= ANIMAL GENETICS ====

Molecular Phylogenetic Study of Several Eelpout Fishes (Perciformes, Zoarcoidei) from Far Eastern Seas on the Basis of the Nucleotide Sequences of the Mitochondrial Cytochrome Oxidase 1 Gene (*Co-1*)

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Abstract—A total of 95 nucleotide sequences of a *Co-1* gene fragment of approximately 650 bp were analyzed for fishes of the orders Perciformes and Scorpaeniformes (outgroup). Gene trees based on four algorithms (BA, NJ, MP, and ML) were similar in topology of solved branches. An emphasis was placed on the species and generic levels, but a significant phylogenetic signal was obtained for higher taxonomic ranks as well. For instance, a monophyletic origin was confirmed for the family Zoarcidae and the subfamily Opisthocentrinae (Stichaeidae). The proportion of different nucleotides in the sequences compared (*p*-distances) significantly increased with increasing taxonomic rank. The *p*-distances were estimated for four hierarchic levels and were (1) 0.15 \pm 0.06% for the within-species hierarchic level, (2) 6.33 \pm 0.37% for the within-genus level, (3) 11.83 \pm 0.06% for the within-family level, and (4) 15.22 \pm 0.05% for the within-order level. The difference in the *Co-1* gene fragments between levels (1) and (2) allows almost errorless species identification on the basis of this kind of a molecular bar code.

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INTRODUCTION

Molecular phylogenetic analysis of taxa at the species-family level is most often based on the nucleotide sequences (hereafter referred to as sequences) of the mitochondrial genes for subunit 1 cytochrome oxidase c(cytochrome oxidase 1, Co-1) and cytochrome b [1, 2]. When taxa of higher ranks are studied, analysis of these genes may yield inadequate results because of mutational saturation due to repeated substitutions or the homoplasy effect. The sequences of the two genes are still useful for species identification and reconstruction of phylogenetic relationships in various taxa [3-5], including fishes, up to the order level [1, 4, 6], 7]. In addition, genetic distances (p-distances) provide information on the taxonomic position of individuals because they characterize a certain level well and substantially increase from lower to higher hierarchic or taxonomic ranks [1, 2, 4-7].

Fish taxonomy still has many disputable issues, in particular, those related to the order Perciformes. The order includes approximately 6,000 species and is one of the largest in fish [8]. Phylogenetic studies are topical for such a numerous group. The taxonomic positions of many families in suborders are still unclear. Moreover, a monophyletic origin cannot be established for certain for both families and suborders, as is required by modern taxonomy [9]. Particular interest is attracted by the family Stichaeidae, which belongs to the suborder Zoarcoidei and currently includes 80 species of six subfamilies and 38 genera [10-12]. Many taxonomic problems are still unsolved at the levels of genera and species for other suborders and families, and monophyly of many taxa remains questionable [8].

Molecular phylogenetic analysis of Stichaeidae has already been performed in several studies [7, 13, 14]. Stepien et al. [13] examined only few individuals to study the evolution of Blennioidei, and their information was insufficient for phylogenetic inferences. Radchenko et al. [14] investigated the total suborder Zoarcoidei and noted a distinctness between the subfamilies Opisthocentrinae and Chirolophinae and between Lumpeniinae and Stichaeinae. According to Kartavtsev et al. [7], Opisthocentrinae similar group with Chirolophinae, but Stichaeinae is a daughter branch of Chirolophinae, which agrees with the conventional taxonomy of the group [15].

The objective of this work was to carry out molecular phylogenetic analysis of several perch-like fishes (Zoarcoidei) on the basis of data on the *Co-1* gene sequences.

Accordingly, the three main tasks of our work were (1) to study genetic divergence (p-distances) within species and in taxa of higher hierarchical ranks on the basis of the *Co*-1 gene; (2) to analyze the nucleotide composition of the sequences of the perch-like fishes

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No.	Species	Individual identifica- tion number	GenBank accession no.	Source of the individual or sequence
1	Acantholumpenus mackayi (Gilbert, 1896)	AM3	HQ704734	Sea of Japan, Russia
2	Alectrias alectrolophus (Pallas, 1814)	317	FJ932610	NCBI GenBank
3	Ammodytes hexapterus Pallas, 1814	308	HQ704752	Bering Sea, Russia
4	A. hexapterus Pallas, 1814	309	HQ704753	The same
5	A. hexapterus Pallas, 1814	310	HQ704754	"
6	Anisarchus medius (Reinhardt, 1837)	281	HQ704777	"
7	A. medius (Reinhardt, 1837)	282	HQ704778	"
8	A. medius (Reinhardt, 1837)	283	HQ704779	"
9	A. medius (Reinhardt, 1837)	284	HQ704780	"
10	A. medius (Reinhardt, 1837)	285	HQ704781	"
11	Anoplarchus purpurescens Gill, 1861	5846	FJ164282	NCBI GenBank
12	A. purpurescens Gill, 1861	5847	FJ164281	The same
13	A. purpurescens Gill, 1861	5848	FJ164280	"
14	Askoldia variegata Pavlenko, 1910	AV1	JF343542	Tatar Strait, Russia
15	A. variegata Pavlenko, 1910	AV2	JF343543	The same
16	A. variegata Pavlenko, 1910	AV3	JF343544	"
17	Bathymaster signatus Cope, 1873	141	HQ704765	Bering Sea, Russia
18	B. signatus Cope, 1873	142	HO704766	The same
19	B. signatus Cope, 1873	143	HO704767	"
20	B. signatus Cope, 1873	144	HO704768	"
21	B. signatus Cope, 1873	145	HO704769	"
22	Bothrocara zestum Jordan & Fowler, 1902	102	HO704771	"
23	<i>B. zestum</i> Jordan & Fowler, 1902	105	HQ704772	"
24	Cehidichthys violaceus (Girard 1854)	MFC387	GU440267	NCBI GenBank
25	Chirolophis decoratus (Jordan & Snyder, 1902)	MFC239	GU440277	The same
26	<i>Chirolophis iaponicus</i> Herzenstein, 1890	CI3	HO704732	Sea of Japan, Russia
27	<i>Chirolophis nugator</i> (Jordan & Williams, 1895)	MFC338	GU440279	NCBI GenBank
28	Dasvcottus setiger Bean, 1890	62	HO704756	Bering Sea, Russia
29	Ernogrammus hexagrammus (Schlegel 1845)	1196	FI932611	NCBI GenBank
30	E hexagrammus (Schlegel, 1845)	F1	HO704722	Sea of Japan Russia
31	E hexagrammus (Schlegel, 1845)	E1 F2	HQ704723	The same
32	E hexagrammus (Schlegel, 1845)	E2 F3	HQ704723	" "
33	E hexagrammus (Schlegel, 1845)	E3 F4	HQ704725	"
34	E hexagrammus (Schlegel, 1845)	E1 F5	HQ704726	"
35	Esselenichthys carli (Follett & Anderson 1990)	MEC200	GU440318	NCBI GenBank
36	Kasatkia seigeli Posner & Lavenberg 1999	SIQ03-92	HO010045	The same
37	Lentoclinus maculatus (Fries 1838)	263	HQ704750	Bering Sea Russia
38	L maculatus (Fries 1838)	263	HQ704751	The same
39	Lobotes suringmensis (Bloch 1790)		HQ704731	Sea of Japan Russia
40	Lumpenella longirostris	441	FI164727	NCBI GenBank
40	(Evermann & Goldsborough, 1907)	771	1 3 10 47 27	I CDI Gendank
41	L. longirostris (Evermann & Goldsborough, 1907)	442	FJ164730	The same
42	L. longirostris (Evermann & Goldsborough, 1907)	448	FJ164729	"
43	Lumpenus sagitta Wilimovsky. 1956	302	HQ704782	Bering Sea, Russia
44	<i>L. sagitta</i> Wilimovsky. 1956	303	HQ704783	The same
45	L. sagitta Wilimovsky, 1956	304	HO704784	"
46	L. sagitta Wilimovsky, 1956	LS1	HQ704731	Sea of Japan, Russia
47	Lycenchelys crotalinus (Gilbert, 1890)	231	HQ704760	Bering Sea, Russia

