

# Relative-rate tests *versus* paleontological divergence data for diatoms and vertebrates

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Molecular divergence rates between taxa can be estimated through two independent methods, namely the relative-rate test and by using divergence dates derived from the fossil record. These two approaches are employed here to elucidate the existence of a regularly ticking ribosomal DNA clock in diatoms and in the vertebrate clade composed of the Actinistia and Tetrapoda. The results obtained from the relative-rate test and the paleontological information are contradictory. The former suggests that the vertebrates diverged at a significantly higher molecular rate than diatoms while the latter indicates that the diatom lineages evolved at about a speed 4.5 fold higher than the Actinistia-Tetrapoda clade. Possible causes of this paradox are discussed. It is concluded that each of these two approaches suffers from weaknesses of its own and that the absolute divergence rates are more reliable than those derived from the relative-rate test.

**Key words:** molecular evolution, divergence time, relative-rate test, diatoms, Actinistia, Tetrapoda, ribosomal RNA.

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## Introduction

This study is dedicated to my mentor, Antoni Hoffman (formerly at the Lamont-Doherty Geological Observatory) who suggested diatoms as an interesting group for the study of rates of evolution. Previous publications (Sorhannus *et al.* 1988, 1991; Fenster *et al.* 1989, 1991; Sorhannus & Fenster 1989; Sorhannus 1990a, b; Fenster & Sorhannus 1991) addressed morphological rates of evolution. The present analysis of molecular rates is a continuation of these studies.

The existence of a universal molecular clock (e.g. Zuckerkandl & Pauling 1965; Wilson *et al.* 1977; Ochman & Wilson 1987), which postulates an approximately constant nucleotide substitution rate per unit time in different groups of organisms, continues to be widely discussed (e.g. Langley & Fitch 1974; Gillespie 1986; Scherer 1990). Evidence that has accumulated for and against the rate constancy hypothesis (e.g. Wu & Li 1985; Britten 1986; Li *et al.* 1987; Easteal 1990; O'hUigin & Li 1992) is based on two approaches to studying molecular rates of evolution, namely the relative-rate test (Sarich & Wilson 1973; Wilson *et al.* 1977) and divergence dates derived from the fossil record. The latter technique relies on comparisons of the number of mutations fixed in various lineages since they diverged from the last common ancestor whereas the former is a method that compares molecular distances between each of the ingroup lineages to the outgroup. The relative-rate test is thus independent of the paleontological record. When both of the previously described approaches are used, the results of the analyses should be confirmatory in order to be reliable.

This study addresses the ribosomal DNA clock in two distantly related taxa, namely the diatoms and the Actinistia-Tetrapoda clade (Maisey 1988). Conclusions derived from the two independent methods have been compared. It turns out that the two approaches yielded contradictory results. Possible causes of this inconsistency are discussed.

## Material and methods

The entire nuclear small subunit ribosomal DNA gene (18srDNA) has been sequenced for representatives of a number of diatom and vertebrate taxa which all have relatively well documented fossil records. All the diatom sequences (*Cylindrotheca closterium*, *Nitzschia apiculata*, *Rhizosolenia setigera*, and *Stephanopyxis cf. broschii*) used in this investigation are obtained from Bhattacharya *et al.* (1992) and the nucleotide data of *Alligator mississippiensis*, *Heterodon platyrhinos*, *Mus musculus*, *Oryctolagus cuniculus* and *Latimeria chalumnae* have been published by Hedges *et al.* (1990), Hedges *et al.* (1990), Raynal *et al.* (1984), Rairkar *et al.* (1988) and Stock *et al.* (1991), respectively. The *Paramecium tetraurelia* sequence is from Sogin & Elwood (1986).

The alignment of the nucleotide sequences (see appendix) was performed 'by eye' on a personal computer, using a program written by Herve Philippe who also wrote the rest of the data management software (Version 1.00, 1991, Laboratoire de Biologie Cellulaire 4, Universite de Paris, Orsay). Nucleotides that I considered ambiguously aligned or/and that were characterized by unknown bases were eliminated from the analysis (661 characters; see appendix). The previous software package was also employed to calculate the corrected number of nucleotide differences (Kimura 1980) from 1243 characters (see Tab. 1). The distance matrix was

Tab. 1. Number of substitutions per nucleotide estimated by Kimura's (1980) two-parameter method.

Oryctolagus									
<i>Mus</i>	0.00								
Heterodon	0.03	0.03							
Alligator	0.03	0.03	0.01						
Latimeria	0.05	0.05	0.04	0.03					
Nitzschia	0.22	0.23	0.23	0.22	0.22				
Rhizosolenia	0.24	0.24	0.24	0.23	0.23	0.08			
<i>Stephanopyxis</i>	0.24	0.24	0.24	0.23	0.23	0.07	0.08		
<i>Cylindrotheca</i>	0.22	0.22	0.23	0.22	0.22	0.01	0.08	0.08	
Paramecium	0.27	0.27	0.27	0.26	0.27	0.20	0.23	0.24	0.19

Tab. 2. Absolute divergence rates within Bacillariophyceae and Actinistia-Tetrapoda. The distance values represent number of nucleotide substitutions/ 100 nucleotides. Time (divergence time) in millions of years. Rate in number of nucleotide substitutions/100 nucleotides/million years. The divergence times are taken from the references indicated:

Taxa	Distance	Time	Rate	Reference
<b>Bacillariophyceae:</b>				
<i>Nitzschia</i> - <i>Cylindrotheca</i>	1.3	40	0.032	Simmons 1979
<i>Rhizosolenia</i> - <i>Stephanopyxis</i>	7.8	180	0.043	Tappan 1980
<i>Rhizosolenia</i> - <i>Nitzschia</i>	8.0	200	0.040	Simmons 1972
<i>Rhizosolenia</i> - <i>Cylindrotheca</i>	7.9	200	0.040	Simmons 1972
<i>Stephanopyxis</i> - <i>Nitzschia</i>	7.2	200	0.036	Simmons 1972
<i>Stephanopyxis</i> - <i>Cylindrotheca</i>	7.7	200	0.038	Simmons 1972
<b>Actinistia-Tetrapoda:</b>				
<i>Mus</i> - <i>Oryctolagus</i>	0.2	60	0.003	Benton 1990
Heterodon - Alligator	1.2	260	0.005	Benton 1990
Heterodon - <i>Mus</i>	3.3	1310	0.011	Benton 1990
Heterodon - <i>Oryctolagus</i>	3.2	310	0.010	Benton 1990
Alligator - <i>Mus</i>	2.6	310	0.008	Benton 1990
Alligator - <i>Oryctolagus</i>	2.5	310	0.008	Benton 1990
Latimeria - <i>Mus</i>	4.8	405	0.012	Benton 1990
Latimeria - <i>Oryctolagus</i>	4.5	405	0.011	Benton 1990
Latimeria - Alligator	2.8	405	0.007	Benton 1990
Latimeria - Heterodon	3.6	405	0.009	Benton 1990

then used to construct an evolutionary tree through the neighbor joining (NJ) method (Saitou & Nei 1987) and also for the molecular rate analysis (Li & Bousquet 1992). I calculated absolute divergence rates by dividing the distance values between the compared taxa by the estimated time period they have evolved as independent lineages (divergence time) (see Tab. 2.)

## Results

The analyses of the distance matrix by the neighbor joining method (Saitou & Nei 1987) yielded the tree shown in Fig. 1. Under the assumption that the dendrogram shown in Fig. 1 is correct, I tested the null hypothesis of rate equality between the subclades including Bacillariophyceae and the Actinistia-Tetrapoda. For this purpose the extended relative-rate test of Li & Bousquet (1992) was used. This procedure uses the standardized normal distribution to test whether the number of nucleotide substitutions (assumed to follow a Poisson process) are the same in the two subclades (diatoms and Actinistia-Tetrapoda). The result indicates that the small subunit ribosomal DNA gene evolved at a significantly faster rate in the vertebrate clade than in the diatoms since the null hypothesis of no rate differences was rejected (test statistics was 3.58;  $P = 0.001$ ).

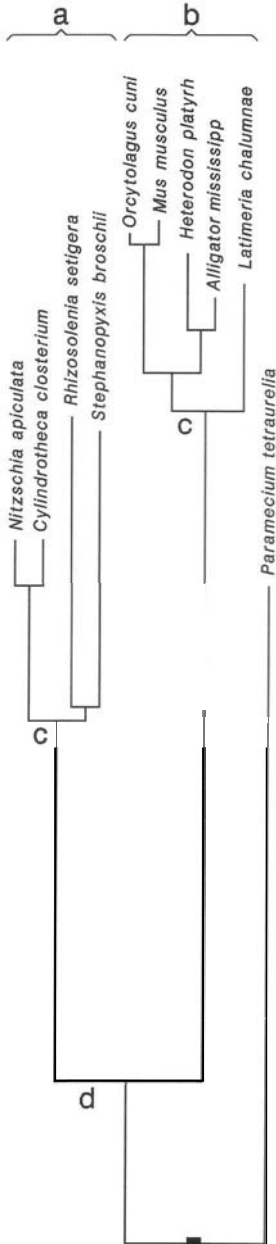
To obtain the absolute divergence rates, the molecular distance values were divided by the divergence times as indicated in Tab. 2. The average divergence rate within diatoms is 0.038 nucleotide substitutions/100 nucleotides/ million years and within the Actinistia-Tetrapoda the corresponding value is 0.008. The results of these calculations clearly show that the small subunit ribosomal DNA gene has changed at about  $0.038/0.008 = 4.75$  times faster in the diatom taxa than in the considered vertebrate lineages.

## Discussion

The results obtained from the relative-rate test and the calculations of absolute rDNA divergence rates are contradictory. The former procedure shows that the vertebrate taxa have diverged at a significantly faster rate than diatoms while the latter approach indicates the opposite. Exactly the same pattern has been observed in an earlier study where partial sequences of the large subunit rRNA were used (Philippe *et al.*, unpublished manuscript), suggesting that the results obtained here are not likely to be a chance phenomenon.

The relative-rate test

A problem, which will confound the relative-rate analysis, is the failure of the proposed relative-rate test to differentiate substitution rate differences 'caused' by the 'stems' leading to the monophyletic subtaxa from those actually existing between the compared subgroups (i.e. *Bacillariophyceae* and *Actinistia-Tetrapoda* subclades). This difficulty arises from the fact



that the analytical technique compares the mean substitution rates in both lineages, that is by averaging the 'stem' and subclade rates (Fig. 1.). Consequently, in this investigation as well as in other similar studies, it is difficult to specify whether the significant rate discrepancy observed between the two taxonomic groups is a result of rate differences between the two subclades or just the 'stems' or by both of these factors.

From a taxonomic point of view, the 'stems' of the phylogenetic tree are not part of the monophyletic groups that are compared since the diatoms and *Actinistia-Tetrapoda* are defined by the nodes from which they radiated and not by the node that unites **all** the lineages within the entire monophyletic group (Fig. 1). This reasoning forms the foundation for splitting up the evolutionary lineages into 'stems' and subclades and also for their separate analysis. Consequently, the result obtained using the proposed test pertains to variation in nucleotide substitution rates within the monophyletic group comprising the diatoms and vertebrates but not to potential discrepancies that may exist between the two subtaxa (i.e. excluding the 'stems').

Despite the inappropriateness of including 'stems', the extended relative-rate test should perform rather well, particularly in situations where the internodes leading to the subclades are short in relation to the terminal branch lengths. As these internodes start to increase in

Fig. 1. A dendrogram derived through the neighbor joining procedure from corrected nucleotide substitutions estimated by the two parameter method of Kimura (1980). Horizontal distances are proportional to evolutionary distances whereas the vertical distances are arbitrary. Subtaxon (a) represents Bacillariophyceae and subtaxon (b) includes Actinistia-Tetrapoda. The 'stems' are located between points (c) and (d).

length relative to the terminal branches, the test will become less sensitive in discerning substitution rate differences between 'stems' from possible rate variation between the compared subtaxa. This problem pertains to any relative-rate test which compares distance values of several terminal taxa within two or more subgroups to an outgroup. Relative-rate analyses between two evolutionary lineages (only 2 terminals are compared) within a single monophyletic group does not suffer from this difficulty since in this situation the 2 lineages of the ingroup share the same immediate 'stem'. That is, however, not the case in this study.

