

# Molecular phylogeny of Baikalian Lumbriculidae (Oligochaeta): evidence for recent explosive speciation

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**Abstract.** The phylogenetic relationships of the Lumbriculidae (Oligochaeta) of Lake Baikal (Siberia, Russia) were investigated with a data set of new mitochondrial COI gene sequences of 26 species, 25 of which belong to Baikalian fauna. Species of the most representative genera inhabiting the lake, *Lamprodrilus* Michaelsen, 1901, *Agriodrilus* Michaelsen, 1905, *Teleuscolex* Michaelsen, 1901, *Stylodrilus* Claperède, 1862, *Styloscolex* Michaelsen, 1901, and *Rhynchelmiss* Hoffmeister, 1843, were included in the data set. The possibility of explosive speciation events was investigated in studying deviations of the observed rates of cladogenesis from a uniform one. In contrast, the monophyly of most genera was recovered, with the exception of *Lamprodrilus* and *Rhynchelmiss*. The polyphyletic nature of *Rhynchelmiss* gave support to the revalidation of the genus *Pseudorhynchelmiss* suggested on morphological characters. The lack of phylogenetic signal in the *Lamprodrilus*-group was interpreted as evidence of explosive radiation of the genera of this group (*Lamprodrilus*, *Agriodrilus*, and *Teleuscolex*), 4 to 3 million years ago, and was put in the context of the Baikal Rift Zone history.

**Key words:** Lake Baikal, Oligochaeta, Lumbriculidae, mtCOI gene, molecular phylogeny.

## INTRODUCTION

During the last decade, phylogenetic studies have come to rely more and more on molecular analysis, an important part of which is the comparative analysis of homologous DNA sequences. The advantage of this approach is the theoretical possibility to estimate the time of lineage divergences based on a molecular clock hypothesis, as well as to correlate speciation processes with different historical events in the ecosystem rearrangements.

Species flocks, i.e. monophyletic groups of closely related species, which underwent an unusually high rate of speciation in confines of a single restricted ecosystem (Brooks, 1950; Coulter,

1991), are the most attractive objects for this kind of study, because the rate and mode of speciation may provide the clues to the mechanisms of generating such a vast biodiversity. Such species flocks are most peculiar to ancient freshwater lakes like Baikal. Lake Baikal is the deepest, largest and the most ancient (at least 28 million years) of all the present-day freshwater reservoirs (Mats, 1987; Logachev, 1993).

The long and complex geological history of the lake enabled a unique and highly endemic fauna to evolve in this environment. The biodiversity of Lake Baikal is almost twice as high as that of Lake Tanganyika (Timoshkin, 1999). In contrast to other ancient lakes (Tanganyika, Malawi etc.), for which molecular studies were mostly restricted to a pe-

cular species flock (such as cichlid fishes for the Great African lakes), many species flocks in Lake Baikal, among the richest in terms of species, have already been studied, using molecular data: flatworms (Kuznedelov, Timoshkin, 1993), sculpine fishes (Slobodyanyuk et al., 1994), amphipods (Sherbakov et al., 1998), and molluscs (Zubakov et al., 1997). Oligochaeta is another interesting object for such study, and especially because of their mode of life and unique distribution. Indeed, their distinction from the other species flocks is their ability to penetrate the sediment substrate down to 20-25cm in Lake Baikal, which results in the relatively less importance of some geographical isolation factors driving evolution of the surface-bound or swimming, more mobile organisms. The fact that the family Lumbriculidae has generated one or possibly several species flocks raises the question about the mechanisms of their speciation.

The Lumbriculidae Vejdovsky, 1884 is one of the most important families of Oligochaeta in Lake Baikal. More than half of the whole world's known lumbriculid taxa indeed inhabit the lake. Sixty-eight lumbriculid species and subspecies have been recently reported from Lake Baikal, 55 of which are endemic (Snimshikova, Akinshina, 1994; Martin et al., 1998; Semernoy, 2004). In Lake Baikal, the family underwent an intense evolutionary radiation, giving rise to a variety of forms including dwarf and giant species (as *Lamprodrilus pygmaeus* Michaelsen, 1902 and *Rhynchelmis brachycephala* Michaelsen, 1901, respectively), and even species flocks (amongst 26 *Lamprodrilus* species known so far, 19 are endemic). The Lumbriculidae dominate the oligochaete biomass in the abyssal and include truly abyssal species with specific morphological adaptations. Accordingly, the family offers a unique opportunity to elucidate questions related to the lake evolution of annelids.

Moreover, lumbriculids occupy a key position in Oligochaeta systematics and have an important

significance for the understanding of oligochaete evolution as a whole (Isossimov, 1962; Brinkhurst, 1989; Brinkhurst, 1992; Ferraguti et al., 1999; Martin et al., 2000; Kaygorodova, Sherbakov, 2006). In contrast to the Tubificidae, another family well represented in Lake Baikal, which benefited from a careful re-examination of its fauna during the last two decades, the family Lumbriculidae was revised many years ago (Isossimov, 1962). The scarcity of external morphological features, as well as the vague descriptions of many species make taxonomic identification and understanding the value of different morphological characters very difficult in this group. With accumulation of new data, it has become quite clear that a revision of the existing classification is essential, especially for the construction of a phylogenetically justified classification of the family.

A molecular phylogenetic study would create a framework for further re-evaluation of the systematic feasibility of morphological traits in this group. In the present study, we aim at obtaining of more detailed knowledge of the evolutionary history of the lumbriculid species flock in Lake Baikal. This will help us to resolve some of phylogenetic and systematic puzzles currently existing, and to pave the way for a full revision of this group, which will be hopefully based on phylogeny of species herewith considered.

