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Evaluation of the Water Reservoirs Ecological State Using the COI Protein of Zoobenthos.

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ABSTRACT

One of the widespread research objects for ecological condition of fresh-water reservoirs along with a zooplankton is zoobentos organisms. The analysis of 79 CO1 proteins indicator the zoobentos organisms from the V. Sladcheka (1973) list revealed existence of 32 unique sites of amino-acid sequences of CO1 for 19 types the zoobentos organisms. Unique variable sites located on CO1 protein surface of zoobentos organisms do not coincide with 36 unique variable sites of indicator zooplanktonic organisms, and can specifically be distinguished by antibodies in one water samples with use of the IFA-method. Researches on use of CO1 protein as marker for identification of zoobenthos along with zooplankton in water samples give more reliable assessment of reservoir ecological condition.

Key terms: COI protein marker, zoobenthos, indicator types

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INTRODUCTION

Currently the ecological status of water objects is estimated by means of saprobity coefficient, paying off a ratio of indicator types in water samples [1]. It is known that one water sample contains a set of animal and vegetable organisms of different taxons, part of which serve as indicators for ecological condition of a reservoir. It is known that a number of requirements developed on the international level is due to indicator types of reservoir saprobity. According to these requirements it is hard to find separate systematic groups of organisms by means of which it would be possible to carry out reservoir monitoring in full.

Bioindication methods for fresh-water reservoirs are based on multilevel biological indicator system. One of the widespread objects for researching the fresh-water reservoirs along with zooplankton is zoobentos organisms. They are often found, large in size for convenience of collecting and processing [2].

Traditionally identification of organism types in samples is carried out visually by highly skilled specialists-zoologists using continuants [3]. At the same time the modern methods of molecular and genetic analysis enable us to define organisms instrumentally by type. Thus, for example, the DNA-bar-code method [4] is used for this purpose. Numerous DNA bar codes by organism types accumulate in the international database of nucleotide sequences of GenBank, and their proteinaceous sequences are stored in the GenPept database [5]. At the heart of the DNA-bar-code method lies the sequence of DNA-bar-code nucleotides identical for specimens of just one type, for example, for animals it is the variable fragment of CO1 600-700 gene base pairs [6, 7]. Whereas results of scientific researches on nucleotide sequences of CO1 for animal organisms often occur in publications [8, 9], possibilities of CO1 gene product use are little-known.

Thus, in our previous work possibility of CO1 protein use as zooplankton identification marker in water samples for assessment of reservoirs ecological condition was shown [10].

At this stage we conducted researches on use of CO1 protein as zoobenthos identification marker which resulted, along with zooplankton in water samples, in more reliable assessment of reservoir ecological condition.

MATERIALS AND METHODS

In this work indicator types of zoobenthos from V. Sladeczek's list are used [1]. Amino-acid sequences of CO1 protein of zoobenthos indicator types are received from the international GenPept database on the NCBI site (www.ncbi.nlm.nih.gov/).

Multiple alignment of CO1 protein amino-acid sequences of zoobenthos indicator types of organisms is performed in on-line mode using the Clustal Omega program [11]. The leveled CO1 protein amino-acid sequences of zoobenthos are analysed in the Jalview 2.8.0b1 program [12].

3D model operation of zoobenthos indicator types of organisms COI proteins is carried out in the SWISS-MODEL program [13], and the analysis of these models in the PDB format is carried out in the Jalview program.

RESULTS AND DISCUSSIONS

Developing ecologic-and-genetic database of zoobentos organisms

In the developed ecologic-and-genetic database results of research on amino-acid sequences cytochrome of c-oxydes 1 (CO1) for 249 zoobenthos indicator types from which for 79 types information on amino-acid sequences of CO1 protein in the international database of amino-acid sequences of GenPept (table 1) is placed are presented.

