

## Genetic Variation in the ND1 Gene and D-loop in Protected and Commercially Exploited European Cisco (*Coregonus albula* L.) Populations\*

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The European cisco (*Coregonus albula* L.) is a species with high environmental requirements. The deterioration of environmental conditions in recent decades has decreased its distribution. Currently the species is conserved by stocking, and the few existing natural populations are at risk of extinction. Therefore, contemporary studies involve not only reporting phenotypic parameters, but also determining the genetic structure of the population. This is an important aspect monitored in the *C. albula* population, which provides information valuable for proper fishing economy. This study included valuable populations from lakes located in Drawa National Park (DNP) and Wigry National Park (WNP), as well as lakes used for commercial fishing. In order to molecularly characterize the European cisco, the control region and ND1 gene were sequenced from 48 individuals from 9 populations from lakes throughout northern Poland. Analysis revealed that populations from two park lakes (Marta, Ostrowieckie) are unique. This was also the case for some sequences originating from Lake Wigry. The mean value of genetic diversity was 0.2% within each region and 0.1-0.3% between the investigated regions. The obtained results demonstrated the necessity to strengthen and protect natural populations of the European cisco, which constitute a valuable element of the European ichthyofauna.

Key words: cisco, *Coregonus albula*, mtDNA fish, sequencing.

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The European cisco (*Coregonus albula* L.) is a valuable species of lake ichthyofauna in Poland (CZERNIEJEWSKI & WAWRZYŃIAK 2006), Finland (VILJANEN *et al.* 2004), England (WINFIELD *et al.* 2004) and Lithuania (KAUPINIS & BUKELSKIS 2010). Due to high environmental requirements for occurrence and reproduction, as well as recently deteriorating environmental conditions, the geographical range of the population is being reduced (BERNOTAS 2002). Also in Poland, in which it is a valuable component of the lake ichthyofauna, the species is threatened with extinction (WITKOWSKI *et al.* 2009), and the number of European cisco individuals caught over the past four decades has decreased by half (MICKIEWICZ 2012). Previous studies of indigenous Polish

populations focused on growth rate, age structure (CZERNIEJEWSKI *et al.* 2006; KOZŁOWSKI *et al.* 2010) and fertility (CZERNIEJEWSKI & WAWRZYŃIAK 2006). Moreover, stocking activities have been conducted for many years in order to conserve the existing populations and stock the endangered populations to ensure that appropriate resources are available for commercial use (MICKIEWICZ & WOŁOS 2012; WOŁOS *et al.* 2016). To date, few studies have investigated the populations of *C. albula* from a genetic perspective. This is important for assessment of the genetic diversity of the fish, so that excessive catches do not lead to inbreeding and a decrease in population fitness. Limiting the size of natural *C. albula* populations in single lakes can lead to the elimination of ge-

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netic variation due to genetic drift, which in turn negatively affects the fitness of the species. Therefore, monitoring allows the assessment of the genetic resources of fish in specific water bodies (PAMMINGER-LAHNSTEINER *et al.* 2009, WINKLER *et al.* 2011), and consequently provides data for rational stocking policy. According to PAMMINGER-LAHNSTEINER *et al.* (2009) current studies should follow this direction.

Our analysis covered very valuable natural populations from the strict protection zone of two national parks, as well as populations from lakes included in the Natura 2000 network. One of them, Lake Wigry, is listed by the International Union for Conservation of Nature (IUCN) among the world's most valuable water bodies (Project Aqua, Wigry National Park 2000-2016a). This is a particularly interesting area of investigation, as it will elucidate the genetic diversity of the populations from lakes located in protected areas.