Table 1. Species under study with their voucher identification numbers, GenBank accession numbers, and collection sites

Table	1.	(Contd.)
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No.	Species	Individual identifica- tion number	GenBank accession no.	Source of the individual or sequence
48	L. crotalinus (Gilbert, 1890)	234	HQ704761	The same
49	Lycodes brevipes Bean, 1890	108	HQ704757	"
50	L. brevipes Bean, 1890	109	HQ704758	"
51	L. brevipes Bean, 1890	110	HQ704759	"
52	L. concolor Gill & Townsend, 1897	226	HQ704773	"
53	L. concolor Gill & Townsend, 1897	227	HQ704774	"
54	L. concolor Gill & Townsend, 1897	228	HQ704775	"
55	L. concolor Gill & Townsend, 1897	230	HQ704776	"
56	Lycodes beringi Andriashev, 1935	59	HQ704755	"
57	Lycodes palearis Gilbert, 1896	236	HQ704762	"
58	L. palearis Gilbert, 1896	237	HQ704763	"
59	L. palearis Gilbert, 1896	238	HQ704764	"
60	Lycodes raridens Taranetz & Andriashev, 1937	311	HQ704785	"
61	L. raridens Taranetz & Andriashev, 1937	312	HQ704786	"
62	L. raridens Taranetz & Andriashev, 1937	313	HQ704787	"
63	L. raridens Taranetz & Andriashev, 1937	314	HQ704788	"
64	Opisthocentrus ocellatus (Tilesius, 1811)	001	HQ704736	Sea of Japan, Russia
65	O. ocellatus (Tilesius, 1811)	OO 11	HQ704741	The same
66	O. ocellatus (Tilesius, 1811)	002	HQ704737	"
67	O. ocellatus (Tilesius, 1811)	003	HQ704738	"
68	O. ocellatus (Tilesius, 1811)	005	HQ704740	"
69	Opisthocentrus tenuis Bean & Bean, 1897	OT5	HQ704739	"
70	O. tenuis Bean & Bean, 1897	OT4	EU200481	"
71	Opisthocentrus zonope Jordan & Snyder, 1902	OZ1	HQ704727	"
72	O. zonope Jordan & Snyder, 1902	OZ2	HQ704728	"
73	O. zonope Jordan & Snyder, 1902	OZ3	HQ704729	"
74	O. zonope Jordan & Snyder, 1902	OZ4	HQ704730	"
75	Ph. dybowskii (Steindachner, 1880)	PhD1	HQ704746	"
76	Ph. dybowskii (Steindachner, 1880)	PhD2	HQ704747	"
77	Ph. dybowskii (Steindachner, 1880)	PhD3	HQ704748	"
78	Plectobranchus evides Gilbert, 1890	04HBL008132	FJ165022	NCBI GenBank
79	Poroclinus rothrocki Bean, 1890	533	FJ165043	The same
80	P. rothrocki Bean, 1890	534	FJ165039	"
81	P. rothrocki Bean, 1890	622	FJ165040	"
82	Ronquilus jordani (Gilbert, 1889)	TZ-06-RICKER-774	FJ165103	"
83	R. jordani (Gilbert, 1889)	TZ-06-RICKER-775	FJ165104	"
84	R. jordani (Gilbert, 1889)	TZ-06-RICKER-779	FJ165105	"
85	R. jordani (Gilbert, 1889)	TZ-06-RICKER-785	FJ165106	"
86	R. jordani (Gilbert, 1889)	TZ-06-RICKER-830	FJ165107	"
87	Stichaeopsis nevelskoi (Schmidt, 1904)	SN1	HQ704733	Sea of Japan, Russia
88	Stichaeus ochriamkini Taranetz, 1935	SO1	HQ704742	The same
89	S. ochriamkini Taranetz, 1935	SO3	HQ704743	"
90	S. ochriamkini Taranetz, 1935	SO4	HQ704744	"
91	S. ochriamkini Taranetz, 1935	SO5	HQ704745	"
92	Stichaeus punctatus (Fabricius, 1780)	210	HQ704770	Bering Sea, Russia
93	Xiphister mucosus (Girard, 1858)	5073	FJ165465	NCBI GenBank
94	X. mucosus (Girard, 1858)	5074	FJ165466	The same
95	X. mucosus (Girard, 1858)	5075	FJ165467	"



Fig. 1. Map with collections sites indicated. The sites where individuals under study were fished in the Japanese and Bering seas are encircled.

examined; and (3) to study the peculiarities of molecular genetic divergence and phylogenetic relationships among the perch-like fishes, with a special emphasis on Stichaeidae and Zoarcidae.