In conclusion, the result obtained through the extended relative-rate test (Li & Bousquet 1992) may be largely affected by evolutionary rate differences in the long 'stems' that are connected to both Actinistia-Tetrapoda and diatoms (Fig. 1) and not by potential speed discrepancies between the two subclades. Since the ultimate goal of this study is to elucidate possible rate variation between the subtaxa, the acquired result is questionable.

### Paleontological information

In order to elucidate the previously discussed problem, the nodes from which the lineages within Bacillariophyceae and Vertebrata radiated should be dated using information from the fossil record. In this way possible rate variations that may exist between the 'stems' will be eliminated. The problem with this approach is the well known fact that the fossil record usually underestimates divergence dates between different lineages which gives rise to overestimated nucleotide substitution rates. In fact, the approximate 4.5 fold higher molecular divergence rate in diatoms could 'be easily explained by the diatom dates being highly underestimated, particularly due to the fact that most of the separation events are from the time of first appearance of diatoms in the fossil record. There is no firm agreement on the time of the origin of Bacillariophyceae since this could have taken place anywhere between the Precambrian and early Jurassic (e.g., Tappan & Loeblich 1971; Ross & Simms 1973; Loeblich 1974; Simmons 1979; Round & Crawford 1981). The geologic record of diatoms extends back to at least the early Jurassic as indicated by the recovery of *Pyxidicula* species in the Toarcian stage (Simmons 1979; Harwood & Gersonde 1990). These forms may, according to Simmons (1979), represent an ancestral stock from which the rest of the diatoms radiated with an explosive increase in diversity and abundance in Late Cretaceous and early Tertiary (Harwood & Gersonde 1990). The abrupt appearance of diatoms in early Jurassic has led some workers to believe that these organisms have a prolonged pre-Jurassic evolutionary history as naked, siliceous scale-covered or weakly silicified cells referred to as the 'pre-diatom' stage by Round and Crawford (1981). Although evidence for such a stage is still lacking, it is a possible scenario of early

diatom evolution. In spite of this, the divergence dates used in this study are most likely underestimated.

The question is by how much are the dates underestimated and what effects will these underestimations have on the analysis? If one considers the *C. closterium* – *N. apiculata* split, which is 'known' to be rather recent (about 40 Myr [Simmons 1979]) and assumes that these two lineages evolved at the same average rate as the considered vertebrates (a molecular clock assumption), their divergence times would have been about  $1.3/0.008 = 162.5$  million years ago (see Tab. 2 for values). This calculation also presupposes that the paleontological record of vertebrates is fairly accurate. Thus, the result of the estimation is that the divergence date of 40 million years is underestimated by about 120 million years. In addition, one would also have to assume that these two raphid pennate genera occurred at a time when, according to the fossil record, no representatives of this diatom group existed (appeared about 65 Myr ago). Even if the considered divergence event was underestimated by for instance 25 million years, the molecular rates between Actinistia-Tetrapoda and diatoms would still differ by a factor of about 2.5. On the same assumptions, the other observed diatom divergence dates (180 and 200 Myr) would be pushed back to about  $7.7/0.008 = 962.5$  million years ago (see Tab. 2 for values), that is, an underestimation of about 700 million years. This is a time when no diatoms existed according to the fossil record but it is still conceivable that these unicellular organisms occurred in the Precambrian but failed to leave a fossil record.

How likely is it that early diatoms left no fossil record? There are taphonomic indications that this is an unlikely event since siliceous unicellular organisms, such as radiolarians, are well preserved in pre-Jurassic deposits. Since diatoms (the focal group) and radiolarians (the taphonomic control group) have similar siliceous shells and both taxa are planktonic, the focal group should also have left a paleontological record if it was present at that time. According to Marshall (1990 and references therein) the absence of the focal group but the presence of the control group in the fossil record should be interpreted as if the focal group did not exist at that time.

## Conclusion

The molecular divergence rates obtained from the paleontological information are probably more accurate than those acquired through the extended relative-rate test. The reason is that the 'stems' leading to the compared taxonomic groups are rather long, thus, making the relative-rate test less sensitive to detecting interclade rate variation. In addition, an underestimation of the diatom divergence dates by as much as 120 million and 700 million years is rather unlikely.

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## Appendix

Alignment of 1904 nucleotides of the 18s rRNA. Only sequences that differ from *Oryctolagus cuniculus* are shown. A dot (.) signifies a nucleotide that is identical to the one observed in *O. cuniculus* while a dash (-) indicates a deletion and a question mark (?) is an unknown base. The regions that were eliminated from both the phylogenetic and rate analyses due to uncertain alignment are indicated by 'boxes'. All the sites that show unknown bases (?) were also excluded from the phylogenetic and rate analyses. The program package PHYLIP Version 3.3 (Felsenstein 1990) was used to print out the aligned sequences.

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Oryctolagus  UACCUGGWG AUCCUGCCAG UAG-CAUUG CWGUCUCA  AGAUUAAGCC AUGCAUGUCU
Mus          .....
Heterodon   -----?..... ?.....???. ..?.....
Alligator   ??.....?.....?.....
Latimeria   ??.....?.....?.....
Nitzschia   A..... ..U...C...C.....
Rhizosolenia A..... ..U...C...C..... ..G.
Stephanopyxis A..... ..UG...C...C.CA.UU..... ..G.
Cylindrotheca A..... ..J...C...C.....
Paramecium   --U..... A..U..... ..U.....

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Oryctolagus  AAGUACGCAC GGCCGGUACA GUGAAACUGC GAAUGGCUCA  UUAAAUCAGU UAUGGUUCCU
Mus          .....
Heterodon   .....A... ..G..UG... ..??? ? ..???..
Alligator   .....A... ..UGC..... ..??? ? ..???..
Latimeria   .....AA... ..UGC..... ..?? ..?...?..
Nitzschia   .....UAA.U AU.U--..U U..... ..C..... ..U..... ..A...UA.
Rhizosolenia .....UAA.U AUUU--..U ..A..... ..AU.AA..UAC
Stephanopyxis C...UUAA.A AUUU-A.UU- ..G...U ..... ..U..... ..A...UA.
Cylindrotheca .....UAA.U AU.U--..U U..... ..C..... ..U..... ..A...UA.
Paramecium   .....UAA.U A.UAUA--.. ..A..... ..A...UA.