## MATERIAL AND METHODS

### Taxa

The material was collected from Lake Baikal (Siberia, Russia), during 1996-1998 expeditions on board the R/V Vereshchagin, by means of a dredge, a Petersen grab and by diving, at different locations ranging between 5 and 420 m deep (Table 1; Fig. 1). The entire material was fixed in 80% ethanol. The sexual segments of the worms were dissected for identification, following a hardening of tissues in 7% neutralized formalin, stained with alcoholic carmine and mounted in Canada

**Table 1.** List of species used in the mtCOI gene fragment analysis, sampling localities and EMBL Accession numbers.

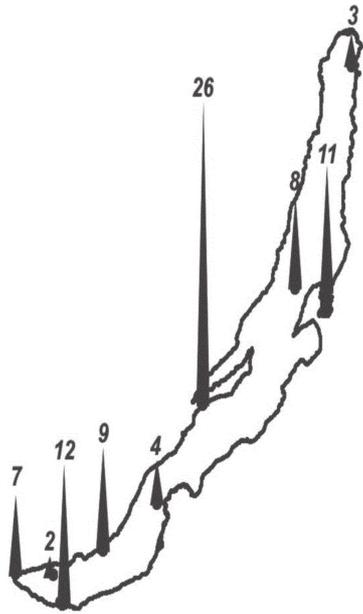
Taxa	Locality	EMBL number
<b>Lumbriculidae Vejdovsky, 1884</b>		
<i>Lamprodrilus achaetus achaetus</i> Isossimoff, 1962	Chivyrkui Bay	AJ577626
<i>L. achaetus hemiachaetus</i> Snimschikova, 1987	Maloe More Strait	AJ577625
<i>L. ammophagus</i> Michaelsen, 1905	Olkhon Gates Strait	AJ577627
<i>L. decathecus</i> Michaelsen, 1901	Olkhon Gates Strait	AJ577619
<i>L. dybowski</i> Michaelsen, 1905	Kultuk Bay	AJ577621
<i>L. sp. prope isoporus</i> Michaelsen, 1901	Maloe More Strait	AJ577624
<i>L. satyriscus</i> Michaelsen, 1901	Olkhon Gates Strait	AJ577620
<i>L. secernus</i> , Semernoy, 2001	Cape Polovinnyi	AJ577623
<i>L. stigmatias</i> Michaelsen, 1901	Chivyrkui Bay	AJ577628
<i>L. polytoreutus</i> Michaelsen, 1901	Cape Frolikha	AJ577618
<i>L. wagneri</i> Michaelsen, 1901	Murinskaya Bank	AJ577622
<i>Agriodrillus vermivorus</i> Michaelsen, 1905	Olkhon Gates Strait	AJ577617
<i>Teleuscolex baicalensis</i> Grube, 1873	Varnachka Bay	AJ577630
<i>T. korotneffi</i> Michaelsen, 1905	Chivyrkui Bay	AJ577629
<i>Styloscolex asymmetricus</i> Isossimoff, 1948	Cape Frolikha	AJ577634
<i>S. baicalensis</i> Michaelsen, 1901	Chivyrkui Bay	AJ577635
<i>S. chorioidalis</i> Isossimoff, 1948	Maloe More Strait	AJ577636
<i>S. kolmakowi</i> Burow, 1931	Kultuk Bay	AJ577637
<i>Stylodrillus asiaticus</i> Michaelsen, 1901	Chivyrkui Bay	AJ577613
<i>St. mirus</i> Cekanovskaja, 1956	Murinskaya Bank	AJ577614
<i>St. opisthoannulatus</i> Isossimoff, 1948	Chivyrkui Bay	AJ577616
<i>St. parvus</i> Hrabé et Čenosvitov, 1927	Maloe More Strait	AJ577615
<i>Rhynchelmis alyonae</i> Martin <i>et al.</i> , 1998	Olkhon Gates Strait	AJ577633
<i>Rh. brachycephala</i> Michaelsen, 1901	Chivyrkui Bay	AJ577631
<i>Rh. shamanensis</i> Martin <i>et al.</i> , 1998	Olkhon Gates Strait	AJ577632
<b>Tubificidae Vejdovsky, 1876</b>		
<i>Tubifex tubifex</i> Müller, 1773	EMBL	U74076

balsam. The remaining segments were used for DNA extraction. The species were identified by I. Kaygorodova, according to existing systematic keys (Chekanovskaya, 1962; Semernoy, 2004) and descriptions of species (Isossimov, 1962; Hrabé, 1982; Martin *et al.*, 1998). All specimens are deposited in the collection of Limnological Institute SB RAS (Irkutsk, Russia).

#### DNA methods

Twenty-five species from 6 genera living in Lake Baikal were taken for molecular analysis

(Table 1). DNA was extracted using a slightly modified CTAB method (Doyle, Dickson, 1987) from the caudal part of the fixed worms. A mitochondrial COI gene fragment of 710 bp long was amplified with the primers universally used for most invertebrates (Folmer *et al.*, 1994). A direct sequencing of the double-chain products of amplification was performed using a sequencing kit from Amersham Biosciences (Thermo Sequenase Primer Cycle Sequencing Kit RPN2436), with the same primers. Cycle sequencing was carried out using 30 cycles of 95° C (36 s; 5 min in the first



**Fig. 1.** Distribution of Lumbriculidae species in Lake Baikal. The height of bars and numbers designate the number of species determined from all samples of the locality. Localities: 2 - Cape Polovinnyy; 3 - Frolikha Bay; 4 - Selenga River Delta; 7 - Kultuk Bay; 8 - Ushkanyi Islands; 9 - Varnachka Bay; 11 - Chivyrkui Bay; 12 - Murinskaya Bank; 26 - Olkhon gates Straight.

cycle), 52° C (36 s). 72° C (80 s; 5 min in the last cycle). The sequencing products were analyzed with an automatic sequencer (ALF-DNA sequencer, Pharmacia Biotech). All sequences used in the present study are available from EMBL (Table 1).