The European cisco populations are found in lakes which are a natural habitat of this species. The lakes (so-called cisco lakes), have a sand and gravel bottom, and are of 1<sup>st</sup> and 2<sup>nd</sup> class purity (CZABAN 2008). The occurrence of the European cisco is closely related to the different intensity of fishing pressure. Before 1990 (date of establishment of Drawa National Park – DNP), the populations of the lakes of DNP (Płociowe, Ostrowieckie, Marta) were fished for commercial purposes. Currently, the lakes are situated in the strict protection zone and fishing is prohibited. The populations are small (personal communication from DNP employees), and in order to protect and renew them, stocking was conducted in 2004-2008 using material from spawning fish from populations of the lakes of DNP. For over 80 years, the population of Lake Wigry (Wigry National Park – WNP) has been maintained by stocking using material from spawning fish from that lake. In the 1990s, the lake would periodically lose part of its European cisco population as a result of eutrophication. Fish were also stocked in Lake Miedwie and Lake Morzycko using material originating from each of the lakes. The European ciscoes from Lake Bytyń, Lake Drawsko and Lake Żerdno are caught commercially and their reintroduction involves material from spawning fish from the same lake or other lakes (personal communication from PZW SZCZECIN 2014; MODEHPOLMO 2013).

To date, very few studies of the European cisco have been conducted in Poland and have regarded only a few populations caught in commercially exploited lakes. The studies were based on mtDNA analysis (ND1, D-loop, ND3/4) (BRZUZAN & CIESIELSKI 2002; BRZUZAN *et al.* 2004). In this study, an analysis of two mtDNA regions, i.e. ND1 (NADH dehydrogenase, subunit 1) and the D-loop

control region, was conducted along with an analysis of relationships based on these two genes.

The study is in line with current European trends in research on endangered populations (BRZUZAN 2000; JACOBSEN *et al.* 2012; KUCINSKI *et al.* 2015). The analysis provided information on the genetic variability of the European cisco populations and on interpopulation relatedness, and may be a basis for reintroduction policy. To date, European cisco populations from most of the investigated lakes have not been studied. It is of particular significance for the restoration of the genetic structure of the European cisco in lakes whose populations have decreased or have become extinct. The aim of the study was to analyse the mtDNA sequences of protected populations of *C. albula* from lakes located in Drawa National Park and heavily populated, commercially utilized populations of north-western Poland (Western Pomerania) with reference to a population widely recognized as the source population, originating from Wigry National Park (north-eastern region of Poland).

## Material and Methods

### Study sites and amplification of biological material

Between 7 and 10 individuals of the European cisco (*C. albula*) representing populations of each of the 9 investigated lakes of northern Poland were analyzed. The investigated area included the following lakes: (i) areas near the Poland-Germany border: Lake Miedwie (M) and Lake Morzycko (R) – Region I; (ii) the strict protection zone of Drawa National Park (DNP): Lake Ostrowieckie (T), Lake Marta (E), Lake Płociowe (C) – Region II; (iii) the centre of Western Pomerania: Lake Drawsko (D), Lake Żerdno (Z) and Lake Bytyń (B) – Region III, and (iv) Lake Wigry located in Wigry National Park in north-eastern Poland – Region IV (Fig. 1). European cisco individuals from the reservoirs of DNP were caught with permission of the Ministry of the Environment, no. DLPpn-4102-229/17717/13/M. As for other lakes, the fish were bycatch in commercial fishing. The study involved taking muscle samples using a sterile technique, which were subsequently stored in Eppendorf-type tubes and frozen until analysis.

### DNA extraction and mtDNA amplification (ND1 gene and D-loop)

DNA was extracted using phenol-chloroform following BERNATCHEZ *et al.* (1988), and kept at -70°C until analysis. Molecular analyses involved two mtDNA fragments, i.e. the ND1 gene (NADH dehydrogenase, subunit 1 (complex I))

