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Molecular phylogeography of Palearctic and Nearctic ciscoes

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We studied mtDNA phylogeography of Palearctic and Nearctic *Coregonus*, including four species of ciscoes (*C. sardinella*, *C. autumnalis*, *C. artedi*, *C. laurettae*), Baikal omul (*C. migratorius*) and related taxa. The *C. sardinella* complex, which includes *C. peled*, is distantly related to the other cisco species. Eurasian *C. sardinella* haplotypes are the most basal in this lineage. *C. autumnalis* and *C. laurettae* are well-differentiated species and belong to a clade that also includes *C. artedi*. This clade likely originated from Baikal omul which is intermediate between the *C. autumnalis* complex and the rest of the network. Haplotypes from Siberian populations of *C. autumnalis* occupy the basal positions of the *C. autumalis* complex. We conclude Siberia is the place of origin for the *C. autumnalis* and *C. sardinella* complexes. Two routes of invasion, along the northern and southern coasts of the Pleistocene Bering land bridge, explain the origin of the North American populations of *C. sardinella* (north), *C. autumnalis* (north), *C. laurettae* (south) and *C. artedi* (probably south).

Introduction

Phylogeography is a relatively new field of biological science dealing with the history and formation of species (Avise 2000). The inventor of the term and founder of the discipline itself, John C. Avise, defines phylogeography as “a field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages, especially those within and among closely related species”. In coregonid fishes (Salmonidae: Coregoninae), relationships among species have been studied by morphology and genetic markers whereas geographic variation within species remains poorly investigated. Phylogeography and macrogeographic variation have been studied in Palearctic and Nearctic whitefishes (coregonids with subterminal mouths) whereas in ciscoes these issues have been studied only at local scales.

Ciscoes were traditionally defined as a group of coregonine fishes with terminal or supraterminal mouths. Formerly they were often treated as separate genera (*Leucichthys* or *Argyrosomus*). Presently ciscoes comprise the subgenus *Leucichthys* of the genus *Coregonus*. Data on

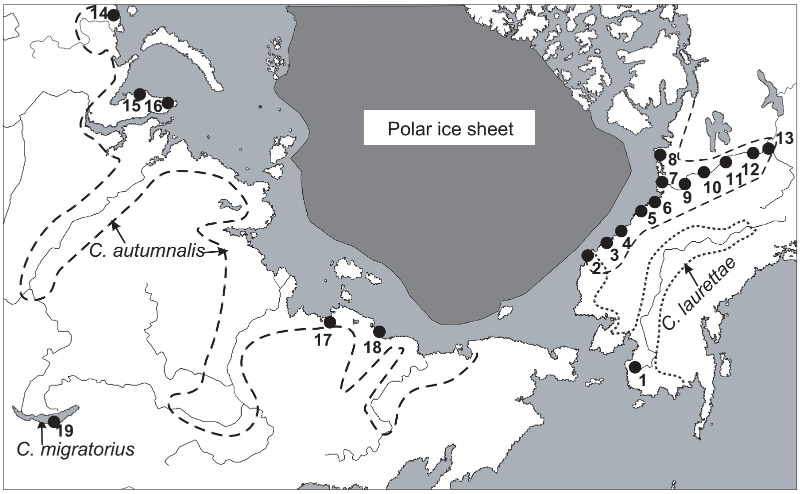

Fig. 1. Species ranges for Arctic cisco (broken line), Bering cisco (dotted line) and Baikal omul. Sampling sites are shown as circles with sample numerical codes corresponding to numbers in Table 1.

genetic differentiation employing molecular markers have shown that this subgenus is not monophyletic. Lineages of the Arctic cisco and least cisco–vendace complexes are not phylogenetically closely related (Bodaly *et al.* 1991, Bodaly *et al.* 1994). However, the relationships among the multiple forms comprising these lineages remain unclear. It is commonly accepted that the Arctic cisco complex includes Arctic cisco (*C. autumnalis*), Bering cisco (*C. laurettae*), and the endemic Penzhina River cisco (*C. subautumnalis*). Molecular data suggest that lake cisco (*C. artedi*) and other ciscoes of the Laurentian Great Lakes basin also belong to this clade. The position of the Baikal omul (*C. migratorius*), which used to be a subspecies of Arctic cisco, has been recently refined. Its similarity with whitefishes (Politov *et al.* 2000, Sukhanova *et al.* 2002) and its probable basal position with respect to Arctic cisco (Politov *et al.* 2000, Politov *et al.* 2002) was hypothesized. Two wide-spread cisco species (Arctic cisco, least cisco) are found in both Eurasia and North America. Along with conspecific populations on both sides of the Arctic Sea coast, a number of local forms known under separate scientific names are described. Until now, the center of origin of these forms was never investigated by mtDNA, which has proven to be a powerful tool in phylogeographic reconstructions (Avise 2000). In this paper we investigate the phylogeographic and evolutionary relationships of ciscoes and related forms using mtDNA restriction analyses.

