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# Morphological and genetic analyses identify a new record of a glacial relict: pygmy whitefish (*Prosopium coulterii*) from northwestern Ontario

P.J. Blanchfield, E.B. Taylor, and D.A. Watkinson

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**Abstract:** The pygmy whitefish (*Prosopium coulterii* (Eigenmann and Eigenmann, 1892)) is a glacial relict species with a disjunct North American distribution that, apart from its most easterly known location in Lake Superior, is predominantly found in northern and western regions of Canada. Here we report on a new finding of pygmy whitefish from Winnange Lake in northwestern Ontario that extends the range of this species ~320 km from its most easterly distribution in Lake Superior and almost 1500 km east of the closest previously known western localities. Genetic analyses confirmed that the fish from Winnange Lake were most closely related to the lineage that includes fish from Lake Superior and likely also originated via postglacial dispersal from a refugium in the upper Mississippi – Missouri river system.

*Key words:* pygmy whitefish, *Prosopium coulterii*, disjunct distribution, clades, refugia.

**Résumé :** Le ménomini pygmée (*Prosopium coulterii* (Eigenmann and Eigenmann, 1892)) est une espèce relique de l'ère glaciaire de répartition nord-américaine disjointe qui, outre sa présence la plus orientale connue dans le lac Supérieur, est principalement présente dans les régions du nord et de l'ouest du Canada. Nous faisons état de la découverte de ménominis pygmées dans le lac Winnange, du nord-ouest de l'Ontario, qui élargit l'aire de répartition de l'espèce à ~320 km du lac Supérieur, sa localité la plus à l'est connue à ce jour, et à près de 1500 km à l'est des localités occidentales les plus proches déjà connues. Des analyses génétiques confirment que les poissons du lac Winnange s'apparentent le plus étroitement à la lignée qui comprend les poissons du lac Supérieur, et qu'ils sont vraisemblablement issus de la dispersion postglaciaire à partir d'un refuge dans le réseau hydrographique supérieur de la rivière Missouri et du fleuve Mississippi.

*Mots-clés :* ménomini pygmée, *Prosopium coulterii*, répartition disjointe, clades, refuges.

## Introduction

Pygmy whitefish (*Prosopium coulterii* (Eigenmann and Eigenmann, 1892)) have a disjunct distribution in North America. West of the Continental Divide, native populations are widely distributed in portions of the Columbia River system in Montana, Idaho, Washington State, and British Columbia; north in the Fraser, Skeena, and Yukon river systems in Canada; and the Chignik and Ugashik river systems in southwestern Alaska (Scott and Crossman 1973; McPhail 2007; Page and Burr 2011; Witt et al. 2011). East of the Continental Divide, they are known to occur in northerly regions that include Lake Athabasca, Great Bear Lake, Elliot Lake (in the Peel River drainage), lakes in the Liard and Peace river systems, the Athabasca River, and most recently Bluefish Lake on the Yellowknife River (Lindsey and Franzin 1972; Scott and Crossman 1973; McPhail 2007; Page and Burr 2011; Alberta Sustainable Resource Development and Alberta Conservation Association 2011; Witt et al. 2011; Vecsei and Panayi 2014). Waterton Lakes in southwest Alberta represent the southern extent of the eastern range of pygmy whitefish. The third, and most easterly, area of this disjunct North American distribution is Lake Superior (Eschmeyer and Bailey 1955). A single population has also been recorded in Ekityki Lake on the Chukotski Peninsula in northeastern Russia (Chereshnev and Skopets 1992).

Recently, Witt et al. (2011) investigated the degree of phylogeographical divergence within pygmy whitefish and the origin of these three disjunct populations within North America. Using mitochondrial and nuclear DNA sequence variation, they found the species is composed of two monophyletic mitochondrial clades in North America. One consists of populations in Alaska, in the Chignik, Ugashik, and Alsek river watersheds and Aishihik Lake. The second consists of remaining portions of the distribution in Cascadia and Mackenzie (Peace) river watersheds and Lake Superior. The authors propose that the most likely explanation for the current range disjunctions of pygmy whitefish in North America was from a combination of isolation, genetic divergence, and selective dispersal from the Beringia and Cascadia Pleistocene glacial refugia, as well as more recent isolation and dispersal from an upper Mississippi refugium (Witt et al. 2011).