Table 1: Ecological and genetic database of indicator zoobenthos species

No.	Species	Saprobity	Indicator weight	Accession number in the database GenPept
Phylum Mollusca, Classis Gastropoda, Ordo Planorboidea, Familia Planorbidae				
1	<i>Ancylus fluviatilis</i>	o-b	1.35	AAQ22736
Ordo Acroloxoidea, Familia Acroloxidae				
2	<i>Acroloxus lacustris</i>	b	2.00	AAQ22735
Ordo Risssoidea, Familia Bithyniidae				
3	<i>Bithynia tentaculata</i>	b	2.00	AFY03556
Familia Amnicolidae				
4	<i>Bythinella austriaca</i>	x	0.10	ADR64561
Ordo Planorboidea, Familia Planorbidae				
5	<i>Gyraulus albus</i>	b	2.00	CAA75012
6	<i>Planorbis corneus</i>	b	2.00	AAQ22744
7	<i>Planorbis planorbis</i>	b	2.00	ABM63214
Familia Physidae				
8	<i>Physa fontinalis</i>	b	2.00	ACJ09165
9	<i>Physa acuta</i>	a	3.00	ACJ09163
Ordo Lymnaeidea, Familia Lymnaeidae				
10	<i>Radix auricularia</i>	b-a	2.50	AJE70572
11	<i>Radix peregra</i>	b	2.00	AET42998
Ordo Neritoidea, Familia Neritidae				
12	<i>Theodoxus fluviatilis</i>	b-o	1.50	AHY24766
Ordo Valvatoidea, Familia Valvatidae				
13	<i>Valvata piscinalis</i>	b	2.00	ACZ97576
Classis Bivalvia, Ordo Unionoidea, Familia Unionidae				
14	<i>Anodonta cygnea</i>	b	2.00	ACZ64217
15	<i>Margaritana margaritifera</i>	o	0.90	AAN31166
16	<i>Unio crassus</i>	b-o	1.50	AAM49791
17	<i>Unio pictorum</i>	b	2.30	AHA43261
18	<i>Unio tumidus</i>	b	2.00	ACZ64219
Phylum Crustacea, Classis Branchiopoda, Ordo Cladocera, Familia Macrothricidae				
19	<i>Acantholeberis curvirostris</i>	o	1.10	ABC41992
Familia Chydoridae				
20	<i>Acroperus harpae</i>	o-b	1.40	ABC42001
21	<i>Alonella excisa</i>	o	1.20	AEL16787
22	<i>Alonella exigua</i>	o	1.20	ABC41996
Familia Bosminidae				
23	<i>Bosmina coregoni</i>	o	0.95	AAL80020
24	<i>Bosmina longirostris</i>	o-b	1.35	ACD86050
Familia Chydoridae				
25	<i>Camptocercus rectirostris</i>	o	1.20	ABC42000
26	<i>Chydorus sphaericus</i>	b	1.75	ACJ01598
27	<i>Dunhevedia crassa</i>	b	1.70	AGL10338
Familia Daphniidae				

No.	Species	Saprobity	Indicator weight	Accession number in the database GenPept
28	<i>Ceriodaphnia laticaudata</i>	b-o	1.60	ACD86115
29	<i>Daphnia atkinsoni</i>	o-b	1.50	ABA54601
30	<i>Daphnia magna</i>	a-p	3.40	AHY19796
31	<i>Daphnia similis</i>	o-b	1.50	AED99113
32	<i>Daphnia cucullata</i>	b-o	1.75	AED99107
33	<i>Daphnia longispina O.F.M.</i>	b	2.05	ABS86783
34	<i>Daphnia obtusa</i>	b-o	1.60	AAQ90455
35	<i>Daphnia pulex DEGEER</i>	a	2.80	AFV14128
36	<i>Daphnia pulex DEGEER</i>	b	2.20	AFV14130
37	<i>Scapholeberis mucronata</i>	b	2.00	ABO28841
Familia Sididae				
38	<i>Diaphanosoma brachyurum</i>	o	1.40	ABO28831
39	<i>Sida crystallina</i>	o	1.30	ABC42010
Familia Eurycercidae				
40	<i>Eurycerus lamellatus</i>	o	1.20	AFC87916
41	<i>Graptoleberis testudinaria</i>	o-b	1.50	ABC42002
Familia Holopedidae				
42	<i>Holopedium gibberum</i>	x-o	0.60	ABK05966
Familia Leptodoridae				
43	<i>Leptodora kindti</i>	o-b	1.63	ADU52938
Familia Moinidae				
44	<i>Moina brachiata</i>	b-a	2.45	AEZ36020
45	<i>Moina macrocopa</i>	a	2.75	AGL10468
46	<i>Moina micrura</i>	b	2.20	AGP50644
47	<i>Moina rectirostris</i>	a-p	3.40	AJP16689
Familia Polyphemidae				
48	<i>Polyphemus pediculus</i>	o	1.30	ADA68671
Classis Maxillopoda, Ordo Copepoda, Familia Cyclopidae				
49	<i>Acanthocyclops vernalis</i>	b	1.63	AFZ64415
50	<i>Cyclops strenuus</i>	b	2.25	AGJ74817
51	<i>Eucyclops macruroides</i>	o	1.00	AGJ74852
52	<i>Eucyclops macrurus</i>	o-b	1.40	AGJ74854
53	<i>Eucyclops serrulatus</i>	b	1.63	AEH50084
54	<i>Macrocylops albidus</i>	b	2.00	AGU02293
55	<i>Macrocylops distinctus</i>	o	1.00	AGU02292
56	<i>Megacyclops viridis</i>	b-o	1.65	AGJ74866
57	<i>Mesocyclops leuckarti</i>	o	1.25	AGU02294
58	<i>Thermocyclops oithonoides</i>	o	1.30	AGU02290
59	<i>Tropocyclops prasinus</i>	o	1.00	AGL10838
Familia Diaptomidae				
60	<i>Acanthodiaptomus denticornis</i>	o	1.20	AGT56886
61	<i>Eudiaptomus gracilis</i>	o	1.25	ABE68707
62	<i>Eudiaptomus graciloides</i>	b-o	1.60	AIN35078