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Oryctolagus  UUGGUCGCUC GCUCCUCUCC UACUUGG-AU AACUGUGGUA AUUCUAGAGC UAAUACAUGC
Mus          .....
Heterodon   ???..... --C.A-.CGU .....C..... ?? ? ..
Alligator   .? ? .. --.AA.CGU ..... ? ? ..
Latimeria   .??A..... --.AA-CGU .....C.....
Nitzschia   ..A.A.-- -UC...A..... ..A..... ..C..A...
Rhizosolenia A..A.AA-- -UC...A..... ..C..A...
Stephanopyxis ..A.A.-- -U...CU.A..... ..C..A...
Cylindrotheca ..A.A.-- -UC...A..... ..C..A...
Paramecium   ..A.A.-- --UGCAAU .....A;..... ..C.....

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Oryctolagus  CGACGG-CGC UGACUCCC-U UUGUGUGGG- --AUGCGUGC AUUUAUCAGA UCAA--AACC
Mus          .....G... ..C...C...CCC.G...G GG.....
Heterodon   .....AG... ..CU...- -G GG..... C.....
Alligator   .....AG... ..CU?...- -G GG..?..? ..C.....
Latimeria   .....AG... ..U...- -G GG..... C.....
Nitzschia   GU-.AAUAC. CW-CUG-. -G G.--UAGUA U...U...UG.....
Rhizosolenia W-AAAUGC. GA--CUG.U. --GCA.AAG G.--AGCA U...U...AG...G..
Stephanopyxis AUCAA.G.C. GA--CUU.- -GAA.G G.--UU..AU U.A--U...U.CAA...
Cylindrotheca GU-.AAUAC. CUU-CUG-. -G G.--UAGUA U...U...UG.....
Paramecium   --G.AAAUA. C.GACG.A.- -A.AAU GGUA..A.UU ...AG-AUU-.A.CC..U.A

```

<i>Oryctolagus</i>	AACCCGGUCA	GCC--UCCCC	GCCGGCCGGG	GGGG---UG	GGGCGGCGGC	UUUGGUGACU
<i>Mus</i>	.....G.	..UCCCU...	.G.UC.G.CC	...GUCGG.	C.C.....	..-.....
<i>Heterodon</i>	...GG.C.??	C..GGC.---	-----	-----	-----	?......
<i>Alligator</i>	..GG.C..G	C..GGC.---	-----	-----	-----	?......
<i>Latimeria</i>	..U.GC.GUC	CG.C.CGG..	-----	-----	-----	.....
<i>Nitzschia</i>	..UU.CU---	-----	-----	-----	UC.G--GU.A	.G.....U.
<i>Rhizosolenia</i>	..U..CU---	---CU..GGA	-----	-----	-----GUAU	.....A.
<i>Stephanopyxis</i>	..U..U---	-----	-----	..CU.U	UA.A...UAG	.G.....A.
<i>Cylindrotheca</i>	....CU---	-----	-----	..UC.	..GU.A	.G.....U.
<i>Paramecium</i>	C.-----	-----	-----	-----	-----	..GUG

<i>Oryctolagus</i>	CUAGUAUACC	UCGGGCCG-A	UCGCA-GCCC	UCCGUGGCGG	CGACGACCCA	WCGAACGUC
<i>Mus</i>	.....	.....	.....C....	C.....	.....	.....
<i>Heterodon</i>	.....	.....	.....	C.....	.....G..	.....
<i>Alligator</i>	.....	?.....	.....?	C?.....	.....G..	.....U..
<i>Latimeria</i>	.....	.....	.....C.U.	..-.....	.....W.C	.....U..
<i>Nitzschia</i>	.AUA...G.	.U-.G...	...U.G.U	.U--GC-...	..U.GAU..	...A.GUU..
<i>Rhizosolenia</i>	.AUA...U	.U-.G...	...U.G.U	CUACGC-...	...AGAU..	...A.GUU..
<i>Stephanopyxis</i>	.AU...GU	.U-.G...	...U.G.U	C.AUGC-...	...AGAU..	.AUA.GUU..
<i>Cylindrotheca</i>	.AUA...G.	.U-.G...	...U.G.U	.U--GC-...	..U.GAU..	...A.GUU..
<i>Paramecium</i>	AAUC...GUA	A.UUA--.CU	GAC.GC.UG-	.AUA-C.U.	UA.--.U.AU	.CA-.GUU..

<i>Oryctolagus</i>	UGCCCUAUCA	ACUUUCGAUG	GUAGUCGCCG	UGCCUACCAU	GGUGACCACG	GGUGACGGGG
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	.....	.....	..C.UU.U.	.....	.....	...A.....
<i>Alligator</i>	?.....	.....?	..C.UU.U.	.....	.....?	...A?.....
<i>Latimeria</i>	.....	.....	..C.UU.U.	.....	.....	...A.....
<i>Nitzschia</i>	.....	G...G...	...GGUAW	G.../...	..CUUUA..	...A...A
<i>Rhizosolenia</i>	.....U.	GA...G...	.CU..GUAUU	G.A.AG...	.UCUUUG.U	...A...A.
<i>Stephanopyxis</i>	.....	G...G...	...GGUAW	G.....	..CAUUA..	...A...A
<i>Cylindrotheca</i>	.....	G...G...	...GGUAW	G.....	..CUUUA..	...A...A
<i>Paramecium</i>	.....	G.....	...GUAW	G.A.....	..CAGU....	...A...A.

<i>Oryctolagus</i>	AAUCAGGGW	CGAUUCCGGA	GAGGGAGCCU	GAGAAACGGC	UACCACAUC	AAGGAAGGCA
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	.....	.....	.....	.....	.....	.....?
<i>Alligator</i>	..?..?..?	.....?..?	.....?	.....	.....	.....?..?..?
<i>Latimeria</i>	.....	.....?	.....	.....	.....	.....
<i>Nitzschia</i>	..U.....	U.....	.....	..G.....	.....	.....
<i>Rhizosolenia</i>	..U.....C	.....	.....	..G.....	.....	.....
<i>Stephanopyxis</i>	G.....	U..C.....	.....	..G.....	.....	.....
