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Reticulate evolution and phenotypic diversity in North American ciscoes, *Coregonus* ssp. (Teleostei: Salmonidae): implications for the conservation of an evolutionary legacy

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Abstract

Coregonine fishes are notorious taxonomic problems due to their extreme morphological and ecological variation. In North America, diversity is particularly baffling among ciscoes, and both morphological and phylogenetic analyses have resulted in major polytomy among the 8 taxa of the "*Coregonus artedi*" species complex. Ciscoes are also a devastated group, accounting for 10% of the fish species listed by the Committee on the Status of Endangered Wildlife in Canada. Here, we complete the genetic characterization of North American ciscoes with mitochondrial and microsatellites markers previously used to analyse populations of *C. artedi* in order to elucidate the evolutionary history and identify appropriate conservation units. Our results revealed a complex evolutionary history marked by postglacial reticulation events coupled with recent and independent evolution of similar phenotypes (taxa). Genetic variation reflects geography rather than taxonomy, and consequently, we recommend that a single taxon, *C. artedi (sensu lato)* be recognized. Local genetic differentiation is often coupled with ecophenotypic diversification, and gill raker polymorphisms, depth-related habitat preference and reproductive behaviour are considered as phenotypic traits with probable adaptive value contributing to the niche expansion of ciscoes. Ecomorphotypes of each particular locale thus represent a unique expression of a diverse genetic pool still undergoing divergence and sorting. Consequently, ciscoes from lakes with distinct ecomorphotypes are recognized as ESUs, as well as each of sympatric forms when they are genetically differentiated. We recommend that an ESU strategy focusing at a very local level be adopted for continental ciscoes as a valid alternative to protect significant evolutionary processes of divergence encountered in polytypic species of newly colonized habitats.

Introduction

Ciscoes are a devastated group of North American fishes (genus *Coregonus*) whose remaining diversity constitutes a fish fauna heritage of obvious and intuitive conservation value. The original center of diversity was located in the Laurentian Great Lakes, but these fishes now account for 10% of the fish species listed by the Committee on the Status of Endangered Wildlife in Canada (seven of 72 species, http://www.cosewic.gc.ca). Some endemic forms have gone extinct (longjaw cisco – *C. alpenae*, deepwater cisco – *C. johannae*), while others are severely threatened (shortjaw cisco – *C. zenithicus*, shortnose cisco – *C. reighardi*), or considered vulnerable (*C. kiyi*). While ciscoes species from northwestern North America are easily identified, the extreme morphological and ecological variation among continental ciscoes has never allowed α-taxonomists to identify diagnostic characters among the eight species that are currently recognized (*C. alpenae*, *C. artedi*, *C. johannae*, *C. hoyi*, *C. kiyi*, *C. nigripinnis*, *C. reighardi* and *C. zenithicus*, Scott and Crossman 1973). Identification keys are not available for these species, and morphological phenetic analyses have consistently resulted in major polytomies (Smith

& Todd 1992) that phylogenetic studies based on molecular markers have not resolved (Bodaly et al. 1991; Bernatchez et al. 1991; Lockwood et al. 1993; Sajdak and Phillips 1997; Reed et al. 1998). The apparent lack of congruence between adaptive phenotypic and genetic divergence poses a serious challenge for the identification of appropriate conservation units in this diverse and threatened group of fish.

The origin of cisco phenotypic diversity is also contentious. Several authors have suggested or implicitly accepted that they recently radiated via a process of ecological divergence driven by trophic and habitat resource specialization (Smith and Todd 1984; Schluter and McPhail 1993; Reed et al. 1998). However, patterns of ecophenotypic and genetic diversity within the last remaining cisco 'species flock' (Lake Nipigon) indicated that historical events involving postglacial contacts between closely related lineages may also play a role (Turgeon et al. 1999). A recent mtDNA phylogeographic study of the presumed ancestral lineage and most widely distributed cisco taxon, *C. artedi*, indicated the existence of two mitochondrial lineages exhibiting contrasted geographical distribution but extensive intermixing over most of the current species range (Turgeon and Bernatchez 2001a). Analysis of microsatellite polymorphisms confirmed the existence of these genetically distinct races and their nearly complete intermixing by neutral secondary contacts via extensive dispersal in the proglacial lakes that formed during the retreat of the last Wisconsinian ice sheet 12-8 Kya BP (Turgeon and Bernatchez 2001b).

In all, it is obvious that the extreme phenotypic variability of North American ciscoes is difficult to appraise with the usual concepts of divergent evolution and reproductive isolation and that ciscoes can not easily fulfill species or ESU conservation criteria: there are no available characters to diagnose separate units, and no agreement regarding the reference group (species) relative to which particular ecomorphotypes must be evaluated. Yet, many potentially introgressed lineages possess sufficient genetic differentiation to be irreplaceable in nature (Smith et al. 1995) and taxonomic confusion conceals diversity at the very level where diversity should be emphasized (Lindsey 1988).

In this study, we complete the genetic characterization of North American ciscoes with the mitochondrial and nuclear (microsatellites) markers used to analyse populations of *C. artedi* (Turgeon et al. 1999; Turgeon and Bernatchez 2001a,b), and pay a special attention to the genetic relationships of Great Lakes endemics and eco/morphotypes with geographically and/or taxonomically related populations. In doing so, we aim to (1) gain insight on the evolutionary mechanisms having promoted phenotypic diversity, (2) assess the validity of current taxonomy, (3) evaluate the evolutionary significance of genetic and ecological characteristic for the identification and listing of ESUs. We confirm that the taxonomy of Northwest ciscoes (least cisco (*C. sardinella)*, Bering cisco (*C. laurettae)*, and Arctic cisco (*C. autumnalis*)) reflects divergent evolutionary lineages, but that biological or phylogenetic species status other than *C. artedi* is unwarranted for all other currently recognized taxa. We provide new or review evidence of historical, behavioral, ecological and morphological differentiation and argue that these observations, coupled with the predominance of geography in patterns of genetic differentiation at neutral genetic markers, is reflecting a recent, incipient process of parallel diversification. We further recommend that an ESU strategy focusing at a very local level be adopted in order to optimize conservation practices for continental ciscoes.