### Phylogenetic analysis

We analysed the data set of 26 sequences, 25 of which belong to six principal genera of Lumbriculidae inhabiting Lake Baikal, *Lamprodrilus*, *Agriodrillus*, *Teleuscolex*, *Stylodrilus*, *Styloscolex*, and *Rhynchelmis*. The choice of an outgroup for Lumbriculidae was problematic because so far both morphological studies have failed to recover the position of this taxon and because putatively valid external groups are scattered among annelids. For this reason, ho-

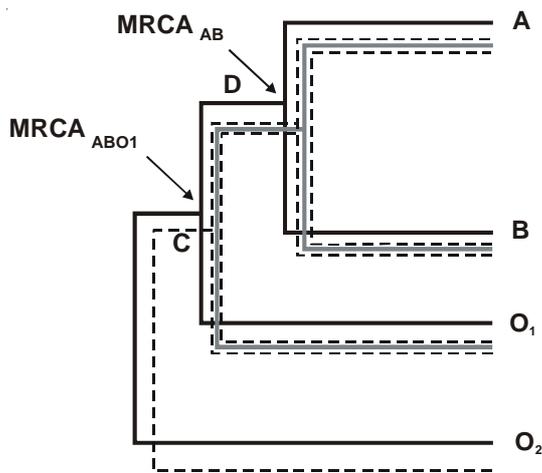
mologous sequence of *Tubifex tubifex* was designated as an unambiguous outgroup in accordance with its morphological and molecular placement among Tubificidae (Brinkhurst, 1992; Martin et al., 2000; Erséus, Källersjö, 2004; Kaygorodova, Sherbakov, 2006), the sister group of the Lumbriculidae.

Set of mtCOI sequences were aligned using DIALIGN (Morgenstern et al., 1996). The translation into amino acid sequences was done with MEGA 3.1 (Kumar et al., 2004) using the *Drosophila* mitochondrial genetic code table for amino acids (Clary, Wolstenholme, 1985). The resulting alignment (EMBL Accession numbers: **AJ577613** - **AJ577637**, **U74076**) was analyzed with the Neighbor Joining method, using PAUP v.4.0b10 (Swofford, 1998) with parameters specified in Table 2. The model of nucleotide substitution which

**Table 2.** Parameters estimated for COI partial sequences of Baikalian lumbriculids, according to a GTR evolutionary model, including a proportion of invariable sites (I) and variable sites distributed according to a Gamma model (lnL: log Likelihood; AIC: Akaike Information Criterion)

Base frequencies	
A	0.3126
C	0.2069
G	0.1481
T	0.3323
Substitutions rate matrix	
A↔C	1.3394
A↔G	4.9307
A↔T	2.2178
C↔G	0.9573
C↔T	9.2905
G↔T	1.0000
Ratio of invariable sites (I)	0.46
Gamma distribution shape parameter	0.9780
-lnL	6249.2080
AIC	12518.4160





**Fig. 2.** The design of Bayesian test for polytomy of OTUs A, B and  $O_1$ .

best fitted the data was chosen with the aid of Modeltest (Posada, Crandall, 1998), which hierarchically compared 56 models of base substitutions, using likelihood ratio test statistics and the Akaike Information Criterion scores (AIC) (Akaike, 1974) associated with the likelihood scores. The support for the tree topology obtained was estimated from an analysis of 500 bootstrap replicates.

Deviations of the observed rate of cladogenesis from a uniform one were analyzed using GENIE 1.0 (Pybus et al., 2001).

### Bayesian test for polytomy

Estimating the nature (mode) of evolution for specific lineages has primarily been accomplished using Bayesian analysis as implemented in MrBayes v3.1.1 (Huelsenbeck, Ronquist, 2001). Model parameters (GTR+I+PINVAR) were estimated during the run, with default values of 4 for the number of chains and 2.0 for the chain temperature parameter. The prior for the shape parameter of the gamma distribution was optimized as the free parameter. Default priors were used for all other parameters. Each run was  $1 \times 10^6$  generations, with sampling every 100 generations, and a burn-in

period of  $2 \times 10^5$  generations. The Bayesian posterior probability (bbp) of each clade was determined as the number of sampled trees, post-burn-in, that contained each observed bipartition (Larget, Simon, 1999). Respective values have been regarded as the measures for statistical support of the clades.

Analysis of true polytomy was designed as testing for zero lengths for all internal branches of binary trees compatible with the data, which appear to be “swallowed” by the polytomic node on the phylogeny resulting from Bayesian analysis. It means that as shown on Fig.2 the expectation of the distribution of the DC lengths must be insignificantly different from zero. This would mean that  $MRCA_{ABO1}$  and  $MRCA_{AB}$  coincided in time and therefore node C is the same as node D and they belong to hard polytomy DC. The distribution in this case is obtained from the list of recorded post-burn-in time trees recorded during the Bayesian inference.

The posterior probability distribution of lengths for all branches was obtained by saving branch lengths for each sampled tree during a Bayesian tree search. For each sampled tree, we then estimated the distance from the most recent common ancestor (MRCA) of our ingroup to each of terminal taxa by summing branch lengths. The ingroup MRCA is identified by ingroup comparison, and is simply the ancestral node shared by all ingroup taxa, with the exception of the outgroup taxon. Extraction of branch length estimates from the log file was done using Cadence v.1.0 (Wilcox et al., 2004).

