

Dr. Robert J. Behnke  
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Wildlife Biology  
Colorado State University  
Fort Collins, CO 80523  
USA

October 15, 1991

Dear Dr. Behnke,

I'm sending you these fishes from Elgygytgyn Lake. Dr. I. Chereshev asked me because he could not to do it himself in the Magadan.

Sincerely yours

S. Frolov  
Institute of Marine Biology  
Far East Branch  
Academy of Sciences of the USSR  
Vladivostok 690032, USSR



COMMENTS OF REFEREE (A guide is given on the reverse)

COMMENTAIRES DE L'ARBITRE (Voir guide au verso)

AUTHOR(S)/AUTEUR(S):

V. V. Efremov

TITLE/TITRE:

Genetic differentiation among species of charrs of  
Kamchatka river basin

RECOMMENDATION/RECOMMANDATION

Accept  
Accepter

Accept after suitable revision  
Accepter après révision adéquate

Reconsider after further <sup>revision</sup> research  
À reconsidérer après de plus amples recherches

Reject  
Rejeter

This paper contains significant new information but requires more information to be acceptable for publication. A comprehensive electrophoretic analysis of *S. leucomaenis* (46 loci in present study) has not yet been published. However, the alleles determined at these loci are not identified. The table lists the proteins, the number of loci and if loci are monomorphic or polymorphic, but the alleles are not identified (by degree of mobility in relation to a standard) nor are percent frequencies of alleles at polymorphic loci given. These data would be necessary for an adequate electrophoretic assessment of *S. leucomaenis* -- which would, by itself, be of great significance and highly recommended for publication.

The highly significant results that two "forms" of *malma* are much more different from each other than is one of the *malma* from *S. albus* also requires elucidation. It has long been known that a distinct form of char, commonly known as kamen golets (in Russian) or "stone char", occurs in Kamchatka. In Lake Azabache of Kamchatka River drainage various forms of char have been documented -- predator, mollusk-eater, benthophage, etc. (besides "stone" char). The identification of the two forms of *malma* and of *albus* used in this study must be made clear in relation to char diversity previously identified. The information given simply states that 37 *S. leucomaenis*, 29 *malma*, and 35 *albus*, from the Kamchatka River and L. Azabache were used. There is not doubt about the identification of *leucomaenis*, but the 29 *malma* specimens should be identified -- each form by precise locality and with reference to what form of char each *malma* sample represents in relation to diversity of described forms. Taxonomic data on gill rakers, pyloric caeca, vertebrae, etc. could be used for evidence of identification. What is the evidence that the specimens identified as *albus* are of the same species as the *albus* described by Glubokovsky?

Besides the distinctive *malma*-like char described from Lake Kronotskoe by Viktorovsky, a new species of *malma*-like char, *S. levanidovi*, was described in 1989 from a few mainland tributaries to the Okhotsk Sea. Can the char identified as *malma* with the distinctive alleles be aligned with any of the previously described char of uncertain affinity known from L. Azabache, L. Kronotskoe, or the Okhotsk Sea coast? If not, why not?

Please type comments on this or a separate sheet, and return the original and one copy. Do not hesitate to write on the manuscript itself.

Prérez de dactylographier les commentaires sur la présente feuille ou sur une page distincte et de retourner l'original ainsi qu'une copie. Ne pas hésiter à écrire sur le manuscrit même.



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J1  
GENETIC DIFFERENTIATION AMONG SPECIES OF CHARRS

OF KAMCHATKA RIVER BASIN

V.V.Efremov

Institute of Marine Biology, Far East Division, Academy of  
Sciences of the USSR, Vladivostok 690032

We examined the level of genetic divergence among sympatric forms of charrs of Kamchatka river basin: Salvelinus leucomaenis, S.malma and S.albus. 20 proteins have been studied. The proteins studied represent at least 46, 47 and 47 loci accordingly. We didn't observe that malma and albus are fixed for alternative alleles and the Nei genetic distance was 0,002. Among charrs morphologically identified as malma we found two groups of individuals are fixed for alternative alleles at three loci (  $D= 0,144$  ). These differences between two sympatric groups are the result of reproductive isolation. S.leucomaenis has a large difference ( at 10 loci ) from albus and malma (  $D= 0,349$  ).