Methods

Our sampling strategy covered most of the range of North American ciscoes, and encompasses all but two of the extant taxa described by Scott and Crossman (1973), i.e. *C. reighardi* and *C. kiyi*. It does, however, focused on cisco taxa associated with the *C. artedi* species complex (McPhail and Lindsey 1970), including *C. zenithicus* and the Great Lakes endemics (Scott and Crossman 1973). Forty-five samples (tissue or heads) were obtained from 34 waterbodies (Table 1, Figure 1). The 27 sites sampled for *C. artedi* in Turgeon and Bernatchez (2001a) are included along with three sites (4 taxa) along the Northwest Arctic coast, one northwestern river site with *C. autumnalis* (Peel R.), one continental site with *C. zenithicus* in allopatry (George L.), and Great Lakes where *C. artedi* does not occur or is extremely rare (L. Michigan, L. Huron) (Table 1). In Lake Superior and Nipigon, as well as in four other interior lakes (Great Slave L., L. Barrow, Cormorant L., La Grande Reservoir), sympatric taxa or reported morphotypes were sampled, usually in different sites. Taxonomic assignments and morphotypes description were recognized on various basis: in Lake Nipigon, we assessed morphological variability ourselves (Turgeon

Table 1. Collection sites, reported taxon and phenotypes of ciscoes

Code	Description		Location		Lat./Long.
Site-sample	Taxon[a]	Phenotype[b]	Waterbody	Drainage	(°N.min)/(°W.min)
1 ar	CoAr	—	L. Champlain, VT	Atlantic	45.03/73.09
2 ar	CoAr	—	Reservoir Poisson Blanc, QC	(Laurentian)	45.55/75.45
3 spring	CoAr	spring spawner	Lac des Écorces, QC		46.32/75.25
4 ze	CoZe	LGR	White Partridge L., ON		45.50/78.06
5 ar	CoAr	—	Kipawa Reservoir, QC		46.50/79.05
6 ar	CoAr	—	L. Opasatica, QC		48.05/79.15
7 ar	CoAr	—	L. Ontario, ON	Atlantic	43.45/78.00
8 ho1	CoHo	—	L. Huron, Georgian Bay, ON	(Great Lakes)	44.41/80.37
8 ho2	CoHo	—	L. Huron (Hammond Bay), MI		45.00/83.50
9 ho	CoHo	—	L. Michigan (Beaver Is), MI		45.40/85.30
10 ar	CoAr	—	L. Superior (Thunder Bay), ON		48.00/88.45
10 ho	CoHo	—	L. Superior (Black Bay), ON		48.10/88.35
10 ze	CoZe	—	L. Superior (Bayfield), WS		48.00/92.00
11 ar	CoAr	—	L. Nipigon, ON		49.45/88.30
11 ho	CoHo	—			
11 ni	CoNi	—			
11 ze	CoZe	LGR			
12 ar	CoAr	—	Three L., QC	Ungava	58.00/68.00
13 ar	CoAr	anadromous	Puvungnituk River, QC	Hudson	60.02/77.10
14 ar	CoAr	—	La Grande Reservoir, QC	(east)	55.00/78.30
14 large	CoAr	large			
15 ar	CoAr	anadromous	Nelson River, MB		56.50/91.00
16 ar	CoAr	anadromous	Rupert River, QC		51.00/78.50
17 np	CoNip	HGR	L. Saganaga, MN	Hudson	48.15/90.52
18 ar	CoAr	—	L. Seul, ON	(west)	50.30/92.02
19 ar	CoAr	—	L. of the Woods, ON		49.18/94.44
20 ar	CoAr	—	L. Winnipeg, MB		50.40/97.15
21 ze	CoZe	LGR	George L., MB		50.15/95.30
22 ar	CoAr	—	L. Winnipegosis, MB		52.45/100.15
23 ar	CoAr	—	L. Cormorant, MB		54.30/100.35
23 dwarf	CoAr	dwarf			
24 ar	CoAr	large	Pasqua L., SK		51.47/103.58
25 ar	CoAr	—	L. La Ronge, SK		55.14/104.57
26 ar	CoAr	—	L. La Biche, AB	Mackenzie	54.45/112.05
27 ar	CoAr	small-HGR	Barrow L., AB		59.15/111.13
27 ze	CoZe	large-LGR			
28 ar	CoAr	dwarf	Peerless L., AB		56.40/114.30
29 ar	CoAr	large	Utikuma L., AB		55.50/115.30
30 ar	CoAr	large	Great Slave L., NWT		61.23/115.38
30 sa	CoSa[c]	small			
31 au	CoAu	—	Peel River, NWT	Arctic	63.13/135.00
32 sa	CoSa	—	Shingle Point, NWT		68.59/137.23
33 la	CoLa	—	Colville River Delta, AK		70.26/150.25
33 au	CoAu	—			70.26/150.25
34 sa	CoSa	—	Avak River (L.), AK		71.13/156.36

[a]Reported taxon: *C. artedi* (CoAr), *C. hoyi* (CoHo), *C. zenithicus* (CoZe), *C. nigripinnis* (CoNi), *C. nipigon* (CoNip), *C. sardinella* (CoSa), *C. laurettae* (CoLa), *C. autumnalis* (CoAu).
[b]: see details in Table 2.
[c]: Reported as *C. artedi*, but with mtDNA and microsatellites alleles typical of *C. sardinella*, see Results.

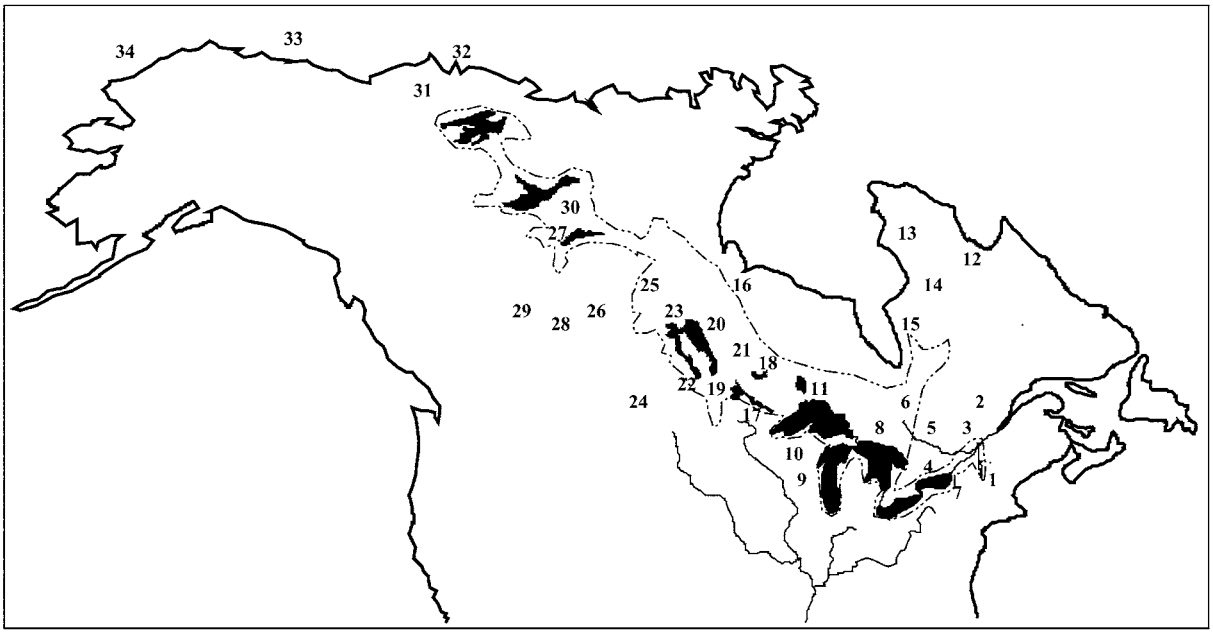

Figure 1. Sampling locations with numeral codes as per Table 1. The broken line indicates the maximal extent of proglacial lakes at the end of the Wisconsinian glaciation.

et al. 1999) and our taxonomic assignments have been confirmed independently (T. Todd, NBS Ann Arbor MI, pers. comm.). In the Great Lakes, taxa were identified by government researchers and managers providing the samples; academic personnel also identified and provided samples for interior lakes. Eco-morphological polymorphisms are reported and detailed in Table 2. On the basis of our results (see below), we will hereafter refer to *C. sardinella*, *C. laurettae*, and *C. autumnalis* as the Northwestern ciscoes, and to *C. artedi*, *C. hoyi*, *C. nigripinnis*, *C. nipigon*, *C. zenithicus*, and ecomorphotypes of *C. artedi* as members of the *C. artedi* complex (and later, as *C. artedi sensu lato*, see discussion.)