<i>Cylindrotheca</i>	..U.....	U.....	.....	..G.....	.....	.....
<i>Paramecium</i>	..U.....	.....	.....	.....	.....U	.....

<i>Oryctolagus</i>	GCAGGCGCGC	AAAWACCCA	CUCCCGACCC	GGGGAGGUAG	UGACGAAAAA	UAACAAUACA
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	.....?	??.....	.....?	.....??...	..?..?..	.....
<i>Alligator</i>	..?..?..?	??.....	.....??	.....??...	..?..?..	.....
<i>Latimeria</i>	.....	??.....	.....G.	.....?	.....	.....
<i>Nitzschia</i>	.....U	.....	A..U..A.	A.....	...A.U..	.....G.C
<i>Rhizosolenia</i>	.....U	.....	A..U..A.	A.....	...A.U..	.....G.C
<i>Stephanopyxis</i>	.....	.....	A..U..A.	A.....	...W..	.....G.C
<i>Cylindrotheca</i>	.....U	.....	A..U..A.	A.....	...A.U..	.....G.C
<i>Paramecium</i>	.....U	.....	A.....W.	.....	...A.G...	..G...CU.G

<i>Oryctolagus</i>	GGACUCUUUC	GAGGCCUGU	AAUUGGAAUG	AGUCCACUW	AAAUCCUUUA	ACGAGGAUCC
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	.....	.....??	.....?	.....	.....	.....??
<i>Alligator</i>	.....???	.....	.....?	.....A	.....	.....??
<i>Latimeria</i>	.....	.....	.....	.....A	.....	.....U
<i>Nitzschia</i>	.G.CU.G-	U..U.UG.C	.....	..AA..A..	..C..C..	U...U...A
<i>Rhizosolenia</i>	.GUCUAC-	A..AU.UG.C	.....	..AA..A..	..C..C..	U...U...A
<i>Stephanopyxis</i>	.G.CU.-	C...U.UG.C	.....	..AA.GA.G.	..CA.CC..	U...C.A
<i>Cylindrotheca</i>	.G.CU.G-	U...U.UG.C	.....	..AA..A..	..C..C..	U...U...A
<i>Paramecium</i>	U.CG...G	--C.UUAC.G	G...C...	..AUA.G.CC	.....G.A.	...UU.A.A

<i>Oryctolagus</i>	AWGGAGGGC	AAGUCUGGUC	GCAGCAGCCG	CGGUAAUUC	AGCUCCAAUA	GCGUAUAWA
<i>Mus</i>	.....	.....G	C.....	.....	.....	.....
<i>Heterodon</i>	.....?	.....???????	.....?????????	.....???????	.....?	.....
<i>Alligator</i>	.....?	.....???????	.....?????????	.....?????????	.....?????????	.....?????????
<i>Latimeria</i>	.....	.....G	C.....	.....??	.....G	.....
<i>Nitzschia</i>	.....	.....G	C.....	.....	.....	.....
<i>Rhizosolenia</i>	.....	.....G	C.....	.....	.....	.....
<i>Stephanopyxis</i>	.....	.....G	C.....	.....	.....	.....
<i>Cylindrotheca</i>	.....	.....G	C.....	.....	.....	.....
<i>Paramecium</i>	.....	.....G	C.....	.....	.....	.....C.U

<i>Oryctolagus</i>	AAGWGCUGC	AGWAAAAAG	CUCGUAGWG	GAUCUUGUGG	AGGGUGCGUA	GCGGGCG---
<i>Mus</i>	.....	.....	.....	.....	.....G.	.....GGC
<i>Heterodon</i>	.....	.....??	.....???	.....	.....G.	.....A..UGG.
<i>Alligator</i>	???????????	???????????	?.?.?.???	..?.?.-..	.....G.	U..A..??G.
<i>Latimeria</i>	.....	.....?	.....?	.....	.....G.	U..A..UGGC
<i>Nitzschia</i>	.....U..	.....	.....	..U.-...	UU.UC.UC-	...C.UGGU
<i>Rhizosolenia</i>	.....U..	.....	.....	A..U.-C..	U...A...A-	U...U.CAUC
<i>Stephanopyxis</i>	.....U..	.....	.....	A..U.-C..	C...A..AA-	C...U.CUUC
<i>Cylindrotheca</i>	.....U..	.....	.....	..U.-...	-U.UACUU--	...CG.CCC
<i>Paramecium</i>	.....u..	.....	.....	A..U.-C.-A	-UCA.UUAC	UA.UUG.CUC

<i>Oryctolagus</i>	-GUCCGCCG	GAGGCAGCC	ACCGCCGUC	CCC GCC----	--CCUUGCCU	CUCGGCGCCC
<i>Mus</i>	G.....	.....U.	.....	.....	.....	.....
<i>Heterodon</i>	.....	.....G	.....??	..A	.....	.....U..U.
<i>Alligator</i>	.....	.....?..CGU	.....?	..?	.....C.-U..	.....?
<i>Latimeria</i>	G.....	.....CGU	.....U..	..A	.....	.....U...U.
<i>Nitzschia</i>	AC.UA.UGC.	.GU..UU..A	G.G..G.C--	.....	AU.....	.....U.GAA
<i>Rhizosolenia</i>	CU..ACUG.U	UG.AACCUGA	UUGU.U.CCG	.....	AU..A..---	---AGAGAA
<i>Stephanopyxis</i>	---ACUU.G	UGUUG.UA.U	UGU.UUGUCU	UG.....	AU.....	---GAGAG
<i>Cylindrotheca</i>	GUCA.UAU.U	..U.GAGCUU	G.U.AAGUCG	.....	..C AU.....	---U.GAU
<i>Paramecium</i>	UUCGUCAGUU	AGUUAUUUA	UU.U..GUCU	-----ACAA	UC...UUG-	-----

<i>Oryctolagus</i>	CCUCGAUGC	CUUAGCUGAG	UGUCCCGCG	GGCCCGAAGC	GWUACUWG	AAAAAAWAG
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	..C....	.....A....	.....U..G?	??..-.....	???	.....
<i>Alligator</i>	..U.....	.....?A.??	.....U.G?	..U.....	.....	.....