MATERIALS AND METHODS

A total of 95 Co-1 gene sequences were used in comparative phylogenetic analysis (Table 1), including 70 original sequences and 25 sequences from Gen-Bank (NCBI, http://www.ncbi.nlm.nih.gov/) [16]. The analysis included 36 species of five perch-like families: Stichaeidae (25 species), Zoarcidae (seven species), Bathymasteridae (two species), Lobotidae (one species), and Ammodytidae (one species), and one species (Dasycottus setiger Bean, 1890) of the order Scorpaeniformes. Material for the study was collected from three trawling catches in the Bering Sea during an expedition onboard the TINRO research chip in 2008. In the Japanese See, material was collected from 2009 to 2010 with the use of gill nets, fry nets, and shrimp traps (Fig. 1). Species identification followed Lindberg and Krasyukova [17]; species of the genera Pholidapus and Opistocentrus were identified using Shiogaki criteria [18]. Species names are given as in [19]. Muscle tissue for genetic analysis was taken from each separate individual and fixed with 96% ethyl alcohol. The majority of the fishes examined were stored in a fixed form in the collection of the Zhirmulsky Institute of Marine Biology (IMB, curator A.A. Balanov) under corresponding catalog (voucher) numbers; their color photographs and complete information on the samples and genetic data were included in the BOLD database [20] (http://www.boldsystems.org/) of the iBOL global program.

Muscle tissue DNA was isolated via phenol-chloroform extraction [21] with minor modification [7]. A mitochondrial Co-1 gene fragment of approximately 650 bp was amplified in the polymerase chain reaction (PCR) with primers FishF1 (5'-TCAACCAACCA-CAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') [4]. The reaction mixture (25 µl) contained 17.4 µl of distilled water, 2.5 µl of a 10× buffer (TaKaRa, Japan), 2.0 µl of a dNTP mixture (2.5 mM each triphosphate), 1 μ l of each primer (20 pmol/ μ l), 0.1 μ l of 5 units/ μ l Taq polymerase, and 1 µl of DNA. PCR was run on Eppendorf Mastercycler and BIORAD My Cycler thermal cyclers. Amplification included denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and synthesis at 72°C for 1 min; and last synthesis at 72°C for 7 min. The PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide, and viewed in transmitting UV light. The amplicon size was estimated against a 100-bp DNA marker. The best samples were selected for subsequent manipulations and purified with 96% and 70% ethanol according to a standard protocol. Cyclic sequencing was then performed. The nucleotide sequences were identified physically on an ABI-3130 sequencer (Applied Biosystems, United States) at the IMB and Far East Federal University (Vladivostok).

Two antiparallel sequences were obtained for each individual. Consensus sequences were formed using the ChromasPro editor [22] and deposited in Gen-Bank [16] and, partly, in BOLD [20]. The best-fit model of nucleotide substitution for subsequent construction of gene trees was selected using the Modeltest 3.7 program [23] and the PAUP* 4.0 package [24]. The GTR+I+G model was selected as optimal for the given data set according to the Akaike information criterion. The topology of a Bayesian (BA) tree was calculated using the MrBayes 3.1 software package [25]. Sequence alignments were constructed using the MEGA 5 software package [25] with Clustal-W in two steps. At the first step, the gap opening penalty was 15, and the gap closing penalty was 5 for both pairwise and multiple alignments. At the second step, the penalties were reduced to 5 and 0.5, respectively. Gaps were removed after each alignment step. Calculation and visualization of neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) trees were carried out using MEGA 5 [26]. Since the program does not utilize the model GTR+I+G to construct an NJ tree, we used the similar Tamura-Nei model of nucleotide substitution with the γ parameter G = 0.9 and the invariable site proportion I = 0.56. When the MP tree was constructed, starting trees were searched by the close-neighbor-interchange (CNI) method and search level 1. Random addition of starting trees (10 replications) was used in the CNI search. The stability of the tree topology was checked by bootstrap analysis with 1000 replications in the case of the MP and NJ algorithms and 500 replications in the case of the ML algorithm. The replications were used to construct consensus trees with a threshold of 50%. BA trees were obtained for 10⁶ model generations. The parameters of the BA program were: MCMCP ngen = 1000000, printfreq = 1000, samplefreq = 1000, nchains = 4, and SUMP burnin = 100; the other parameters were set at default values. Analysis vielded 19,652 trees. A 50% consensus tree was constructed on their basis and included 9,851 trees.

RESULTS

The total length of the sequences under study was 524 bp after alignment. The BA, NJ, MP, and ML consensus trees were basically similar in topology at the levels of genera and species, differing only in boot-strap support of nodes. At higher hierarchical levels, the topology of the trees was only partly resolved in most cases and, consequently, differed among the algorithms. However, the branches of the family Zoarcidae and subfamily Opisthocentrinae had a good sup-

port in all of the four reconstruction algorithms. The BA tree was the best resolved topologically and, consequently, was taken as an illustration (Fig. 2).

When necessary, the resulting trees were rooted using *Dasycottus setiger* as an outgroup. The species grouped with *Ammodytes hexapterus* and *Lobotes surinamensis* to form a separate branch, which was supported in different algorithms (98/54/59/72) (Fig. 2). The percent support is hereafter given for the BA, NJ, MP, and ML trees, respectively; a dash indicates a lack of support in the corresponding algorithm. *Ammodytes hexapterus* individuals separate into two branches, while their common node is supported (98/54/59/72).

At the level of families, a distinct monophyletic branch is formed by representatives of the family Zoarcidae (99/100/99/99). Lycenchelys crotilinus is the first to produce a separate branch in the resulting topology. Then, Bothrocara zestum separates from the main tree (57/67/65/–). This is followed by division into two branches (92/54/59/62), of which one is formed by the only representative of Lycodes beringi, and the other combines L. brevipes, L. palearis, L. raridens, and L. concolor. In the latter branch, L. concolor (99/89/69/80) and L. raridens (91/51/56/54) consecutively form separate lineages, and then a common branch is formed by L. palearis and L. brevipes (91/51/54/54), which are the most similar genetically in the family according to the given topology.