We assessed genetic variability using two types of markers. Mitochondrial polymorphisms were detected by D-loop Single-Strand-Conformation-Polymorphisms (SSCP) and sequence analysis, and nuclear polymorphisms were assayed at seven microsatellite loci. The methodological details are given in Turgeon and Bernatchez (2001a) and Turgeon et al. (1999), respectively. The polymorphisms detected in non-*artedi* populations are described in relation to those encountered in these previous studies. For mitochondrial data, the phylogenetic relationships among all cisco sequences are established by building a Neighbor-Joining (NJ) phenogram with Kimura's two-parameter model using Mega 1.01 (Kumar et al. 1993). A single individual of *C. autumnalis* (MacKenzie delta, NWT) and *C. autumnalis pollan* (Loch Neagh, Ireland) used in Bernatchez et al. (1991) were also analysed. The geographic distribution of new haplotypes are examined in relation to that of the two major *C. artedi* clades identified as Mississippian (A) and Atlantic (B) refugial races (Turgeon and Bernatchez 2001a).

For microsatellites, analyses of intrapopulation genetic variation (conformance to HW proportions, heterozygosity levels, linkage disequilibrium) were performed with Genepop 3.1 (Raymond and Rousset 1995). Interpopulation genetic variation was first appraised by carrying a cluster analysis using Cavalli-Sforza and Edwards (1967) chord distance (D_{ce}) and the Neighbor-Joining tree-building method in Phylip 3.5 (Felseinstein 1993). As expected from mtDNA results, the three Northwestern cisco taxa were clearly discriminated, but the phylogenetic signal was extremely weak in the rest of the tree (see results). We thus attempted to partition genetic diversity within the *C. artedi* complex on the basis of geography and taxonomic status. These analyses were performed over three different geographic scales. First, for sympatric pairs of interior lakes (Sites 14, 23 and 27), we assessed the significance of allelic frequency differ-

Table 2. Phenotypic polymorphisms reported in samples of the *C. artedi* complex

Sample		Phenotype[a]				Data source[b]
Site No. (Table 1)	Reported Taxon	Type	N	Length (mm) mean (sd) (range)	Gill raker # mean (sd) (range)	
3	*C. artedi*	Spring spawner	30	212 (14) (182–253)	42.7 (2.1) (38–48)	1, 2
4	*C. zenithicus*	LGR[c]	49	—	35.1 (1.0) (33–37)	1
11	*C. artedi*	planktivorous	45	219 (26) (174–302)	46.6 (2.6) (40–54)	3
	C. hoyi	deep form	50	231 (17) (182–284)	45.3 (1.9) (42–50)	
	C. nigripinnis	HGR[c]	50	290 (38) (228–407)	51.0 (2.0) (47–55)	
	C. zenithicus	LGR	57	321 (34) (239–407)	38.3 (4.1) (31–44)	
14	*C. artedi*	normal	25	140 (15) (122–189)	—	4
		large	46	399 (42) (301–380)	—	
17	*C. nipigon*	HGR	123	—	55.9 (3.4) (49–70)	5
21	*C. zenithicus*	LGR		—	33.8 (1.9) (30–37)	6
23	*C. artedi*	normal	29	72.2 (7.6)[d] (58–87)	52.8 (2.1) (49–58)	1
		dwarf	22	52.2 (5.3)[d] (43–60)	48.4 (3.1) (43–53, 57)	
27	*C. artedi*	small, HGR	74	—	47.8 (2.2) (43–52)	6
	C. zenithicus	large, LGR	19	—	40.5 (1.5) (38–43)	
28	*C. artedi*	dwarf	30	274 (58.9) (195–427)	—	7
29	*C. artedi*	large	30	419 (23) (369–466)	—	7
30	*C. artedi*	large	1 9	58.6 (6.0)[d] (60–80)	55 (49–59)	1
	C. sardinella[d]	small	39	48.7 (4.3)[d] (38–58)	43 (39–46)	

[a]Double lies indicates significant differences between sympatric forms ($p < 0.01$).
[b]Data source: 1: this study; 2: Hénault and Fortin (1989); 3: Turgeon et al. (1999); 4: R. Verdon, Hydro-Quebec; 5: D.A. Etnier, U. Tennessee; 6: Todd and Steinhilber, in press; 7: D. Brown, Alb. Envoronmental Protection.
[c]HGR/LGR: High/Low total number of Gill Raker.
[d]Head length (tip of lower jaw to posterior end of operculum).
[e]Identification revised on the basis of mtDNA and microsatellite results of this study, see Results and Figures 2 and 4.

ences (Fisher exact tests), and then evaluated if the extent of differentiation (as estimated by θ) was significant using 2000 permutations in Arlequin 1.1 (Schneider et al. 1997). Secondly, for the Great Lakes ciscoes, we performed an analysis of molecular variance (AMOVA) with Arlequin 1.1, and successively used taxa and lake as top grouping variables. In order to use the same set of samples for both hierarchical analyses, the unique sample of *C. nigripinnis* (Lake Nipigon) was not used. Thirdly, we evaluated the genetic relationships among samples of *C. artedi* and putative *C. zenithicus* over the entire range of distribution using similar hierarchical schemes, with drainage (Mackenzie, Hudson, Great Lakes, and Laurentian) and taxa as primary grouping variables. In order to balance the analytical design as much as possible, we used, within each drainage, each sample of *C. zenithicus* and its sympatric *C. artedi* sample (when available), as well as one or two samples of the geographically closest allopatric population of *C. artedi*. (Mackenzie: 30-ar, 30-ze, 34-ar; Hudson: 21-ze, 20-ar, 22-ar; Great Lakes: 11-ar, 11-ze, 10-ar, 10-ze, 7-ar; Laurentian: 4-ze, 2-ar, 5-ar, see Table 1).

The geographic origin of samples of the *C. artedi* complex proved to be more important than taxonomy in explaining their genetic affinities (see results). Consequently, we positioned samples with distinct taxonomic status or ecomorphological characters (i.e., different than 'pure' *C. artedi*) along the gradient of clinal variation documented in *C. artedi* populations located in/near the area formerly covered by proglacial lakes (Figure 1). This was achieved by first determining the proportion of alleles typical of the eastern race at four clinal loci in each individual, and then by evaluating the mean hybrid index of each sample in relation to its location along the proglacial lake axis defined as the distance to Lake Champlain (detailed in Turgeon and Bernatchez 2001b).