## RESULTS

### COI sequences

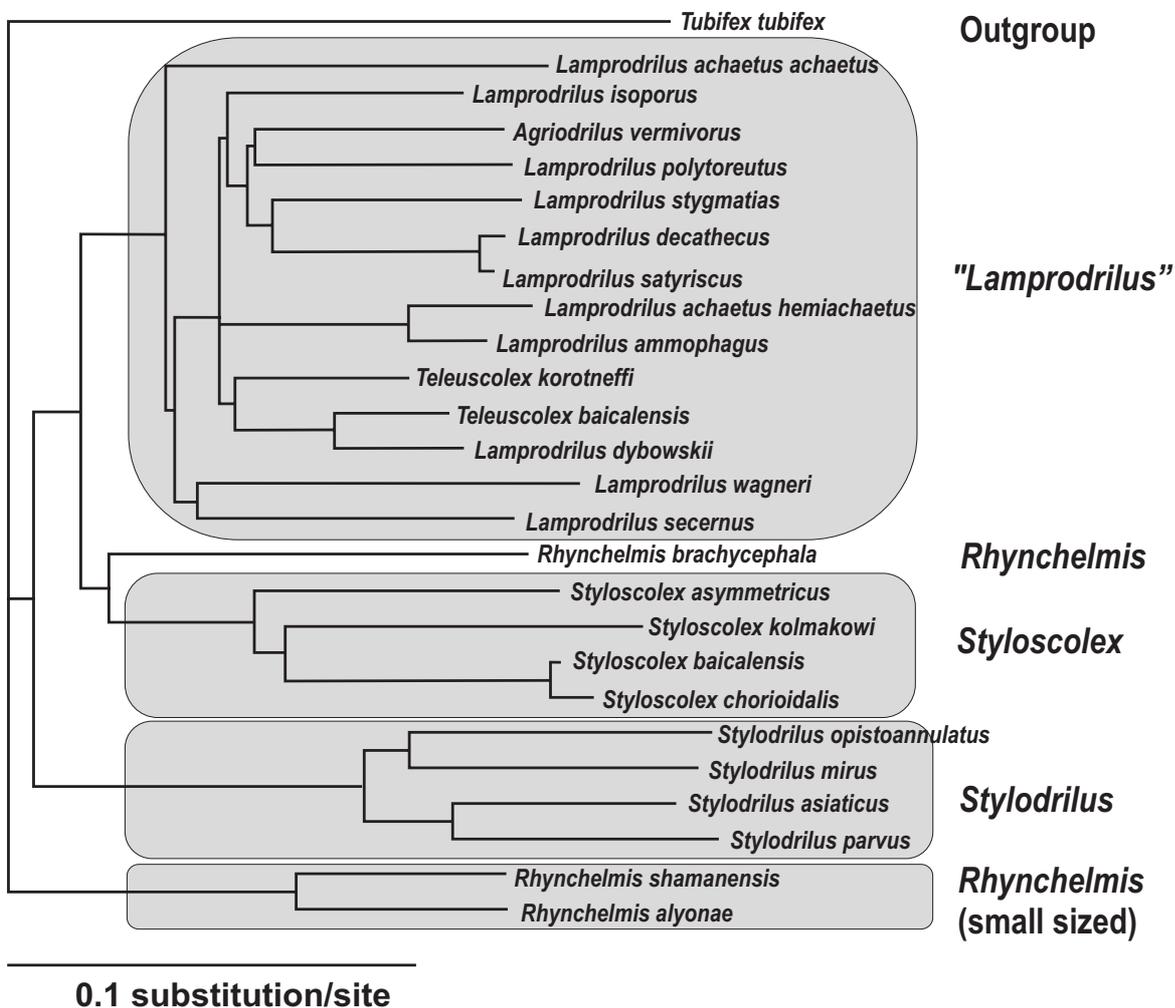
Alignment of the 26 studied taxa resulted in a fragment of 642 bp of the mitochondrial cytochrome oxidase C subunit I gene, among which 320 sites (49.84%) are informative. Translation into amino acid sites gave a single uninterrupted reading frame in the expected position. The ob-

tained amino acid sequences were highly homologous with the known COI sequences of different invertebrate groups. The protein-coding fragments did not show insertions or deletions. Therefore, the sequences could be aligned easily. The sequences had complex composition bias, thus making difficult to choose the base substitution model appropriate for evolutionary inferences, which, while retaining the most of the phylogenetically relevant information, would be the least sensitive to possible saturation of some kinds of substitutions.

Modeltest identified the General Time Reversible Model (GTR) (Rodriguez et al., 1990) with variable sites according to a gamma distribution and a proportion of invariable sites as significantly better than any simpler model. The parameter values estimated are summarized in Table 2.

### Phylogenetic inferences

The Baikalian Lumbriculidae appear an assemblage of at least four well supported clades, which we designate as the *Styloscolex*, the *Stylodrilus*,



**Fig. 3.** Phylogeny of Baikalian lumbriculids inferred from their partial COI sequences. Neighbour joining tree built from a distance matrix estimated from General Time Reverse Model with gamma-distributed rates and a proportion of invariable sites (see Material and Methods).

**Table 3.** Age estimations for times of radiation of the major lumbriculid taxa

Taxon	Mean branch length in percentage of base substitutions, %	Age, million years
<i>Lamprodrilus</i> -group	8.50	4.3-3.3
<i>Styloscolex</i>	7.29	3.6-2.8
<i>Stylodrilus</i>	7.16	3.6-2.8
<i>Rhynchelmis</i>	12.57	6.3-4.8
“small-sized” <i>Rhynchelmis</i>	8.35	4.2-3.2

the “small-sized” *Rhynchelmis* (*R. shamanensis* and *R. alyonae*), and the *Lamprodrilus*-group lineages (Figs 3 and 4).

