RESEARCH ARTICLE

# The correlation between saprobity and mitochondrial genes of indicator fish species based on molecular phylogeny

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## Abstract

**Aim and Scope:** The aim of the work is to reveal the correlation between saprobity and mitochondrial genes of COI-5p and 12S rRNA of indicator fish species based on phylogenetic tree reconstruction. **Material and Methods:** The article was made a first attempt to reveal the correlation between the saprobity and the mitochondrial genes of indicator freshwater fish species based on phylogenetic trees reconstruction of the COI-5p and 12S rRNA genes by neighbor -joining method and maximum parsimony method. **Result and Discussion:** Preliminary results show the clusterization of freshwater fish species by saprobity zones. The statistical significance of phylogenetic tree clusters containing indicator fish species according to COI-5p and 12S rRNA genes was evaluated by bootstrap analysis in 100 replicas. **Conclusion:** The article shows the possibility of modern methods of molecular genetics and bioinformatics use to assess the ecological status of water bodies. The identification of organism species by molecular genetics and the appropriation of a saprobic indicator using bioinformatics method provide a new modern tool to assess the ecological status of water bodies and monitor water bodies. Further research in this direction will be carried out for other hydrobionts.

**Key words:** 12S rRNA, COI-5p, environment, fishes, genes, indicator species, lakes, phylogenetic reconstruction, saprobity

## INTRODUCTION

At present, the scientists around the world reconstruct phylogenetic trees according to genes (by one gene or complex of genes) to establish the hypothetical way of evolution and/or to identify the species organisms.[1] For example, Radchenko describes mitochondrial genes, which are used for fish species systematics and phylogeny.[2] The variability of mitochondrial genes increases in the following order: rRNA (12S and 16S), tRNA, COI, COII, COIII, cytochrome b, two subunits of adenosine triphosphate synthetase, and seven subunits of NADH dehydrogenase, D-loop sequence (control region).[3] Besides, the mitochondrial gene COI-5p is used to identify fish species in DNA-barcoding technology.[4]

The correct identification of indicator fish species along with other indicator organisms is very important to assess the ecological status of water bodies by hydrobiological indicators (including the saprobity index) since the indicator species react immediately to the whole complex of impacts. The identification of the species as an indicator and its saprobity requires a lot of time and efforts from the researchers. Hence, now, most of the existing species do not have the status of indicator organisms. For the evaluation of the water quality by hydrobiological indicators, Pantle–Buck saprobic index (in the version by Sladeček) is used.[5] Sladeček presented 28 species of fishes, which are the indicators of different zones of freshwater reservoirs saprobity from clean to dirty ones.[6]

Based on the definitions of saprobity as the ability of freshwater organisms to live in water containing various amounts of organic substances[7] and mitochondria as the energy station of a cell, the main function of which is the

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oxidation of organic substances,[8,9] we assumed that there is a correlation between saprobity and mitochondrial genes of the indicator organism.

## MATERIALS AND METHODS

The correlation between saprobity and genes was carried out by the molecular phylogeny methods for 28 indicator species of freshwater fish from the list of indicator species, given by Sladeček. The search and analysis of the nucleotide sequences of COI-5p and 12S rRNA genes of indicator fish species in freshwater reservoirs were performed in the International Database GenBank Nucleotide Sequences on NCBI website.[10] Multiple alignment of the nucleotide sequences of the COI-5p and 12S rRNA genes of indicator species of freshwater fishes was carried out in the Clustal Omega program.[11] The reconstruction and the view of molecular phylogenetic trees by neighbor-joining method (NJ) and the maximum parsimony method (MP) were carried out using the MEGA7 program.[12]

Table 1 shows the indicator fish species from the Sladeček's list which are used to assess the ecological state of freshwater reservoir and the fish species that inhabit in Sredny Kaban (S. Kaban) lake, with saprobity, indicator weight, and accession number of CO1-5p and 12S rRNA genes in the international database GenBank.