Results

As reported in previous studies, phenotypic variation among ciscoes of the *C. artedi* complex was related to size, gillraker number, diet, and reproductive season. In all cases of sympatry, at least one of these characters was significantly different between (among) morphotypes (Table 2), and the polymorphism reported in allopatry were also biologically meaningful: ciscoes of Lac des Écorces are the only spring spawner known out of the Great Lakes (Hénault and Fortin 1989, 1991), the Lake Saganaga population identified as *C. nipigon* is characterized by a remarkably high gillraker number (mean of 56), and the mean adult length of 'large' cisco populations was above 400 mm. With very few exceptions, the small and large individuals from Great Slave Lake were characterized by non-overlapping number of gillrakers, and were clearly characterized by mitochondrial haplotypes and microsatellite alleles typical of *C. sardinella* and *C. artedi*, respectively. These were reclassified accordingly for all further analyses.

The relationships among sequenced mtDNA haplotypes are depicted in Figure 2, where five major cisco lineages are identified. As expected, three distinct lineages coincided with the taxonomic designations of Northwestern ciscoes. The two other lineages corresponded to clades that have been equated with Mississippian (Clade A) and Atlantic (Clade B) refugial races of *C. artedi* (Turgeon and Bernatchez 2001a). Both lineages included haplotypes documented in *C. hoyi* and/or *C. zenithicus*, and these taxa contributed only two new sequences to those already documented in *C. artedi* (sequences 18.K and 19.0, Figure 3). The scattering of these taxa across lineages indicates that they are not characterised by distinct mtDNA lineages (Figure 2). The geographical distribution of Clade A and B haplotypes was invariable among taxa but significantly heterogeneous among drainages. For taxa documented in more that one site (*C. artedi*, *C. hoyi*, *C. zenithicus*), the frequency of individuals belonging to Clade B was nearly equal across taxa (30%). By contrast, X^2 tests with Monte Carlo simulations (REAP, McElroy et al. 1992) indicated that the frequency of occurrence of Clade B was significantly different among drainages in *C. artedi* and *C. zenithicus*. ($p < 0.005$). In *C. zenithicus*, this heterogeneity was only due to the absence of Clade B in the MacKenzie drainage, and Clade B was equally represented in the other drainages where this taxon was present ($p = 0.06$).

Microsatellite polymorphisms paralleled mtDNA results in clearly discriminating the Northwestern ciscoes species and they confirmed the lack of correspondence between taxonomy and genetics within the *C. artedi* complex. The allelic arrays of Northwestern species were clearly distinct, while those of *C. hoyi*, *C. nigripinnis*, *C. nipigon* and *C. zenithicus* were encompassed within that of 'pure' *C. artedi* populations. Indeed, the D_{ce} phenogram indicated that the Northwestern species form distinct, well-supported groups (Figure 4). By contrast, the other taxa were

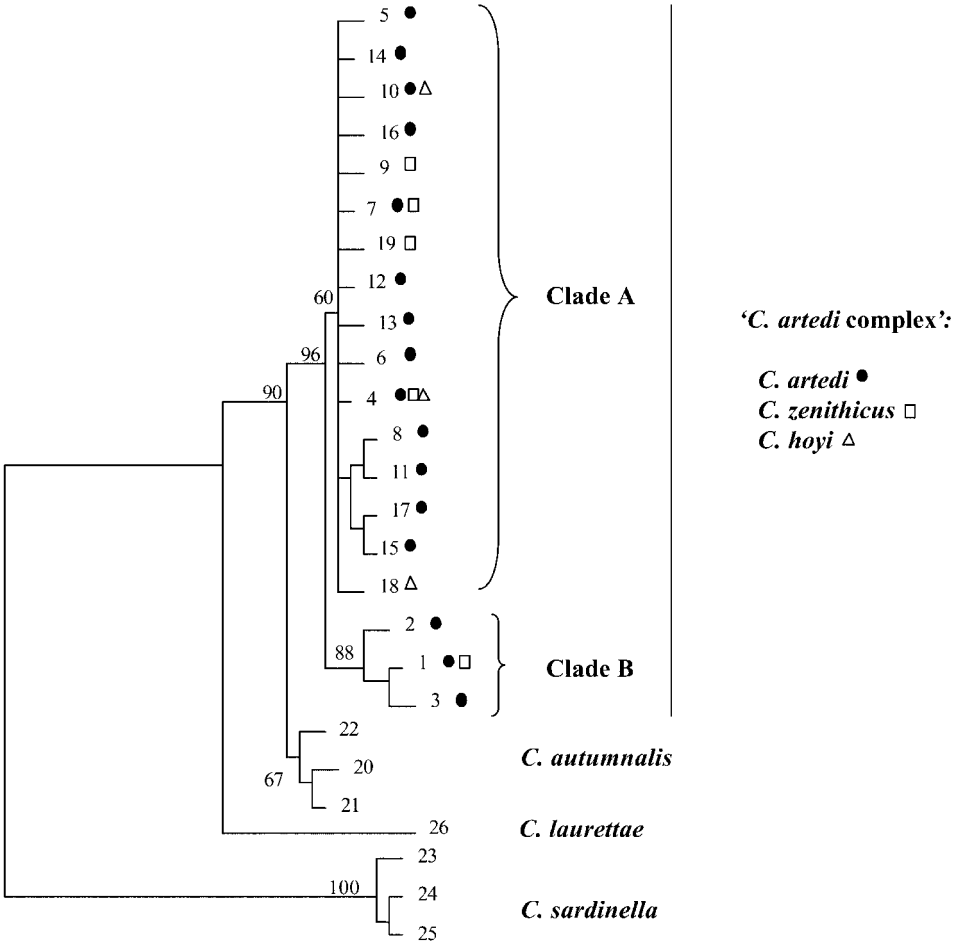

Figure 2. Neighbour-joining phenogram of 26 composite mitochondrial sequences (codes as per Figure 2) using Kimura two-parameter model. Percent bootstrap values > 50 are given for major nodes.

scattered over the rest of the tree, where most nodes were poorly supported and separated by very short branches. It is worth noting, however, that several terminal subgroups were well supported, and that they all included sympatric taxa/forms (Sites 11, 14, 23 and 27) or samples from nearby locations (sites 2 and 3, 15 and 16, 19 and 21). Despite the low bootstrap values, there was a clear geographic trend in clustering order: samples from the Laurentian Great Lakes and most of eastern Hudson Bay drainages clustered together and formed an 'eastern group' that was separated from a 'western group' encompassing all samples from the western Hudson Bay and Mackenzie drainages (Figure 4). The only exceptions to this pattern were Lake Nipigon samples (Great Lakes drainage) and one sample from eastern Hudson Bay (Rupert River), which clustered with the western group.