Material and methods

Fish samples

Tissue samples of coregonid fishes were collected during fieldwork conducted between 1988 and 1998 (Fig. 1 and Table 1). Most Eurasian samples were collected during the Swedish–Russian Expedition "Tundra Ecology-94" (Grønlund & Melander 1995) and described earlier (Novikov *et al.* 2000, Politov *et al.* 2000, Politov *et al.* 2002). Fish were identified using existing keys (Berg 1948, McPhail & Lindsey 1970, Reshetnikov 1980). Reference specimens of each species from several localities were fixed in formalin and later deposited in the Texas Cooperative Wildlife Collection (Texas A&M University, College Station, TX, USA), the Department of Ichthyology, Moscow State University (Russia), and the Swedish Museum of Natural History (Stockholm). Tissues taken for genetic analysis (skeletal muscle, heart and liver) were stored frozen at –80 °C or fixed in ethanol or buffer (20% DMSO in saturated NaCl) at the Laboratory of Population Genetics, Vavilov Institute of General Genetics, Russian Academy of Sciences (Moscow, Russia) or the Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, USA.

DNA extraction

Deep-frozen or ethanol preserved tissues were used for analysis of DNA polymorphisms. Prior to DNA extraction, frozen tissues (heart, skeletal muscle or liver) were transferred into 20% DMSO in saturated NaCl to enhance DNA recovery. Total DNA was extracted by lysis of tissues for 1 hour at 60 °C in 0.65 ml extraction

Table 1. Sample size and location. Numbers in the first column correspond to numbers of sampling sites given in Fig. 1.

No.	Species and location(s)	Coordinates	Sample size (*n*)
	Bering cisco, *C. laurettae* Bean		10[a]
1	USA, Alaska, Yukon River	62°35´N, 151°57´W	10
	Arctic cisco, *Coregonus autumnalis* Pallas		300 (+ 139)
2	USA, N Alaska, Point Barrow	71°16´N, 152°24´W	10
3	USA, N Alaska, Kogru River mouth	70°46´N, 152°10´W	8
4	USA, N Alaska, Colville River mouth	70°24´N, 151°14´W	10 (+ 61)[b]
5	USA, N Alaska, Sagavanirktok River mouth	70°21´N, 148°01´W	30 (+ 29)[b]
6	USA, N Alaska, Kaktovik Lagoon	70°04´N, 144°18´W	9
7	Canada, Yukon Province, Stokes Point	69°16´N, 138°18´W	25
8	Canada, NW Territories, Atkinson Point	69°53´N, 131°31´W	24
9	Canada, NW Territories, Peel River	67°37´N, 134°39´W	30 (+ 16)[b]
10	Canada, NW Territories, Arctic Red River	67°17´N, 133°36´W	30 (+ 33)[b]
11	Canada, NW Territories, Mountain River	65°42´N, 128°49´W	20
12	Canada, NW Territories, Carcajou River	65°22´N, 127°26´W	21
13	Canada, NW Territories, Liard River	60°45´N, 124°47´W	30
	(Total North American Arctic cisco)		237 (+ 139)[b]
14	Russia, NE Europe, W to Pechora River delta	68°49´N, 53°42´E	1
15	Russia, W Siberia, W Yamal Peninsula	70°20´N, 67°50´E	3
16	Russia, W Siberia, N Yamal Peninsula	72°51´N, 70°55´E	8
17	Russia, E Siberia, E to Yana River mouth	72°24´N, 140°57´E	50
18	Russia, E Siberia, Indigirka River delta	71°26´N, 149°45´E	1
	(Total Eurasian Arctic cisco)		63
	Baikal omul, *C. migratorius* (Georgi)		40
19	Eurasia, Russia, Lake Baikal	53°50´N, 108°37´E	40
	Least cisco, *C. sardinella* Valenciennes		6 (+ 43)[c]
	Eurasia, Russia, 6 localities, NE Europe and Siberia	*see* Politov *et al*. 2000	3 (+ 43)[c]
	N. America, Canada, Mackenzie River basin		3
	Peled, *C. peled* Gmelin		3 (+ 10)[c]
	Eurasia, Russia, Yenissey River basin	65°47´N, 87°56´E	3 (+ 10)[c]
	Siberian whitefish, *C. lavaretus pidschian* Pallas		8 (+ 16)
	Eurasia, Russia, 5 localities in Siberia	*see* Politov *et al*. 2000	3 (+ 16)
	N. America, Canada, Mackenzie River basin	66°58´N, 135°08´W	5
	Muksun, *C. muksun* Pallas		2 (+ 15)[c]
	Eurasia, Russia, 3 localities in Siberia	*see* Politov *et al*. 2000	2 (+ 15)[c]
	Humpback whitefish, *C. clupeaformis*		2 (+ 15)[c]
	N. America, Canada, Mackenzie River basin	66°58´N, 135°08´W	2 (+ 15)[c]
	Broad whitefish, *C. nasus* Pallas		8 (+ 31)[c]
	Eurasia, Russia, 5 localities in Siberia	*see* Politov *et al*. 2000	3 (+ 31)[c]
	N. America, Canada, Mackenzie River basin	66°58´N, 135°08´W	5
	Inconnu/nelma, *Stenodus leucichthys nelma* Güldenstädt		4
	N. America, Canada, Mackenzie River basin	66°58´N, 135°08´W	3
	Eurasia, Russia, Yenissey River basin	65°47´N, 87°56´E	1