No.	Species	Saprobity	Indicator weight	Accession number in the database GenPept
Classis Malacostraca, Ordo Isopoda, Familia Asellidae				
63	<i>Asellus aquaticus</i>	a	2.80	AJD09438
Ordo Gammarus, Familia Gammaridae				
64	<i>Gammarus balcanicus</i>	x	0.20	AGX00128
65	<i>Gammarus pulex</i>	x-o	0.65	ABX59177
Classis Ostracoda, Ordo Cypridoidea, Familia Cyprididae				
66	<i>Cypridopsis vidua</i>	b	1.80	AHM63680
Phylum Arthropoda, Classis Arachnida, Ordo Hydracarina, Familia Hygrobatidae				
67	<i>Hygrobates fluvialtilis</i>	b-o	1.60	ACV82349
Phylum Annelida, Classis Oligochaeta, Ordo Tubificina, Familia Tubificidae				
68	<i>Limnodrilus hoffmeisteri</i>	p-a	3.60	AAQ10449
69	<i>Lumbricus rubellus</i>	a	3.00	CCE60882
70	<i>Tubifex tubifex</i>	p	3.80	AAQ10450
Familia Naididae				
71	<i>Stylaria lacustris</i>	b	2.00	AAQ10445
Ordo Lumbriculida, Familia Lumbriculidae				
72	<i>Stylodrilus heringianus</i>	o-b	1.75	AGT55935
Classis Hirudinea, Ordo Archynchobdellea, Familia Haemopidae				
73	<i>Haemopsis sanguisuga</i>	b	1.70	AAL69897
Ordo Rhynchobdella, Familia Glossiphoniidae				
74	<i>Helobdella stagnalis</i>	a	2.60	AJD19671
Familia Piscicolidae				
75	<i>Piscicola geometra</i>	b	2.00	AAB88043
Phylum Porifera, Classis Demospongiae, Ordo Haplosclerida, Familia Spongillidae				
76	<i>Ephydatia fluvialtilis</i>	b	2.00	AEA11208
Phylum Cnidaria, Classis Coelenterata, Ordo Anthoathecata, Familia Hydridae				
77	<i>Pelmatohydra oligactis</i>	b	1.75	ABK58274
Phylum Platyhelminthes, Classis Turbellaria, Ordo Seriata, Familia Planariidae				
78	<i>Crenobia alpina</i>	x	0.10	AJO64714
79	<i>Dendrocoelum lacteum</i>	b	2.00	AAK19063

Apparently from the above-stated table, 79 types of zoobenthos are indicators of different zones of reservoir saprobity: from "clear" (xenosaprobic weighing 0.1) to "dirty" (the polisaprobic weighing 3.8).

We believe that zoobenthos indicator organisms can be, as well as zooplanktonic organisms, identified by means of CO1 marker genes products. Thus, identification of zoobenthos along with zooplankton by means of CO1 protein in water sample will increase reliability of reservoir ecological condition assessment.

Identification of CO1 proteins epitopes of zoobenthos organisms

For identification of unique sites of CO1 proteins in zoobenthos organisms multiple alignment of 79 amino-acid sequences of CO1 protein indicators in zoobenthos organisms is carried out (Rice 1).

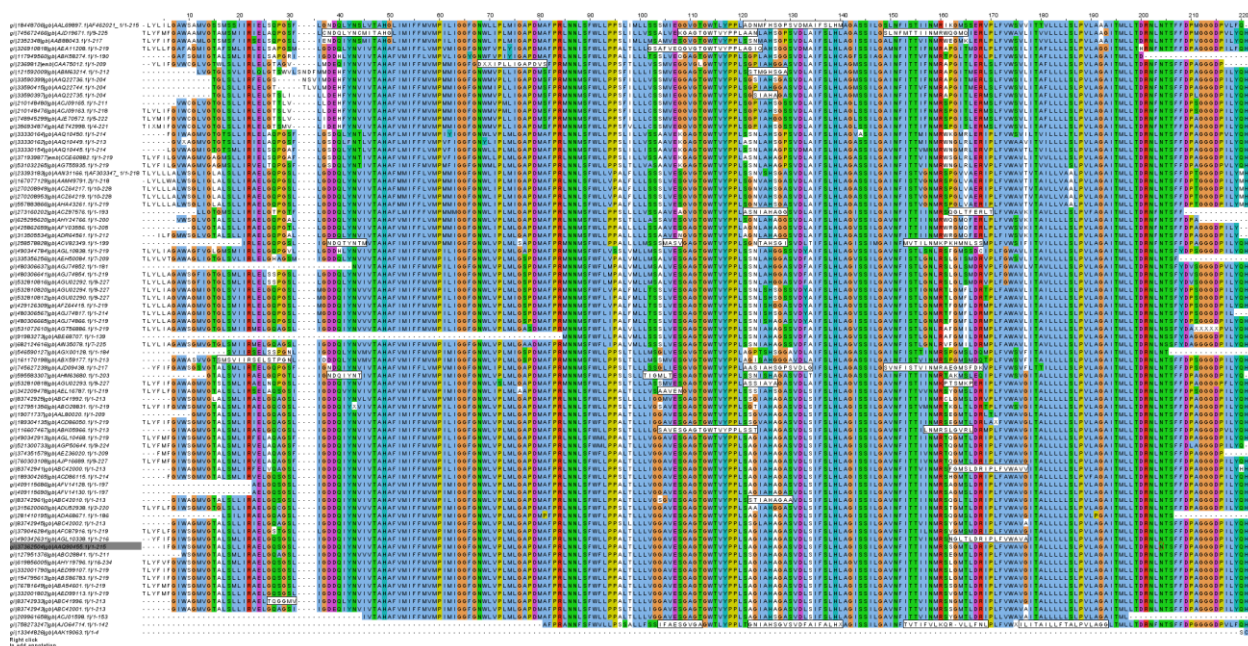


Figure 1: Multiple alignment of amino-acid sequences of zoobentos organisms CO1 protein.