<i>Latimeria</i>	..U.....	.....A....	.....U.G.	..U.....	.....	.....
<i>Nitzschia</i>	..GUG..GC	A...G.UGU	C..G.A.G.	AUG..C.-U.	A.....G..	.....
<i>Rhizosolenia</i>	..U.AUC..GC	A...AG.UGU	C.GGUU.GCA	AU.UUC.-U.	U.....G..	.....
<i>Stephanopyxis</i>	..GUG..GC	A...AG.UGU	C.GG.G.G.	AU..C.-U.	.....G..	..G.....
<i>Cylindrotheca</i>	..GUG..GC	A...AG.UGU	C..G.A.G.	AUG..C.-U.	.....G..	.....
<i>Paramecium</i>	-----CGC	U...G.-UU	GCAG.U.G.C	.AGUA..C-A	A.....C..	.....

*Oryctolagus* AGUGWCAAA GCAGGCCCGA GCCGCCUGGA UACCGCAGCU AGGAAUAAUG GAAUAGGACC  
*Mus* .....  
*Heterodon* .....--CG .U...G.A. ...UC..... U  
*Alligator* .....--UG .U...G.A. ...UC..... U  
*Latimeria* .....--CG .U...U... ..W..... U  
*Nitzschia* .....W A U ...-U. A. .UGUU...A U.....A AG.....  
*Rhizosolenia* .....UUAU ....-U. A. .UAUU...A U.....A AG.....U  
*Stephanopyxis* .....UUAU ....-..A. .AUU...A U.....A AG.....  
*Cylindrotheca* .....W A U ...-U. A. .UAUU...A U.....A .G.....  
*Paramecium* .....C.G .....U.UUC ..--.G.A. ...AUU...A U.....U

*Oryctolagus* GCGGWCUAU UUUGUUGGUU UUCGGAAACUG AGGCCAUGAU UAAGAGGGAC GGCCGGGGGC  
*Mus* .....  
*Heterodon* C..... C. G?..... -?.?.????  
*Alligator* C..... G..... -?.?.???  
*Latimeria* C..... G..... ?  
*Nitzschia* UU...A..... .G.C.C.-A ...UA..... .A..... A.UU.U...U  
*Rhizosolenia* AU...A..... .GU.C.C.-A U...UA..... .U..... A.UU.....U  
*Stephanopyxis* UU...A..... .G.C.U.-A ...UA..... .U..... A.UU.....U  
*Cylindrotheca* UU...A..... .G.C.C.-A ...UA..... .U..... A.UU.....U  
*Paramecium* UG.....-C. ....A...CU.. ..UA..... .U..... A.AU.....

*Oryctolagus* AWCGUAWG GCCCGUAGA GGUGAAAUUC WGGACCJGGC GCAAGACGGG CCAGAGCGAA  
*Mus* .....  
*Heterodon* ?..... U..... ?..... AC.....  
*Alligator* ..... U..... A.....  
*Latimeria* ?..... U..... A.A.....  
*Nitzschia* .....C .AUU.UC... ..UUUUU .G..... A. .U.CU.....  
*Rhizosolenia* .....C .AUU.UC... ..WUAU .G..... A. .U.CU.....  
*Stephanopyxis* .....C AAUU.UC... ..UUUCU .G..... A. .U.CU.....  
*Cylindrotheca* .....U .AUU.UC... ..UWU .A..... A. .U.CU.....  
*Paramecium* ...A.....U AAUU.UC... ..UUUAU UA..... UA. .UUAU.....

*Oryctolagus* AGCAUUUGCC AAGAAUGUW UCAUUAAUCA AGAACGAAAG UCGGAGGUUC GAAGACGAUC  
*Mus* .....  
*Heterodon* .....?..... ?..... ?.....  
*Alligator* ..... ?..... ?.....  
*Latimeria* .....  
*Nitzschia* .....A. .G..... .UA.G.A. ....U...U  
*Rhizosolenia* .....A. .G..... .UAAG.A. ....U...U  
*Stephanopyxis* .....A. .G..... .U.G.A. ....U...U  
*Cylindrotheca* .....A. .G..... .UA.G.A. ....U...U  
*Paramecium* .....G..... .UA.G.A. A.....

*Oryctolagus* AGAUACCGUC GUAGWCCGA CCAUAAACGA UGCCGACUGG CGAUGCGGCG GCGUUAUUCC  
*Mus* .....  
*Heterodon* .....A. .C..... ?.....  
*Alligator* .....A. .C..... ??  
*Latimeria* .....A...AC .C..... u.  
*Nitzschia* .....A. ....CUUA. ....U. ....AA. G...UG.CG. -A...UCAUU  
*Rhizosolenia* .....A. ....CUUA. ....U. ....A...A. A...CG.CA. ---.G. AA  
*Stephanopyxis* .....A. ....CU.A. ....U. ....A...A. G...UA.CG. -UCG.U.AUU  
*Cylindrotheca* .....A. ....CUUA. ....U. ....AA. G...UG.UG. -A...UCGUU  
*Paramecium* .....CUUA. .U.....U. .A.....C. A...CG.AA. .GUAAUA .AU

*Oryctolagus* CAUGACCCGC CGGGCAGCUU CCGGAAACC AAAGUCUUUG GGUUCCGGGG GGAGUAUGGU  
*Mus* .....  
*Heterodon* .....?A.....?.....  
*Alligator* ????.?.....??.....?  
*Latimeria* .....?..G.....  
*Nitzschia* WGUCU.... .A.CACCU.A UGA.A..U.A C.....  
*Rhizosolenia* U....A.UG UC....U... AU.A...U.....  
*Stephanopyxis* AG....U.CG UUA...C... AU.A...U.....  
*Cylindrotheca* AUGUCU..AU .A.CACCU.A UGA.A..U.A C.....  
*Paramecium* U.GUC..UUU ...CAUCG.A AGA.A..U.A .....U....

*Oryctolagus* UGCAAAGCUG AAACWAAAG GAAWGACGG AAGGGCACCA CCAGGAGUGG AGCCUGCGGC  
*Mus* .....  
*Heterodon* ?????????? .....?.....--AG.....  
*Alligator* .....?.....--A???.  
*Latimeria* .....-CAUG.....  
*Nitzschia* C...G.... A.....  