INTRODUCTION

There are two species of charrs in Kamchatka river basin according to Savvaitova (1973) - Salvelinus alpinus and S.leucomaenis. The former contains some sympatric ecological forms such as anadromous predatory and benthic feeding charrs, river residential and lake predatory and benthic feeding charrs, creek residential form and "stone charr".

On the other hand, Glubokovskii (1977a) has identified a morphological group of benthic feeding charrs with Pacific species S.malma using craniological characters. He also, has

described predatory group of charr as a new species - *S.albus* (Glubokovskii, 1977b). So, there are three species of charrs in Kamchatka river basin: *S.leucomaenis*, *S.malma* and *S.albus*.

A genetic variation among these species was examined by proteins electrophoresis.

#### MATERIALS AND METHODS

Samples of *S.leucomaenis*, *S.malma* and *S.albus* have been collected from downstream of Kamchatka river and from Azabachia lake. 37 individuals of *S.leucomaenis*, 29 of *S.malma* and 35 of *S.albus* muscle and liver were analysed by starch and polyacrilamide electrophoresis.

#### RESULTS AND DISCUSSION

The following 20 proteins gave good electrophoretic resolution (Table): Malate dehydrogenase (MDH), Malic enzyme (ME), Glycerol-3-phosphate dehydrogenase (G3PDH), Diaphorase (DIA), Aconitase (ACO), Phosphoglucomutase (PGM), Phosphoglucose isomerase (PGI), Aspartate aminotransferase (AAT), Lactate dehydrogenase (LDH), Creatine phosphokinase (CK), Isocitrate dehydrogenase (IDH), Alcohol dehydrogenase (ADH), Superoxide dismutase (SOD), Esterase (EST), 6-Phosphogluconate dehydrogenase (PGD), Glucose-6-phosphate dehydrogenase (G6PDH), Esterase D (EST D), Peptidase (PEP), Formaldehyde dehydrogenase (FDH), General proteins (GEN).

The proteins examined represent at least 46, 47 and 47 loci for *S.leucomaenis*, *S.malma*, *S.albus* respectively. Five loci for *albus* and three for *malma* are polymorphic. We didn't observe any variation among *leucomaenis* individuals (Table, Fig.1).

The results of investigation of electrophoretic variation of charrs from Kamchatka basin were rather unexpected. Firstly, ~~we didn't observe qualitative differentiations between albus and malma~~ <sup>No</sup> ~~although 47 loci were examined~~ <sup>were found</sup> ~~Otherwise, we didn't observe that malma and albus are fixed for alternative alleles, and the Nei genetic distance was 0,002 (Fig.2 ).~~ The absence of ~~these~~ <sup>the loci</sup> differences in ~~studied features~~ <sup>studied</sup> features, obviously, can't unambiguously indicate ~~the absence of differences~~ <sup>that</sup> in general. Such a situation is a result of either weak reproductive isolation between *S.malma* and *S.albus*, or consequence of their recent divergency, ~~and following expansion of loci number will reveal the discriminative genetic features by which these species can be identified.~~ Secondly, with the help of electrophoresis we found two groups of individuals among charrs, morphologically identified as malma, the differences between those can't be explained by intraspecific variation. The differences at 2 loci of IDH and 1 locus of ADH have been observed between groups (  $D = 0,144$  ). All ~~these~~ 3 loci are monomorphic (Fig.1,2). These differences between two sympatric groups are the result of reproductive isolation. ~~Obviously, sibling species we observed.~~ These sibling species have a similar external morphological traits but unsimilar genetic traits ~~or independent genetic fund.~~ As expected, *S.leucomaenis* has a large differences ( at 10 loci ) from albus and malma, and  $D = 0,349$  (Fig.1,2).