The monophyletic nature of the genera *Styloscolex*, *Stylodrilus* is confirmed with the notable exception of *Lamprodrilus* which forms a mixing of species typical for the genus and species classically assigned to other genera (*Agriodrilus vermivorus* and *Teleuscolex* spp.). Although there is nearly no resolution within this cluster of species, this association of different genera receives a bootstrap support as high as 86, suggesting that these genera are closely related. The genus *Rhynchelmis* seems to be polyphyletic but the absence of support for basal branches gives no firm evidence of this assertion (Figs 3 and 4). The relationship between *R. shamanensis* and *R. alyonae* is strongly supported, but their sister relationship with *R. brachycephala* is not recovered in the present tree.

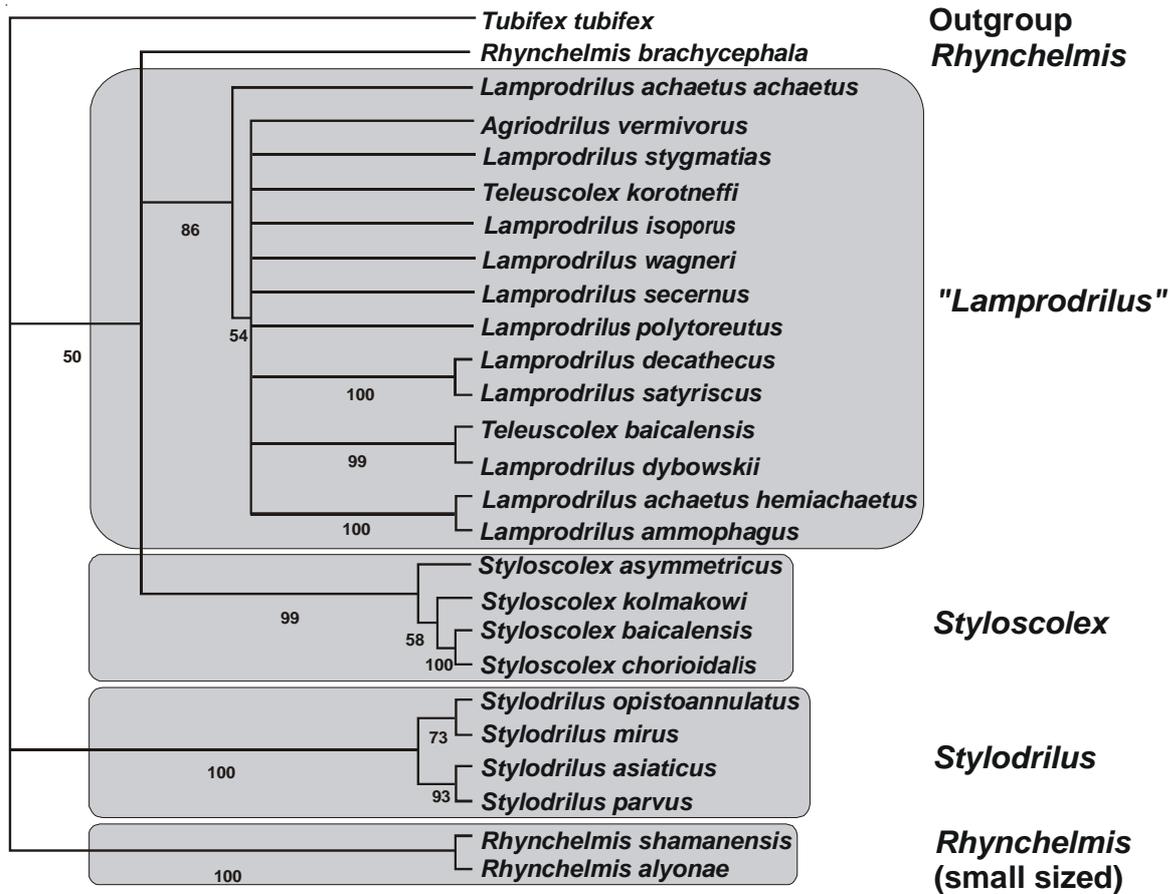
Branching order within the first three clades (*Styloscolex*, *Stylodrilus* and “small-sized” *Rhynchelmis*) appears to be well resolved. In contrast, the *Lamprodrilus*-group is badly resolved, suggesting that this species flock could result from a “crown-like” radiation. The branching pattern within this group is not significantly improved even if these taxa are analyzed separately from the rest with one or two outgroups, and using only third codon positions, and using different algorithms of phylogenetic analysis (data are not

shown).

### Polytomy of *Lamprodrilus*-group

Phylogenetic analysis of mtCOI sequences reveals possible polytomy at the base of the *Lamprodrilus*-group (Figs 3 and 4). Intriguing possibility is that this reflects realistically the peculiarity of evolutionary events at the beginning of the *Lamprodrilus*-group adaptive radiation. Therefore we needed to test whether nucleotide divergence data support significantly the simultaneity of the speciation processes in this area of the tree. This goal was achieved by inferring Bayesian tree, which was compatible with the tree in Fig. 3. The 50% major rule consensus tree contained the apparent polytomy in question, but this tree is superposition of fully resolved binary trees, one of which having the highest posterior probability is given at Fig. 5, a. On the base of this tree the test on polytomy of *Lamprodrilus*-group was designed. First, we assigned as the MRCA the node connecting the ingroup to the outgroup. In total 5 outgroups were used in this analysis as shown in Fig. 5 (numbered arrows).