A separate stable branch includes representatives of the subfamily Opisthocentrinae of the family Stichaeidae. One member of the subfamily (*Plectobranchus evides*) is basal relative to the total subfamily, forming an unresolved branch of the tree. The subfamily is represented by five genera and seven species. *Kasatkia seigeli* is the first to separate from the main branch (99/91/74/92). The branch then divides into two separate groups (96/87/60/75), which include representatives of the genus *Opisthocentrus* and the species *Ascoldia variegata* and *Pholidapus dybowskii*, which, in turn, form two species branches (51/72/60/75). In the genus *Opisthocentrus*, *O. zonope* is the first to separate (99/97/84/98) and is followed by *O. ocellatus* and *O. tenius* (90/99/88/54).

In addition, several representatives of the subfamilies Stichaeinae, Chirolophinae, and Xiphisterinae of the family Stichaeidae separate to form individual branches, which were not topologically resolved. Separation of several branches at the suprafamily level was noted in the BA tree, but these branches did not have a high statistical support and were not topologically resolved in the other algorithms. A clustering was observed for the species *Alectrias alectrolophus* and *Anoplarchus purpurescens* (99/86/69/94) and branches of the genus *Chirolophis*, with consecutive separation

Fig. 2. Rooted consensus tree of phylogenetic relationships of perch-like fishes as based on the *Co-1* nucleotide sequence. Support is indicated for the nodes that were resolved in more than 50% of bootstrap replications (ML, MP, and NJ) or had an a posteriori probability of more than 50% (BA). Statistical support is given for the BA, NJ, MP, and ML trees, respectively.

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of *Ch. japonicus* (100/95/87/92) and *Ch. nugatory* with *Ch. decoratus* (99/100/99/100). The other branches included individual species: *Stichaeopsis nevelskoi, Ernogrammus hexagrammus*, and *Xiphister mucosus*, and lacked substantial (more than 70–80%) support.

In the subfamily Lumpeninae of the family Stichaeidae, *Leptoclinus maculatus* and *Anisarchus medius* form a common branch (54/-/-/-) and group with representatives of the family Zoarcidae. The branches of *Lumpenella longirostris, Poroclinus rothrocki, Acantholumpenus mackayi*, and *Lumpenus sagitta* are unresolved.

The family Bathymasteridae (*Bathymaster signatus* and *Ronquilus jordani*) does not form a separate branch, representing a polyphyletic group. The tree includes several unresolved branches; in particular, such branches are formed by the other representatives of the subfamily Xiphisterinae of the family Stichaeidae, which produce a common node with *Cebidichthys violaceus* and *Esselenichthys carli* (98/87/90/91).

To estimate divergence at various taxonomic levels, mean *p*-distances were calculated from a matrix of pairwise distances (see Appendix). In this analysis, values were taken from the triangular matrix and divided into four groups according to the taxon rank, an arithmetic mean was calculated for each group, and differences between the means were evaluated by oneway analysis of variance with the use of Statistica 6.0 [27]. This gave a picture of nucleotide diversity at four different hierarchical levels: (1) within species, among individuals of one genus; (3) within family, among individuals of one family; and (4) within order, among individuals of one order (Fig. 3). The mean *p*-distances for the corresponding taxonomic levels were, % (1) 0.15 ±



Fig. 3. One-factor analysis of variance of p-distances based on the *Co-1* nucleotide sequences for several hierarchic levels. Mean values were obtained from the triangular matrix of p-distances (Appendix) for each of the groups.

0.06, (2) 6.00 ± 0.37 , (3) 12.00 ± 0.06 , and (4) 15.00 ± 0.05 (mean \pm standard error).

One-factor analysis of variance (ANOVA) of the variation of *p*-distances within groups and between groups showed that the mean values of the four groups differed significantly: F = 1075.5, d.f. = 3, P < 0.0001. The above mean *p*-distances and their errors and analysis of variance indicate that different *p*-distances are characteristic of the within-species, within-genus, within-family, and within-order groups in the suborder Zoarcoidei.

Nucleotide proportions were heterogeneous (Table 2), which was mostly due to a predominance of pyrimidines in both our *Co-1* sequences (Group I) and Gen-Bank sequences (Group II) (Fig. 4). Two-way analysis of variance (ANOVA) for the variation of purine and pyrimidine proportions in the two groups revealed a significant difference between mean values for the main factor, which was the proportions of the four nucleotides: F = 5.9635, d.f. = 3, P = 0.00056. The proportions of similar nucleotides did not differ among sequences within groups I and II (Fig. 4).

DISCUSSION

The topologies of the *Co-1* gene trees indicate that the 524-bp sequences under study were not informative enough to allow a resolution of all branches and to reveal the relationships for the majority of taxonomic groups. As was described in Results, many branches of higher hierarchical levels were not topologically resolved in the molecular phylogenetic trees (Fig. 2). At the same time, the *Co-1* sequences of individuals from one species clustered close together and displayed minimum *p*-distances within all of the genera. The distances were $0.15 \pm 0.06\%$ on average (Fig. 3). Sequences from different species of one genus formed



Fig. 4. Two-factor analysis of variance of the nucleotide composition in groups I (original sequences) and II (Gen-Bank sequences).

Nucleotide	T(U)	С	Α	G
GenBank data	30.5 ± 0.24	27.6 ± 0.24	23.2 ± 0.24	18.7 ± 0.24
Original data	29.7 ± 0.14	28.5 ± 0.14	23.0 ± 0.14	18.9 ± 0.14

Table 2. Mean nucleotide composition (%) of the Co-1 sequences under study (mean \pm standard error)

separate branches with a mean *p*-distance of $6.00 \pm 0.37\%$ (Fig. 3). The monophyletic origin of the genera under study was supported well in all four algorithms of tree reconstruction (Fig. 2). These features of the tree and the distance of more than one order of magnitude between the *p*-distances of the within-species and within-genus levels provide a basis for developing a DNA-based bar code of species. In other words, the *Co-1* sequences under study are informative enough for species diagnosis at the level of genera, which agrees with published data [1, 4, 6, 7].

In addition, the topology of the Co-1 tree has a good support at the levels of one family and two subfamilies. The family Zoarcidae and subfamily Opisthocentrinae have the highest support in nodes and form monophyletic branches. With the rather short length of the sequences examined, the finding indicates that the two taxa are the most evolutionarily separate in the group under study. In the subfamily Opisthocentrinae, species branches have a good support and group to produce monophyletic genera. The monophyletic origin of the subfamily has a high support of 74-99% (Fig. 2). A similar topology was observed in a study of a smaller taxon sample, which included only the genera Opisthocentrus and Pholidapus, and sequences of a slightly different length [7]. At the same time, the topological position of the monotypal genus *Plectobranchus* in the subfamily could not be resolved on the basis of the *Co-1* gene fragment.