Analysis of multiple amino-acid sequences alignment of CO1 protein in zoobentos organisms showed the existence of 32 unique sites of 19 types of zoobenthos from 4 to 22 amino-acid remains long (a.o.) with an optimum length from 6 to 17 a.o. for antibodies discernment (table 2).

Table 2: Unique variable sequences of zoobenthos species COI protein indicator

Species, Accession number in database GenPept	Saprobity*	Indicator weight	Unique fragments of amino acid sequences of the COI protein in zoobenthos with positions in alignment
<i>Ephydatia fluviatilis</i> AEA11208	b	2.00	GSFVFEQGVGTGWTVPPLAGIQ (117-139)
<i>Haemopsis sanguisuga</i> AAL69897	b	1.70	ADNMFHSGPSVDMAIFSLHM (136-155)
<i>Helobdella stagnalis</i> AJD19671	a	2.60	CNDQLYNCMITAHG (52-65) KGAGTGWTVYPPLAAN (123-138) SLNFMTTIINMRWQGMQ (164-180)
<i>Gyraulus albus</i> CAA75012	b	2.00	DXXIPLLIGAPDVS (49-65)
<i>Planorbis planorbis</i> ABM63214	b	2.00	STMGHSGA (136-144)
<i>Acroloxus lacustris</i> AAQ22735	b	2.00	SIAHA (138-142)
<i>Valvata piscinalis</i> ACZ97576	b	2.00	ASNIAHAGG (136-144) QGLTFERT (177-185)
<i>Hygrobates fluviatilis</i> ACV82349	b-o	1.60	NDQTYNTM (53-60) MASV (120-123) TAHSGI (139-144) MVTILNMKPKHMLNLS (168-183)
<i>Gammarus pulex</i> ABX59177	x-o	0.65	SMSVIIRSELSTPGN (31-45)
<i>Aesellus aquaticus</i> AJD09438	a	2.80	AASIAHSGPSVDLG (136-149) SVNFISTVINMRAEGMSFDK (164-183)
<i>Cypridopsis vidua</i> AHM63680	b	1.80	NDQIYNT (53-59) TIGMLT (116-121)
<i>Macrocyclus albidus</i> AGU02293	b	2.00	ASSIAYA (136-142) PTSMKP (176-181)
<i>Alonella excise</i> AEL16787	o	1.20	AAVEN (119-123)

<i>Holopodium gibberum</i> ABK05966	x-o	0.60	SAVESGAGTGWTVYPPPLSST (119-138) LNMRS LGVRL (172-181)
<i>Camptocercus rectirostris</i> ABC42000	o	1.20	FGMSLDRIPLFVWAVV (177-192)
<i>Sida crystallina</i> ABC42010	o	1.30	STIAHAGAA (137-145)
<i>Dunhevedia crassa</i> AGL10338	b	1.70	NGLTLDRIPFVWAVA (177-192)
<i>Alonella exigua</i> ABC41996	o	1.20	TQGGMV (41-46)
<i>Crenobia alpina</i> AJO64714	x	0.10	IFAESGVGA (119-127) GNIAHSGVSVDFAI FALH (137-154) TVTIFVLKQR-VLLFNL (168-184) ILITAILLFTALPVL AGG (191-208)


* x-xenosaprobity; o-oligosaprobity; b – beta-mesosaprobity; a – alpha-mesosaprobity; p – polysaprobity

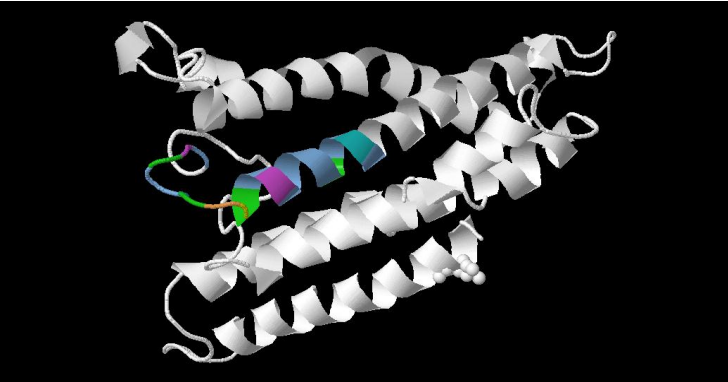
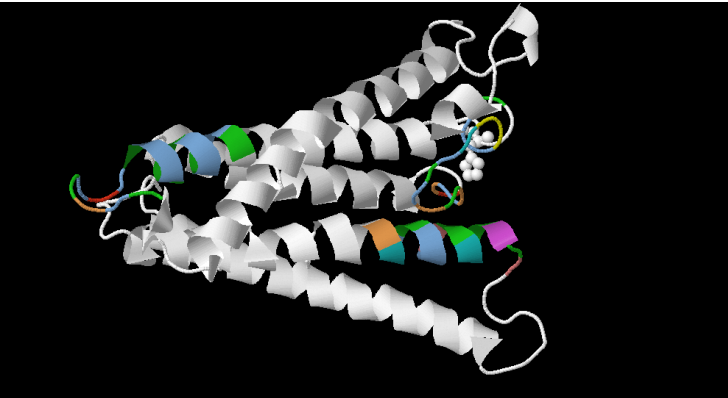
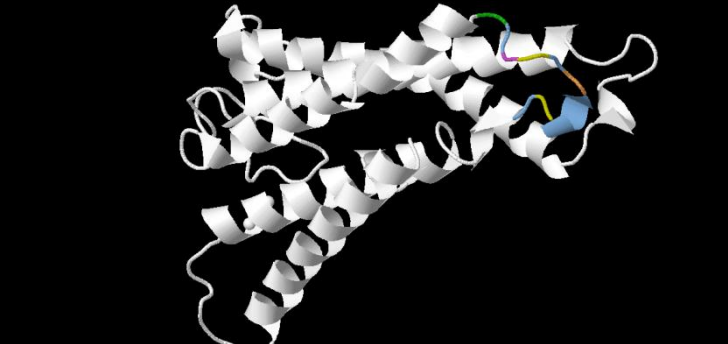