Pygmy whitefish is a glacial relict species, and a common habitat feature among the disjunct areas of their distribution is their presence only in cold, well-oxygenated lakes. Similar to other glacial relict species, such as deepwater sculpin (*Myoxocephalus thompsonii* (Girard, 1851)), lake trout (*Salvelinus namaycush* (Walbaum,

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1792)), and the opossum shrimp (*Mysis diluviana* (Audzijonyte and Väinölä, 2005)), pygmy whitefish is well suited to habitat present in proglacial lakes that formed in central Canada during the retreat of the glaciers (McPhail and Lindsey 1970). Pygmy whitefish was first documented in Lake Superior in 1952 (Eschmeyer and Bailey 1955), and because of the extreme discontinuity in its distribution that this eastern finding presented, it has been postulated for some time that the possibility exists they are distributed more widely in proglacial lakes in North America and not yet collected (McPhail and Lindsey 1970). Similar to deepwater sculpin, the pygmy whitefish is a small fish with adults ranging in size from 65 to 250 mm (McPhail 2007), typically found near the bottom of deep, cold glacial lakes that are rarely sampled using gear that can capture fish of this size (Sheldon et al. 2008). As such, the disjunct nature of their distribution, especially in the eastern part of the Hudson Bay drainage basin where many deep, glacial lakes exist, may be from limited sampling of the habitats where pygmy whitefish could occur. In this study, we report the occurrence of pygmy whitefish in a lake located approximately 320 and 1500 km from its closest previously known eastern and western locations, respectively. Secondly, we provide meristic, morphometric, and genetic comparisons on this new occurrence of pygmy whitefish to representative samples from other regions of their disjunct distribution.

## Materials and methods

### Field collections and laboratory identification

Winnange Lake (also known as Lake 660) is located in the Experimental Lakes Area (ELA) region in northwestern Ontario (Fig. 1), although it is not one of the designated 58 research lakes (Blanchfield et al. 2009). The ELA is remote from nearby towns and is characterized by numerous Boreal Shield lakes surrounded by jack pine (*Pinus banksiana* Lamb.) dominated forest (Cleugh and Hauser 1971). Winnange Lake is a medium-sized (2613 ha), deep ($Z_{max}$ = 115 m) oligotrophic lake that contains a diverse fish community, which includes glacial relict species, such as lake trout, and *Mysis*, as well as lake whitefish (*Coregonus clupeaformis* (Mitchill, 1818)) and cisco (*Coregonus artedi* Lesueur, 1818). Round whitefish (*Prosopium cylindraceum* (Pennant, 1784)) is not currently known from Winnange Lake, and no vouchered specimens have been collected in the Winnipeg–English river system (Scott and Crossman 1973).

Fish were collected by overnight sets of a single gang of variable mesh experimental gill net 1.8 m high and consisting of six duplicate panels (each 7.5 m long) of three different mesh sizes—13 mm (0.50″), 19 mm (0.75″), and 25 mm (1.00″). The net was set on bottom at depths of 25–40 m, parallel to shore following specific depth contours in the southern portion of Winnange Lake, near the deep basin (Fig. 1B). Sampling occurred in the summer and fall of 2008, initially to capture cisco, but with the presence of pygmy whitefish, we conducted additional sampling in 2009 and 2010. In summer 2010, fish were also captured using overnight sets of small-mesh gill nets as part of the broad-scale monitoring (BsM) program of the Ontario Ministry of Natural Resources (Sandstrom et al. 2010). The nets were 1.8 m high and consisted of two gangs, each 12.5 m long that consisted of five 2.5 m panels of the following mesh sizes: 13 mm, 19 mm, 25 mm, 32 mm (1.25″), and 38 mm (1.50″). Gill nets were set on bottom at four depth strata (1–3, 3–6, 6–12, 12–20 m) and were perpendicular to the depth contours of the lake (Sandstrom et al. 2010). A total of 16 overnight sets occurred at random locations in Winnange Lake from 15 to 18 June 2010.