## RESULTS AND DISCUSSION

The phylogenetic trees were constructed on the variable gene CO1-5p and the conservative gene 12S rRNA of indicator fish species by NJ method and the MP method for 28 indicator species of fishes from the Sladeček's list. The bootstrap analysis of the formed clusters was performed.[13]

### The Correlation between Saprobity and the Mitochondrial CO1-5p Gene of Indicator Fish Species

16 clusters are formed on a phylogenetic tree developed according to the variable COI-5p gene of indicator fishes by the NJ method, of which about 70% have a bootstrap value >50% (the clusters with numbers 1–2, 4–5, 8–12, and 14–15) and <50% (the clusters with numbers 3, 6, 7, 13, and 16) [Figure 1]. At the same time, the phylogenetic tree constructed by the MP method, half of the clusters with the same organisms were formed in comparison with the NJ method with a bootstrap value >50% [Figure 1].

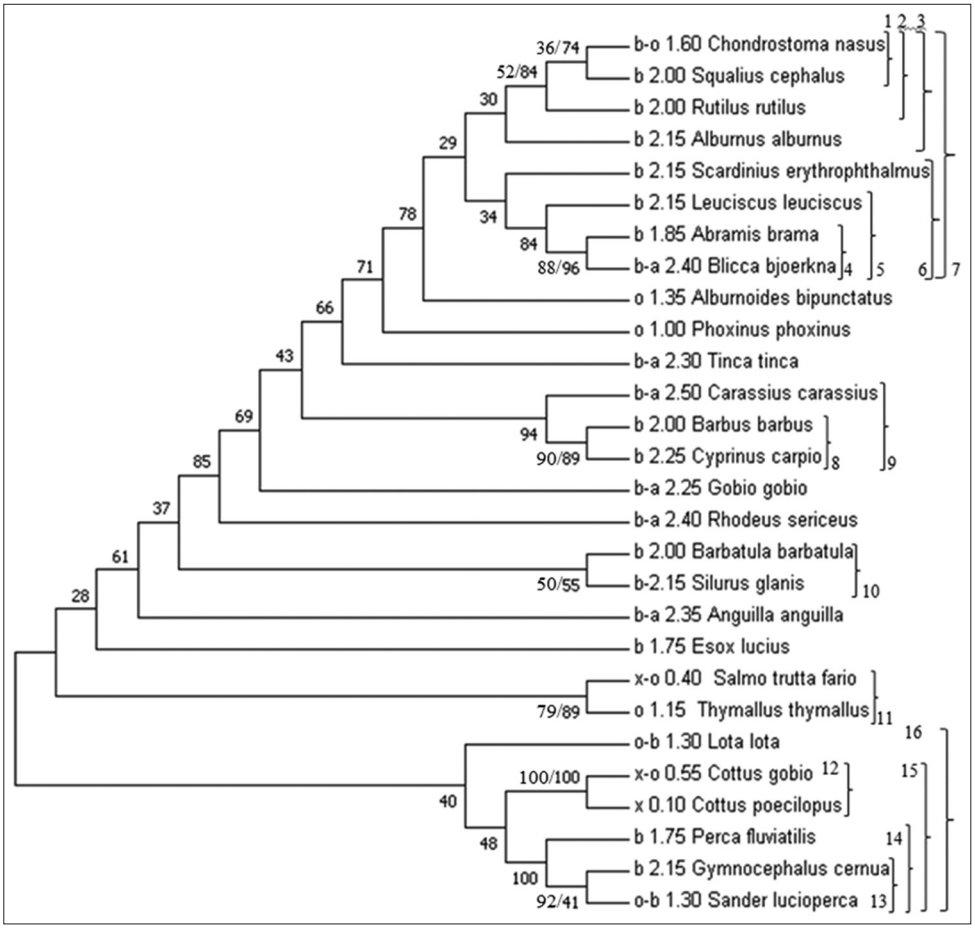


**Figure 1:** Phylogenetic tree constructed using COI-5p gene of indicator fish species

**Table 1:** Accession numbers of CO1-5p and 12S rRNA genes in GenBank database of fish species with the saprobity and indicator weight