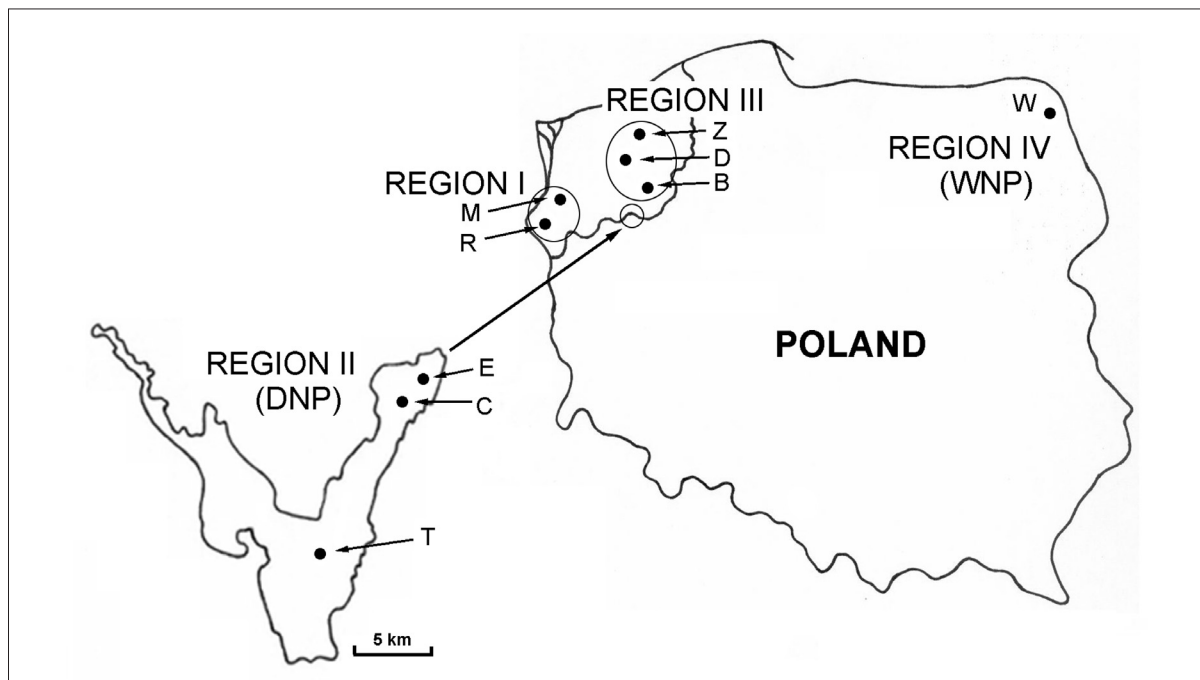


Fig.1. Map of sampling locations in Poland. Research area: Lake Miedwie (M) and Lake Morzycko (R) – Region I; strict protection zone of Drawna National Park (DNP): Lake Ostrowieckie (T), Lake Marta (E), Lake Płociowe (C) – Region II; centre of Western Pomerania: Lake Drawsko (D), Lake Żerdno (Z) and Lake Bytyń (B) – Region III, and Lake Wigry located in Wigry National Park (WNP) in north-eastern Poland – Region IV.

amplified using the primer pair Tt-ND1-F1 and Tt-ND1-R1 (approx. 1100 bp; PAMMINGER-LAHNSTEINER *et al.* 2009), and a non-coding control region (D-loop) amplified using the primer pair L19 and H17 (approx. 1300 bp; BERNATCHEZ *et al.* 1992).

For the PCR, the Phusion High-Fidelity DNA polymerase (Finnzymes, Finland) was used at a concentration of 0.5 U/20 µl of mixture. The final reagent concentrations were 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub> for ND1 and 2.4 mM MgCl<sub>2</sub> for the D-loop region, 200 µM of each deoxynucleoside triphosphate (dNTP), 10 pM of each primer and a DNA template. PCR conditions were adapted for the Phusion High-Fidelity DNA Polymerase, according to producer's indications, and the annealing temperatures were 52°C for ND1 gene and 54°C for D-loop. The results of PCR amplification were visualised using electrophoresis on 1.5% agarose gels with GPB Gold View Nucleic Acid Stain (GenoPlast, Biochemicals, Poland).

#### Sequencing of ND1 gene and D-loop

From each of the nine investigated populations, 7 to 10 individuals (except for Lake Wigry – 13 individuals) were selected for the sequencing of the ND1 and D-loop amplicons. In the final analyses,

identical sequences within a given population were collapsed.

The sequencing was outsourced to Macrogen Inc. (Seoul, Korea). Sequencing was performed with a set of primers identical to those used in the PCR, and the results were compared with each other and with sequences deposited at GenBank.