Fish were removed from gill nets during retrieval and placed on ice. At shore, fish were individually placed in Whirlpak bags and taken to the ELA field station, where they were immediately frozen, and later transported to the Freshwater Institute, Winnipeg, for identification and further processing.

Two of the initial three specimens collected in 2008 had a pelvic fin removed and preserved in 95% ethanol for genetic analysis. The third specimen was sent to the Royal Ontario Museum (ROM) to confirm the identification and catalogue the collection (ROM 84998). The fish were placed in 10% formalin for 2 weeks and then transferred to a water bath for 2 days before transfer to 70% ethanol for long-term storage. The collections made in 2010 were thawed, had a section of dorsal muscle tissue removed, and placed in 95% ethanol. The remainder of the specimen was preserved following the same procedure as described above. Identification keys and description of whitefish from Scott and Crossman (1973), McPhail (2007), and Holm et al. (2009) were used to confirm the identifications of the specimens.

In total, 12 fish were in good enough condition to take meristic counts and morphometric measurements. This included two fish from October 2008, four fish from June 2010, and six fish from September or October 2010. Fish were given unique identification numbers and the following morphometric measurements (in millimetres) were made following Hubbs and Lagler (1958): total length (TL); fork length (FL); standard length (SL); head length (HL); snout length (SNL); and eye diameter (ED); as well as meristic counts of dorsal, anal, pelvic, and pectoral rays, lateral-line scales (when scales were missing, scale pockets were counted), pyloric caeca, and gill rakers (lower, upper, and total). Sex and maturity status was determined by direct observation of the gonads.