The mean genetic distance between genera within the subfamily vary from 7 (between Ascoldia and Phol*idapus*) to 11% (between *Kasatkia* and *Opisthocentrus*), while the genetic distance between the genus *Plecto*branchus and the other genera of the subfamily reaches 15-17%, which is higher than the mean *p*-distance within the subfamily (12%). Gilbert and Thompson [28], who described *P. evides*, noted its similarity to species of the subfamily Lumpeninae; the Pacific subspecies L. maculates diaphanocarus was described as a species of the genus *Plectobranchus* [29]; and Jordan and Evermann [30] isolated the species in a separate monotypal subfamily. It is possible that the species appeared to be separate from the other members of the subfamily in our study because we had no data for species of the genus Lumpenopsis, which is close to Plectobranchus. Makushok [15] believes that Plectobranchus combines the features of Lumpeninae and Opisthocentrinae, uniting them in one taxonomic entity. To address these issues, it is necessary to analyze a more representative sample, including species of the genus Lumpenopsis, and to make the reference sequence more informative by using the full-length Co-1 sequence (approximately 1,500 bp) and including other genes in the analysis.

The genus *Pholidapus* cluster with *Ascoldia* to produce a separate branch with a good support in our tree, and this finding disagrees with the concept that *Pholidapus* is a subgenus within the genus *Opisthocentrus* [31].

The subfamily Chirolophinae similarly has a high statistical support. All of the three its species form a monophyletic branch and belong to one genus (Fig. 2), reflecting the natural position of the group in the system [10]. It is noteworthy, however, that an extremely low *p*-distance of 0.01 was observed between *Chirolophis nugator* and *Ch. decoratus*. Such a distance is characteristic of the within-species level in the group under study (see table in the Appendix). This finding makes it possible to assume that the two sequences, which were extracted from GenBank, belong to one species, indicating that the species was misidentified for one of the two individuals used to obtain these sequences.

Sequences of the family Zoarcidae do not group in the *Co-1* gene trees in accord with the natural system [32], which suggests that the genus *Lycodes* is the most primitive in the subfamily Lycodinae. However, only a limited sample of species represented the subfamily in our study, and, consequently, it is impossible to make reliable conclusions on the relationships within the group. The genetic distances between genera within the family Zoarcidae display only a minor variation, from 7 (between *Bothrocara* and *Lycodes*) to 8% (between *Bothrocara* and *Lycodes*). Radchenko et al. [33] considered the taxonomic relationships of the genera *Bothrocara* and *Lycodes*. However, the results cannot be compared with our findings because of the sample specifics (other species were examined).

The topology of our trees disagrees with the common views of the system of Stichaeidae. The subfamilies Lumpeninae, Stichaeinae, and Xiphisterinae formed unresolved branches and, accordingly, their branching was unclear. A high support was obtained only for within-species groups of these taxa. In view of the above, it is possible to conclude either that the system of the family Stichaeidae needs further development or that the phylogenetic signal based on the Co-1 gene fragment is insufficient for reliable conclusions. Interestingly, the sequence of a L. sagitta individual from the Sea of Japan did not cluster closely with individuals from the Bering See. Gene genetic distances between the sequences of these individuals were 0.05, somewhat higher than usual at the within-species level (0.01). Since the samples were collected at the margins

of the species area, our findings suggest a high interpopulation difference in the *Co-1* gene for *L*. *sagitta*.

The family Bathymasteridae formed two distant groups (Fig. 2). Anderson [32] believes that the family is the most primitive in the suborder Zoarcoidei, and this is supported by molecular data [14]. It seems that genes with a higher extent of conservation should be examined to study the taxonomic relationships within the family. We intend to perform such analysis in our further work.

The families Ammodytidae and Lobotidae were in polytomy relative to *Dasycottus setiger*, but formed a separate group distant from the other taxa. This result supports the natural positions of the group in the conventional system [8] and indicates that the outgroup was selected adequately.

Since *p*-distances increased with increasing taxonomic rank (Fig. 3), a geographical model of speciation and phyletic evolution may be assumed to dominate in the perch-like taxa under study, as in other groups [3, 6]. However, relative genetic distances are an insufficient basis for identifying the model of speciation, and several parameters or descriptors should be analyzed [1, 5]. The above assumption is thereby only preliminary. Extremely low *p*-distances within species testify to a high sequence homology. This is indicative of correct species identification and, as mentioned above, is broadly used to construct a DNA-based bar code of species [20].

The nucleotide composition of the sequences under study was biased from the uniform composition 1 : 1 : 1 : 1 towards a predominance of pyrimidines (Fig. 4). This may be assumed to reflect the hydrophobic properties of protein-coding genes [34], including *Co-1*. However, taxonomic differences in nucleotide composition are also possible, pointing to evolutionary specifics of different phyletic lineages [6].

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4	.12	.12	.13	.13	.13	.13	.13	.14	.13	.13	.11	.11	.12	.12	.12	.12	.15	.15	.01	.14	.13	.14	.14	.13	.13	.13	.12	.12	.12	.12	.12	.12	.12	.12
з	.12	.12	.13	.13	.13	.13	.13	.14	.13	.13	.12	.12	.12	.12	.12	.12	.16	.16	00.	.15	.13	.14	.15	.13	.13	.13	.12	.12	.12	.12	.12	.12	.12	.12
2	.14	.14	.13	.13	.13	.13	.13	.16	.15	.15	.13	.13	.13	.14	.14	.14	.17	.17	.16	.16	.16	.16	.16	.14	.15	.14	.15	.15	.15	.14	.14	.14	.14	.14
-	.12	.13	.12	.12	.12	.12	.12	.12	.12	.12	.11	.11	.12	.12	.12	.12	.14	.14	60.	.13	.14	.14	.14	.13	.14	.13	.14	.14	.14	.12	.12	.12	.12	.12
No.	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67