#### Data analysis

Multiple alignment, distance matrix calculation and phylogenetic trees were done using MEGA6 (TAMURA *et al.* 2013). Bootstrap values were obtained from 1,000 randomly generated trees. We analysed relationships among haplotypes on the basis of the combined sequences of ND1 and D-loop for each individual, using several phylogenetic methods (Neighbor Joining – NJ, Unweighted Pair Group Method Using Arithmetic Average – UPGMA, Maximum Parsimony – MP). The dendrogram presented in this paper was created using the Neighbor-Joining (NJ) method and based on Kimura's biparametric model. The average genetic distance between the analysed regions, as well as within each region, was calculated using Kimura's biparametric method.

To analyse relationships based on the ND1 gene, two sequences deposited in GenBank, DQ399869 (Narie Lake, Poland) and DQ399870 (Stechlin Lake, Germany) were used (KOHLMANN *et al.* 2007).

Table 1

Characterization of the nucleotide variability of the analysed ND1 (dehydrogenase, subunit 1) gene fragment of 1010 bp in *C. albula*; abbreviations: TZ – transitions; TW – transversions; nt – nucleotide; AA – amino acid

Position in sequence	9	42	129	155	180	330	390	465	501	539	582	801	933	956	961	962	975
Substitution	T/C	T/C	A/G	A/G	T/C	A/G	A/G	A/G	A/C	A/G	A/G	T/C	C/G	A/C/G	C/G	C/G	A/C
Mutation type	TZ	TZ	TZ	TZ	TZ	TZ	TZ	TZ	TW	TZ	TZ	TZ	TW	TW/TZ	TW	TW	TW
Codon number (numbers of nucleotides within the codon; bold-substituted nucleotide)				<u>52</u> (154, <b>155</b> , 156)						180 (538, <b>539</b> , 540)				319 (955, <b>956</b> , 957)	321 ( <b>961</b> , <b>962</b> , 963)		
Protein substitutions AA				Y/C						S/N				A/E/G	A/P/G		

## Results

ND1 and D-loop sequences were obtained for 48 individuals from 9 populations caught in different lakes in northern Poland.

All original sequences obtained for the ND1 gene and D-loop control region were submitted to GenBank under accession numbers: KT426542-KT426556 (ND1) and KT426557-KT426561 (D-loop).

Analysis of ND1 nucleotide sequences covered a 1010-bp mtDNA fragment (975-bp gene and flanking sequences). Seventeen nucleotide substitutions were found in the analysed sequences. Most mutations (64.7%) were transitions (A/G: 41.2%; T/C: 23.5%). Transversions constituted 35.3% and regarded A/C (3) and C/G (3) substitutions. Among all observed changes, 14 sites were parsimony-informative (Table 1).

Analysis of amino acid (AA) sequences was also made based on the amplified nucleotide sequence. Among the 17 substitutions observed in the analysed fragment, 4 of them caused substitutions of amino acids in the encoded protein (Table 1).

Based on the analysis of nucleotide sequences, 15 variants of the ND1 gene were identified and named with consecutive numbers (Vn1-Vn15) (Table 2). Using the authors' own sequences and sequences of comparable length deposited in GenBank (DQ399869 and DQ399870), a similarity analysis was performed. Variants Vn6, Vn7 and Vn8 from the fish caught in Lake Marta, located in the protected area of Drawa National Park (DNP), formed a separate clade in the phylogram (Fig. 2). This was also the case for variants Vn2 (Lake Bytyń) and Vn14 (Lake Żerdno). A sequence derived from *C. albula* from Germany was located between these clades. The remaining two clades

Table 2

List of variants of the ND1 gene in the *C. albula* individuals from each lake, taking into account the region of origin, Wigry National Park (WNP), Drawa National Park (DNP)

ND1 gene variant	Lake / Region
Vn1	Bytyń, Drawsko/ Region III; Płociowe (DNP)/ Region II
Vn2	Bytyń/Region III
Vn3	Płociowe (DNP) /Region II
Vn4	Płociowe (DNP)/Region II
Vn5	Drawsko/Region III
Vn6	Marta (DNP) /Region II
Vn7	Marta (DNP) /Region II
Vn8	Marta (DNP) /Region II
Vn9	Miedwie, Morzycko/Region I
Vn10	Ostrowieckie (DNP) /Region II
Vn11	Wigry (WNP) /Region IV
Vn12	Wigry (WNP) /Region IV
Vn13	Wigry (WNP) /Region IV
Vn14	Żerdno/Region III
Vn15	Żerdno/Region III

were formed by haplotypes originating from different lakes.