The results of genetic differentiation tests between sympatric taxa/morphotypes occurringin three interior lakes are reported in Table 3. In Barrow Lake and La Grande Reservoir, the existence of two different gene pools was corroborated by the significant heterozygote deficit only when samples were pooled (Walhund effect). In these two lakes, allelic frequencies were significantly different between sympatric forms, and the extent of this differentiation (θ) was also significantly different from zero. In Lake Cormorant, however, there was no significant difference in allelic composition between dwarf and normal individuals. Moreover, heterozygote deficits in each morph (as well as for pooled samples) were strong indications that our samples were not from a single panmictic population and that the classification of these individuals as dwarf and normal was genetically inappro-

Sequence Code	Taxa	Dloop																																
		29	46	51	61	69	75	79	81	84	86	87	91	93	118	125	142	143	145	147	167	190	194	206	212	232	271	272	274	275	276	285	301	311
1. B	CoAr/Ze	A	G	G	T	T	C	C	T	A	T	G	G	A	G	A	T	T	T	A	C	A	T	C	T	T	C	T	G	A	T	A	A	C
2. B	CoAr	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.
3. B	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4. A	CoAr/Ze/Ho	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
5. A	CoAr	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
6. A	CoAr	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
7. A	CoAr/Ze	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
8. A	CoAr	G	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
9. A	CoZe	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	C	.	.	.	.	.	.	.	.	.	.	.
10. C	CoAr/Ho	.	.	.	.	.	.	.	.			.	.	.	A	G	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
11. D	CoAr	G	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	C	.	.	.	.	.	.
12. H	CoAr	.	.	.	.	.	.	.	.			.	.	G	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
13. I	CoAr	.	.	.	.	.	.	.	C			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
14. L	CoAr	.	.	.	.	.	.	.	.			C	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
15. M	CoAr	.	.	.	.	.	.	.	.		C	.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
16. N	CoAr	.	.	.	.	.	.	.	.			.	A	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
17. T	CoAr	G	.	.	.	.	.	.	.		C	.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
18. K	CoHo	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	G	.	.
19. O	CoZe	.	.	.	.	.	.	.	.	G		.	.	.	.	.	.	.	.		A	.	.	.	.	.	.	.	.	.	.	.	.	.
20. B	CoAu	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.
21. C	CoAu	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.
22. D	CoAu *pollan*	.	.	.	.	.	.	.	.			.	.	.	.	.	.	.	.		.	.	.	T	.	.	.	.	.	.	.	.	.	.
23. E	CoSa	.	A	.	.	C	T	.	.			.	.	.	.	.	C	.	.		.	G	.	.	C	.	A	C	A	G	G	.	G	A
24. F	CoSa	.	A	.	.	C	T	.	.			.	.	.	.	.	C	.	.		A	G	.	.	C	.	A	C	A	G	G	G	G	.
25. G	CoSa	.	A	.	.	C	T	.	.			.	.	.	.	.	C	.	.	C	A	G	.	.	C	.	A	C	A	G	G	.	G	A
26. J	CoLa	.	.	C	C	C	.	T	.			.	.	.	.	.	.	A	C		.	.	.	.	.	del	.	.	.	.	.	.	.	.

[a] : Sequence code: number.SSCP pattern (Nos 1-17 as in Turgeon & Bernatchez 2001a)

[b] : Taxa where sequence was observed (Code as in Table 1)

Taxa	ND1																													
	7	20	32	39	56	68	71	74	80	83	86	89	95	122	128	149	173	188	191	197	215	251	284	308	317	326	332	335	338	344
CoAr/Ze	A	C	C	G	A	T	C	T	C	G	A	T	C	C	T	C	T	T	C	T	G	T	C	A	A	G	T	G	A	C
CoAr	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr/Ze/Ho	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr/Ze	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoAr	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CoZe	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
CoAr/Ho	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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CoHo	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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CoAu	.	T	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
CoAu	.	T	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
CoAu *pollan*	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
CoSa	G	T	T	.	G	.	.	C	T	.	G	.	.	.	C	T	C	C	T	C	T	C	T	G	G	A	C	.	.	G
CoSa	G	T	T	.	G	.	.	C	T	.	G	.	.	.	C	T	C	C	T	C	T	C	T	G	G	A	C	.	G	G
CoSa	G	T	T	.	G	.	.	C	T	.	G	.	.	.	C	T	C	C	T	C	T	C	T	G	G	A	C	.	G	G
CoLa	.	T	.	A	.	C	.	.	T	.	.	C	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.

Figure 3. Variable nucleotides for the D-loop, ND1, and ATPase 6 mitochondrial segments (375, 354, and 489 nucleotides, respectively) relative to the reference sequence (1.B.WPT-1) deposited in GenBank (Accession Nos: AF246932-34, respectively). Numbers refer to nucleotide positions within each segment.

Sequence Code	Taxa							1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4
			1	2	3	8	9	0	1	1	1	5	7	2	2	4	5	6	6	9	9	1	1	2	3	4	4	6	7	8	1	2	3	4	5	5	5	6	7	8	8
		9	7	9	8	6	1	7	0	2	9	4	6	6	9	4	3	5	8	1	4	5	8	4	3	2	5	2	6	0	4	0	5	9	0	3	8	5	9	0	6
1. B	CoAr/Ze	T	C	G	G	G	G	C	T	T	C	G	T	A	A	G	C	G	T	C	C	T	C	G	C	T	T	T	C	T	C	T	T	T	T	T	C	C	G	G	G
2. B	CoAr	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
3. B	CoAr	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
4. A	CoAr/Ze/Ho	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
5. A	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	T	.	.	.	.
6. A	CoAr	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
7. A	CoAr/Ze	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
8. A	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
9. A	CoZe	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
10. C	CoAr/Ho	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
11. D	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
12. H	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
13. I	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
14. L	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
15. M	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
16. N	CoAr	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
17. T	CoAr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
18. K	CoHo	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
19. O	CoZe	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
20. B	CoAu	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	C	.	.
21. C	CoAu	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
22. D	CoAu *pollan*	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.
23. E	CoSa	.	T	.	.	A	T	.	.	.	T	.	C	G	G	A	.	A	G	.	T	.	T	A	T	.	C	C	T	G	T	C	.	C	C	C	.	T	.	.	T
24. F	CoSa	.	T	C	.	A	T	.	.	.	T	.	C	G	G	A	.	A	G	.	T	.	T	A	T	.	C	C	T	G	T	C	.	.	C	C	.	T	.	A	T
25. G	CoSa	.	T	.	.	A	T	.	.	.	T	.	C	G	G	A	.	A	G	.	T	.	T	A	T	.	C	C	T	G	T	C	.	.	C	C	.	T	.	A	T
26. J	CoLa	.	.	.	.	.	.	T	C	.	.	.	.	G	.	.	T	.	C	.	.	.	T	.	.	C	.	.	.	G	T	.	C	.	C	.	.	.	.	A	.

ATPase 6

Figure 3. Continued

priate. Although this result remains unexplained at the moment, it has no consequences on the rest of our analyses and on our conclusions.