#### REFERENCES

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electrophoretic forms of transferrins, albumins, pre-albumins and plasma esterases of horses. *Genetics*. 53: 681-687.

Glubokovskii M.K. 1977. Taxonomic relationships of chara of genus *Salvelinus* in the basin of the Kamchatka river. *Sov. J. Mar. Biol.* 3: 182-190.

Glubokovskii M.K. 1977. *Salvelinus albus* sp.n. from the basin of the Kamchatka river. *Sov. J. Mar. Biol.* 3: 281-286.

Guyomard R., Krieg F. 1983. Electrophoretic variation in six population of brown trout (*Salmo trutta* L.). *Can. J. Genet.* 25: 403-413.

Markert C.L., Faulhaber I. 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* 159: 319-332.

Moon T.W., Hochachka P.W. 1972. Temperature and the kinetic analysis of trout isocitrate dehydrogenase. *Comp. Biochem. Physiol.* 42B: 724-730.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*. 89: 583-590.

Ridgway G.J., Sherburne S.W., Lewis R.D. 1970. Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99: 147-151.

Savvaitova K.A. 1973. Ecology and systematics of freshwater's charra of genus *Salvelinus* (Nilsson) Richardson from some basin of Kamchatka. *Vopr. Iktiolog.* 1(78): 67-68. (In Russian).

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MONOMORPHIC AND POLYMORPHIC LOCI OF CHARRS OF KAMCHATKA RIVER

Protein	Number of loci			Buffer	Tissue
	S.leucom.	S.albus	S.malma		
MDH	6	6,1P	6	2	M
ME	2	2	2	2	M
G3PDH	3	3	3	2	M
DIA	1	1	1	1	L
ACO	2	2,2P	2,1P	2	M
PGM	2	2	2	1	M
GPI	3	3,1P	3,1P	1	M
AAT	2	2	2	2	M
LDH	3	4	4	1,5,6	M
CK	2	2	2	1	M
IDH	2	2	2	1	L
ADH	2	2	2	1,2	L
SOD	1	1,1P	1,1P	1	L
EST	4	4	4	1	L
PGD	1	1	1	2	M
G6PDH	1	1	1	2	L
EST D	1	1	1	3	M
PEP	1	1	1	1	M
FDH	1	1	1	1,5	M
GEN	6	6	6	1	M
General number of loci	46	47,5P	47,3P		

P- polymorphic locus, M- muscle, L- liver. Buffer- starch gel: 1- Ridgway et al., 1970; 2- Shaw, Prasad, 1970; 3- Markert, Faulhaber, 1965; 4- Moon, Hochachka, 1972; 5- Guyomard, Krieg, 1983; 6- Gahne, 1966.

Fig.1.