From Table 2 it is visible that different types the zoobentos organisms have from one to four unique variable sites of CO1 amino-acid sequences. For example, CO1 *Dunhevedia crassa* protein 216 a.o long, has one site 15 a.o. long, and CO1 *Crenobia alpina* protein 259 a.o. long has four sites 8 a.o. long, 17 a.o., 16 a.o. and 17 a.o. respectively. Presence of more than one unique variable a site of CO1 amino-acid sequence expands possibilities of identification of this organism.

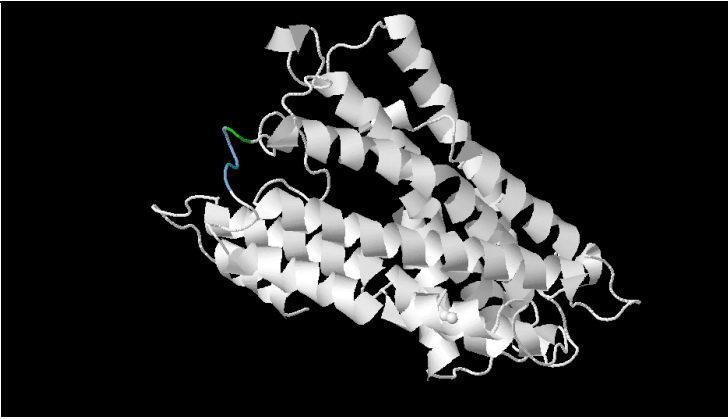
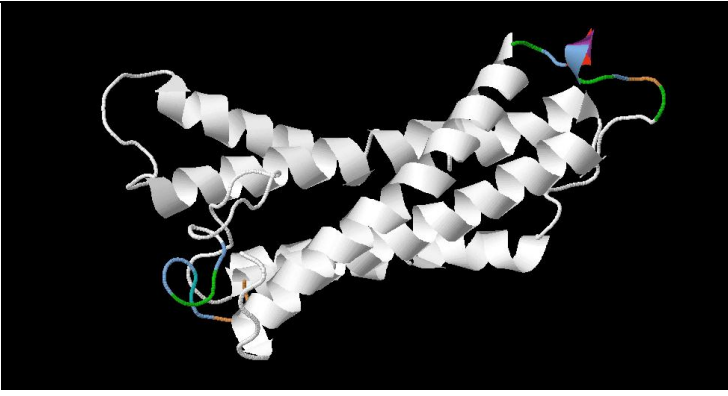
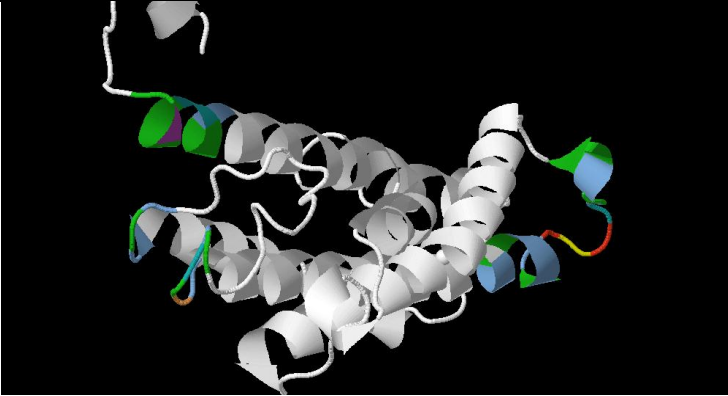
Verification of epitopes availability for antibodies discernment