[a] Yukon River is a type habitat for Bering cisco, these fishes were also identified as *C. laurettae* by morphology, so they were treated here as reference specimens while all other North American ciscoes collected from the Arctic Sea basin were formally classified as Arctic cisco; some of them in fact had Bering cisco mtDNA.

[b] In Arctic cisco sizes of additional samples analysed only by diagnostic *Dde* I, *Hinf* I restrictions of *ND1* fragment are given in parentheses.

[c] In species other than Arctic cisco sizes of additional samples analysed only by *ND1* fragment or by *ND1* and *ND2* fragment are given in parentheses.

buffer (540 μl 0.1 M Tris pH 7.8, 70 μl 10% SDS, 40 μl 1% proteinase K). Successive extractions with phenol: chloroform: isoamyl alcohol (24:24:1) and methylene chloride: isoamyl alcohol (24:1) were followed by ethanol precipitation of the aqueous phase, centrifugation for 5 min at 12 000 rpm and resuspension of the DNA pellet in 100 μl of TE buffer.

PCR

The polymerase chain reaction (PCR) was used to amplify three fragments of mtDNA. The first fragment NADH-dehydrogenase-1 gene (‘*ND1*’) together with flanking regions of 16S ribosomal RNA and tRNAs was amplified using primers LGL381 (forward): 5´-ACCCCGCCTGTT TACCAAAAACAT-3´ and LGL563 (reverse): 5´-GGTTCATTAGTGAGGGAAGG-3´ (Cronin *et al*. 1993). The second fragment included the NADH-dehydrogenase-2 gene (‘*ND2*’) and was amplified with primers LGL562 (forward): 5´-TAAGCTATCGGGCCCATACC-3´ and LGL452B (reverse): 5´-GTGGTGTTTAGRTT TCGGTC-3´ (Cronin *et al*. 1993). The third fragment included the NADH-dehydrogenase-5 and NADH-dehydrogenase-6 (*ND 5/6*) genes (hereafter called ‘*ND5/6*’) and was amplified with primers LGL763 (forward): 5´-AAATCCAAG-TAGCAGCTATGCA-3´ and LGL764 (reverse): 5´-TTACAACGATGGTTTTTCATRTCA-3´ (Cronin *et al*. 1993). The PCR reactions were performed in 60 μl volumes using a Perkin-Elmer DNA Thermal Cycler (Perkin Elmer Applied Biosystems, Foster City, CA). PCR was carried out in 32 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 1 min 45 sec. Sizes of the PCR products were approximately 2.1 kb (*ND1*), 2.0 kb (*ND2*), and 2.4 kb (*ND5/6*).

