
t[17]	0.3719	193.89	0.3697	240.29	0.4417	267.81
t[18]	**0.9877**	**514.95**	**0.979**	**636.27**	**0.8791**	**533.04**
t[19]	0.4472	233.16	0.5415	351.94	0.6426	389.65
t[20]	**0.9962**	**519.39**	**0.9913**	**644.23**	**0.9155**	**555.14**
t[21]	1	521.36	1	649.9	1	606.35

troponin c



	CLOCK		EXP		OUP	
	relative	absolute	relative	absolute	relative	absolute
t[14]	0.3523	297.75	0.3623	312.58	0.429	289.9
t[15]	0.1636	138.24	0.1437	124	0.319	215.62
t[16]	0.3618	305.79	0.3833	330.67	0.4941	333.95
t[17]	0.4789	404.7	0.4703	405.76	0.5858	395.92
t[18]	0.0815	68.883	0.0606	52.252	0.1162	78.499
t[19]	0.5064	428	0.4961	428	0.6333	428
t[20]	0.9294	785.48	0.9695	836.44	0.6565	443.69
t[21]	0.4888	413.13	0.236	203.63	0.4326	292.33
t[22]	**0.6431**	**543.47**	**0.5369**	**463.18**	**0.6729**	**454.73**
t[23]	0.9587	810.27	0.9858	850.5	0.8707	588.43
t[24]	**0.9883**	**835.24**	**0.9948**	**858.27**	**0.9444**	**638.23**
t[25]	1	845.14	1	862.75	1	675.82