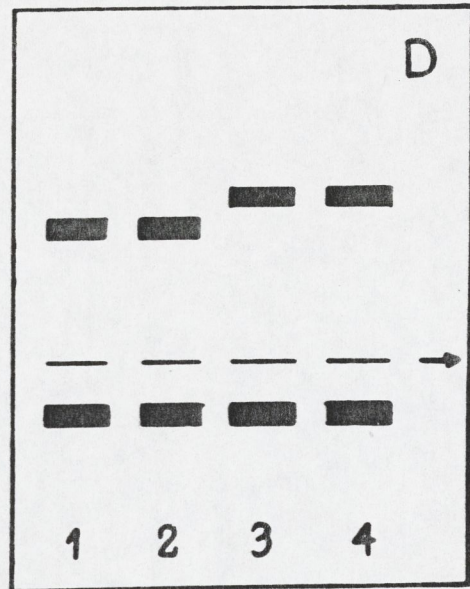
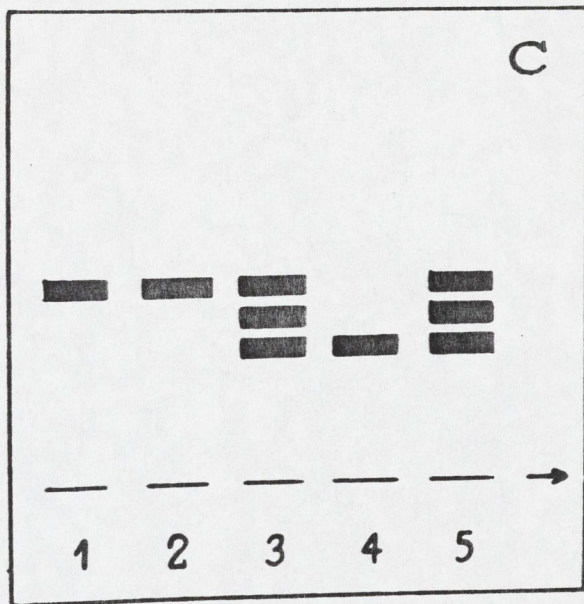
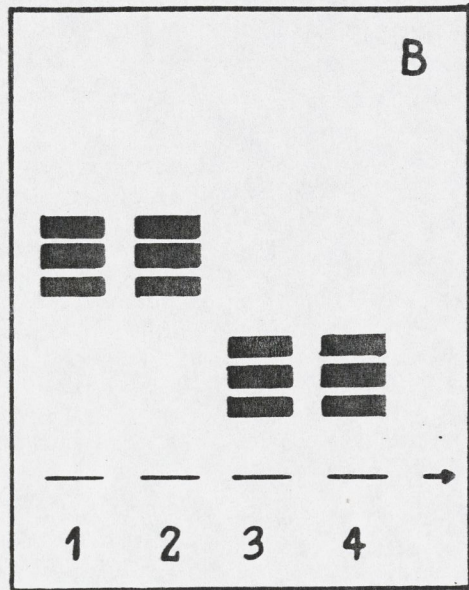
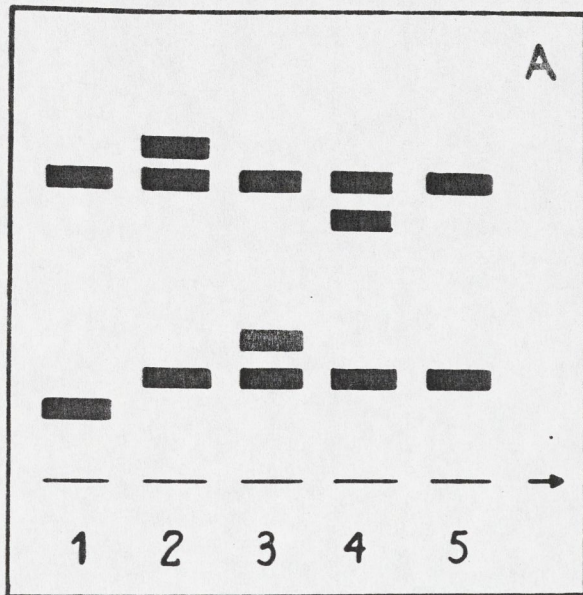
A. Aconitase: 1- *S.leucomaenis*; 2,4-5- *S.albus*, *S.malma*; 3-  
*S.albus*.