33	.13	.13	.13	.13	.13	.14	.13	.13	.13	.12	.12	.13	.13	.13	.14	.14	.14	.14	.14	.14	.13	.13	.13	.14	.16	.16	.16	.22	
32	.13	.13	.13	.13	.13	.14	.14	.14	.14	.13	.13	.14	.14	.14	.15	.14	.16	.15	.15	.15	.15	.15	.15	.15	.18	.18	.18	.22	
31	.13	.13	.13	.13	.13	.17	.17	.17	.17	.15	.15	.16	.16	.16	.16	.17	.16	.16	.16	.16	.17	.17	.17	.17	.18	.18	.18	.21	
30	.14	.13	.13	.13	.13	.17	.16	.16	.16	.15	.15	.17	.17	.17	.16	.17	.17	.16	.16	.16	.17	.17	.17	.17	.18	.18	.18	.20	
29	.13	.13	.13	.13	.13	.17	.17	.17	.17	.15	.15	.16	.16	.16	.16	.17	.16	.16	.16	.16	.17	.17	.17	.17	.18	.18	.18	.21	
28	.13	.13	.13	.13	.13	.17	.17	.17	.17	.15	.15	.16	.16	.16	.16	.17	.16	.16	.16	.16	.17	.17	.17	.17	.18	.18	.18	.21	
27	.14	.14	.14	.14	.14	.17	.17	.17	.17	.16	.16	.17	.17	.17	.17	.17	.17	.17	.17	.17	.18	.18	.18	.18	.18	.19	.18	.21	
26	.13	.13	.13	.13	.13	.16	.17	.17	.17	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.18	.18	.18	.21	
25	.12	.12	.12	.12	.12	.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.19	.19	.19	.22	
24	.12	.13	.13	.13	.13	.15	.14	.14	.14	.14	.14	.14	.14	.14	.14	.14	.16	.15	.15	.15	.15	.15	.15	.15	.18	.18	.18	.23	
23	.12	.12	.12	.12	.12	.15	.14	.14	.14	.14	.14	.14	.13	.13	.14	.14	.15	.15	.15	.15	.14	.14	.14	.15	.18	.18	.18	.23	
22	.12	.13	.13	.13	.13	.15	.14	.14	.14	.14	.14	.14	.14	.14	.14	.14	.15	.15	.15	.15	.14	.14	.14	.15	.18	.18	.18	.23	
21	.13	.13	.13	.13	.13	.15	.14	.14	.14	.14	.14	.14	.14	.14	.14	.14	.16	.15	.15	.15	.15	.15	.15	.15	.18	.19	.18	.23	
20	.11	.11	.11	.11	.11	.16	.13	.13	.13	.14	.14	.13	.13	.13	.14	.14	.15	.14	.14	.14	.14	.14	.14	.14	.18	.18	.18	.22	
19	.13	.13	.13	.13	.13	.14	.14	.14	.14	.15	.15	.14	.14	.14	.16	.15	.16	.16	.16	.16	.15	.15	.15	.15	.17	.17	.17	.23	
18	.13	.13	.13	.13	.13	.14	.14	.14	.14	.15	.15	.14	.14	.14	.16	.16	.16	.16	.16	.16	.15	.15	.15	.15	.17	.17	.17	.23	
17	.13	.13	.13	.13	.13	.14	.14	.14	.14	.15	.15	.14	.14	.14	.16	.16	.16	.16	.16	.16	.15	.15	.15	.15	.17	.17	.17	.23	
16	.14	.14	.14	.14	.14	.16	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.23	
15	.14	.14	.14	.14	.14	.16	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.23	
14	.14	.14	.14	.14	.14	.16	.16	.16	.16	.16	.16	.15	.15	.15	.16	.16	.17	.16	.16	.16	.16	.16	.16	.16	.18	.18	.18	.23	
13	.13	.13	.13	.13	.13	.17	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.17	.17	.17	.22	
12	.13	.13	.13	.13	.13	.17	.16	.16	.16	.16	.16	.17	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.17	.17	.17	.22	
11	.13	.13	.13	.13	.13	.17	.15	.15	.15	.15	.15	.15	.15	.15	.16	.16	.17	.16	.16	.16	.16	.16	.16	.16	.19	.19	.19	.23	
10	.13	.13	.13	.13	.13	.17	.15	.15	.15	.15	.15	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.19	.19	.19	.23	
6	.13	.13	.13	.13	.13	.17	.15	.15	.15	.15	.15	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.19	.19	.19	.23	
8	.13	.13	.13	.13	.13	.17	.15	.15	.15	.15	.15	.15	.15	.15	.16	.16	.17	.16	.16	.16	.16	.16	.16	.16	.19	.19	.19	.23	
7	.13	.13	.13	.13	.13	.16	.15	.15	.15	.15	.15	.16	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.22	
9	.13	.13	.13	.13	.13	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.17	.16	.16	.16	.17	.17	.17	.17	.17	.17	.17	.23	
5	.13	.13	.13	.13	.13	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.17	.16	.16	.16	.17	.17	.17	.17	.17	.17	.17	.23	
4	.13	.13	.13	.13	.13	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.17	.16	.16	.16	.17	.17	.17	.17	.17	.17	.17	.23	
з	.13	.13	.13	.13	.13	.16	.15	.15	.15	.15	.15	.16	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.22	
7	.15	.15	.15	.15	.15	.17	.17	.17	.17	.18	.18	.17	.17	.17	.18	.18	.17	.17	.17	.17	.17	.17	.17	.17	.20	.20	.20	.22	
1	.13	.13	.13	.13	.13	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.17	.18	.18	.18	.23	
No.	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	