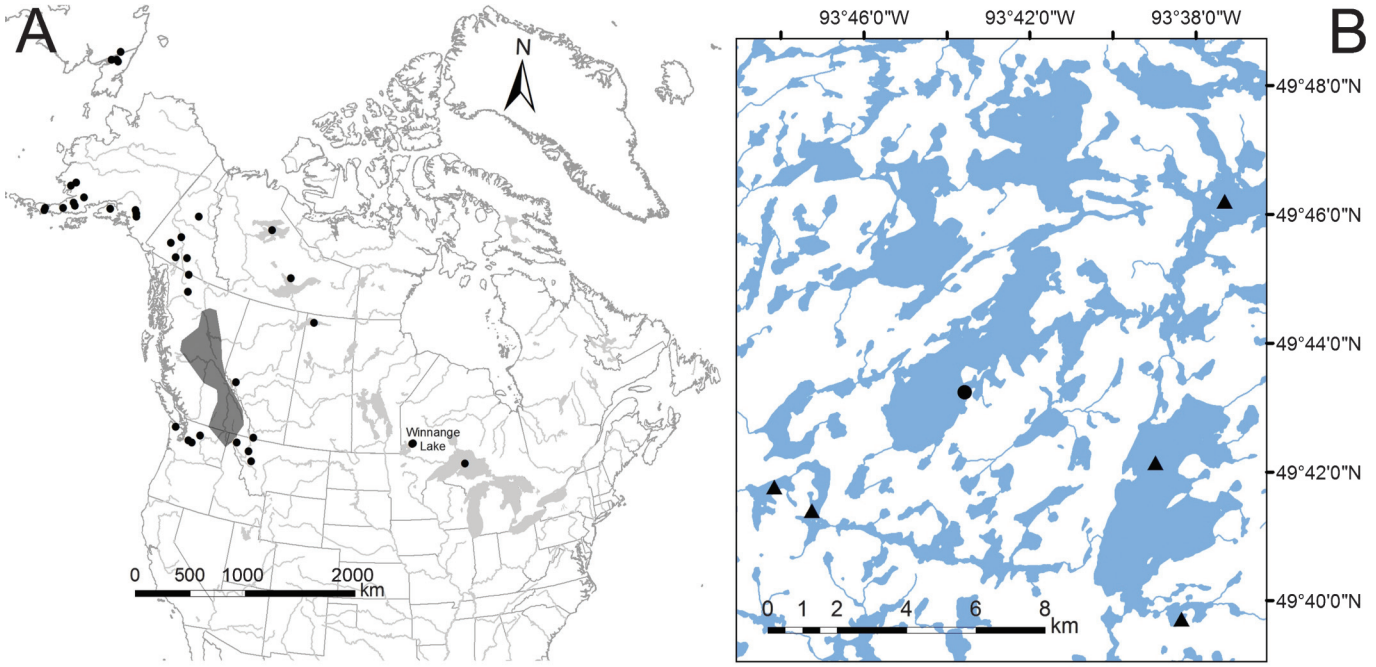
### Genetic-based identifications

We extracted genomic DNA from the Winnange Lake samples (n = 15) using the spin-column based Qiagen DNeasy extraction kit and confirmed our morphological identification of pygmy whitefish using genetic techniques and comparison of DNA sequences to various databases (see below). First, we amplified a 652 base pair (bp) segment of the mitochondrial DNA (mtDNA) cytochrome oxidase I gene using the polymerase chain reaction (PCR) and the primers FishF1 (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FishR1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (Ward et al. 2005). Cycling conditions during PCR were 1 cycle of 95 °C denaturation for 3 min, 50 °C annealing for 30 s, 72 °C extension for 1.5 min; 4 cycles of 95 °C for 1 min, 50 °C for 30 s, 72 °C for 1.5 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; followed by a final extension step at 72 °C for 10 min. Second, we amplified a 627 bp fragment of the ATPase VI gene of mtDNA using primers and procedures outlined in Witt et al. (2011) to help identify which of the two major mtDNA clades identified by Witt et al. (2011) the Winnange Lake pygmy whitefish positive samples belonged to. Finally, we identified six fish collected in 2010 at a nuclear-encoded locus by amplifying the first nuclear ribosomal internal transcribed spacer region (ITS-1; 630 bp) using the KP-2 (5′-AAA AAG CTT CCG TAG GTG AAC CTG CG-3′) and 5.8S (5′-AGC TTG GTG CGT TCT TCA TCG A-3′) primers described by Sajdak and Phillips (1997). Cycling conditions were 1 cycle of 95 °C denaturation for 3 min, 53 °C annealing for 2 min, 72 °C extension for 2.5 min; 4 cycles of 95 °C for 1 min, 52 °C for 2 min, 72 °C for 2.5 min; 30 cycles of 92 °C for 30 s, 52 °C for 2 min, 72 °C for 2.5 min; followed by a final extension step at 72 °C for 10 min. All PCRs were conducted in 50 μL volumes using 1 U (1 U ≈ 16.67 nkat) of *Taq* polymerase (New England Biolabs; NEB) using NEB reaction buffer (at 2 mmol/L $MgCl_2$).

Products from PCR were isolated from PCR reaction reagents using Qiagen PCR purification kit, diluted to 15 ng/μL, and sequenced using the FishF1 (CO1), the L8558 primer (ATPase VI), or the KP-2 primer (ITS-1) on an ABI 3730 sequencer.