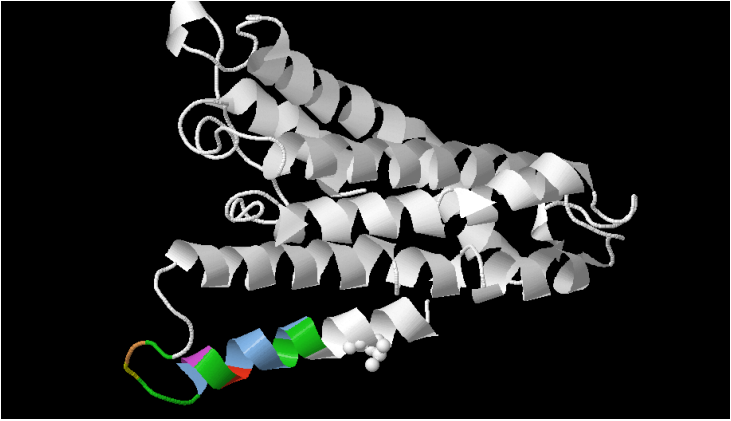
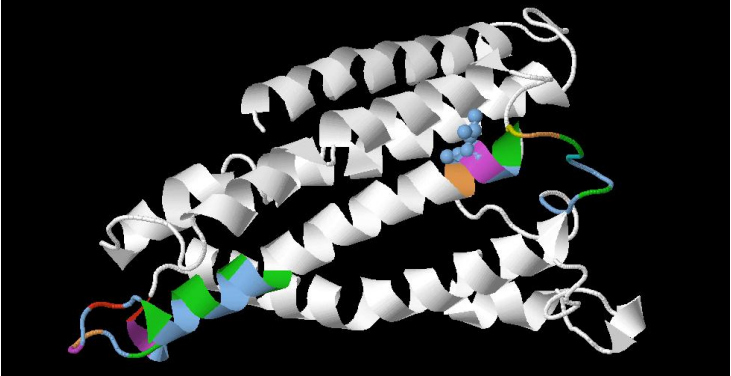
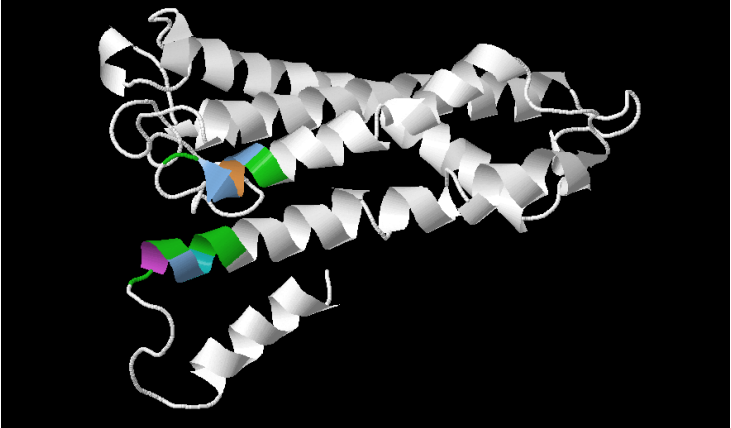
3D models are constructed for verification of 32 unique sites of CO1 proteins indicator in zoobentos organisms availability for antibodies discernment (Tabl 3).


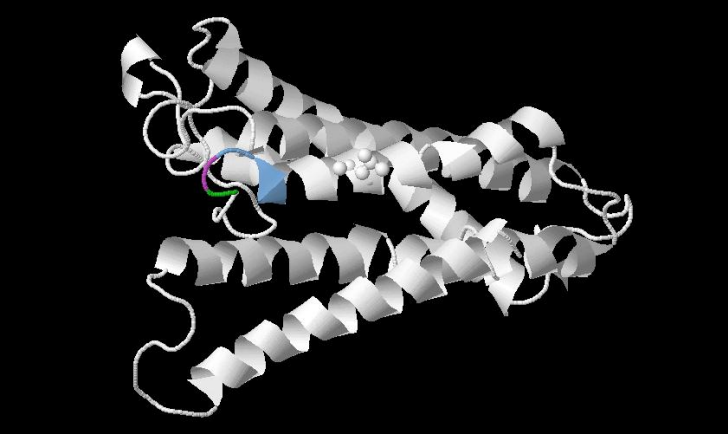
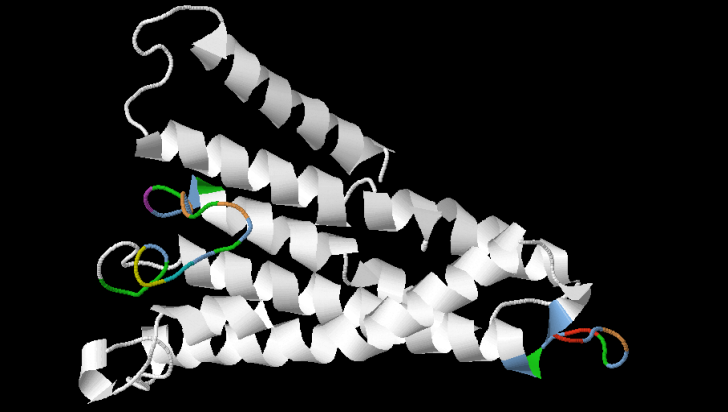
Table 3: 3D-models of amino acid sequences COI of some indicator species of zoobentos