| Species | Saprobity | Indicator weight | Accession number in GenBank database | | Species of S. Kaban lake |
|---|---|---|---|---|---|
| | | | COI 5p (Sladeček) | 12S pPHK (Sladeček) | |
| *A. brama* | b | 1.85 | KR476989 | KC894466 | + |
| *A. bipunctatus* | o | 1.35 | KM286435 | - | - |
| *A. alburnus* | b | 2.15 | KM373683 | NC_008659 | + |
| *A. anguilla* | b-a | 2.35 | KM286458 | KJ564270 | - |
| *B. barbatula* | b | 2.00 | KM373684 | NC_027192 | + |
| *B. barbus* | b | 2.00 | KM286499 | NC_008654 | - |
| *B. bjoerkna* | b-a | 2.40 | KR477008 | NC_020355 | + |
| *C. carassius* | b-a | 2.50 | KM286503 | JQ911695 | + |
| *C. gibelio* | - | - | - | GU170401 | + |
| *C. nasus* | b-o | 1.6 | KM286519 | - | - |
| *C. taenia* | - | - | - | - | + |
| *C. gobio* | o-x | 0.55 | KM373675 | - | - |
| *C. poecilopus* | x | 0.10 | HQ961093 | NC_014849 | - |
| *C. carpio* | b | 2.25 | KM492736 | JN105352 | - |
| *E. lucius* | b | 1.75 | HM563702 | NC_004593 | + |
| *G. gobio* | b-a | 2.25 | KM373667 | AB239596 | - |
| *G. cernua* | b | 2.15 | KR477204 | NC_025785 | + |
| *L. delineatus* | - | - | - | NC_020357 | + |
| *L. idus* | - | - | - | KF913024 | + |
| *L. leuciscus* | b | 2.15 | KM286751 | - | - |
| *L. lota* | o-b | 1.30 | KM286761 | KM201364 | - |
| *M. fossilis* | - | - | - | - | + |
| *N. melanostomus* | - | - | - | KU755530 | + |
| *P. fluviatilis* | b | 1.75 | KR477077 | NC_026313 | + |
| *P. glenii* | - | - | - | KM657956 | + |
| *P. phoxinus* | o | 1.00 | KR477082 | KC992395 | - |
| *R. sericeus* | b-a | 2.40 | HQ557338 | NC_025326 | - |
| *R. rutilus* | b | 2.00 | KM287069 | - | + |
| *S. trutta fario* | x-o | 0.40 | FJ907951 | KT633607 | - |
| *S. lucioperca* | o-b | 1.30 | KR477271 | KM410087 | + |
| *S. erythrophthalmus* | b | 2.15 | KJ554942 | NC_031561 | + |
| *S. glanis* | b | 2.15 | KP237865 | NC_014261 | - |
| *S. cephalus* | b | 2.00 | KM287150 | NC_031540 | - |
| *T. thymallus* | o | 1.15 | KR477290 | FJ853655 | - |
| *T. tinca* | b-a | 2.30 | KR477138 | NC_008648 | + |

*A. brama: Abramis brama, A. bipunctatus: Alburnoides bipunctatus, A. alburnus: Alburnus alburnus, A. Anguilla: Anguilla Anguilla, B. barbatula: Barbatula barbatula, B. barbus: Barbus barbus, B. bjoerkna: Blicca bjoerkna, C. carassius: Carassius carassius, C. gibelio: Carassius gibelio, C. nasus: Chondrostoma nasus, C. taenia: Cobitis taenia, C. gobio: Cottus gobio, C. poecilopus: Cottus poecilopus, C. carpio: Cyprinus carpio, E. lucius: Esox Lucius, G. gobio: Gobio gobio, G. cernua: Gymnocephalus cernua, L. delineatus: Leucaspius delineatus, L. idus: Leuciscus idus, L. leuciscus: Leuciscus leuciscus, L. lota: Lota lota, M. fossilis: Misgurnus fossilis, N. melanostomus: Neogobius melanostomus, P. fluviatilis: Perca fluviatilis, P. glenii: Perccottus glenii, P. phoxinus: Phoxinus phoxinus, R. sericeus: Rhodeus sericeus, R. rutilus: Rutilus rutilus, S. trutta* fario*: Salmo trutta* fario*, S. lucioperca: Sander lucioperca, S. erythrophthalmus: Scardinius erythrophthalmus, S. glanis: Silurus glanis, S. cephalus: Squalius cephalus, T. thymallus: Thymallus thymallus, T. tinca: Tinca tinca*