*Rhizosolenia* C...G.... A.....  
*Stephanopyxis* C...G.... A.....  
*Cylindrotheca* C...G.... A.....  
*Paramecium* C...G....

*Oryctolagus* UUAUWGC UCAACACGGG AAACCUCACC CGCCCCGGAC ACGGACAGGA UUGACAGAW  
*Mus* .....  
*Heterodon* ..?..????? ?? .....A... ???.....C  
*Alligator* ..U....??? ?? .....?.....A... ??.....  
*Latimeria* .....?? ?? .....A.....  
*Nitzschia* .....A..U... A..U..A... UA.UG.....  
*Rhizosolenia* .....A..U... A..U..A... UA.UG.....  
*Stephanopyxis* .....A..U... A..U..A... UA.UG.....  
*Cylindrotheca* .....A..U... A..U..A... UA.UG.....  
*Paramecium* .....G..A..U... A..U.AAA.. U...UG.....

*Oryctolagus* GAUAGCUCW UCUCGAWCU GUGGGUGGUG GUGCAUGGCC GWCUUAGW GGUGGAGCGA  
*Mus* .....C.....  
*Heterodon* .....?..? .....? ??.  
*Alligator* .....???? .....U.....  
*Latimeria* .....?.....  
*Nitzschia* ..G..... ..u..... A..... ..U..  
*Rhizosolenia* ..G..... ..u..... A..... ..UU..  
*Stephanopyxis* ..G..... ..u..... A..... ..u..  
*Cylindrotheca* ..G..... ..u..... A..... ..u..  
*Paramecium* ..A..... ..u..... A..... ..u..

*Oryctolagus* UWGUCUGGU UAAUCCGAU AACGA?CGAG ACUCUGGCAU GCUAACUAGU **UACGGACCC**  
*Mus* .....A.....  
*Heterodon* .....A.....?.....U.....  
*Alligator* .....A.....U.....  
*Latimeria* .....A.....?CUC.....A.....  
*Nitzschia* .....U.....A.....C.CU.C.....A..C .UGCAU.GUG  
*Rhizosolenia* .....C.....G..U.....C.CC.C.....A..A C...U..AUG  
*Stephanopyxis* .....U.....A.....C.CC.C.....A... CCU.U..AUG  
*Cylindrotheca* .....U.....A.....C.CU.C.....A..G .UGC.U.GUG  
*Paramecium* .....A.....CU.AA.C.....U-----

<i>Oryctolagus</i>	CCGAGCGGUC	GGCGUCCCC	AAUCUCU	JAG	AGGGA	AAGU	GGCGWCAGC	CACCCG----
<i>Mus</i>	.....	.....	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	..?.....	..??.....	..????..	..?	.....	.....	.....	.....
<i>Alligator</i>	..?..?..?	..?..?..?	..?..?..?	..?	.....	.....	.....	.....
<i>Latimeria</i>	..U....	.....	.....	.....	.....	.....	..A...U... ..A.....	.....
<i>Nitzschia</i>	AAUU-UUCA.	U.-UGUGAAG	----	.....	.....	GU.C	AUU--CU.--	-UUAGAUGCA
<i>Rhizosolenia</i>	AAUU-AUCAU	U.AUGA-UGG	U-.....	.....	.....	UUU.	..U.ACU.A-	-C.AA-...A
<i>Stephanopyxis</i>	AAW-UUCAU	U.ACAA--GG	U-.....	.....	.....	U W C	..U.ACU.A-	-C.GA-...A
<i>Cylindrotheca</i>	AAU--UUCA.	U.-GUGAAU	----	.....	.....	GU.C	AUU--CU.--	-UUAGAUGCA
<i>Paramecium</i>	G.UU.U.AA.	AA.AGGUAUA	.....	.....	.....	U.U-	..UA.GU.A-	-GUG.AUGGA

<i>Oryctolagus</i>	---AGA-UUG	AGCAAUAACA	GGUCUGUGAU	GCCCUAGAU	GUCGGG-GCU	GCACGCGCGC
<i>Mus</i>	.....	.....	.....	.....	..C..G..	.....
<i>Heterodon</i>	.....?	.....?	.....?	.....?	..?????	.....?
<i>Alligator</i>	.....	.....?	.....?	.....?	..C..G..	.....?
<i>Latimeria</i>	.....	.....?	.....	.....	..C..G..	.....
<i>Nitzschia</i>	GGA...UAG.	G.....	.....	.....	..CU.G..C	.....
<i>Rhizosolenia</i>	GGA..UUGG.	G.....	.....	.....	..UCU.G..C	.....
<i>Stephanopyxis</i>	GGA..UUGG.	G.....	.....	.....	..CU.G..G	.....
<i>Cylindrotheca</i>	GGA...UAG.	G.....	.....	.....	..CU.G..C	.....
<i>Paramecium</i>	AGUUU--AA	G.....	.....	..C...C	..CU.G..C	.....

<i>Oryctolagus</i>	UACACUGACU	GGCUCAGCGU	GUGCCUACCC	UACGCCGGCA	GGCGGGGUA	ACCCGWGAA
<i>Mus</i>	.....	.....	.....	.G.....	.....	.....
<i>Heterodon</i>	.?.....	.....?	?.U.....	.....A..	..U.....?	..U.....
<i>Alligator</i>	.....	.....	.....U.....	.....A..	..U.....	.....
<i>Latimeria</i>	.....A..	..A... ..	..U.....	..A..A..	..U.....	.....
<i>Nitzschia</i>	.....UG	UAU...A..A	..UU--UA..	..UG...AG.	..CU...C.	..U.UU....
<i>Rhizosolenia</i>	.....UG	CAU...A..A	..CUUA..A..	..UG...AG.	..UG..CA.	..U.UU..A..
<i>Stephanopyxis</i>	.....UG	CAU...A..AA	..CAUA..A..	..UG...AG.	..CU.....	..U.UU.GA..
<i>Cylindrotheca</i>	.....UG	CAU...A..A	..U.--UA..	..UG...AG.	..UG..-C.	..U.UU....
<i>Paramecium</i>	.....A	C.U.....A	..CUUA.UUA.	CUGU..C.A.	A.G..AC.GG	..AAUC...UU