Genetic variation among Great Lakes cisco taxa was structured by lake rather than by taxonomic designation (Table 4). Genetic variance among lakes was significant when lakes were used as a top or nested grouping criterion. This indicates that genetic differentiation was more pronounced between samples of a given taxon from different lakes than between sympatric taxa from a given lake. Similarly, the partitioning of genetic diversity documented in the two taxa (*C. artedi* and *C. zenithicus*) present in several drainages (Arctic, Hudson West, Great Lakes, and Laurentian) indicated that geography better reflected the structuring of genetic variation than current taxonomy (Table 5). *C. zenithicus* was genetically more similar to sympatric and/or nearby *C. artedi* than to *C. zenithicus* from other drainages.

The allelic composition of all samples of the *C. artedi* complex located in/near former proglacial areas varied clinally, with the proportion of alleles previously identified as those of an eastern refugial race (Turgeon and Bernatchez 2001b) gradually diminishing from Lake Champlain to Great Slave Lake (R = –0.88, $p < 0.0001$, Figure 5). This clinal pattern held for 'pure' *C. artedi* sample (R = –0.93, $p < 0.0001$), as well as for all samples identified as belonging to other taxon or ecomorphotype (R = –0.70, $p < 0.0001$).

Discussion

Evolutionary history

Mitochondrial and microsatellites confirmed the distinct evolutionary origin of *C. sardinella* and *C. laurettae*, as well as the sister species status of *C. autumnalis* with the *C. artedi* complex. Similar results have already been obtained with allozymes (Bodaly et al. 1991) and mitochondrial DNA restriction analyses (Bernatchez et al. 1991). By contrast, taxonomy was not commensurate with evolutionary history within the *C. artedi* complex. All analyses clearly demonstrated that genetic variation reflects geography rather than taxonomy, and there were no indications that any of the putative taxa formed distinct evolutionary lineages. Indeed, microsatellite loci indicated that the evolution of phenotypes characterizing these taxa postdates the contact and admixture of two cisco refugial races following the Wisconsinian

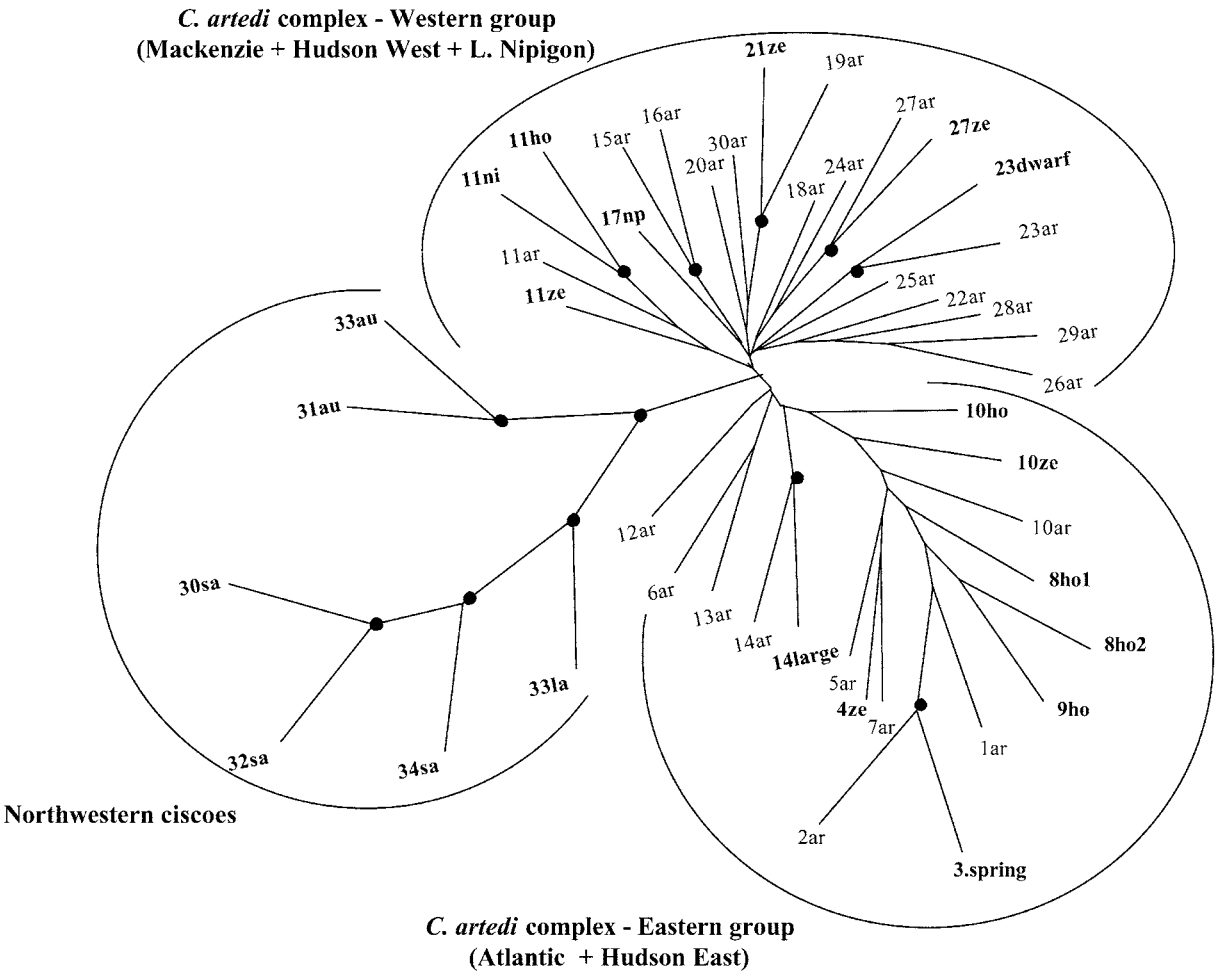

Figure 4. Neighbour-joining D_{ce} phenogram among 45 samples of North American cisco. Filled circles indicate percent bootstrap values > 50. Bold characters indicate taxa/morph other than *C. artedi*, while samples from the Great Lakes are underlined. Sample/taxon codes are as in Table 1.

Table 3. Genetic differentiation between sympatric cisco taxa/morphotypes in three interior lakes

Lake (site No.)	Taxa/Morph	Heterozygote deficit		Allele frequency difference	θ	p value[a]
		by morph	pooled	(p value*)		
La Grande Res. (14)	large	0.13	0.008**	0.001**	0.03	<0.0001**
(*C. artedi*)	normal	0.26				
Lake Cormorant (23)	dwarf	0.01*	<0.0001**	0.59	0	0.64
(*C. artedi*)	normal	0.001**				
Lake Barrow (27)	CoAr	0.10	0.01*	<0.0001**	0.08	0.0001**
	CoZe	0.79				

[a]p vlaues are corrected for multilocus tests by the Fisher's method; *α = 0.05, **α = 0.01.

Table 4. Hierarchical analysis of genetic variance among Great Lakes ciscoes. (A) Taxonomy as top level, (B) Geography (lake) as top level

Source of variation	d.f.	% variance	θ	p value[a]
(A) Taxonomy				
Among taxa	2	0	0	0.543 ns
Among lakes within taxon	7	0.50	0.045	<0.0001**
Within sample	642	95.5	0.043	<0.0001**
(B) Geography (lake)				
Among lakes	4	4.13	0.041	0.014*
Among taxa within lake	5	1.04	0.011	<0.0001**
Within sample	741	94.83	0.052	<0.0001**

[a]p vlaues are corrected for multilocus tests by the Fisher's method; *$\alpha = 0.05$, **$\alpha = 0.01$.

