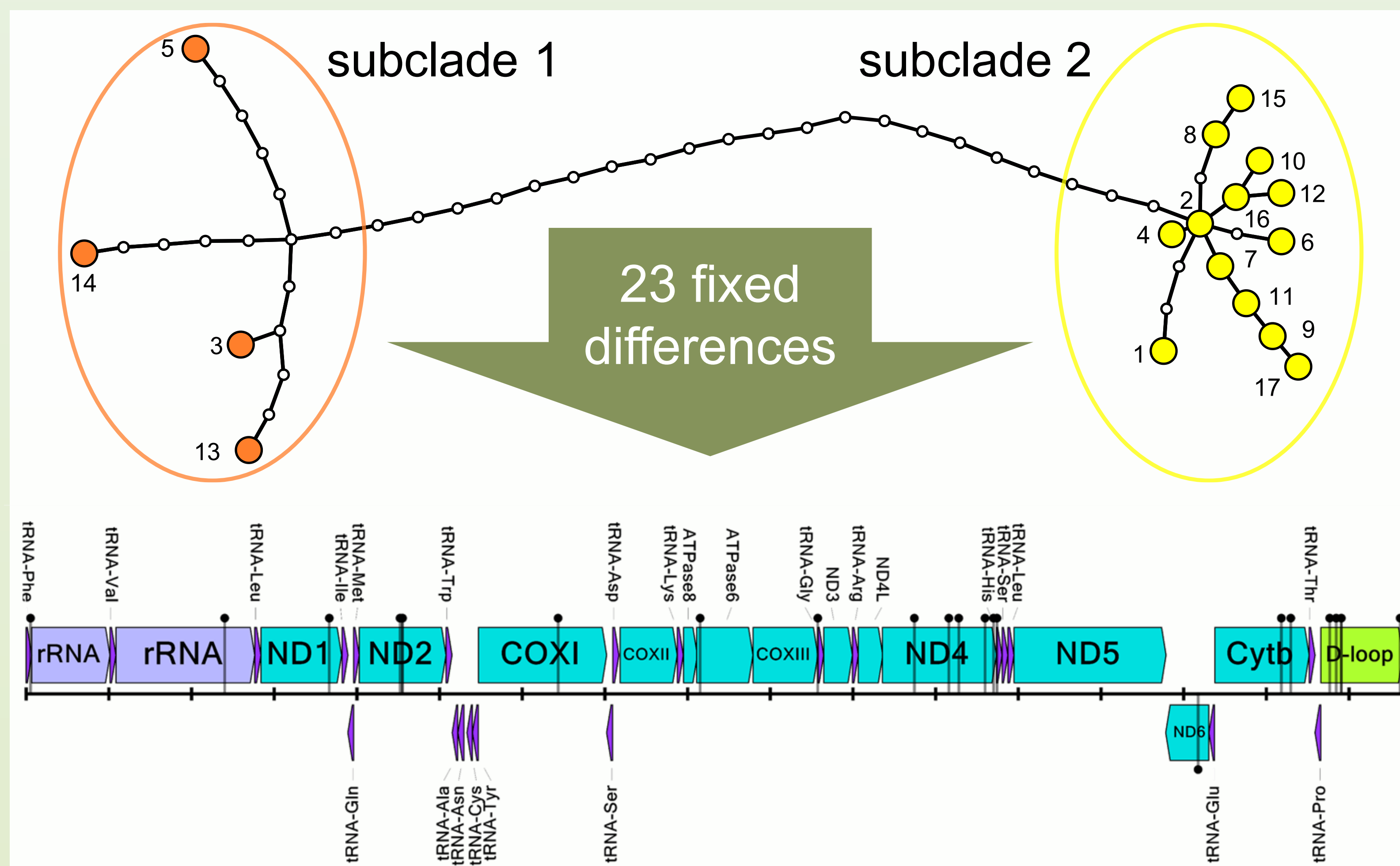


## 1 Introduction

- Mitogenome variation can covary with performance and is a taxonomic tool allowing reconstructing of population histories.
- North Atlantic Arctic charr whole mitogenome variation has revealed an Arctic and an Atlantic clade, the latter containing two subclades that may have diverged 60,000 years ago<sup>1</sup>.
- Previous haplotype occurrences indicate that eastern Greenland harbours subclades 1 and 2, but western Greenland, Norway, and Iceland only subclade 2 (Fig. 1)<sup>1</sup>.
- Can we expect only one Atlantic subclade in Iceland?