RFLP analysis

Aliquots (2 to 2.5 μl) of PCR products were cut by a set of restriction enzymes. The *ND1* fragment was cut by 18 restrictases: *Ase* I, *Ava* II, *Bsa*J I, *Bsp*1286 I, *Bst*N I, *Bst*U I, *Dde* I, *Dpn* II, *Hae* III, *Hha* I, *Hinc* II, *Hinf* I, *Hph* I, *Msp* I, *Nci* I, *Rsa* I, *Ssp* I, *Taq* I; the ND-2 fragment by 20 enzymes: *Acc*I, *Alu* I, *Ase* I, *Bsa*J I, *Bsp*1286, *Bst*N I, *Bst*U I, *Dde* I, *Dpn* II, *Dra* I, *Hae* III, *Hha* I, *Hinc* II, *Hinf* I, *Hph* I, *Msp* I, *Nci* I, *Rsa* I, *Sau*96 I, *Taq* I, and the ND-5/6 fragment by 16 enzymes: *Ase* I, *Bsa*J I, *Bsp*1286, *Bsr* I, *Bst*N I, *Dde* I, *Dpn* II, *Dra* I, *Hae* III, *Hha* I, *Hinf* I, *Hph* I, *Nci* I, *Sau*96 I, *Ssp* I, *Taq* I. Restriction digests were incubated for at least 2 hours under conditions specified by the manufacturer (New England BioLabs, Beverly, MA). Restriction products were separated electrophoretically on 2%–2.5% agarose gels, stained with ethidium bromide and screened under UV-light. Fragment size was determined using GIBCO BRL 100 bp DNA size marker (Life Technologies, Gaithersburg, MD). Restriction banding patterns were transformed into a matrix of presence or absence of cut sites. Numbers of non-shared restriction sites (hereafter called “pairwise differences”) were counted among haplotypes and a minimum-spanning network was constructed using Arlequin 2.0 software (Schneider *et al*. 2000). All specimens were initially screened with the *ND1* fragment. Subsequently, all Arctic cisco, Bering cisco, and most Baikal omul specimens were additionally analyzed by *ND2* and *ND5/6* fragments. For least cisco, peled and other species used as outgroups only representative haplotypes (including most divergent or/and most common *ND1* haplotypes) were screened by these two additional mtDNA fragments.

Analysis of mtDNA variation

Expectations from population genetic modelling predict that the patterns and distribution of genetic variation are to a great extent determined by the demographic histories of populations (Avise *et al*. 1984, Slatkin & Hudson 1991, Nee *et al*. 1995). In a population that has undergone recent exponential growth, its mtDNA haplotype networks should appear star-like in shape as a consequence of little incidental loss of novel haplotype. Consequently, since most haplotypes are thought to originate from one of a few closely related ancestral haplotypes, in such populations the distribution of pairwise differences among these haplotypes will approximate a