The D-loop sequence was 560 bp. This sequence was highly conserved and showed only five variable sites corresponding to 0.9% of the total sequence length. Three of the observed substitutions were transitions (2 T/C and 1 A/G), while the other two were transversions (G/C and G/T). Four substitutions were parsimony-informative (Table 3).

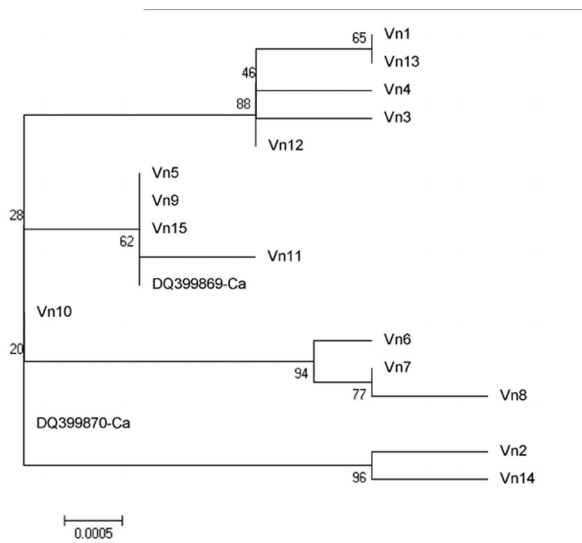


Fig. 2. Phylogram based on the nucleotide sequences of the ND1 (dehydrogenase) gene in nine *C. albula* populations in the investigated lakes (as described in Table 2), created using the Neighbor-Joining (NJ) method and based on Kimura's biparametric model. Two sequences of the ND1 gene deposited in GenBank were used in the comparison (DQ399869 and DQ399870; KOHLMANN *et al.* 2007).

Due to the low variability, a separate phylogram dedicated to this gene region was not built.

In the phylogeny, two main groups were observed (Fig. 3). The first group consisted of sequences derived from the European cisco individuals caught in Lake Żerdno and a single sequence from the ciscoes of Lake Bytyń (Region III). The second group was more varied and contained sequences from all four regions. The nucleotide sequences obtained from the fish caught in DNP (Region II) formed two separate clades (Lake Marta and Lake Ostrowieckie), just like most (69%) sequences derived from the fish from Wigry National Park (WNP), Region IV. However, some of the sequences of the WNP European cisco populations (Region IV) grouped together with the sequences from Lake Płociowe (DNP), and further with the sequences from Lake Bytyń. The nucleotide sequences originating from the populations of Lake Miedwie and Lake Morzycko, located at the western end of Western Pomerania (Region I) are grouped together in one clade, which may indicate a common origin.

The average genetic diversity of the European ciscoes was 0.2% within each region and 0.001 to 0.003 between regions (Table 4).

**Discussion**

Mitochondrial DNA (mtDNA) is commonly used in population genetic studies in fish (BERNATCHEZ *et al.* 1996; HANSEN *et al.* 1999; DEL-