<i>Oryctolagus</i>	CCCCAU--UC	GUGAUGGGGA	UCGGGGAWG	CAAWAWCC	CCAUGAACGA	GGAAUCCCA
<i>Mus</i>	.....	.....	.....	.....	..U.....	.....
<i>Heterodon</i>	.....	.....	.....	.....	..U.....	.....
<i>Alligator</i>	.....	.....?	.....?	.....?	..??..?..?	.....
<i>Latimeria</i>	.....	.....A...	.....	.....U..	..G.....	.....
<i>Nitzschia</i>	..GUGCA...	.....A...	..A.AUU...	.....AA	U.U.....	.....U.
<i>Rhizosolenia</i>	..GUGCA...	.....A...	..A.AUU...	.....AA	U.U.....	.....U.
<i>Stephanopyxis</i>	..GUGCA...	.....A...	..A.AUU...	.....AA	U.U.....	.....U.
<i>Cylindrotheca</i>	..UGCA...	.....A...	..A.AUU...	.....AA	U.U.....	.....U.
<i>Paramecium</i>	AGGACGUG..	..C.....	..A.AUCU..	.....AGA	U.U.....	.....UU

<i>Oryctolagus</i>	GUAAGUGCGG	GUCAUAAGCU	UGC GWGAW	AAGUCCCGC	CCUUUGUACA	CACCGCCCGU
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	.....	.....?	.....?	.....?	.....?	.....U????.
<i>Alligator</i>	.....	.....	.....C.....	.....??..?..?	.....??..?..?	.....????.
<i>Latimeria</i>	.....	.....	.....C.....	.....	.....	.....
<i>Nitzschia</i>	..AC..A..	A...C.AUC	..A.....	..C.....	.....	.....
<i>Rhizosolenia</i>	..AC..A..	A...C.AUC	..A.....	..C.....	.....	.....
<i>Stephanopyxis</i>	..AC..A..	A...C.AUC	..A.....	..C.....	.....	.....
<i>Cylindrotheca</i>	..AC..A..	A...C.AUC	..A.....	..C.....	.....	.....
<i>Paramecium</i>	.....CA.A.	.....C...C	..U.C...A.	..C.....	.....	.....

<i>Oryctolagus</i>	CGCUACUACC	GAWGGAUGG	UUUAGUGAGG	CCCUCG-GAU	CGGCCCCGCC	GGGUCGGCC
<i>Mus</i>	.....	.....	.....	.....	.....	.....
<i>Heterodon</i>	?.....	.....	.....	U?.U.....	?.....U.?	.....u.....
<i>Alligator</i>	.....	.....	.....	U.....	?.....??...	.....?..-?
<i>Latimeria</i>	.....	.....	.....	U.....	...A.....	.....U..
<i>Nitzschia</i>	..AC.....	.....A.....	.CCG...A.	-.....G...	U-----,UG	.C.AGUUC..
<i>Rhizosolenia</i>	..AC.....	.....A.....	.CCG.....	-.....G...	U-----,UG	.UCUGGUUG.
<i>Stephanopyxis</i>	..AC.....	.....A...U	.CCG.....	-A.....G...	U-----,AU	.UUAG.UU..
<i>Cylindrotheca</i>	..AC.....	.....A.....	.CCG.....A.	-.....G...	U-----,UG	.C.AGUUC..
<i>Paramecium</i>	...C.....	.....-UCGA.	.GAU...GU.	-AA-.UAUC.	G.A.U---G	C.-----

<i>Oryctolagus</i>	CACGGC-CUG	GCGGAGCGCU	GAGAAGACGG	UCGAACUUGA	CUAUCUAGAG	GAAGUAAAAG
<i>Mus</i>	.....C...	.....	.....	.....	.....	.....
<i>Heterodon</i>	-U?....C..?	?????....C	.....	.?....???	.....	.....??????
<i>Alligator</i>	??....C..?	?????....C	.....	.....???	.....	.....C?????
<i>Latimeria</i>	-G....C...	.....	.....	.....A	..A....?..	.....
<i>Nitzschia</i>	UUUAUUGGAA	.UUUGU...-	.....CUU.U	CUA...C.U.	UC..U.....	.....GUG...
<i>Rhizosolenia</i>	UUUAUUGGAA	C.U.-.UG.	.....CCU.U	C.A...C.U.	UC..U.....	.....GUG...
<i>Stephanopyxis</i>	UUU.UUGGGA	CUU..UA-A.	.C...CU..U	C.A...C.U.	UC..U.....	.....GUG...
<i>Cylindrotheca</i>	UU.AUUGGGA	.UUUGU...-	.....CUU.U	CUA...C.U.	UC..U.....	.....GUG...
<i>Paramecium</i>	AUG...U.GA	A.CAUUU...-	.G...UUUC	GUA...C.U.	UC.CU.....	.....G.G...

<i>Oryctolagus</i>	UCGUACAAG	GUUUCGUG	GUGAACUCG	GGAAGGAUCA	UUA?
<i>Mus</i>	.....	.....	.....	.....	.....
<i>Heterodon</i>	???????????	???????????	???????????	???????????	???
<i>Alligator</i>	???????????	???????????	???????????	???????????	???
<i>Latimeria</i>	...???????	???????????	???????????	???????????	???
<i>Nitzschia</i>	.....	.....	.....	A.....	AGC.
<i>Rhizosolenia</i>	.....	.....	.....	A.....	AGC.
<i>Stephanopyxis</i>	.....	.....	.....	A.....	AGC.
<i>Cylindrotheca</i>	.....	.....	.....	A.....	AGC.
<i>Paramecium</i>	.....	.....	.....	.....	..UA

### Streszczenie

Średnie tempo substytucji nukleotydów w mniejszej podjednostce rybosomalnego DNA wynosi 0.038 substytucji na sto nukleotydów na milion lat wśród okrzemek i odpowiednio 0.008 w grupie obejmującej ryby trzonopłetwe i czworonogi. Tempo ewolucji molekularnej okrzemek było więc w tym przypadku 4,5 raza większe niż kręgowców.