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99																																	00.
65																																00.	00.
64																															00.	.01	.01
63																														00.	00.	.01	.01
62																													.13	.13	.13	.13	.13
61																												0.	.13	.13	.13	.13	.13
60																											00.	00.	.13	.13	.13	.13	.13
59																										.12	.12	.12	.14	.14	.14	.14	.14
58																									00.	.12	.12	.12	.14	.14	.14	.14	.14
57																								00.	00.	.12	.12	.12	.14	.14	.14	.14	.14
56																							.15	.15	.15	.13	.13	.13	.13	.13	.13	.13	.13
55																						.11	.15	.15	.15	.14	.14	.14	.14	.14	.14	.14	.14
54																					.15	.16	60.	60.	60.	.12	.12	.12	.12	.12	.12	.13	.13
53																				.13	.14	.13	.12	.11	.12	.12	.12	.12	.12	.12	.12	.12	.12
52																			.15	.13	.14	.15	.13	.13	.13	.12	.12	.12	.12	.12	.12	.12	.12
51																		.15	60.	.13	.13	.14	.13	.13	.13	.12	.12	.12	.14	.14	.14	.14	.14
50																	00.	.15	60.	.13	.13	.14	.13	.13	.13	.12	.12	.12	.14	.14	.14	.14	.14
49																.12	.12	.12	.13	.12	.14	.15	.13	.13	.13	.11	.11	.11	.12	.12	.12	.12	.12
48															00.	.12	.12	.12	.13	.12	.14	.15	.13	.13	.13	.11	.11	.11	.12	.12	.12	.12	.12
47														00.	00.	.12	.12	.12	.13	.12	.14	.15	.13	.13	.13	.11	.11	.11	.12	.12	.12	.12	.12
46													.08	.08	.08	.13	.13	.12	.13	.13	.14	.13	.13	.13	.13	.12	.12	.12	.10	.10	.10	.10	.10
45												00.	.08	.08	.08	.13	.13	.12	.12	.13	.14	.13	.12	.13	.12	.12	.12	.12	.10	.10	.10	.10	.10
44											00.	00.	.08	.08	.08	.13	.13	.12	.12	.13	.14	.13	.12	.13	.12	.12	.12	.12	.10	.10	.10	.10	.10
43										60.	60.	60.	60.	60.	60.	.12	.12	.13	.13	.12	.15	.14	.12	.12	.12	.11	.11	.11	.12	.12	.11	.11	.11
42									00.	60.	60.	60.	60.	60.	60.	.12	.12	.13	.13	.12	.15	.14	.12	.12	.12	.11	.11	.11	.12	.12	.11	.11	.11
41								.01	.01	60.	60.	60.	.10	.10	60.	.12	.12	.13	.13	.13	.15	.14	.12	.13	.12	.12	.12	.12	.12	.12	.12	.12	.12
40							.10	60.	60.	.08	.08	.08	60.	60.	60.	.13	.13	.13	.14	.12	.15	.15	.11	.12	.11	.12	.12	.12	.13	.13	.13	.13	.13
39						00.	.10	60.	60.	.08	.08	.08	60.	60.	60.	.13	.13	.13	.14	.12	.15	.15	.11	.12	.11	.12	.12	.12	.13	.13	.13	.13	.13
38					00.	00.	.10	60.	60.	.08	.08	.08	60.	60.	60.	.13	.13	.13	.14	.12	.15	.15	.11	.12	.11	.12	.12	.12	.13	.13	.13	.13	.13
37				00.	00.	00.	.10	60.	60.	.08	.08	.08	60.	60.	60.	.13	.13	.13	.14	.12	.15	.15	.11	.12	.11	.12	.12	.12	.13	.13	.13	.13	.13
36			00.	00.	00.	00.	.10	60.	60.	.08	.08	.08	60.	60.	60.	.13	.13	.13	.14	.12	.15	.15	.11	.12	.11	.12	.12	.12	.13	.13	.13	.13	.13
35		.07	.08	.07	.07	.07	.08	.08	.08	.07	.07	.08	60.	60.	60.	.13	.13	.12	.15	.13	.15	.15	.13	.13	.13	.12	.12	.12	.11	.11	.11	.11	.11
34	.01	.08	.08	.08	.08	.08	60.	.08	.08	.08	.08	.08	60.	60.	60.	.13	.13	.12	.14	.13	.15	.15	.13	.13	.13	.11	.11	.11	.11	.11	.11	.11	.11
No.	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	99	67