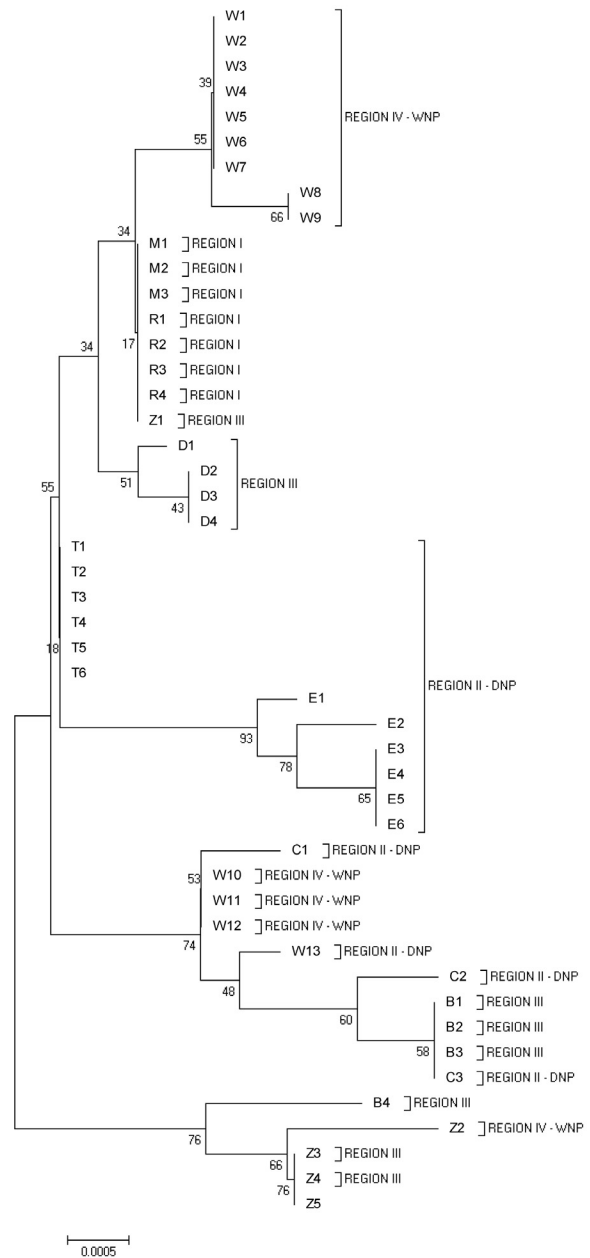


Fig. 3. Dendrogram based on a combined analysis of the sequences of ND1 and the D-loop control region in nine *C. albula* populations from the four investigated regions (as described in Fig. 1), created using the Neighbor-Joining (NJ) method and based on Kimura's biparametric model.

Table 3

Substitutions in the non-coding region of D-loop in a fragment of approximately 560 bp in *C. albula*; abbreviations: TZ – transitions; TW – transversions; nt – nucleotide; AA – amino acid

Position in DNA	19	203	254	449	462
Substitution	G/C	T/C	A/G	T/G	T/C
Mutation type	TW	TZ	TZ	TW	TZ

Table 4

Estimates of average evolutionary divergence over sequence pairs within groups *C. albula* of the analyzed regions. Analyses were conducted using the Kimura 2-parameter model; description of the Regions as in Fig. 1. Drawa National Park (DNP), Wigry National Park (WNP)

	Region III	Region II – DNP	Region I
Region III			
Region II – DNP	0.003		
Region I	0.003	0.002	
Region IV – WNP	0.003	0.003	0.001

LING *et al.* 2014). Compared to nuclear DNA, mitochondrial DNA has some advantages. It is present in many copies per cell, is maternally inherited and is haploid with no recombination (RFE). Mitochondrial DNA lacks intronic regions and mutates more often than nuclear DNA (ncDNA) due to e.g., lack of repair systems (MEYER 1993).

To date, several studies have analysed mitochondrial DNA in fish of the Coregoninae subfamily. In studies of one species, the main markers are the D-loop control region and genes encoding the consecutive NADH dehydrogenase subunits, i.e., ND1, ND1/ND2, ND3/4, and ND5/ND6 (BRZUZAN *et al.* 2004, SCHULZ *et al.* 2006; GORDEEVA *et al.* 2008; WINKLER *et al.* 2011; DELLING *et al.* 2014).

In Poland, genetic characterization of the European cisco from the north-eastern region was based primarily on sequences of the D-loop and a fragment of the gene encoding NADH dehydrogenase subunits 3 and 4 (ND3/4) (BRZUZAN & CIESIELSKI 2002; BRZUZAN *et al.* 2002; BRZUZAN *et al.* 2004). To our knowledge, the ND1 marker has not yet been used to characterize *C. albula*.