Table 5. Hierarchical analysis of genetic variance between *C. artedi* and *C. zenithicus*. (A) Taxonomy as top level, (B) Geography (drainage) as top level.

Source of variation	d.f.	% variance	θ	p value[a]
(A) Taxonomy				
Between taxa	1	0	0	0.809 ns
Among drainages within taxon	12	11.66	0.122	<0.0001**
Within sample	776	88.34	0.109	<0.0001**
(B) Geography (lake)				
Among drainages	3	6.58	0.066	0.0005**
Between taxa within drainage	10	6.32	0.068	<0.0001**
Within sample	776	87.09	0.129	<0.0001**

[a]p vlaues are corrected for multilocus tests by the Fisher's method; **$\alpha = 0.01$.

glacial retreat. Recent parallel and local divergence is the most plausible scenario for the origin of significant morphological and ecological polymorphisms observed within that group. Moreover, the occurrence of a common phenotypic variant, namely the low gillraker form (*C. zenithicus*) in several widespread locales, as well as its close genetic relationship with sympatric or nearby high gillraker forms (*C. artedi*) strongly indicates that it has evolved repeatedly. Similar conclusions can be drawn for the deep-bodied form occurring in the deep waters of Laurentian Great Lakes (*C. hoyi*). Parallel evolution has been reported for several other fish species of postglacial lakes (reviewed in Schluter 1996), including other coregonines (Pigeon et al. 1997; Douglas et al. 1999), and this study adds to the evidence that recent ecological divergence has been an important evolutionary process in these fishes.

Taxonomy and conservation

Our results provide evolutionary insights that also have important implications for the taxonomy and conservation of North American ciscoes. The most important finding of this study is that reticulate evolution has marked the postglacial history of continental ciscoes over most of their range. The recent episode of 'genetic convergence' (secondary contacts of *C. artedi* refugial races) blurs the genetic signal of recent divergence processes that have repeatedly and independently given rise to similar ecomorphotypes across the entire range. In regard to taxonomy, these results imply that morphological and genetic markers will most likely always fail to identify diagnostic characters for currently recognized taxa. In order for taxonomy to better reflect the evolutionary reality of the group, we recommend that binomial taxonomic designations of these different forms be discarded, and that all ciscoes of the *C. artedi* complex (including *C. zenithicus* and the Great Lakes endemics) be referred to as *C. artedi (sensu lato)*.

The recognition of a single taxon, *C. artedi (s.l.)*, is also more appropriate for conservation purposes. In the light of their evolutionary history, species status is not likely to be ever confirmed for taxa other that *C. artedi (s.l.)*, no matter the species concept used. Awaiting multiple cisco species recognition and taxonomy-dependent listing is clearly inappropriate and can only be a source of confusion hindering and disserving their conservation. In order to define conservation units, the ESU concept seems more warranted. The lack of congruence between phenotypic and genetic divergence can be reconciliated by adopting the general principle advocated by Adaptive Evolutionary Conservation (AEC) (Fraser and Bernatchez 2001), which allows for the dynamic and context-based combination of various ESU criteria. Given the information at hands for ciscoes, we feel that the original recommendations of Waples (1991, 1995) to identify ESUs on the basis of the distinctiveness and contribution of populations with unusual ecophenotypic characteristics to the overall genetic/ecological diversity of the

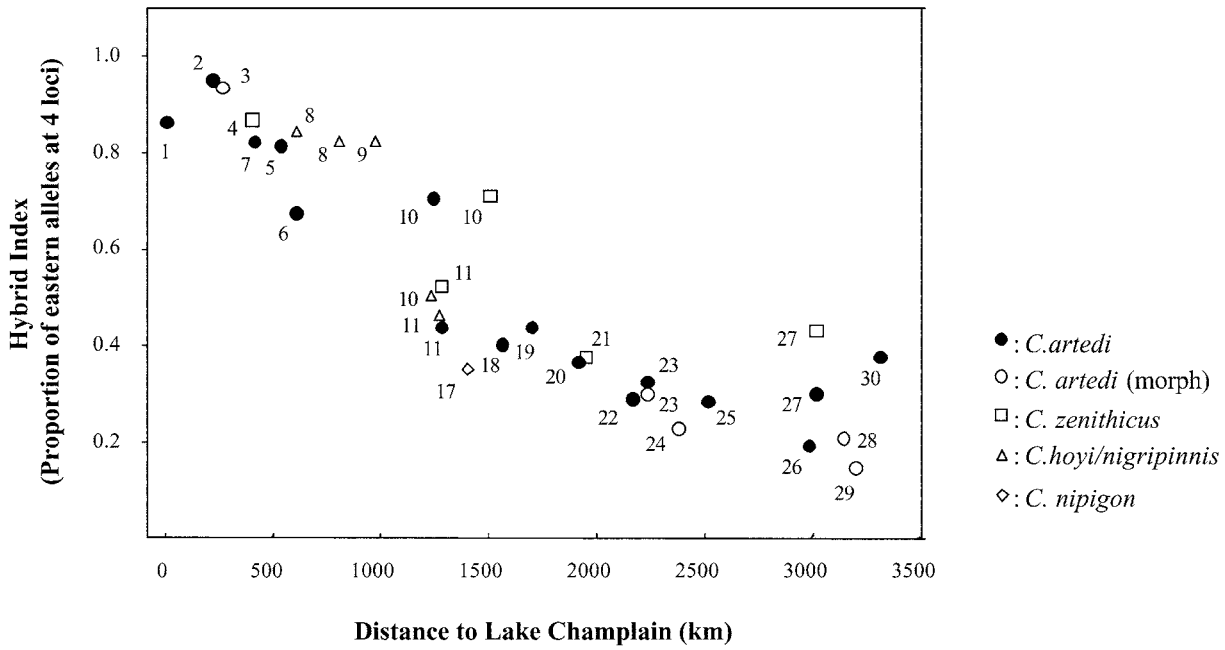

Figure 5. Mean population Hybrid Index (i.e., proportion of eastern alleles at four loci) for samples of the *C. artedi* complex in relation to their position along the axis of former proglacial lakes (codes as per Table 1).

species can be gradually integrated using the general method proposed by Bernatchez (1995). Briefly, this method progresses from defining the smallest natural assemblages of populations to demonstrating the adaptive uniqueness of populations within these. It implies that "neutral" genetic markers are reliable in defining cohesion among populations, that is assessing their evolutionary relationships, while their utility declines as potentially selected phenotypic traits become increasingly important in discriminating uniquely adapted populations within each natural assemblage.