## 2 Methods

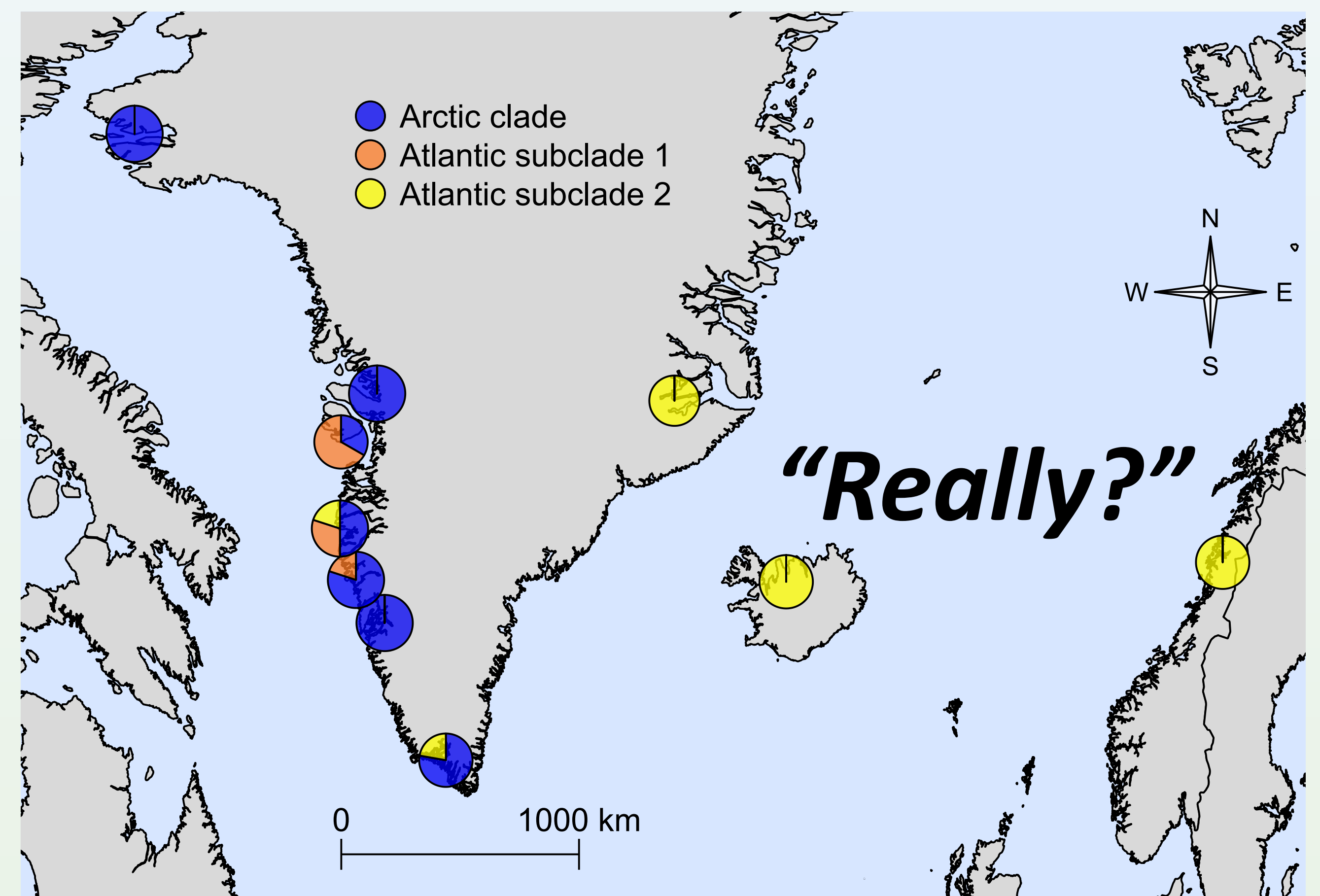
- *PacBio HiFi* whole-genome sequencing of a single Icelandic Arctic charr female followed by mitogenome assembly using *MitoHiFi*<sup>2</sup> and annotation using *MitoAnnotator*<sup>3</sup>.
- Additional mitogenome assemblies from whole-genome short-read sequences of 1,001 pedigreed individuals from the breeding programme using *GetOrganelle*<sup>4</sup>.
- Pedigree-based reconstruction of haplotypes for breeding programme wild-origin founder females.