ND1 is a gene encoding subunit 1 of NADH dehydrogenase located on the outer strand (H). The product of the ND1 gene belongs to enzyme complex I (BALLARD & WHITLOCK 2004). The complex is one of several enzyme complexes necessary for oxidative phosphorylation. According to POLITOV *et al.* (2000), the ND1 gene in the Coregoninae subfamily exhibits inter- and intra-specific variability, therefore it is successfully used to identify species, hybrids and ecological forms of Coregoninae, especially in the cases in which it is difficult to distinguish taxa based on morphology (BOROVIKOVA & MAKHROV 2009; BOROVIKOVA *et al.* 2012).

The Polish haplotypes were compared to the ND1 gene haplotypes (of similar length) obtained by KOHLMANN *et al.* (2007), and deposited in GenBank: DQ399869 and DQ399870. The sequences are derived from individuals caught in Lake Narie (Poland) and Lake Stechlin (Germany), respectively. The genetic distance between these sequences and the sequences obtained in our study is small, and their position in the phylogeny indicates a monophyletic origin of the populations from northern Poland and the selected *C. albula* population from Lake Stechlin in Germany (SCHULZ *et al.* 2006).

The mtDNA control region, D-loop, is particularly useful in fish population studies (SUKHANOVA *et al.* 2002). The non-coding D-loop region is the most variable fragment of mtDNA (BERNATHEZ *et al.* 1992; BRZUZAN *et al.* 2002; GORDEEVA *et al.* 2008; REED *et al.* 1998). This applies to both nucleotide substitutions and different lengths, even within the same species (BRZUZAN 2000). The obtained sequences of D-loop were compared to the Polish sequences submitted to GenBank (300-500 bp), which showed slight variability that regarded only 5 nucleotides (BRZUZAN & CIESIELSKI 2002; BRZUZAN *et al.* 2002). In German populations of *C. albula*, SCHULZ *et al.* (2006) demonstrated 5 different haplotypes in a fragment of 328 bp, with the haplotypes differing by 1 to 6 nucleotides. The sequences, corresponding to individuals caught in different lakes, i.e. Lake Stechlin (Germany), Lake Breiter Lutzin (Germany), Lake Onkamo (Finland), Lake Kuohijaervi (Finland), were identical with the Polish sequences with one exception. The sequence submitted under accession no. AY277981 derived from the ciscoes of Lake Breiter Lutzin (Germany) differed from the Polish sequences. Genetic variability in European ciscos is quite low and thus possibly reflects a recent recolonization event of Northern Europe (MEHNER *et al.* 2010).

In many studies of the variability of Coregoninae, phylogenetic trees were built on the basis of single mtDNA fragments, such as D-loop (BRZUZAN & CIESIELSKI 2002; BRZUZAN *et al.* 2004), as well as cytochrome *b* (SUKHANOVA *et al.* 2002) and ND1 (NIELSEN *et al.* 1998; BOROVIKOVA & MAKHROV 2009; BOROVIKOVA *et al.* 2013) genes. However, it has been shown that multilocus comparisons increase phylogenetic resolution and hence the possibility to detect e.g. recent population structure and gene flow (JACOBSEN *et al.* 2012). Therefore a combined tree was built using sequences of the ND1 gene and D-loop.

The results of the phylogenetic analysis using the combined mtDNA fragments revealed an interesting population structure of *C. albula* in northern Poland. On the one hand, there are lakes

such as Lake Ostrowieckie (T) or Lake Marta (E), whose populations are monophyletic, without mitochondrial haplotypes shared among any other populations. It cannot be excluded that a rapid decrease in the number of individuals has occurred in these populations and the bottleneck effect caused reduction in the number of alleles. The effect was amplified by sporadically conducted stocking with fish from spawning individuals originating from the same lake. In populations occurring in protected areas, this can lead to positive effects in terms of preserving precious natural and unique populations. On the other hand, in many lakes, including Lake Płociowe (Drawa National Park – DNP), the populations of the European cisco are characterized by significant genetic variability. This may be a result of commercial use of these lakes and stocking of material originating from other lakes. In the case of Lake Płociowe, such activity had to take place before the national park was established in 1990 (OSEWSKI 2003). It is also plausible that in the case of Lake Płociowe and Lake Bytyń, there may have been stocking using material from Lake Wigry in the distant past (personal communication, WIGRY NATIONAL PARK – WNP). The stocked lakes (D, Z, B) are characterized by a large number of cisco individuals, while the spawning fish originated from different lakes, which resulted in the observed variability.