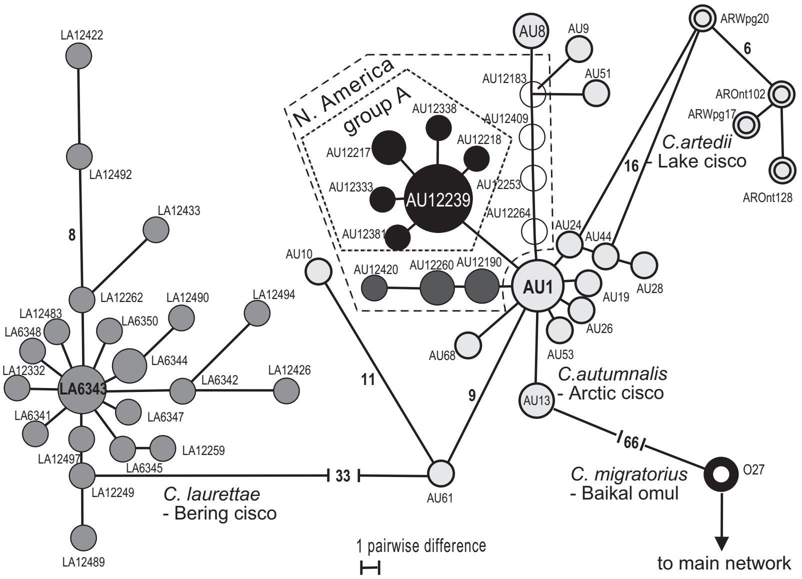

Fig. 2. MSN among haplotypes for Arctic cisco complex. Lengths of connecting lines are proportional to pairwise differences (PD) among haplotypes, numbers at lines are PD (except numbers within broken lines in cases of high PD). Circle shading varies for different species or haplotype groups within Arctic cisco. Letters in haplotype names correspond to species codes in Table 1. Circle sizes correspond to haplotype frequencies. This part of MSN connects to the rest of the network at Baikal omul haplotype O27.

Poisson distribution in fast-growing populations. Populations that are stable or decreasing in size are characterized by more structured haplotype networks and a multimodal distribution of pairwise differences. To some extent and with minor reservations, the first situation can be interpreted as a case of recently derived populations. The second is typical of more ancient, or basal gene pools. To investigate which of these patterns pertains to the ciscoes we constructed a Minimum Spanning Network (MSN) among haplotypes using Arlequin ver. 2.000 software (Schneider *et al.* 2000). We assessed the order of connections and compared the shape of individual species networks from which the following conclusions regarding phylogeographic relationships can be made.

Results

Altogether, 322 cut sites have been observed that allowed to discern 134 different haplotypes in 11 studied species. The constructed MSN among haplotypes shows that the two major cisco lineages, the Arctic cisco complex (*C. autumnalis, C. laurettae, C. artedi*) and the least cisco complex (*C. sardinella, C. peled*) are not closely related based on mtDNA (Figs. 2 and 3).

The Baikal omul (*C. migratorius*) occupies a position between the Arctic cisco complex and the rest of the coregonids in the haplotype network (Figs. 2 and 3). This species is closer than others to the Arctic cisco complex and can be considered as a possible ancestor of that clade.

Within the *C. autumnalis* complex (Fig. 2) the most basal haplotype directly connected to Baikal omul belongs to one of the Arctic cisco lineages, called the B-group by Bickham *et al.* (1989) based on its distinctive *Dde* I haplotype. Four Arctic cisco clades connect to the most frequent Eurasian haplotype AU1 (Fig. 2). One of these clades leads to Bering cisco through the divergent haplotype AU61. The distance from AU61 to the closest Bering cisco haplotype LA12249 is 33 pairwise differences. That is much higher than the maximal divergence within Arctic cisco including both Old and New World populations (18 pairwise differences). Bering cisco are easily identified by several

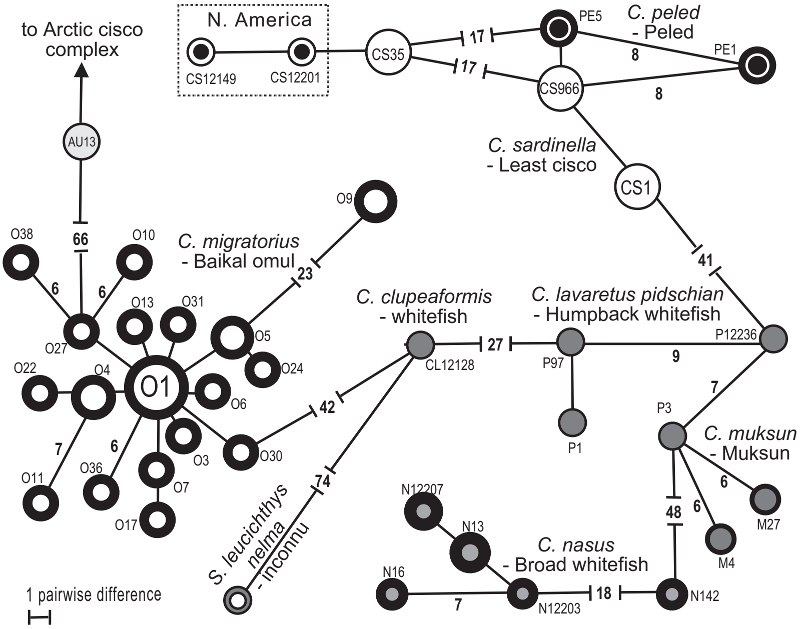

Fig. 3. MSN among haplotypes for the studied coregonids (except Arctic cisco complex). Circle shading is specific for different species or least cisco regions. For more legend *see* Fig. 2 caption. This part of MSN connects to Arctic cisco complex MSN shown on Fig. 2 at haplotype A13.