**Fig. 2:** Estimated haplotype network for Icelandic breeding programme founders and the linearized mitogenome with physical location for 23 variants, indicated as pins, that are fixed between Atlantic subclade 1 and 2.

## 4 Discussion

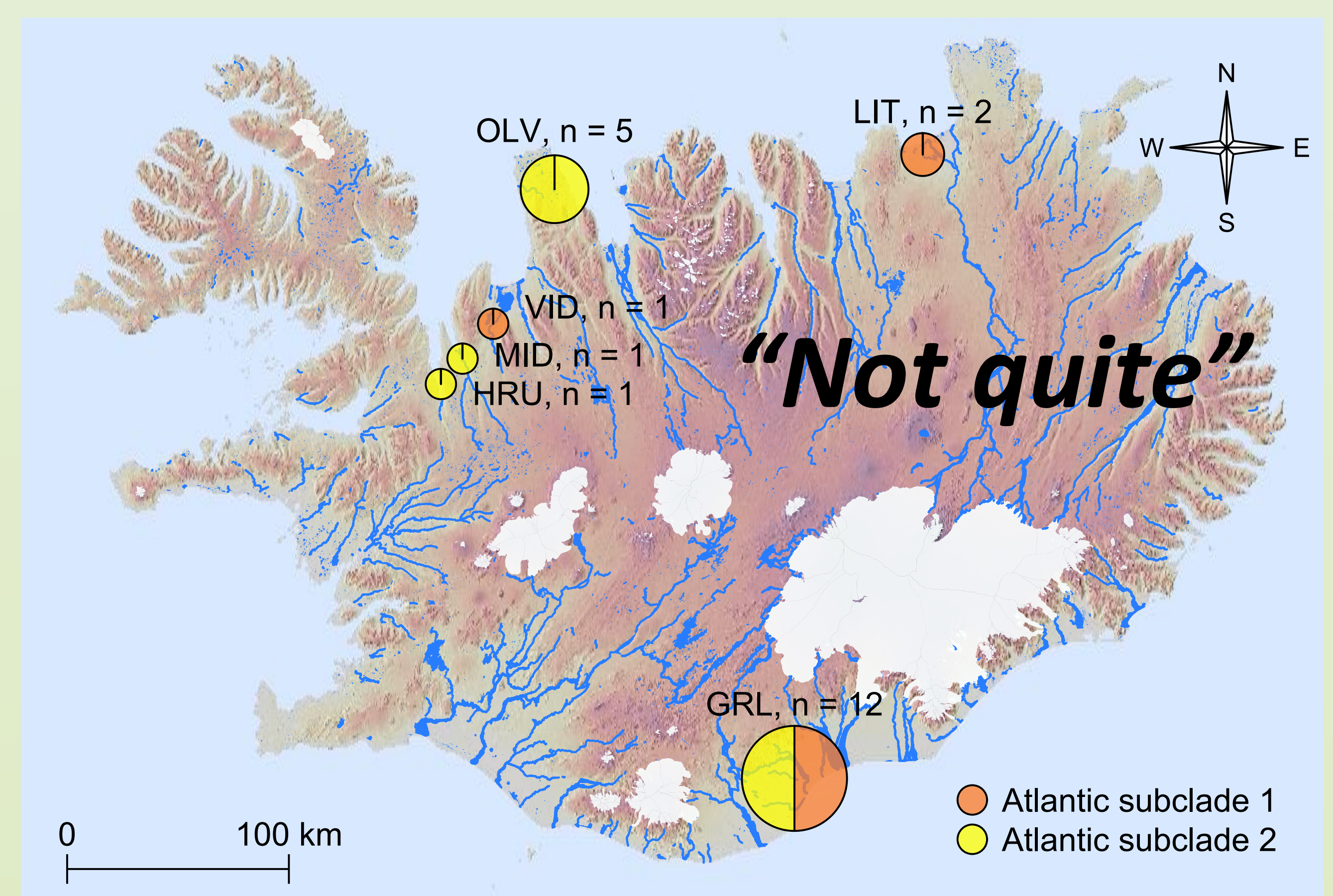
- Atlantic whole mitochondrial subclades diverge genetically, but since most of these are silent mutations in protein-coding regions, equal performance is expected.
- However, the effects of variants in non-coding regions remain to be evaluated.
- The presence of two divergent subclades suggests that they may have originated from two different glacial refugia.
- All populations in the current and previous studies lacking subclade 1 are landlocked.
- Mixed origin appears more likely for anadromous than for landlocked populations.
- Small sample sizes limit inferences about the relationship between subclade identity and colonisation history.
- If the subclades originated from two refugia, nuclear genetic diversity can be expected to be higher in populations of mixed origin.