Thanks to a hatchery existing for more than 80 years and systematic stocking of material from the same lake, the population of Lake Wigry was rebuilt and probably spread westward in recent decades without any stocking activities (BIAŁOKOZ *et al.* 1999). The European cisco of Lake Wigry is a dominating species among the ichthyofauna of this lake and is caught for commercial purposes (BIAŁOKOZ *et al.* 2004). It is characterized by high genetic variability, therefore its population has probably not been rapidly reduced to the extent that would cause a bottleneck. According to KOZŁOWSKI *et al.* (2010), it is the most widely distributed population in Poland. Its genetic influence on the other Pomeranian cisco populations could be seen in this study.

For many years, Lake Miedwie has been restocked with fish derived from the spawning individuals of that lake (communications from fishermen; MODEHPOLMO, 2013). The population is characterized by particularly good condition and growth rate (CZERNIEJEWSKI *et al.* 2004). Also in Lake Morzycko, stocking is based on material from the same lake, and the slightly worse results obtained are a consequence of an increasing trophic level of this lake (communications from fishermen; PZW SZCZECIN 2014). The populations of Lake Miedwie and Lake Morzycko (Region I) have also been stocked using material from fish

spawning at the same lake for many years. According to current good fishing practice, the material for stocking should be derived from spawning fish of the same lake, as it protects populations adapted to specific environmental conditions (OREHA & ŠKUTE 2009; WIGRY NATIONAL PARK 2000-2016b). The presented analysis, however, indicates that the juvenile individuals stocked into both lakes have a common origin.

Currently, preserving the existing populations depends on the trophic status of the lakes and fishing policy. In order to preserve the population of the European cisco, reintroduction is conducted, e.g., in Poland, Scotland, England and Germany (MAITLAND & LYLE 1991; SWEETMAN *et al.* 1996; TURKOWSKI 2002). The improving ecological conditions of lakes, e.g., in the Drawa Lakeland, encourage further activities aimed to strengthen or rebuild the population of the European cisco. These actions should be supported by genetic monitoring in order to prevent disruption of the genetic structure of the population. Supporting the protection and continuous management of indigenous breeding lines is the aim of studies conducted in the population of Coregoninae in Austria (PAMMINGER-LAHNSTEINER *et al.* 2009; WINKLER *et al.* 2011). It is particularly important to support natural populations, as they are adapted to the environmental conditions. Maintaining genetic integrity in ecosystems is one of the most important tools for protecting indigenous populations. Inclusion of further European cisco populations in the monitoring programme, as well as performing the planned stocking activities in Lake Marta and Lake Ostrowieckie of DNP are necessary actions to preserve these valuable populations (personal communication from DNP ichthyologist). Our study is important for the stocking of the DNP lakes planned for the near future (application for funds has been submitted) and will be used for future comparative analyses. The protection of such populations is an important contribution to the protection of the European cisco gene pool, also at a European scale. In recent years, due to deteriorating ecological conditions, there has been a decline in the population of this species in Finland (NÖGES *et al.* 2008) and England (WINFIELD *et al.* 2004), and further decline is expected in the following years (ELLIOT & BELL 2011). Therefore, the best possible investment in the protection of threatened species are measures taken in order to preserve their natural populations and habitat, which has been confirmed in studies (KIDD *et al.* 2009; WINKLER *et al.* 2011). The improving status of some "cisco" lakes combined with rational fishing economy supported by monitoring are the basis for the conservation of the European cisco populations, also those constituting valuable ichthyofauna resources.

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