The value of the bootstrap estimate (% of 100 bootstrap replicas) for the MP_ and NJ_ trees, respectively, is shown next to the node on Figure 1.

According to the results of the phylogenetic tree reconstruction for the COI-5p gene, it can be seen that the organisms of different genus and species are grouped into clusters corresponding to the same or close saprobic zone:

Mainly beta-mesosaprobic zone (b):
- *Chondrostoma nasus, Squalius cephalus, Rutilus rutilus, Alburnus alburnus, Abramis brama, Blicca bjoerkna, Leuciscus leuciscus, and Scardinius erythrophthalmus* (cluster 7);
- *Barbus barbus, Cyprinus carpio, and Carassius carassius* (cluster 9);
- *Barbatula barbatula, C. carpio* (cluster 10);
- *Gymnocephalus cernua, Sander lucioperca,* and *Perca fluviatilis* (cluster 14).

Mainly xeno-oligosaprobic zone (x-o):
- *Cottus gobio and Cottus poecilopus* (cluster 12).

Mainly oligosaprobic zone (o):
- *Salmo trutta fario and Thymallus thymallus* (cluster 11);
- *Alburnoides bipunctatus and Phoxinus phoxinus.*

Mainly beta-alpha-mesosaprobic zone (b-a):
- *Gobio gobio and Rhodeus sericeus.*

Thus, during the comparison of trees constructed according to COI-5p gene by two methods, we observe the clusterization of indicator species of organisms according to saprobity, and the clusters with different values of the saprobity zone do not overlap.

## The Correlation between Saprobity and the Mitochondrial 12S rRNA Gene of Indicator Fish Species

The phylogenetic trees were constructed on the conservative 12S rRNA gene as an alternative to the variable CO1-5p gene of indicator fish species [Figure 2].

Nine clusters are formed on a phylogenetic tree constructed from the conservative 12S rRNA gene of indicator fish species by the NJ method, of which about 80% have a bootstrap value of about 100% (the clusters with the numbers 1–2 and 4–8) and <50% are the clusters with the numbers 3 and 9 [Figure 2]. At the same time, 7 clusters with the same organisms are formed on a phylogenetic tree constructed by the MP method, as compared to the NJ method with a bootstrap value about 100% [Figure 2].

The value of the bootstrap estimate (% of 100 bootstrap replicas) for the MP_ and NJ_ trees, respectively, is shown next to the node on Figure 2.

The comparative analysis of phylogenetic trees using COI-5p and 12S rRNA genes by two methods shows stable clustering