Sequences were assembled, aligned, and trimmed to 630 bp (CO1), 510 bp (ATPase VI), and 600 bp (ITS-1) to remove sequencing ambiguities at the beginning and ends of reads, using BioEdit version 7 (Hall 1999). Sequences have been deposited in GenBank under accession Nos. KF536940–KF536952. To identify the sequences taxonomically, we used the 'Identification' module of Barcode of Life Database Systems (BOLD Systems, available from http://www.boldsystems.org/ [accessed 17 July 2013]; see also Ratnasingham and Hebert

**Fig. 1.** (A) Revised distribution of pygmy whitefish (*Prosopium coulterii*) in North America showing the occurrence in Winnange Lake (Lake 660), northwestern Ontario, Canada. Modified from Alberta Sustainable Resource Development (2011) and updated to include Bluefish Lake, Northwest Territories (Vecsei and Panayi 2014). (B) Pygmy whitefish were primarily captured in the deep, south basin of Winnange Lake (●). Also shown are several nearby lakes sampled for deepwater sculpin (*Myoxochephalus thompsonii*) (Sheldon et al. 2008), but where pygmy whitefish were not detected (▲).




2007) to calculate Kimura-2-parameter (K2P) genetic distances and clustered these by neighbor-joining (NJ) tree analysis and provide a visual representation sequence identity against BOLD (Saitou and Nei 1987; Hubert et al. 2008). We also assessed each sample from 2010 against our ATPase VI database to identify the major evolutionary lineage (sensu Witt et al. 2011) to which the Winnange Lake fish belong by calculating K2P distances and clustering these estimates using NJ. The sequences for ITS-1 were submitted to the BLASTn search engine of GenBank using the "Nucleotide BLAST" program against the Nucleotide collection (nr/nt) database (available from http://www.ncbi.nlm.nih.gov/BLAST, accessed 17 July 2013). For all sequences, we also reported genetic identity scores (GIS) and *E* values. The GIS is the percentage of aligned compared nucleotide sites that are the same between the Winnange Lake samples and the genetic database samples. The *E* value describes the number of different sequence alignments with matching scores equal to or better than that observed and that is expected to occur in a database search by chance; the lower the *E* value (to a minimum of 0), the more significant the observed match.

## Results and discussion

Overall, low numbers of pygmy whitefish ($n = 17$) were caught in Winnange Lake using the experimental small-mesh nets and BsM nets. A total of 3, 1, and 10 pygmy whitefish were captured from all overnight sets of experimental nets in 2008, 2009, and 2010, respectively. These nets were set to target cisco as part of a contaminants monitoring program, and this was the only other species captured along with pygmy whitefish. Similarly, of the 16 overnight sets of small-mesh BsM gill nets in 2010, a total of three pygmy whitefish were captured in a single net that was set at 13.3–14.4 m in depth. In this net, 13 cisco (90–160 mm FL) and a single yellow perch (*Perca flavescens* (Mitchill, 1814)) (50 mm FL) were also captured.

All fish were clearly identifiable as *Prosopium* by the presence of a single flap of skin between the nostrils and the parr marks (patches of dark pigmentation along the lateral body surface); *Coregonus* species have two flaps of skin and lack parr marks in adult-sized fish (Scott and Crossman 1973). The pyloric caeca (10–17) and lateral-line scale counts (51–56) clearly distinguished the Winnange Lake specimens from round whitefish, which have counts of 50–130 and 74–108, respectively (Table 1; McPhail and Lindsey 1970).

The TL of the specimens subject to morphological and meristic analyses ranged from 67 to 81 mm ($n = 12$). Ranges in other morphometric measurements were as follows: 61–76 mm FL, 57–72 mm SL, 13.9–16.2 mm HL, 3.1–4.0 mm SNL, and 4.1–5.2 mm ED. Four fish were immature and the sex could not be determined; the remaining eight fish were mature and had a 50:50 sex ratio (Table 1).

Ranges in meristic counts were similar to other values reported for pygmy whitefish with the exception of pectoral rays (11–14) that were lower than the reported ranges of 13–17 and pyloric caeca (10–17) that were lower than the reported range of 13–33. Lateral-line scale counts were within the reported range of 50–70 but at the lower end of that range with fish having between 51–56 lateral-line scales (Table 1).