Thus, we first focus on genetic variation to identify monophyletic/cohesive reference group. Here, mitochondrial polymorphisms clearly indicate that *C. artedi (s.l.)*, i.e., including all associated taxa and forms, constituted a monophyletic assemblage differing from other (Northwestern) cisco species by several apomorphies. This monophyletic group thus constitutes the 'whole species' relative to which the genetic and ecological contribution of any putative ESU must be evaluated (Waples 1991). Second, we consider the historical events relevant to the observed genetic structure. In *C. artedi (s.l.)*, the geographical distribution of mitochondrial and nuclear genetic variation reveals the extensive and clinal intermixing of two refugial races (Turgeon and Bernatchez 2001a,b, this paper). Genetic differentiation primarily depends on geographical distance between compared units instead of revealing the temporal extent of isolation, and this persisting historical gene flow signal is an inappropriate measure of population isolation. This information is crucial in justifying the inadequacy of using genetic criteria that assume long-term divergent evolution such as reciprocal monophyly of mtDNA alleles (Moritz 1994) to identify cisco ESUs. Third, we identify distinct units by considering significant phenetic groupings. In ciscoes, variation at microsatellite loci defined groups that all comprised sympatric or geographically proximate cisco populations including one or many populations exhibiting significant phenotypic polymorphisms. Microsatellites also revealed that sympatric morphological differentiation is often paralleled by significant genetic divergence. These results clearly show that isolation has been sufficient for phenotypic differentiation to proceed repeatedly on a very local scale (lakes or cluster of lakes). Although a center of increased phenotypic diversity is obviously located in the Laurentian Great Lakes, divergent phenotypes are often restricted to single lake in any particular region, suggesting that their emergence is intimately linked and dependant upon specific local habitat conditions. Finally, we

privilege phenotypic characteristics to assess the evolutionary significance of each distinct unit by considering traits that contribute to the ecological diversity and niche expansion of the whole species. In *C. artedi (s.l.)*, the ecophenotypic diversity of the group is obviously enhanced by populations exhibiting unusual morphology (gill raker counts, adult body size), trophic niche, depth distribution, and reproductive behaviour (spawning season, anadromy).

Extreme or significant differences in gillraker numbers are the most commonly reported phenotypes in ciscoes, as well as in several other species pairs of postglacial fishes. There is compelling evidence in coregonine fishes that differences in gillraker counts results from divergent natural selection associated with the exploitation of alternative trophic niches (Bernatchez 2002). In L. Nipigon (Turgeon et al. 1999) and L. Barrow (M. Steinhilber, pers. comm.), the diet of low gillraker forms (*C. zenithicus*) did not include significant portion of plankton as in sympatric higher gillraker forms (*C. artedi* (*sensu stricto*)). Similarly, the deeper niche of one form in Lake Nipigon (*C. hoyi*) was clearly associated with a distinct benthic diet, albeit its overlapping gillraker distribution with the planktonic form indicated that divergence proceed along the depth niche axis as much as along the trophic axis (Turgeon et al. 1999).

Results of some transplant experiments of *Coregonus* populations have suggested that gill raker count is partially plastic (Lindsey 1981), but plasticity in itself can be an heritable and relevant to adaptive evolutionary changes (Thompson 1991). Nevertheless, mass cross experiments also indicated the high heritability of gill raker traits and a clear potential for response to selection (Svärdson 1952, 1957). In ciscoes, this trait has also proven to be less dependant on environmental conditions than growth, shape and longevity (Shields and Underhill 1993). Maximum lake depth is positively related to mean gill raker count in lake cisco, suggesting that variation for that trait is not random (Phillips and Ehlinger 1995). Moreover, lake depth is also correlated with the number of forms defined on the basis of gillraker numbers (Clarke 1973), and we present evidence that this phenotypic divergence is accompanied by significant genetic divergence. Thus, the parallel evolution of similar gillraker and depth ecotypes strongly suggests that natural selection has played a role in the emergence of these traits in ciscoes, thus pointing to their adaptive significance.

Adult size polymorphisms appear to be more environmentally dependant and, contrary to other postglacial fishes, seems to be partially decoupled from gillraker numbers in ciscoes. Indeed, processes of phenotypic divergence among cisco forms appear to be different and more complex than in other fish species pairs. For example, the occurrence of small morphs bearing *fewer* gill rakers than larger sympatric forms in Lake Cormorant (Table 2) and Lake Ryan AB (M. Steinhilber, pers. comm.), is opposite to prediction (Schluter and McPhail 1993). Likewise, the common occurrence of large or dwarf ciscoes in allopatry has not been associated with distinct trophic niches or habitats. This trait, then, is apparently of less evolutionary significance, but the significant genetic differentiation of a large form in La Grande Reservoir (Site 14) calls for a closer examination of the ecological niches (habitat, trophic ecology, reproductive season) of these populations.

Finally, variation in reproductive behaviour must bear some evolutionary consequences. Allochrony among sympatric forms, as reported for several Great Lake forms (Smith and Todd 1984) is, if heritable, most certainly a factor of isolation. The distinct spring spawning season of ciscoes from Lac des Écorces (QC) is associated with a peculiar thermal regime in that lake (lack of a summer 4 °C stratum and colder than usual winter temperature, Hénault and Fortin 1991), indicating adaptation to a unique embryogenetic habitat. Along with the anadromy of northern populations, these reproductive behaviour contribute to the niche expansion of the whole species.

Thus, the entire cisco population complex of lakes inhabited by ciscoes with exceptional/multimodal gill raker count, distinct depth distribution and/or reproductive behaviour should be considered ESUs. On the basis of this study, these are L. Barrow, L. Cormorant, L. George, L. Saganaga , L. Nipigon, L. Superior, White Partridge Lake, and Lac des Écorces (Table 1), but other lakes are likely to be added to that list. When one of these forms is genetically differentiated from sympatric form(s), each genetic pool should also be considered as separate ESUs. These are the low gillraker forms (shortjaw cisco) of Lake Barrow and Lake Nipigon (Turgeon et al. 1999).

Conclusions

This extensive survey of genetic variation in North American ciscoes revealed the unusual evolutionary history of the *C. artedi* complex. Past reticulation

events coupled with the recent and independent evolution of similar phenotypes explains why morphological and molecular systematics previously failed to identify diagnostic characters. A conservative review of taxonomy is now in order, and we proposed that *C. artedi* be recognized as the sole legitimate taxon for North American ciscoes distributed in Central Canada and Northern United States. The recognition of a unique cisco species and the consideration of their evolutionary history allow for the definition of appropriate conservation units. Ecomorphotypes of each particular locale represent a unique expression of a diverse genetic pool still undergoing divergence and sorting, and gillraker polymorphisms, depth habitat and reproductive behaviour are considered as phenotypic traits with probable adaptive value contributing to the ecological diversity and niche expansion of the whole species. Consequently, ciscoes from lakes with distinct ecomorphoytpes are recognized as ESUs, as well as each of sympatric forms when they are genetically differentiated. A local populational approach has recently been applied in a similar case of recent postglacial radiation in *Gasterosteus* (British Columbia). Limnetic and benthic threespines sticklebacks of individual lakes are now listed separately for conservation in Canada (COSEWIC, http://www.cosewic.gc.ca), and we recommended that a similar this approach be employed for *C. artedi (s.l.)* This conservation strategy should be considered as a valid alternative for polytypic species of newly colonized latitudes in order to protect significant evolutionary processes of divergence.

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