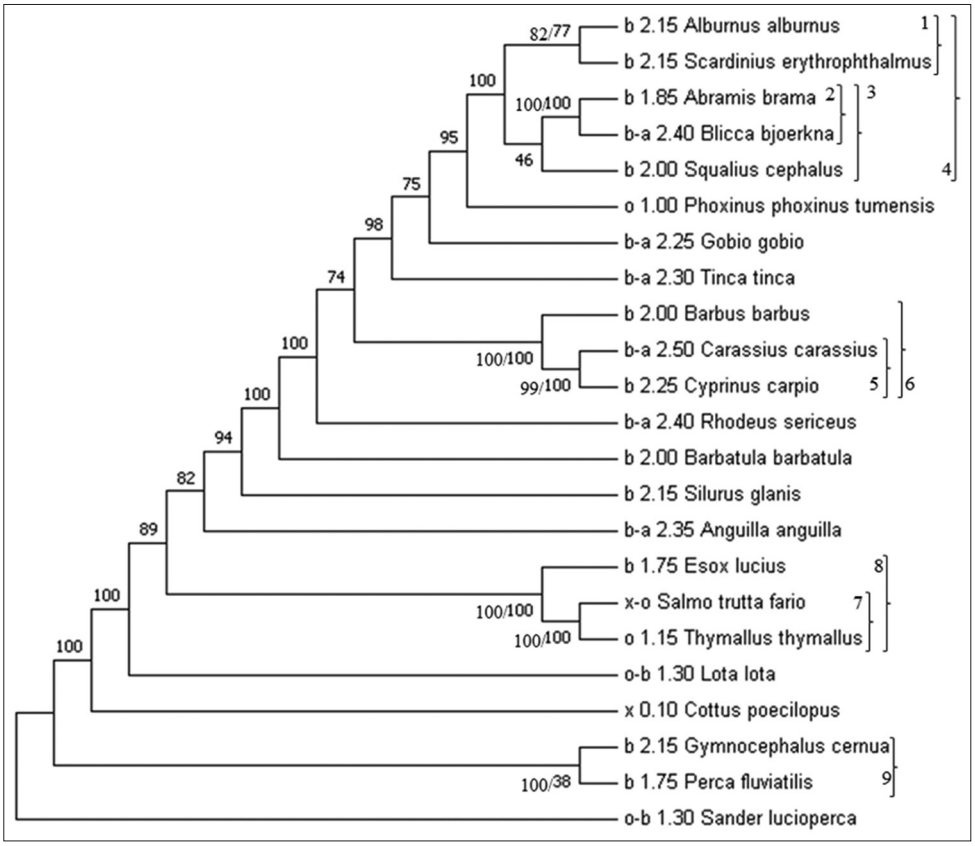


**Figure 2:** Phylogenetic tree constructed using 12S rRNA gene of indicator fish species

of the same indicator species of organisms with the same and/or close saprobity with a high degree of bootstrap support.

Thus, preliminary results have been obtained that show the correlation between saprobity and mitochondrial genes of indicator fish species and provide the optimistic grounds for further research.

### Validation of Correlation between Saprobity and Mitochondrial Genes COI-5p and 12S rRNA of Indicator Fish Species

To validate the correlation between saprobity and mitochondrial genes COI-5p [Figure 3] and 12S rRNA [Figure 4] of indicator fish species, phylogenetic trees were constructed by two methods for indicator fish species and species of fish that inhabit in S. Kaban lake of Kazan city (Russia). According to the ecologists' research, the S. Kaban lake refers to the beta-mesosaprobic (b) by trophic scale.[14]

Below there are trees where 5 clusters for COI-5p gene and 4 clusters for 12S rRNA gene are isolated, these clusters include indicator fish species and fish species from the S. Kaban lake, which do not currently have a specific saprobic indicator signification: *Carassius gibelio, Leucaspius delineatus, Leuciscus idus, Cobitis taenia, Misgurnus fossilis, Percottus glenii,* and *Neogobius melanostomus.* The value of the bootstrap estimate (% of 100 bootstrap replicas) for the MP_ and NJ_ trees, respectively, is shown next to the node on Figure 3.

As can be seen from Figure 3, the above-mentioned species by COI-5p gene are grouped with the indicator species mainly of the beta-mesosaprobic zone with a high bootstrap estimate:

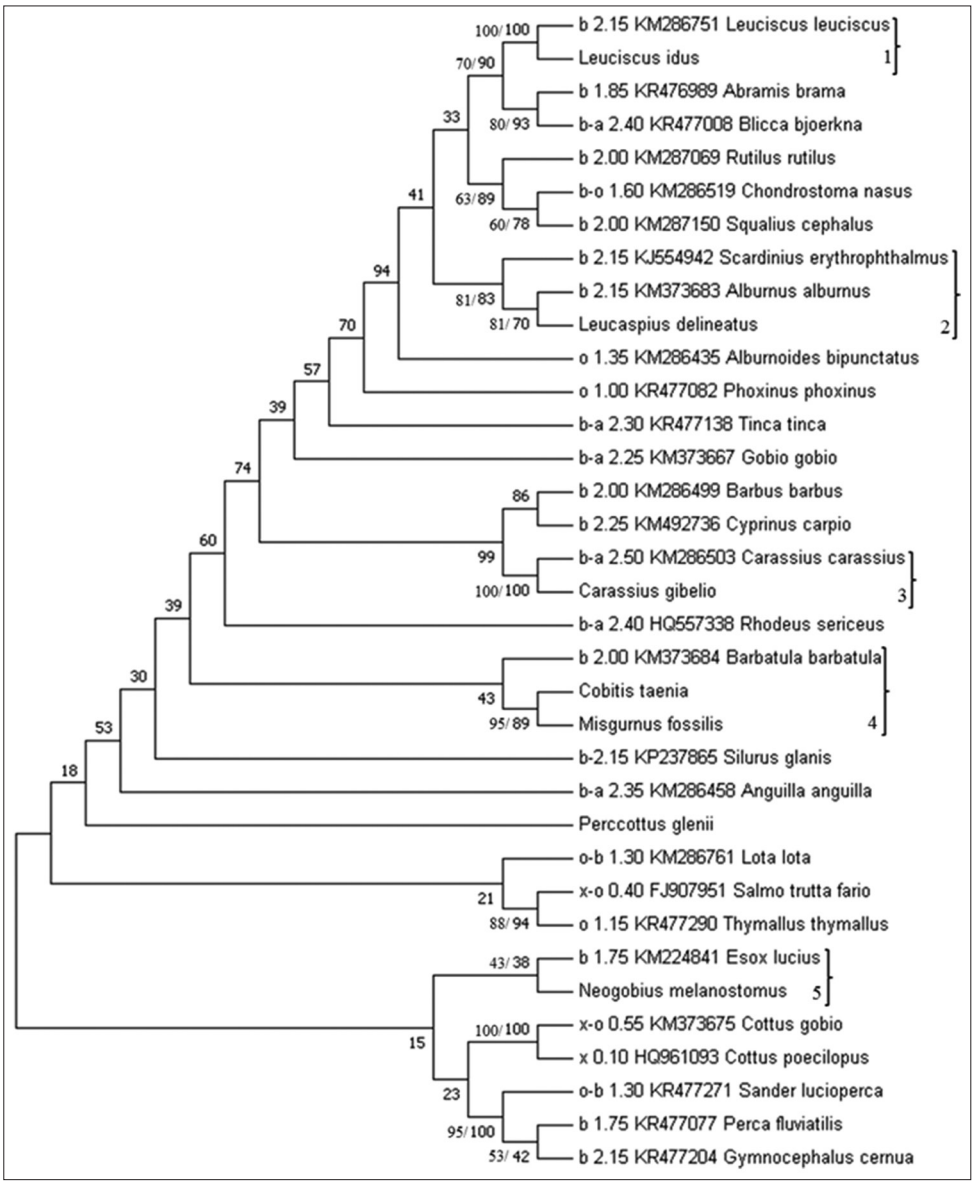


**Figure 3:** Phylogenetic tree constructed using COI-5p gene of indicator fish species with the fish species from S. Kaban lake