### Genetic analyses

The two samples collected in 2008 were of poor quality and the resultant DNA sequences produced low but detectable signals. Despite the poor quality, these two fish were clearly identified as bearing pygmy whitefish mtDNA with >97% sequence identity against the COI database (Table 2, supplementary Fig. S1[1]). The

[1]Supplementary Figs. S1 and S2 are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2013-0252.



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**Table 1.** Meristic measurements of pygmy whitefish (*Prosopium coulterii*) reported from major basins in the literature and specimens ($n$ = 12) from Winnange Lake, northwestern Ontario.

| | Fin rays | | | | | | Gill rakers | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Specimen location | Dorsal | Anal | Pelvic | Pectoral | Lateral-line scales* | Pyloric caeca† | Total | Upper | Lower | References |
| British Columbia | 9–13 | 10–14 | | 14–17 | | 14–31 | 12–17 | | | McCart 1970; McPhail 2007 |
| Mackenzie Basin | 9–13 | 9–14 | 9–11 | 13–18 | 50–70 | 13–33 | 11–21 | 3–7 | 8–13 | McCart 1970; McPhail and Lindsey 1970; Vecsei and Panayi 2014 |
| Lake Superior | 10–12 | 12–14 | 9.5–11 | 13–16 | 54–62 | 15–23 | 16–20 | | | Eschmeyer and Bailey 1955 |
| Alaska | 9–13 | 10–14 | 9–10.5 | 14–18 | 51–81 | 14–33 | 12–21 | | | McCart 1970; Vecsei and Panayi 2014 |
| Columbia Basin | 11–15 | 11–15 | 9–11 | 14–18 | 54–71 | 16–33 | 12–19 | | | McCart 1970; Vecsei and Panayi 2014 |
| Winnange Lake | 10–11 | 10–15 | 10–11 | 11–14 | 51–56 | 10–17 | 14–19 | 4–6 | 10–13 | This study |
| Round whitefish | 11–15 | 10–13 | 9–11 | 14–17 | 74–108 | 50–130 | 14–21 | 5–8 | 9–13 | McPhail and Lindsey 1970; Norden 1970 |

**Note:** Round whitefish (*Prosopium cylindraceum*) are included for comparison.
*$n$ = 8, scales missing and lateral-line scale pockets could not be counted.
†$n$ = 10; pyloric caeca from two specimens could not be counted.

**Table 2.** Genetic identity (percent similarity to reference specimen; GIS) and $E$ (a measure of the probability of obtaining a match with the reference sequences by chance) for Winnange Lake specimens assessed against the BOLD Systems DNA barcoding database (mitochondrial DNA cytochrome oxidase I; COI) or the GenBank internal transcribed spacer region 1 gene (ITS-1) database.

| | COI | | | | ITS-1 | | |
|---|---|---|---|---|---|---|---|
| Specimen | GIS (%) | $E$ | BSAN | GBAN | GIS (%) | $E$ | GBAN |
| Sample 1 | 97.9 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| Sample 2 | 97.9 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-01 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-02 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-03 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-04 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-05 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-06 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-07 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-08 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-09 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-10 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-11 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | 98 | 0 | HQ616474 |
| PWF-666-12 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |
| PWF-666-13 | 100 | 0 | BCFB941–07.COI-5P | EU525104 | NT | NT | |

**Note:** Also shown are the BOLD Systems database accession number (BSAN) and GenBank database accession numbers (GBAN) for the reference specimens with which the Winnange Lake fish identified most closely. NT, not tested.