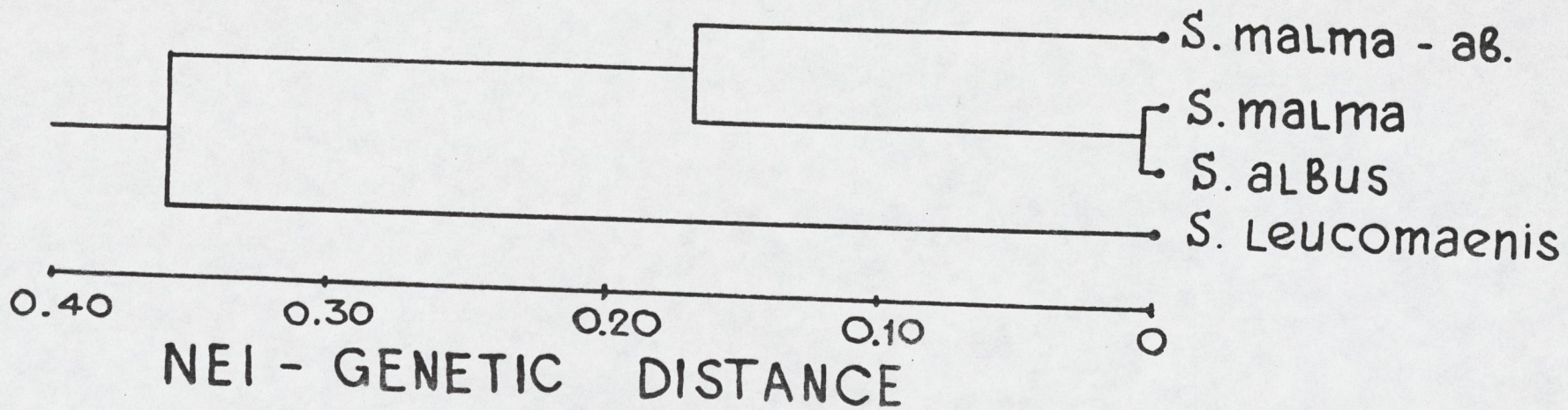
B. Isocitrate dehydrogenase:*S.malma*- 1,2- usual phenotype; 3,4-  
aberrant phenotype.

C. Superoxide dismutase: 1,2- *S.leucomaenis*, 3-5- *S.albus*,  
*S.malma*.

D. Alcohol dehydrogenase:*S.malma*- 1,2- usual phenotype; 3,4-  
aberrant phenotype.







S 14287  
.073

Svärdson, G. 1985. Pleistocene age of the  
spring-spawning cisco, Coregonus tybomi. Nordic J.  
Freshw. Res. 64:101-112

- C.t. in 4 Swedish, 2 Finnish lakes, in all  
sympatric w/ C. albula, also C. ferax (low water) whitefish in  
Swedish lakes. Sweden, lakes in 3 river systems 2 → Baltic  
1 → Kattegat. Pleistocene-holocene transition: Baltic ice lake  
drains in 2 stages. Receding glacial filled  
Vättern basin, which had been inhabited by Baltic ice lake  
fauna, incl. 3 Coregonus sp. Coregonus pop. in South.  
Sweden prob. occ. w/ glaciation of south. Vättern, 11,200-10,400  
B.P. - boloto Svärdson's hypoth. of "regressive" speciation  
Postglacial invasion of genetically distinct stocks, evolved in  
glacial or preglacial time.

- smelt only in lakes a marine limit of ice lake max. -  
- Pontoporeia dist. by ice lake.

Spring-spawning cisco and char in Ören L. → L. Vättern

<u>C. albula</u>	Scanderava	39.9 - 47.5	× values	} similar in sympatris lakes
<u>C. tybomi</u>		42 - 44		

Ryman (pers. comm.) found no electroph. dif. between  
sympat. albula - tybomi (Ören) - Vuorinen (1958) - Finn. Fishes  
found slight dif. Ören pop. in 32 loci - Finnish,  
G.D. 014 L. Sokojärvi - but greatest dif. between 2  
spring spawners (tybomi) - L. Hagoonjärvi and Sokojärvi.

\* - tybomi not recognizable on dendrogram.

Amundsen, P.A. Ibid: 67-73. Habitat and food  
segregation of two sympatric populations of whitefish (Coregonus  
lavaretus L. s.l.) in Storaöjavi, northern Norway.

benthivore  $\bar{x}$  24.0 planktivore  $\bar{x}$  33.5

Ibid: 5-53 Jensen, J.W. Crustacean plankton  
and fish during the first decade at a subalpine,  
man-made reservoir. - Record char prod. - in 60 kg/ha. - -

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Dr. Robert J. Behnke  
Department of Fishery and  
Wildlife Biology  
Colorado State University  
Fort Collins, CO 80523, USA

June 3, 1993

Dear Dr. Behnke,

Thank you for your consent to offer recommendation for my support to the International Science Foundation. I hope Prof. R.B. Phillips (University of Wisconsin-Milwaukee) will give me her recommendation too.