mtDNA restriction patterns (e.g., *ND1* digests with *Ase* I, *Bsa*J, *Bsti*N I, *Dpn* II, *Hinf* I, *Msp* I and *Taq* I). The connection of *C. laurettae* to the main *Coregonus* haplotype network through *C. autumnalis* (Fig. 2) may indicate that this species was derived from Arctic cisco.

Three other Arctic cisco lineages originate directly from the common Eurasian haplotype AU1. Two of these three clades are restricted to North America (A and BA which stands here for B-American). The A-group and its central haplotype AU12239 dominate all American populations by frequency. The basal haplotype of this lineage (AU12239) is separated from the basal Eurasian haplotype (AU1) by a distance of 6 pairwise differences. Lineage BI (B-Intercontinental) includes four basal North American haplotypes and three terminal Eurasian haplotypes. These Eurasian haplotypes appear to be derived from an American haplotype lineage and are all found in Lake Ledyanoye east to the Yana River delta in Eastern Siberia. This may indicate a reintroduction of Arctic cisco from North America to Siberia. All other haplotypes are found exclusively in Eurasia and are combined here into group B that seems to be the most basal of all Arctic cisco complex lineages. It is that lineage that connects directly to the Baikal omul haplotype network. A more detailed analysis of Arctic cisco mtDNA haplotype distribution throuout the range will be presented elsewhere.

Lake cisco, *C. artedi*, represented in our material by samples from Lake Ontario and Lake Winnipeg, is a member of the Arctic cisco complex. This observation indicates a relatively recent origin of lake cisco from Arctic cisco. The shortest connection of *C. artedi* to the rest of the network again, as in case of Bering cisco, is with Siberian Arctic cisco haplotypes belonging to the B-group.

Among least cisco, the haplotype most basal to the main network was CS1 which is found in the western part of the Yamal Peninsula in Western Siberia (Fig. 3). CS1 is connected to CS966 found exclusively in the Pechora River population (northeastern European Russia). Haplotype CS35 from the Kolyma River, the easternmost of our Eurasian localities, links the Eurasian and North American least cisco lineages. Differentiation between the Kolyma River haplotype CS35 and the closest American haplotype CS12201 (3 pairwise differences) was much lower than between CS35 and European least cisco haplotype CS966 (17). The peled (*C. peled*) haplotypes PE1 and PE5 also join the network at CS966. Thus, this species not only belongs to the least cisco complex but does not even connect to the most basal haplotype (CS1). The distances within peled and least cisco lineages are not only comparable to interspecific peled–least cisco values, but European and West Siberian *C. sardinella* seem to be more distant from East Siberian least cisco than from peled.

Discussion

Major cisco lineages

The observed high levels of divergence among major cisco clades in *ND1*, *ND2*, and *ND5/6* mtDNA fragments (Figs. 2 and 3) support earlier findings of other authors by means of various genetic markers (Bernatchez *et al.* 1991, Bodaly *et al.* 1991, Lockwood *et al.* 1993, Bodaly *et al.* 1994, Sajdak & Phillips 1997, Reist *et al.* 1998, Vuorinen *et al.* 1998, Sendek 2002). The accumulated extensive molecular data on the polyphyletic nature of the subgenus *Leucichthys* suggest the need for a re-evaluation of the morphological characters on which the distinctions of subgenera have been made and show the need for a taxonomic revision of *Coregonus*.