13 samples from 2010 were of much better quality and also were clearly identified as pygmy whitefish in the BOLD analysis, all with 100% sequence identity with pygmy whitefish (Table 2, supplementary Fig. S2[1]). No COI or ATPase VI sequence variation was detected among the 13 fish collected in 2010 and all were nested within the Cascadia – southwest Mackenzie – Alsek – Yukon rivers (CMAY) and Lake Superior (LS) ATPase VI lineage (clade 2), which differs from the southeast Alaskan lineage (clade 1) group by about 2.4% net sequence divergence (cf. Witt et al. 2011). The Winnange Lake fish ATPase VI sequences were identical to the haplotype from Lake Superior (H20) and this haplotype is 1.4% divergent (K2P distance) from the most distinct other haplotype (H1) from within the CMAY–LS clade of pygmy whitefish resolved by Witt et al. (2011). The six samples assayed at the ITS-1 locus were all positively identified as pygmy whitefish with >97% sequence identity to accessions within the GenBank database (Table 2). For each of COI, ATPase VI, and ITS-1, all $E$ scores were 0 and any variation from database sequences of pygmy whitefish consisted of single nucleotide substitutions or occasional sequence ambiguities.

Although our sampling effort was concentrated on a single system, the presence of pygmy whitefish beyond Winnange Lake is a distinct possibility. Specifically, we note that several lakes containing lake trout, a glacial relict species that requires similar cold-water habitat, are directly upstream and downstream of Winnange Lake. However, it is interesting to note that in a survey of deepwater sculpin that included five lakes in the ELA region that were in close proximity to Winnange Lake (Fig. 1B), as well as nearby Lake of the Woods, no pygmy whitefish were discovered (Sheldon et al. 2008). The deepwater sculpin study used gear types similar to those that have captured pygmy whitefish in other locales. Specifically, small-mesh gill nets, similar to our study on Winnange Lake, and deep-water trawls, similar to the surveys in Lake Superior (Eschmeyer and Bailey 1955), successfully captured deepwater sculpin in nearby lakes. It is possible that pygmy whitefish are not found as deep as deepwater sculpin, but given that pygmy whitefish are known to occupy depths to 90 m in Lake Superior (Eschmeyer and Bailey 1955), it seems probable that their presence would be detected in this survey of deep, cold, oligotrophic lakes. Nonetheless, these and several similar deep lakes are in close proximity to Winnange Lake, and therefore future sampling of these systems would be beneficial to determine whether the distribution of pygmy whitefish is more widespread than pres-

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ent sampling indicates, especially given that in other systems distinct populations of pygmy whitefish occur among interconnected lakes (Taylor et al. 2011).

Although smaller overall, the North American geographic range of pygmy whitefish is similar to other cold-water salmonid fishes such as the Arctic grayling (*Thymallus arcticus* (Pallas, 1776)) and its eastern North American distribution like that of the cisco. The Arctic grayling has an extensive distribution in northwestern Canada and Alaska, a southern disjunct assemblage of populations in northwestern Montana, and previously included a now extinct set of populations in northwestern Michigan (Scott and Crossman 1973). The cisco has a range that includes Lake Superior and extends to the northwest among a myriad of lakes into the Northwest Territories (Scott and Crossman 1973). The distribution of cisco was likely achieved via postglacial lakes and rivers that covered these areas following the Wisconsinan glaciation, similar to the pygmy whitefish. The main difference is that pygmy whitefish are now found in an apparently smaller subset of lakes, perhaps owing to poorer dispersal or competitive abilities or more stringent habitat requirements.

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) will evaluate the status of pygmy whitefish in Canada in the near future. Such assessments use quantitative criteria developed by the International Union for Conservation of Nature and Natural Resources (IUCN) related to geographic distribution, population sizes, abundance trends, and threats to the species to assess its status (IUCN 2012). Two of the criteria used in such assessments involve thresholds for measures of size of the geographic range: the extent of occurrence (EO) and the index of area of occupancy (IAO). Given that pygmy whitefish have highly disjunct eastern and western distributions and comprise two highly divergent phylogeographic lineages (Witt et al. 2011), the species will likely be assessed as two distinct designatable units (DUs), which are intraspecific groupings that are assessed as wildlife species under Canada's *Species at Risk Act* (COSEWIC 2009). The discovery of pygmy whitefish in Winnange Lake significantly increases both EO and IAO of a putative eastern DU and illustrates how a better understanding of the natural range will be an important contribution to effective assessment of this species.

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