I see you have not received yet my manuscript with *Salvethymus* karyotype when sent me the letter. It's a much enlarged variant of paper published in Doklady of Russian Academy of Sciences (1993, vol.329, N 3). Such publication is permitted by Doklady's instruction for authors. I will send you a reprint of published paper when have it, of course. It's also concerned the paper with Kamchatkan *Salvelinus* karyotypes, which is "in press" from 1990, will be published (I hope) at the end of 1993 and have in title 1991.

Of course, I try synthesize data of charr karyotypes and presented once the results at the conference of our Institute (without publication). Some time ago I have sent a request for oral presentation titled "Comparative karyology and karyotype evolution in the charrs" to International Charr Symposium 1994. It will be the best presentation I think. Manuscripts of presentations will be published in an international journal. I also hope International Science Foundation will help me with covering my travel and living expences.

Unfortunately, I have not any *Salvethymus* specimens used for

Dr. R.J.Behnke

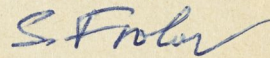
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June 3, 1993

karyotype analysis (excluding fixed with formalin). But we collected in 1991 samples for DNA analysis and sent them to Dr. K.A.Savvaitova. I don't know results. As I know, Dr. I.A.Chereshnev is planning organize complex expedition in Elgygytgyn Lake this summer.

If you are interested, according biochemical data (32 loci) *Salvelinus*, *S.elgyticus* and *S.boganidae* are close related, but have some fixed alleles. Differences of all three species are at one level and less, than between these species and *S.albus* (Glubokovsky M.K., Frolov S.V., Efremov V.V., Rybnikova I.G., Katugin O.N. 1993. Phylogenetic relations and the systematics of charr's fishes in the Elgygytgyn Lake. In: The Nature of the Elgygytgyn Lake Hollow (the Problems of Study and Reservation) (Bely V.F., Chereshnev I.A., eds). Magadan: FEB Russian Acad. Sc. P.149-177 - in Russian).

Sincerely,



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Dr. R. Behnke  
Department of Fishery and  
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November 1, 1993

Dear Dr. Behnke,

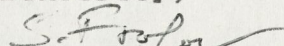
Thank you for invitation to submit our paper for publication in Journal of Ichthyology. I don't know why, but Dr. Chereshev have not inform me about this possibility.

Unfortunately, allozyme data can not be extended. Dr. Efremov who collected and analyzed this material gives us only this information. So, this unique material was actually spoiled by him.

I and Dr. Glubokovsky are ready modify the paper which will consist only morphological and karyological data under two coauthors. I think we can prepare the manuscript for the short time if such paper will be suitable.

Dr. N. Romanov who have sent you the manuscript of his paper in September is interested if you received it? He also asked me about the name of your E-mail.

Sincerely,

  
S. Frolov

S.V.Frolov  
Institute of Marine Biology  
Far East Branch  
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Vladivostok 690041  
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Par avion