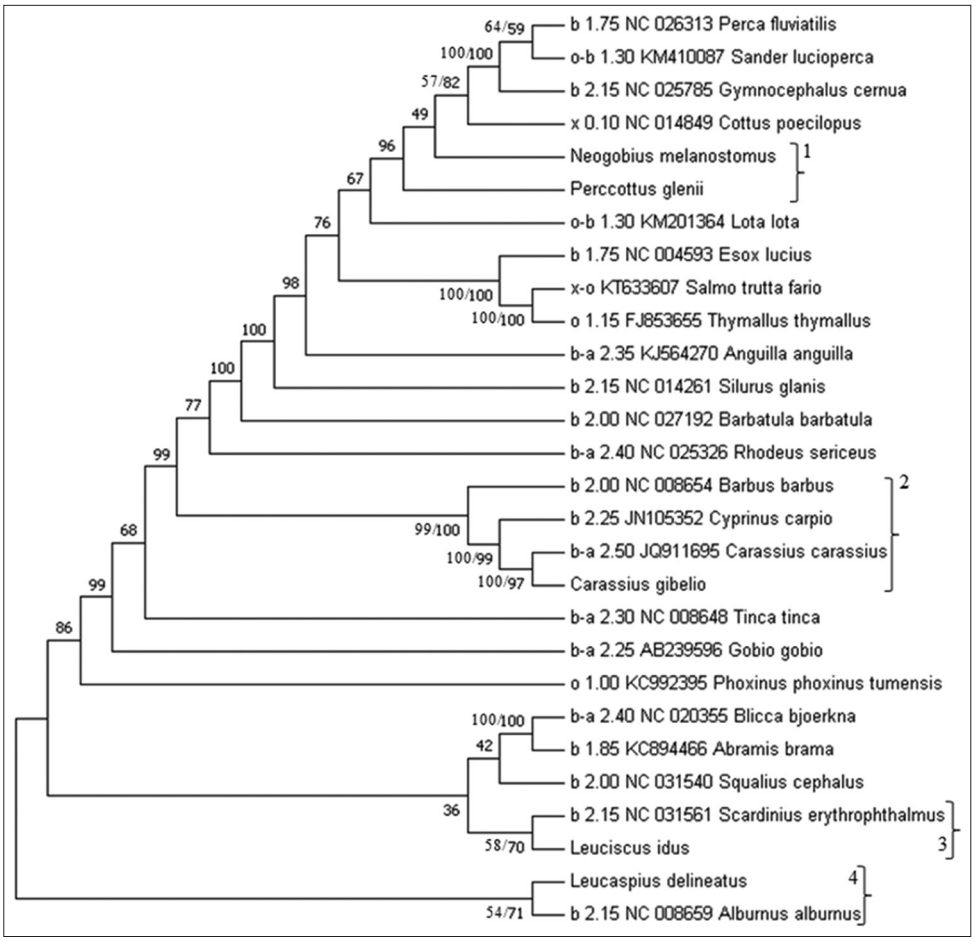


**Figure 4:** Phylogenetic tree constructed using 12S rRNA gene of indicator fish species with the fish species from the S. Kaban lake

- Beta-mesosaprobic zone (b): *L. idus* and *L. leuciscus (cluster 1); L. delineatus, S. erythrophthalmus,* and *A. alburnus* (cluster 2); *C. taenia, M. fossilis, B. barbatula* (cluster 4); *N. melanostomus,* and *Esox lucius* (cluster 5);
- Beta-alpha-mesosaprobic zone (b-a): *C. gibelio* and *C. carassius* (cluster 3).

As can be seen from Figure 4, the fish species from S. Kaban lake according to 12S rRNA gene are also grouped with the indicator species of mainly beta-mesosaprobic zone (b): *C. gibelio* (cluster 2), *L. idus* (cluster 3), and *L. delineates* (cluster 4) with the bootstrap estimate of more than 50%. The value of the bootstrap estimate (% of 100 bootstrap replicas) for MP_ and NJ_ trees, respectively, is shown next to the node on Figure 4.

Thus, above-mentioned results show that the species without the saprobic indicator, living in the beta-mesosaprobic (b) S. Kaban lake, are grouped on phylogenetic trees by mitochondrial genes with the indicator species of the same beta-mesosaprobic zone (b).

## CONCLUSIONS

Based on the results of the study, the correlation between saprobity and mitochondrial genes of freshwater indicator fish species was shown for the first time based on phylogenetic trees reconstruction of COI-5p and 12S rRNA genes by NJ method and MP method. Preliminary results on stable clustering of indicator fish species from freshwater reservoirs are obtained by saprobity zones. The validity of the correlation between saprobity and mitochondrial genes using the example of fish species inhabiting in the S. Kaban lake has been performed, and satisfactory results have been obtained, which are of great scientific and practical interest for further research.

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