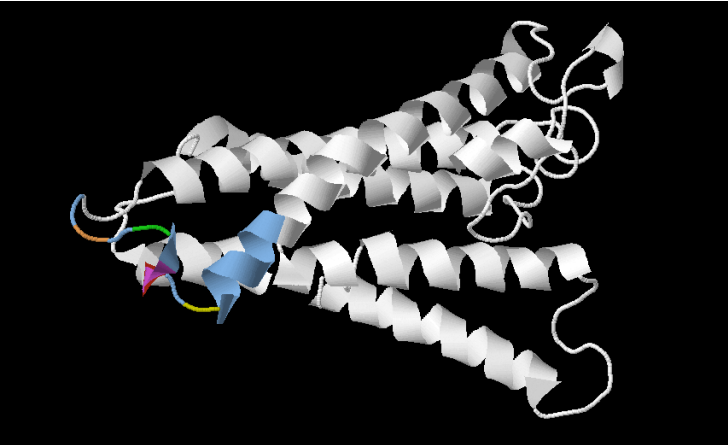
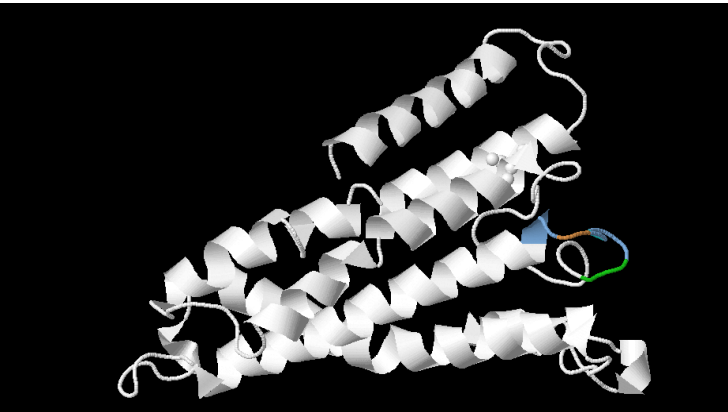
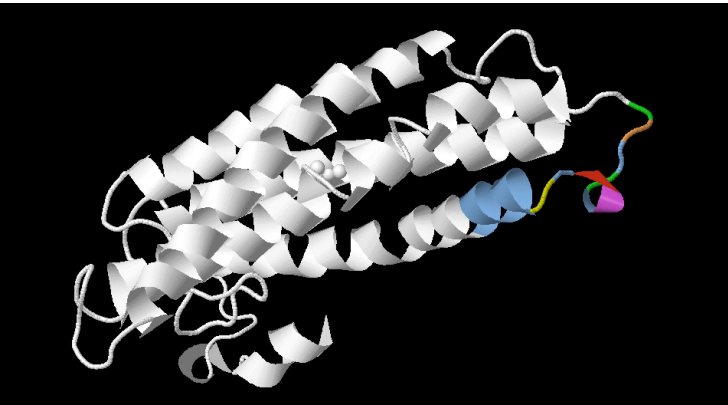
The indicator species of zoobentos / Accession number in the databank GenPept / saprobity*/ indicators weigh	3D-models of amino acid sequences COI
<p><i>Ephydatia fluviatilis</i> AEA11208 b / 2.00</p>	

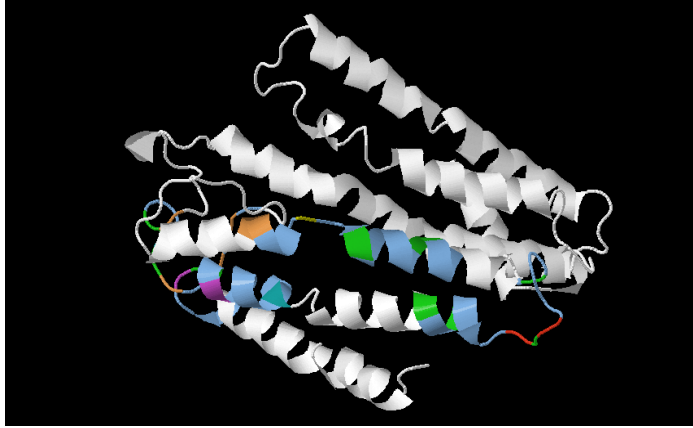
The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weigh	3D-models of amino acid sequences COI
<p><i>Haemopsis sanguisuga</i> AAL69897 b / 1.70</p>	
<p><i>Helobdella stagnalis</i> AJD19671 b / 2.00</p>	
<p><i>Gyraulus albus</i> CAA75012 b / 2.00</p>	
<p><i>Planorbis planorbis</i> ABM63214 b / 2.00</p>	

The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weigh	3D-models of amino acid sequences COI
<p><i>Acroloxus lacustris</i> AAQ22735 b / 2.00</p>	
<p><i>Valvata piscinalis</i> ACZ97576 b / 2.00</p>	
<p><i>Hygrobates fluviatilis</i> ACV82349 b-o / 1.60</p>	

The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weigh	3D-models of amino acid sequences COI
<p><i>Gammarus pulex</i> ABX59177 x-o / 0.65</p>	
<p><i>Asellus aquaticus</i> AJD09438 a / 2.8</p>	
<p><i>Cypridopsis vidua</i> AHM63680 b / 1.80</p>	