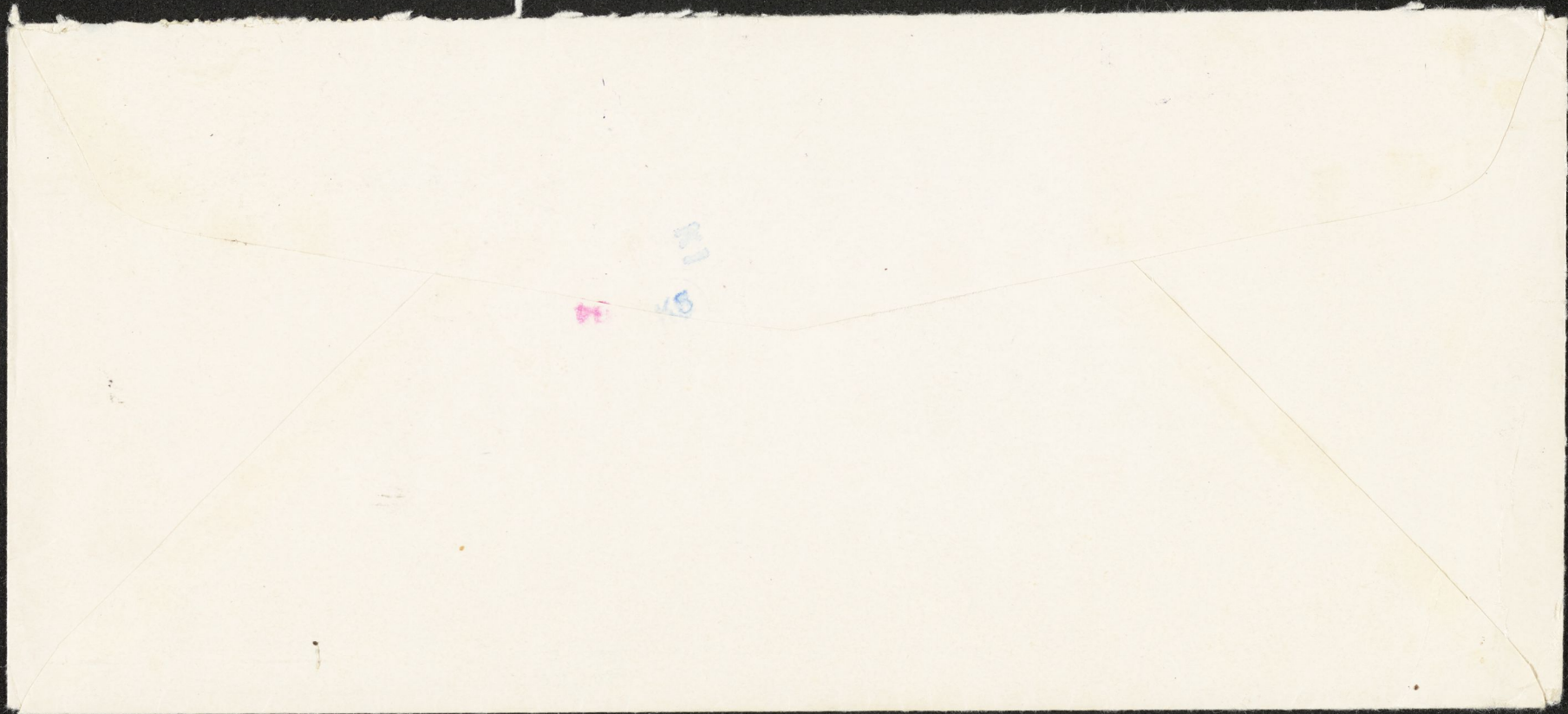


→ 11024mm  
32 loci  
Solenostomus, S. elongatus, →  
S. bispinatus - closely related

Karyotype

2N=56 n=28

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# Colorado State University

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August 2, 1993

Dear Dr. Cheresnev:

My sincere thanks for copy of the Elgygytyn book. I would like to publish English translations of your paper coauthored with M. B. Skopets and the Glubokovsky, Frolov, et al. paper in the Journal of Ichthyology. A problem for this concerns legal aspects that permission might be required from the Russian Copyright Agency. This would likely result in long delays.

What I suggest is that you and Skopets and also Glubokovsky, Frolov., et al. submit "original" manuscripts of these papers for publication in the Journal of Ichthyology (text in Russian, do not attempt English translation). You may want to edit, modify, and rewrite the paper somewhat so that you are credited with two separate publications rather than one publication (in the L. Elgygytyn book and its English translation). This would not only greatly speed-up publication in the Journal of Ichthyology but could be used as evidence for publication in an international journal to support applications for international science funding.

A few months ago Dr. Frolov contacted me regarding listing me as a reference to support his application for funds from an International Science Foundation. He submitted an "original" paper on the Salvethymus karyotype for publication in the Jour. Ichthyol. (modified from his Doklady paper). I told him that both Ruth Phillips and I would support his application for funding.