C. migratorius and *C. autumnalis*

Baikal omul possess high levels of haplotypic and nucleotide variability. For example, there are 23 pairwise differences between haplotype O9 and its closest related haplotype (Figs. 2 and 3). Omul is intermediate between the Arctic cisco and whitefishes and is the species to which the rest of the coregonid haplotype network joins. The high divergence of Baikal omul mtDNA shown in our study supports a previously suggested hypothesis (Politov *et al.* 2000, Politov *et al.* 2002) that Baikal omul cannot have descended from recent (Pleistocene or post-Pleistocene) Arctic cisco migrants from the north, and moreover, should not be treated as a subspecies of *C. autumnalis*. Rather, the omul of Lake Baikal, one of the most ancient lakes on Earth, is more likely the ancestor of the Arctic cisco complex. In our data, Baikal omul shares a number of restriction sites with whitefishes and is generally closer to them (minimum 42 pairwise differences and maximum 57 with *C. lavaretus pidschian* and 61 pairwise differences with *C. nasus*) than to any Arctic cisco haplotype (minimal distance is 66 pairwise differences). Previously, it was shown with isozyme data that the Baikal omul is closer to whitefishes than to Arctic cisco (Politov *et al.* 2000, Politov *et al.* 2002). It was also reported (Gąsowska 1960, 1964) that the shape and proportions of *supramaxillare* in Baikal omul resemble those of whitefishes but not Arctic cisco. Craniological data also suggest Baikal omul is distinct from Arctic cisco (Shaposhnikova 1968, 1970, Chereshnev 1994). These morphological data further support the hypothesis that Baikal omul is an ancient species close to the root of at least the Arctic cisco complex but also perhaps close to the origin of the whole genus *Coregonus*. Some authors (Pirozhnikov *et al.* 1975) supposed the Baikal Lake depression to be one of the centers of origin for coregonids. New molecular data suggest that this cisco form that is usually treated as endemic and represents an offshoot of coregonid evolution is, in fact, ancient and basal for *Coregonus*. Full species status of Baikal omul is also supported by our mtDNA data and we recommend retaining the species name *C. migratorius* (Georgi). Other mtDNA data (Sukhanova *et al.* 2002) suggest that Baikal omul is closer to Baikal whitefishes than to Arctic cisco. Considering the phylogeographic consequences of the placement of Baikal omul at the base of the Arctic cisco clade it is evident that Siberian Arctic cisco are likely ancestral to the Nearctic populations. This is supported by the observation (Fig. 2) that most Siberian haplotypes belonging to the B-group occupy the basal position of the haplotype network (the exception is a set of three haplotypes of the BI-group that seemed to be derived from American haplotypes). The A-group of haplotypes, comprising about 86.5% of all North American Arctic cisco populations, is a terminal, derived lineage because Eurasian populations are closer to the MSN root. Moreover, the distinctive *Dde* I A-type is unique to American Arctic ciscoes while the B-type pattern is typical for all other *Coregonus* species and is considered to be the ancestral condition. Low divergences among haplotypes and the existence of a single central haplotype (AU12239) suggests a recent bottleneck or founder effect with a subsequent rapid increase in population size.

C. laurettae and *C. artedi*

Based on mtDNA data, the two other Nearctic cisco forms — Bering cisco and lake cisco — also seem to be derived from Siberian *C. autumnalis*. This pattern of variation requires several routes and waves of cisco migrations from Siberia to American waters. Bering cisco spawn in the Yukon River but are found also in small numbers at coastal sites in the Beaufort Sea and in the Mackenzie River tributaries (McPhail 1966, McPhail & Lindsey 1970, Bickham *et al*. 1989, 1997, Morales *et al*. 1993). Two Bering cisco males were recently described in the Chegitun River on the Chukotka Peninsula (Chereshnev 1984) further extending the range of *C. laurettae* to the eastern (Eurasian) coast of the Bering Strait. The origin of this form can be attributed to an ancient Beringian land bridge (Lindsey & McPhail 1986). The present day Yukon River is the remnant of a Pleistocene basin putatively lying on the more southern migration routes of the Beringian land bridge. The melted glacial ice in the late Pleistocene formed a network of freshwater lakes that might have served as a conduit for cisco migration along this route (Chereshnev 1979, Lindsey & McPhail 1986). The high level of intraspecific divergence of Bering cisco mtDNA was likely caused by a relatively long period of isolation of this form on the southern side of Beringia separated from Arctic cisco to the north. The high level of genetic divergence between *C. autumnalis* and *C. laurettae* is evidence in support of the recognition of full species status for Bering Cisco. The observed 33 pairwise differences (Fig. 2) are much higher than between recognized full cisco species *C. autumnalis* and *C. artedi* (a minimum is 16 pairwise differences) (Fig. 2) or *C. sardinella* and *C. peled* (minimum 1 pairwise difference) (Fig. 3). The Bering cisco is presently considered a distinct species (Bean 1882, McPhail 1966) but some authors have suggested including this form into *C. autumnalis* as a subspecies (Reshetnikov 1980, Dillinger *et al*. 1992). The two species have only slight morphological differences basically restricted to different numbers of gill rakers on the lower gill branch: 18–25 in *C. laurettae* and 26–31 in *C. autumnalis* (McPhail 1966, McPhail & Lindsey 1970). Bering cisco also have fewer pyloric caeca and fewer scales on the lateral line but values of these characters overlap (McPhail 1966, McPhail & Lindsey 1970, Chereshnev & Skopets 1992). Unlike Arctic cisco, Bering cisco have small teeth on the premaxillary bone and on the anterior end of the lower jaw (McPhail 1966, Shaposhnikova 1968, McPhail & Lindsey 1970, Chereshnev 1984). It was also demonstrated (Chereshnev 1994) that the two species are clearly distinct in several craniological traits. Considering these findings, the hypothesis of conspecificity of these forms (Dymond 1943, Walters 1955, Reshetnikov 1980, Dillinger 1989) should be rejected in spite of the fact that introgression of Bering cisco genes into Mackenzie River Arctic ciscoes has been documented (Bickham *et al*. 1997).