With regard to each outgroup the average distances of each length branch of ingroup to this node were calculated. Consequently, the internal branch lengths were measured as the distance between 2 MRCAs as described in “Material and Methods”. In case of true (hard) polytomy the combined lengths of these branches must be insignificantly different from zero. Since shape of distribution in



**Fig. 4.** 50% majority rule consensus tree of 500 bootstrap replicates obtained from partial COI sequences of Baikalian lumbriculids. Each phylogeny is built from a distance matrix estimated from General Time Reverse Model with gamma-distributed rates and a proportion of invariable sites (Table 2). Numbers under nodes represent bootstrap support.

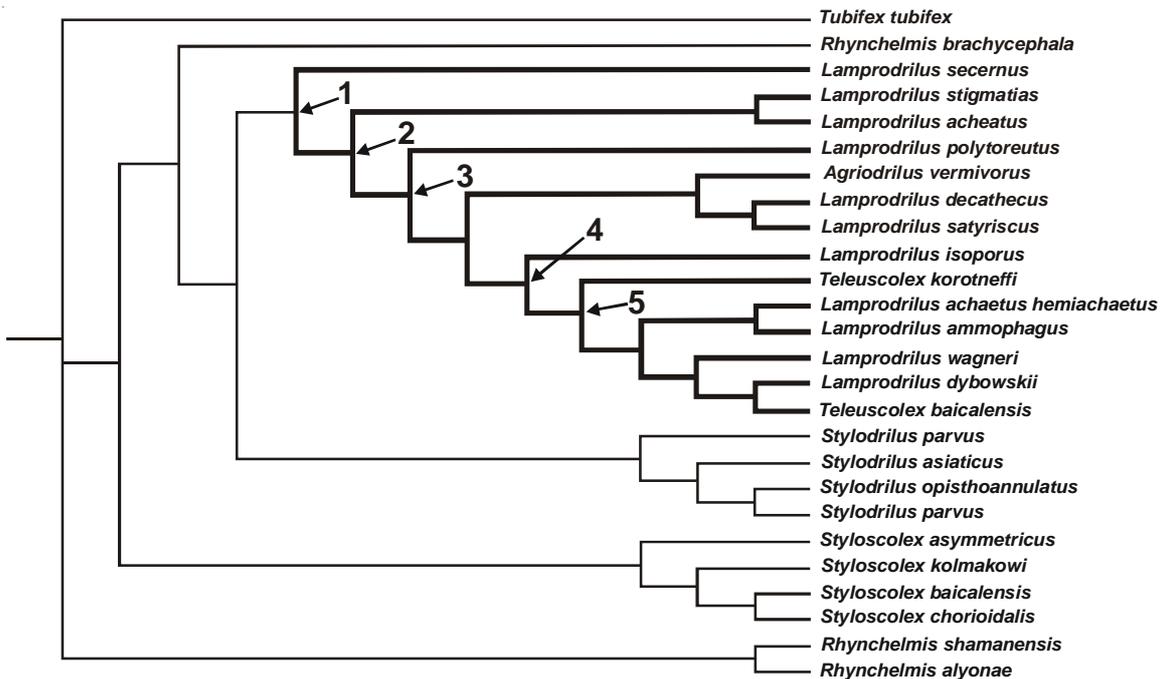
the replicates of Bayesian analysis is not known, we obtained the lengths distribution from log files of MrBayes after the burn-in time. As result we have received that lengths of 1 and 2 branches are significantly different from zero (broken curve in Fig. 6), whereas the lengths of branches 3, 4 and 5 combined are extremely close to zero (black curve in Fig. 6). We regard this as the proof of simultaneity of speciation events. Unfortunately it is impossible to determine the spatial parameters of these events, but the most parsimonious hypothesis would be that all these processes took part in confines of Lake Baikal or even in small part of the lake.