Attached is Ruth Phillips review of Frolov's paper. Note her interest in the Elgygytyn chars. You should communicate with Dr. Frolov about what he has learned about funding from the International Science Foundation. You may use my name (and Ruth Phillips also) in support of your application for an international cooperative study of Far Eastern chars.

You may write to me in Russian (any paper submitted to the Journal of Ichthyol. should be in Russian). I have no formal training in Russian (I've had no university courses). I only learned to translate ichthyology literature by learning basic rudiments of grammar and much practice with a dictionary (many years ago). I cannot communicate in the spoken language (except to purchase food at a store or rynok), but I can translate most

Dr. Robert J. Behnke  
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and Wildlife Biology  
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Fort Collins, Colorado  
80523 USA

June 3, 1993

Dear Dr. Behnke:

Recently, I have received copies of charr-fanatics articles from Johan Hammar. The book is wonderful. I appreciate very much your contribution to the preparation of the book and my article, in particular. I am glad that it was you who have edited my paper because nobody knows my works on charrs than you do. Thank you so ever much.

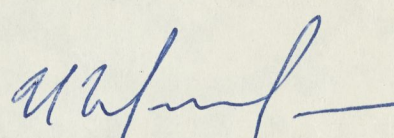
You'll find enclosed a copy of the book on the environment in the area around the Elgygytgyn Lake and the charrs dwelling the lake. Currently, we are trying to achieve the establishment of a reservation in the area. I hope to visit the lake again this summer and to continue the studying charrs. I also hope that another book on charrs from the Far East will be published by the end of this year. It includes articles by researchers from Moscow, Magadan and Vladivostok. I will send it to you as soon as it is issued.

So, as you can see, we do not stop our research despite financial difficulties. I think, it will be just financial difficulties that do not permit us to attend the symposium in Trondheim, Norway.

Unfortunately, our contacts have been broken off because of bad postal service in this country. Currently, the situation seems to changing for the better. So I would like to resume our agreements on carrying out joint research on the systematics and phylogeny of charrs from the Pacific Ocean. Dr. M. Glubokovsky is ready to participate in the research. Is it possible to receive international grant for the conducting this research? And who of US and Canadian Scientists could participate?

In late June I will go to the Anadyr River for field work and stay there till October. You can contact me over fax: (41322)2-47-30, or over E-mail: syarc@orca.alaska.edu. Please inform me on your E-mail, if any. Can I write to you in Russian? It would simplify our contacts.

Sincerely yours,

  
Igor A. Chereshev



To: Bob Behnke

From Ruth Phillips

*Ruth Phillips*

Date: July 6, 1993

Subject: Frolov's MS

Message: I read S.V. Frolov's manuscript: "Unusual chromosome set and constitutive heterochromatin in a new endemic salmonine fish *Salvelinus svetovidovi*" with great interest. The cytogenetic work is excellent and it clearly shows that this fish has a diploid number of  $2n=56$  and an NF number of 98. C banding reveals the presence of large blocks of heterochromatin on the telomeres of many chromosomes and he also reports multiple satellites (presumably NORs) at telomeric locations. These traits are also characteristic of the karyotypes of *S. namaycush* and *S. alpinus*. As he points out the low diploid chromosome number with the NF number of 98 implies that numerous Robertsonian fusion translocations have occurred in the line leading to this species, so that it has a highly derived karyotype. Because it shares an NF number of 98 with *S. alpinus* and *S. malma* as well as the banding characteristics described above, I would conclude from this karyotype data that it is related to *S. alpinus*, possibly within the *S. alpinus*-*S. malma* complex. Although the NF number could be the result of independent inversions, such inversions seem to be relatively rare in *Salvelinus*, so the conservative interpretation is that the same chromosome pair is involved. However we need improved banding techniques in order to resolve this point. It will be important to obtain molecular data on this fish to help determine whether it is a primitive or derived member of the genus *Salvelinus*.

I would be very interested in sequencing the ribosomal RNA genes in this species. We are starting to work with preserved material, so I would like to get tissue samples from the three types of chars in Lake Elgygytgyn.