L	12	12	12	12	12	15	15	15	15	15	15	15	15	15	16	16	15	15	15	15	16	16	16	16	17	8	17	50	
6 6	2					5	5	5	5	5	5	5	5	5	9	9	5.	5	5.	5.	9	9	9	9		<u>8</u>	. 7	0.0	
5 6	3.1	3.1	3.1	3.1	3.1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	6 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	7 .1	8.1	7 .1	0 .2	
4 6	3.1	3 .1	3 .1	3 .1	3 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	6 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	7 .1	8 .1	7 .1	1 .2	
3 64	3 .1	3 .1	3 .1	3 .1	3 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	6 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	5 .1	6 .1	7 .1	8 .1	7 .1	1 .2	
5 63	2	5	2	5	2	5 .1.	4	4	4	5.1.	5	5.1.	4	4	4 .1	4	5	5.15	5 .1.	5.1.	5 .1.	5 .1.	5 .1.	5	.1	<u>.1</u>	.1	1 .2	
62	2	.1.	2	2	2	.1.	+ .1	+ 	<u>+</u>	5	5	5	<u>+</u>	<u>+</u>	<u>+</u>	<u>+</u>	5 .1	5.15	5 .1	5.1.	5 .1.	5 .1	5 .1	5	.1.	6	6	1 .2	
) 61	2 .T.	.1.	2	2	2		.1 .1	.1	.1		. <u>.</u>	. <u>.</u>	.1	+ .1	4.1	1.	. <u>.</u>	. <u>.</u>	.1.	. <u>.</u>	.1:	:T:	. <u>.</u>	. <u>.</u>	.1.6	.10	.1.6	.2	
60	.13	<u> </u>			<u>.</u>	+ .1;	.12	.12	.12	+ .1.	+ .1.	.1.	.12	.12	+ .1 ²	F .12		.1.	.15		.15	.15	.1.	.1.	.19	.19	.19	2]	
59	.12	.12	.12	.12	.12	.14	.15	.15	.15	.14	.14	.15	.15	.15	.14	.14	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.22	
58	.12	.12	.12	.12	.12	.15	.15	.15	.15	.14	.14	.16	.15	.15	.14	.14	.16	.15	.15	.15	.15	.15	.15	.15	.19	.19	.19	.22	
57	.12	.12	.12	.12	.12	.14	.15	.15	.15	.14	.14	.15	.15	.15	.14	.14	.15	.15	.15	.15	.15	.15	.15	.15	.19	.19	.19	.22	
56	.13	.13	.13	.13	.13	.17	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.20	.20	.20	.20	
55	.13	.13	.13	.13	.13	.17	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.17	.20	.21	.20	.21	
54	.14	.14	.14	.14	.14	.15	.15	.15	.15	.16	.16	.15	.15	.15	.13	.13	.15	.15	.15	.15	.15	.15	.15	.15	.20	.20	.20	.21	
53	.13	.13	.13	.13	.13	.17	.17	.17	.17	.16	.16	.17	.17	.17	.16	.16	.17	.17	.17	.17	.17	.17	.17	.18	.19	.19	.19	.22	
52	.13	.13	.13	.13	.13	.16	.15	.15	.15	.15	.15	.15	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.17	.17	.17	.22	
51	.14	.14	.14	.14	.14	.17	.16	.16	.16	.15	.15	.16	.16	.16	.15	.15	.17	.17	.17	.17	.18	.18	.18	.18	.18	.18	.18	.23	
50	.14	.14	.14	.14	.14	.17	.16	.16	.16	.15	.15	.16	.16	.16	.15	.15	.17	.17	.17	.17	.18	.18	.18	.18	.18	.18	.18	.23	
49	.13	.13	.13	.13	.13	.14	.14	.14	.14	.13	.13	.14	.14	.14	.15	.15	.14	.14	.14	.14	.15	.15	.15	.16	.20	.20	.20	.22	
48	.13	.13	.13	.13	.13	.15	.14	.14	.14	.13	.13	.15	.14	.14	.15	.15	.15	.14	.14	.14	.16	.16	.16	.16	.20	.20	.20	.22	
47	.13	.13	.13	.13	.13	.15	.14	.14	.14	.13	.13	.15	.14	.14	.15	.15	.15	.14	.14	.14	.16	.16	.16	.16	.20	.20	.20	.22	
46	.12	.12	.12	.12	.12	.15	.15	.15	.15	.15	.15	.15	.15	.15	.16	.15	.15	.14	.14	.14	.15	.15	.15	.16	.17	.17	.17	.23	
45	.12	.12	.12	.12	.12	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.16	.15	.14	.14	.14	.16	.16	.16	.16	.18	.18	.18	.23	
44	.12	.12	.12	.12	.12	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.16	.15	.14	.14	.14	.16	.16	.16	.16	.18	.18	.18	.23	
43	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.14	.13	.13	.13	.13	.13	.13	.13	.18	.18	.18	.22	
42	.13	.13	.13	.13	.13	.13	.12	.12	.12	.12	.12	.12	.12	.12	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.18	.18	.18	.22	
41	.14	.14	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.14	.14	.14	.14	.13	.13	.13	.13	.18	.18	.18	.22	
40	.13	.13	.13	.13	.13	.16	.15	.15	.15	.14	.14	.15	.15	.15	.16	.16	.15	.15	.15	.15	.16	.16	.16	.16	.17	.17	.17	.21	
39	.13	.13	.13	.13	.13	.16	.15	.15	.15	.14	.14	.15	.15	.15	.16	.16	.15	.15	.15	.15	.16	.16	.16	.16	.17	.17	.17	.21	
38	.13	.13	.13	.13	.13	.16	.15	.15	.15	.14	.14	.15	.15	.15	.16	.16	.15	.15	.15	.15	.16	.16	.16	.16	.17	.17	.17	.21	
37	.13	.13	.13	.13	.13	.16	.15	.15	.15	.14	.14	.15	.15	.15	.16	.16	.15	.15	.15	.15	.15	.15	.15	.16	.18	.18	.18	.21	
36	.13	.13	.13	.13	.13	.16	.15	.15	.15	.14	.14	.15	.15	.15	.16	.16	.15	.15	.15	.15	.16	.16	.16	.16	.17	.17	.17	.21	
35	.13	.13	.13	.13	.13	.15	.14	.14	.14	.14	.14	.14	.14	.14	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.17	.17	.17	.21	
34	.13	.13	.13	.13	.13	.15	.15	.15	.15	.14	.14	.14	.14	.14	.15	.15	.15	.15	.15	.15	.15	.15	.15	.15	.18	.18	.18	.21	
No.	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	

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95																												
94																											.21	
93																										.01	.21	
92																									.01	00.	.21	
91																								.21	.21	.21	.21	
90																							00.	.20	.21	.20	.21	
89																						00.	00.	.20	.21	.20	.21	
88																					00.	00.	00.	.20	.21	.20	.21	
87																				.04	.04	.04	.04	.20	.20	.20	.21	
86																			00.	.04	.04	.04	.04	.20	.20	.20	.21	
85																		00.	00.	.04	.04	.04	.04	.20	.20	.20	.21	
84																	00.	00.	00.	.04	.04	.04	.04	.20	.20	.20	.21	
83																.07	.07	.07	.07	.06	.06	.06	.07	.20	.20	.20	.22	
82															00.	.07	.07	.07	.07	.07	.07	.07	.07	.20	.20	.20	.22	
81														.08	.08	.05	.04	.04	.04	.03	.03	.03	.03	.19	.20	.19	.22	
80													00.	.08	.08	.05	.04	.04	.04	.03	.03	.03	.03	.19	.20	.19	.22	
62												00.	00.	.08	.08	.05	.04	.04	.04	.03	.03	.03	.03	.19	.20	.19	.21	
78											.07	.07	.07	.08	.08	.08	.08	.08	.08	.08	.08	.08	.08	.19	.19	.19	.22	
77										00.	.07	.07	.07	.08	.08	.08	.08	.08	.08	.08	.08	.08	.08	.19	.19	.19	.22	
76									.08	.08	.03	.03	.03	.07	.07	.04	.04	.04	.04	.03	.03	.03	.03	.20	.20	.20	.21	
75								00.	.08	.08	.03	.03	.03	.07	.07	.04	.04	.04	.04	.03	.03	.03	.03	.20	.20	.20	.21	
74							00.	00.	.08	.08	.03	.03	.03	.07	.07	.04	.04	.04	.04	.03	.03	.03	.03	.20	.20	.20	.21	
73						.08	.08	.08	.08	.08	.07	.07	.07	.08	.08	.07	.07	.07	.07	.07	.07	.07	90.	.20	.21	.20	.23	
72					.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.18	.19	.18	.21	
71				00.	.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.18	.19	.18	.21	
70			00.	00.	.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.18	.19	.18	.21	
69		00.	00.	00.	.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.18	.19	.18	.21	
68	.00	00.	00.	00.	.17	.15	.15	.15	.16	.16	.15	.15	.15	.16	.16	.16	.16	.16	.16	.16	.16	.16	.16	.18	.18	.18	.21	
No.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	

MOLECULAR PHYLOGENETIC STUDY

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