The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weigh	3D-models of amino acid sequences COI
<p><i>Macrocyclops albidus</i> AGU02293 b / 2.00</p>	
<p><i>Alonella excise</i> AEL16787 o / 1.20</p>	
<p><i>Holopedium gibberum</i> ABK05966 x-o / 0.60</p>	

The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weigh	3D-models of amino acid sequences COI
<p><i>Camptocercus rectirostris</i> ABC42000 o / 1.20</p>	
<p><i>Sida crystallina</i> ABC42010 o / 1.30</p>	
<p><i>Dunhevedia crassa</i> AGL10338 b / 1.70</p>	
<p><i>Alonella exigua</i> ABC41996 o / 1.20</p>	

The indicator species of zoobenthos / Accession number in the databank GenPept / saprobity* / indicators weight	3D-models of amino acid sequences COI
<p><i>Crenobia alpina</i> AJ064714 x / 0.10</p>	

* x-xenosaprobity; o-oligosaprobity; b – beta-mesosaprobity; a – alpha-mesosaprobity; p – polysaprobity

The analysis of 3D models on CO1 protein indicator for zoobenthos organisms showed that all 32 unique sites are located on molecule surfaces, of which 26 sites are hydrophylic. They are located on alpha spirals and can be therefore accessible to antibodies discernment.

It should be noted that these zoobenthos organisms are used as bioindicators for reservoir saprobity in the wide range, from xeno-saprobic zone weighing 0.1, for example *Crenobia alpina*, to an alpha saprobic zone weighing 2.8, for example *Asellus aquaticus*.

Comparative analysis of epitopes zoobenthos and zooplanktonic organisms

Earlier as a result of CO1 protein amino-acid sequences researches of indicator zooplanktonic organisms we received 36 unique sites which can be used for identification of 23 types of zooplankton, indicators of reservoir saprobity from oligo-("clear") to an alpha polisaprobic ("dirty") [10].

Sharing of 32 epitopes for identification of 19 types of zoobenthos and 36 epitopes for identification of 23 types of zooplankton in water samples will allow to increase reliability of reservoir ecological condition assessment.

For collateral check of CO1 proteins indicator sites uniqueness the zoobenthos and zooplanktonic organisms multiple alignment of their CO1 protein amino-acid sequences (Fig. 2) is carried out.

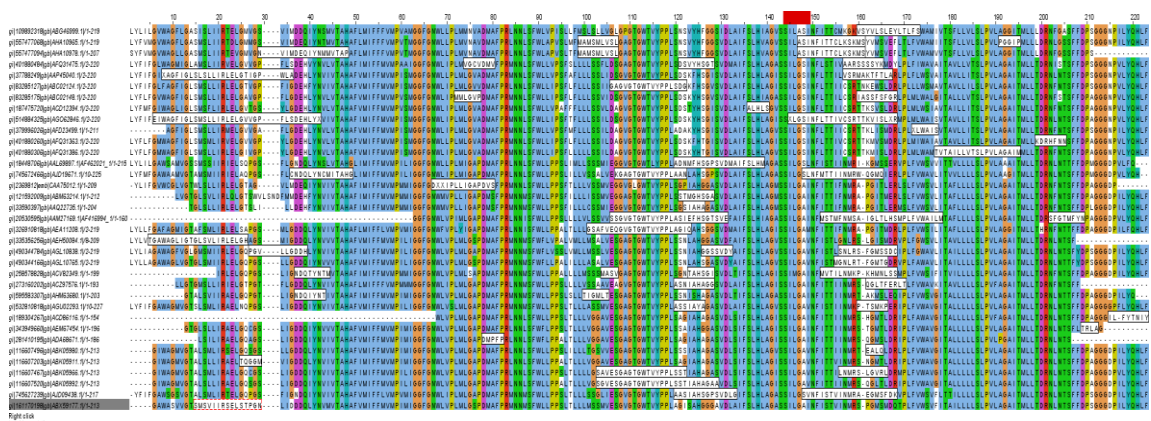


Figure 2: Multiple alignment of CO1 amino-acid sequences of the zoobenthos and the zooplanktonic organisms containing unique sites.

The comparative analysis of 32 and 36 unique sites of CO1 amino-acid sequences of zoobentos and zooplanktonic organisms showed that they do not coincide and can be used for recognition by antibodies, for precise identification of zoobenthos and a zooplankton types in water samples and their sharing will allow to increase reliability of reservoir ecological condition assessment.

CONCLUSION

The analysis of 79 CO1 proteins indicator of zoobentos organisms from the V. Sladcheka (1973) list revealed existence of 32 unique sites of CO1 amino-acid sequences for 19 types of zoobentos organisms. Unique variable sites are located on CO1 protein surface of zoobentos organisms, they do not coincide with 36 unique variable sites of indicator zooplanktonic organisms, and can specifically be distinguished by antibodies for precise identification of zoobenthos and a zooplankton types among organisms of one water sample with use of the IFA-method.

CONCLUSION

The studied organisms are indicators of fresh-water reservoirs saprobity in the wide range: from "clear" to "dirty", and, therefore, their identification on marker CO1 protein will allow to estimate reservoir ecological condition in shorter terms authentically.

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