## DISCUSSION

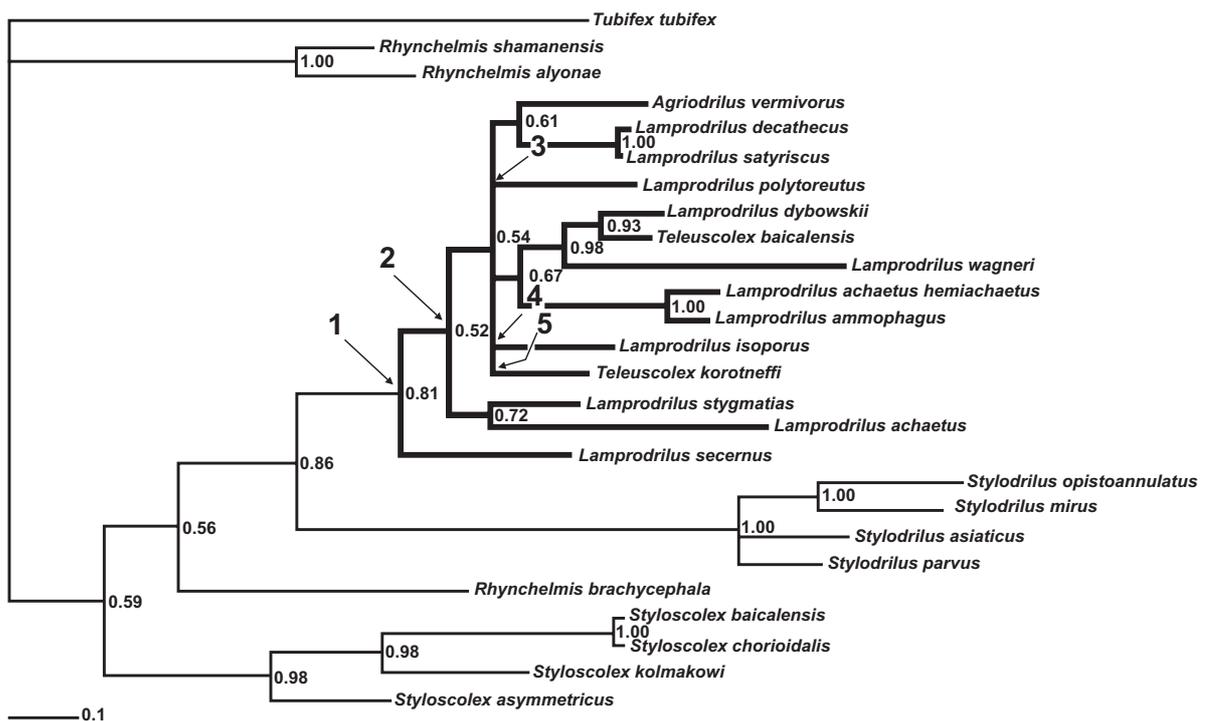
### Lumbriculidae phylogeny

The investigated mtCOI gene fragment supports the monophyly of most genera within the Lumbriculidae, with the exception of *Lamprodrilus* and *Rhynchelmis*. In contrast, practically no resolution is obtained for relationships between genera, suggesting, in accordance with Nylander et al. (1999), that this part of the gene is probably not useful for inferring high level relationships among the annelids.

If the monophyly of the genera *Styloscolex* and *Stylodrilus* is recovered, the obtained phylogeny

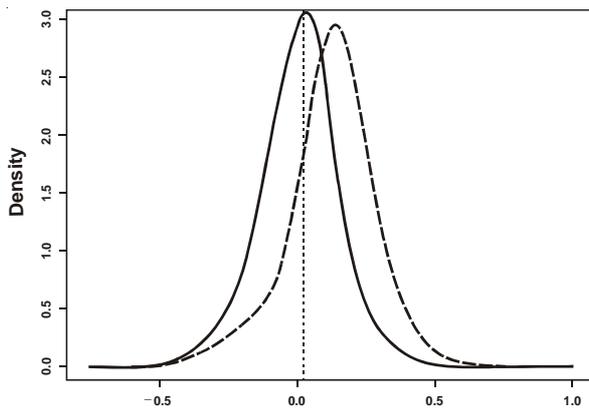


**a**



**b**

**Fig. 5, a, b.** Resulting trees of Bayesian analysis. Numbered arrows point at the internal branches which lengths were measured as described in “Material and Methods”. **a** - fully resolved binary tree, one of the highest posterior probability. **b** - 50% major rule consensus tree. Numbers near nodes represent posterior probability support.



**Fig. 6.** Density of distribution of branch lengths for *Lamprodrilus*-group on MrBayes tree (Fig. 5). Broken curve corresponds to 1 and 2 branches of that tree. Black one is the combined 3, 4 and 5 branches.

strongly suggests that the genera *Agriodrillus*, *Telescolex* and *Lamprodrilus* are closely related. The idea of uniting these three genera into a single genus was already put forward earlier by other authors on the basis of morphological characters (Cook, 1971) and a subsequent cladistic analysis (Brinkhurst, 1989), and receives additional support with the present analysis. Moreover, the branching pattern within this group strongly suggests that speciation results from an approximately synchronous radiation. The *Lamprodrilus*-group shares a common ancestor and therefore represents a genuine species flock.

#### Estimating time elapsed since speciation

The set of lumbriculid species used for phylogenetic inference in the present investigation suggests a few periods of a more intense speciation in some groups. It is therefore of interest to attempt to determine the time at which these events occurred.

Unfortunately, as far as oligochaetes are concerned, there is no possibility of using data from paleontological records. For the calibration of the

rate of substitution accumulation, therefore, the only possibility is to use available estimates of the rates of molecular evolution available in the literature. Unfortunately, there is no molecular clock available for oligochaetes at present. For COI, Knowlton et al. (1993) proposed an approximate rate of molecular divergence of 2.2 to 2.6 % per  $10^6$  years for snapping shrimps of the Atlantic and Pacific coasts of Panama. This calibration will also be used here, even if assuming similar rates of nucleotide substitutions among oligochaetes and crustaceans should be viewed with caution. In order to estimate the times of radiations, giving rise to the major clades of lumbriculids in the lake, we averaged estimated branch lengths leading to a common ancestor of a clade (Table 3). Time intervals thus obtained strongly depend on the rate of substitution accumulation accepted, but evidently overlap.

The polytomy appearing at the beginning of the *Lamprodrilus*-group clade suggests increased rate of diversification in their ancestor(s) during relatively short time of the evolutionary history of the Baikalian lumbriculids. Even the assumption of simultaneous fast multiple speciation is compatible with the data. In order to check if there was indeed a significant burst in base substitution rate, we used a molecular demographic analysis employing an estimate of the number of ancestral lineages from an ultrametric tree (Nee et al., 1995). The plot of the logarithm of the number of ancestral lineage versus time is expected to be linear in case of constant ratio of “speciation/extinction”, and have a sharp increase during the period of time when this ratio increases. If branch lengths on the phylogenetic tree given on figure 2 are estimated with strict molecular clock enforced, this analysis as implemented in GENIE (Pybus et al., 2001), maximum likelihood ratio test prefers the “one step” model at 1% significance over any other model of demographic history (Fig. 7). Therefore, it is possible to conclude that depending on the calibration for molecular clock accepted, there was

a diversification burst in lumbriculids 4.3–3.3 million years ago.

### Analysis of the possible causes and mechanisms of the evolution of the Lumbriculidae in Lake Baikal

Investigation on the origin of Baikalian Oligochaeta is closely related to estimating of their age. High level of endemism of oligochaete fauna implies its development in relatively retired ecological conditions. As it was demonstrated by recent molecular phylogenetic studies of Baikalian endemic sculpine fishes (Kirilchik, Slobodyanyuk, 1997) and molluscs (Zubakov et al., 1997), a wide species radiation can be attained in relatively short geological terms, during several millions of years.