C. artedi directly joins to the B-group of Palearctic *C. autumnalis*. From this it can be concluded that dispersal of this species into the Nearctic basins including the Laurentian Great Lakes might have occurred through a southern Beringian migration route. However, B-haplotypes are also present among Mackenzie River ciscoes, and we cannot exclude an additional northern route of dispersal.

C. sardinella

The least cisco complex seems also to have originated in the Palearctic. The basal haplotypes are from West Siberia and Europe where the highest diversity of *C. albula* and *C. sardinella* is observed. Multiple semianadromous and lake forms, including sympatric populations of small and large sized least cisco, are found throughout northern Europe, Western, and Central Siberia. In Eastern Siberia, least cisco are represented mainly by semianadromous riverine forms. In addition, the most discriminative allozyme locus in *Coregonus*, creatine kinase *CK-1,2**, carries alleles typical for whitefishes and other species (other than the Arctic cisco complex and Baikal omul) in the western least cisco populations, while "pure" Siberian least cisco have a high frequency of two species-specific alleles at *CK-1,2** coding for fast creatine kinase electromorphs (Perelygin 1988, 1989, Ermolenko 1989, Sendek 1998, 2002, Politov *et al*. 2002).

C. peled

Peled, an important subject of cold-water aquaculture was usually regarded to be close to the Arctic cisco complex (Reshetnikov 1980). However, isozyme data indicate that this species seems to be more closely related to vendace and least cisco (Bodaly *et al*. 1994, Vuorinen *et al*. 1998, Sendek 2002). A previous study on the relationships of peled and least cisco based on RFLP analysis of the *ND1* mtDNA fragment (Politov *et al*. 2000) is in agreement with the isozyme studies, as well as with molecular genetic data obtained by other authors (Bernatchez *et al*. 1991, Reist *et al*. 1998, Brzuzan & Ciesielski 2002). The present mtDNA analysis, based on three mtDNA fragments, fully supports the previous molecular studies and demonstrates an even closer relation between peled and the *C. sardinella* complex. The fact that peled is closer to West Siberian and European haplotypes of least cisco indicates that these regions could be the possible centers of origin for *C. peled*.

Conclusions

For both Arctic cisco and least cisco complexes, the most basal haplotypes are found in Siberia rather than in North America. In our opinion, this indicates a Eurasian center of origin for both major cisco clades. Most likely, this hypothetical center was located in Central and East Siberia which is an area of high species diversity (nine species) of *Coregonus*. Despite the fact that the Great Lakes of North America have more (10) species (Reshetnikov 1985), most of them are representatives of the *C. artedi* species flock and are likely of relatively recent origin. Siberian coregonid forms, especially ciscoes, represent ancient lineages having long evolutionary histories. Thus, mtDNA has proven to be a powerful tool in revealing phylogeographic patterns in Arctic cisco, Baikal omul, least cisco and peled and can be recommended for use in similar research in other widespread coregonid species.

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