**Fig. 1:** Arctic and Atlantic clades with the two Atlantic subclade haplotype distribution recovered by Jacobsen *et al.*<sup>1</sup> including the Icelandic Vatnshlíðarvatn landlocked lake population. The Icelandic and Norwegian populations are from landlocked lakes; all others are anadromous. Sample sizes (n) per population are 8-10 fish. Redrawn from Jacobsen *et al.*<sup>1</sup>.

## 3 Results

- We assembled a 16,654 bp mitogenome with the typical features of fish mitogenomes (Fig. 2).
- We assigned the 1,001 Icelandic samples to 17 haplotypes linked to 22 founder females, categorised as either Atlantic subclade 1 or 2 (Fig. 2).
- Although the subclades differ by 23 variants, the 14 variants in protein-coding regions do not alter amino acid sequences (silent mutations).
- As in a previous study<sup>1</sup>, subclade 1 was more prevalent in anadromous populations (Figs. 1, 3).



**Fig. 3:** Inferred Atlantic subclade distribution of some of the founder females of the Icelandic Arctic charr breeding programme. The population abbreviations stand for GRL – Grenlækur, HRU – Hrótafjörður, LIT – Litlaá, MID – Miðfjarðará, OLV – Ölfesvatn, VID – Víðidalá. Only OLV is a landlocked lake population, all others are anadromous.

- 1 Jacobsen, M. W., N. W. Jensen, R. Nygaard, K. Præbel, B. Jónsson *et al.*, 2021 A melting pot in the Arctic: Analysis of mitogenome variation in Arctic char (*Salvelinus alpinus*) reveals a 1000-km contact zone between highly divergent lineages. *Ecology of Freshwater Fish* 31: 330-346.
- 2 Uliano-Silva, M., J. Ferreira, K. Krasheninnikova, C. Darwin Tree of Life, G. Formenti *et al.*, 2023 MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads. *BMC Bioinformatics* 24: 288.
- 3 Zhu, T., Y. Sato, T. Sado, M. Miya and W. Iwasaki, 2023 MitoFish, MitoAnnotator, and MiFish Pipeline: Updates in 10 Years. *Molecular Biology and Evolution* 40: msad035.
- 4 Jin, J. J., W. B. Yu, J. B. Yang, Y. Song, C. W. Depamphilis *et al.*, 2020 GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology* 21: 241.

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