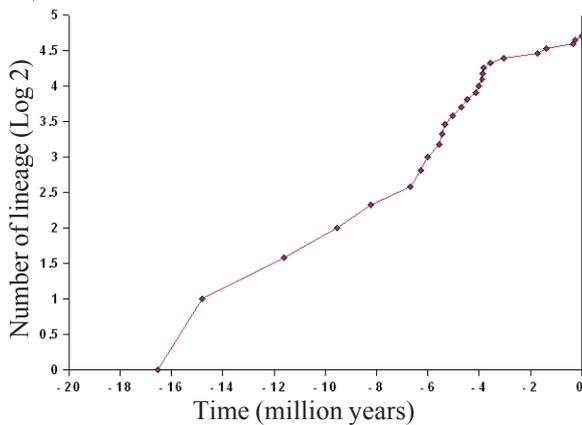
The most striking event in the evolutionary history of Baikalian lumbriculids, according to the data presented here, is the suggestion that an outburst of genetic diversity accumulation occurred somewhere between 4.3 and 3.3 million years ago (Table 3). The best accordance found is between the *Lamprodrilus* and the small-size *Rhynchelmis* groups. They both consist mostly of local endemics inhabiting Olkhon Gates Strait, which is the richest site of Lake Baikal in terms of lumbriculid species (Fig. 1). It is an interesting possibility then that some very local conditions in this straight area were responsible for a rapid accumulation of genetic diversity in these groups.

Descendants of different lines, which were formed at that time, live at different depths and substrates. At the same time, it should be mentioned that similar speciation outbursts were observed approximately in the same time for molluscs of the family Baicaliidae (Zubakov et al., 1997; Sherbakov, 1999) the study of which was based on DNA sequences of the same mitochondrial gene fragments. On the other hand, according to recent data, the organisms living in the water body at similar depths (fishes, amphipods) did not undergo considerable evolutionary transfor-

mations at that time (Kirilchik, Slobodyanyuk, 1997; Ogarkov et al., 1997). It is therefore difficult to imagine that climate changes (cooling), which had only begun at that time (Mats, 1992; Logachev, 1993; Kozhova, Izmet'seva, 1999) could by themselves play directly a significant role.

The burst of speciation in the major clades (*Lamprodrilus*-group, *Styloscolex*, *Stylodrilus*, and “small-sized” *Rhynchelmis*) happened in approximately at the same period (between 4.3–3.3 million years ago). This period of Baikal history was very rich in geological events. For example, about 6 million years ago the thermo-haline regime of the lake changed dramatically due to the periodic seasonal freezing of lake surface (Kuimova, Sherstyankin, 1999). Until that time the deep zone of Baikal below approximately 100 m probably was almost lifeless and anoxic. After that, the cool superficial waters saturated with oxygen started to penetrate deeper, oxygenating the abyssal zone of the lake. Hence, this zone became available for colonization, and this still makes Lake Baikal unique by comparison with other ancient lakes. For instance, waters of lakes Tanganyika and Malawi are anoxic below approximately 250 m (Coulter, 1991). The lowest age estimate (3.3 million years ago) coincides with a period of active tectonic processes at the Baikal Rift Zone, which corresponds to “Neobaikalian” sub-stage according to Mats (1992).

Indeed, some of Baikalian lumbriculids seem to be genuine deep-water species. *Lamprodrilus bythius* and *Lamprodrilus inflatus* possess morphological traits peculiar to deep-water animals, such as transparent and band-like body (Isosimov, 1962). Other species of the *Lamprodrilus*-group display a wide variety of morphological adaptations which could play a major part in their diversification. For instance, *L. wagneri*, *L. achaetus hemiachaetus* and the extreme case of *L. achaetus* have reduced setae, which could increase their ability to burrow dense sediments. Some representatives of the major lumbriculid



**Fig. 7.** Number of ancestor lineages plotted versus time as estimated from a Neighbour joining tree built from a distance matrix estimated from General Time Reverse Model with gamma-distributed rates and a proportion of invariable sites, with strict molecular clock enforced.

genera have a reduced body size, that could possibly counter predation (“small-sized” *Rhynchelmis*-group, many species of the genus *Stylo-drilus*, *Lampro-drilus pygmaeus*, *L. achaetus*, *Styloscolex baicalensis*, *S. chorioidalis*, and *S. asymmetricus*). Within the *Lamprodrilus*-group, *Agriodrilus vermivorus* is carnivorous, which might be a consequence of very high densities of oligochaetes during some periods, in corroboration with the sharp increase of the number of ancestral lineages of recent lumbriculids (Fig. 4) in Lake Baikal.

According to the hypothesis of the burst speciation within some Baikalian lumbriculids, it is important to consider that intense speciation must be preceded by just an intense extinction (e.g. Eldridge, 1996; Hallam, Wignall, 1997 and many others), although for each case, this assumption requires special investigations. Such an extinction could affect not only lumbriculids, but also other benthic invertebrates, thus emptying the ecological niche. The appearance of new adaptive zones as well as the enlargement of already existing zones

would have helped the lumbriculids in particular, as the group of animals best adapted to the grim habitat conditions of a cold mountain lake.

There is no reason to consider Lake Baikal to be the cradle of lumbriculids. Lake Baikal is the site of a massive and recent speciation of lumbriculids, but it is not a sanctuary of relicts. For instance, the genus *Trichodrilus* Claparède, 1862, which is more ancient than the genera found in the lake, as suggested by the morphological cladogram (Brinkhurst, 1989), is absent from Lake Baikal. However, it is still possible that some taxa spread across the holarctic area from the lake.

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