Identification of Knotweed taxa through DNA analysis: a study to determine the dispersal methods of highly invasive knotweed species.

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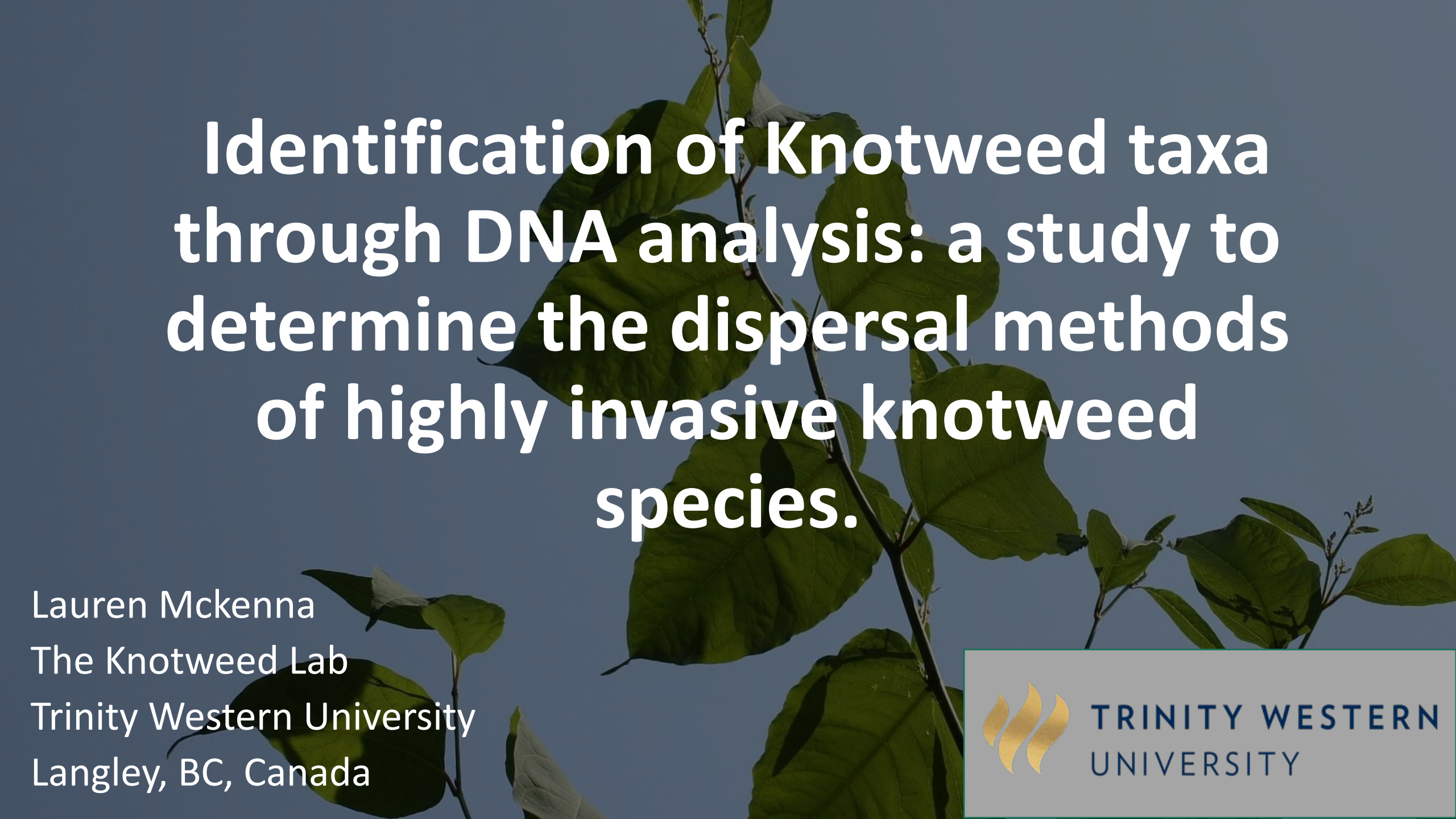
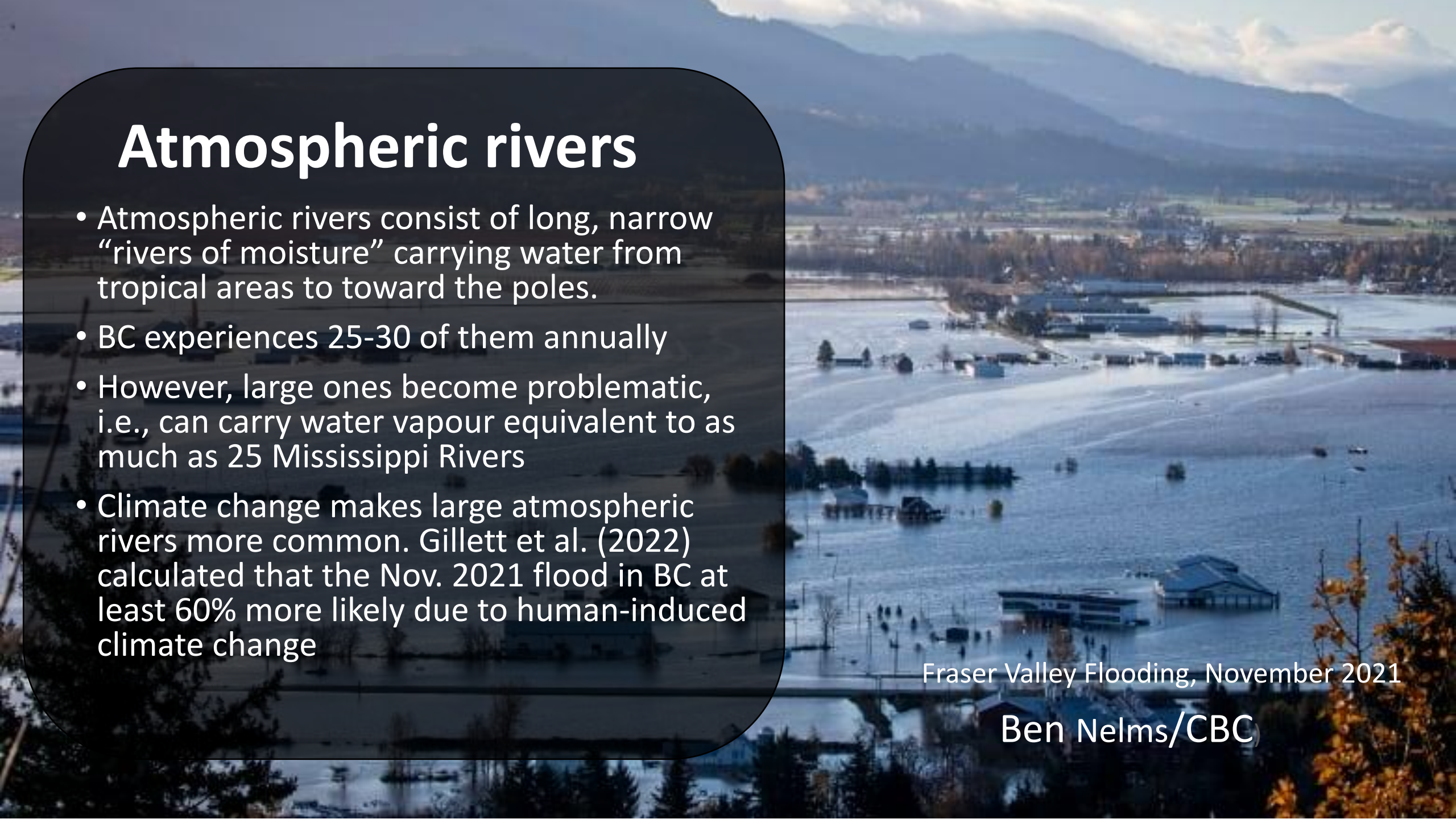


Atmospheric rivers

- Atmospheric rivers consist of long, narrow “rivers of moisture” carrying water from tropical areas to toward the poles.
- BC experiences 25-30 of them annually
- However, large ones become problematic, i.e., can carry water vapour equivalent to as much as 25 Mississippi Rivers
- Climate change makes large atmospheric rivers more common. Gillett et al. (2022) calculated that the Nov. 2021 flood in BC at least 60% more likely due to human-induced climate change



Fraser Valley Flooding, November 2021

Ben Nelms/CBC

Chilliwack-Vedder River November 2021

Sarah D. 2023

Chilliwack-Vedder River 2021 & 2022

Sarah D. 202

Clonal Growth and Spread by Knotweed spp.

Japanese knotweed was introduced to North America in the 1870's for ornamentation and is native to China, Korea and Japan. first known locations of knotweed in Canada include Ontario, Quebec, and British Columbia specifically in Chilliwack. However what was sold was the female clone of Japanese knotweed! Therefore the spread of knotweed at first was solely due to asexual spread like rhizomes.

(Grimsby et al. 2007; Duquette et al. 2015)


Giant Knotweed

The Path to Seed Production and Introgression

Japanese knotweed

Reynoutria japonica

Highly invasive

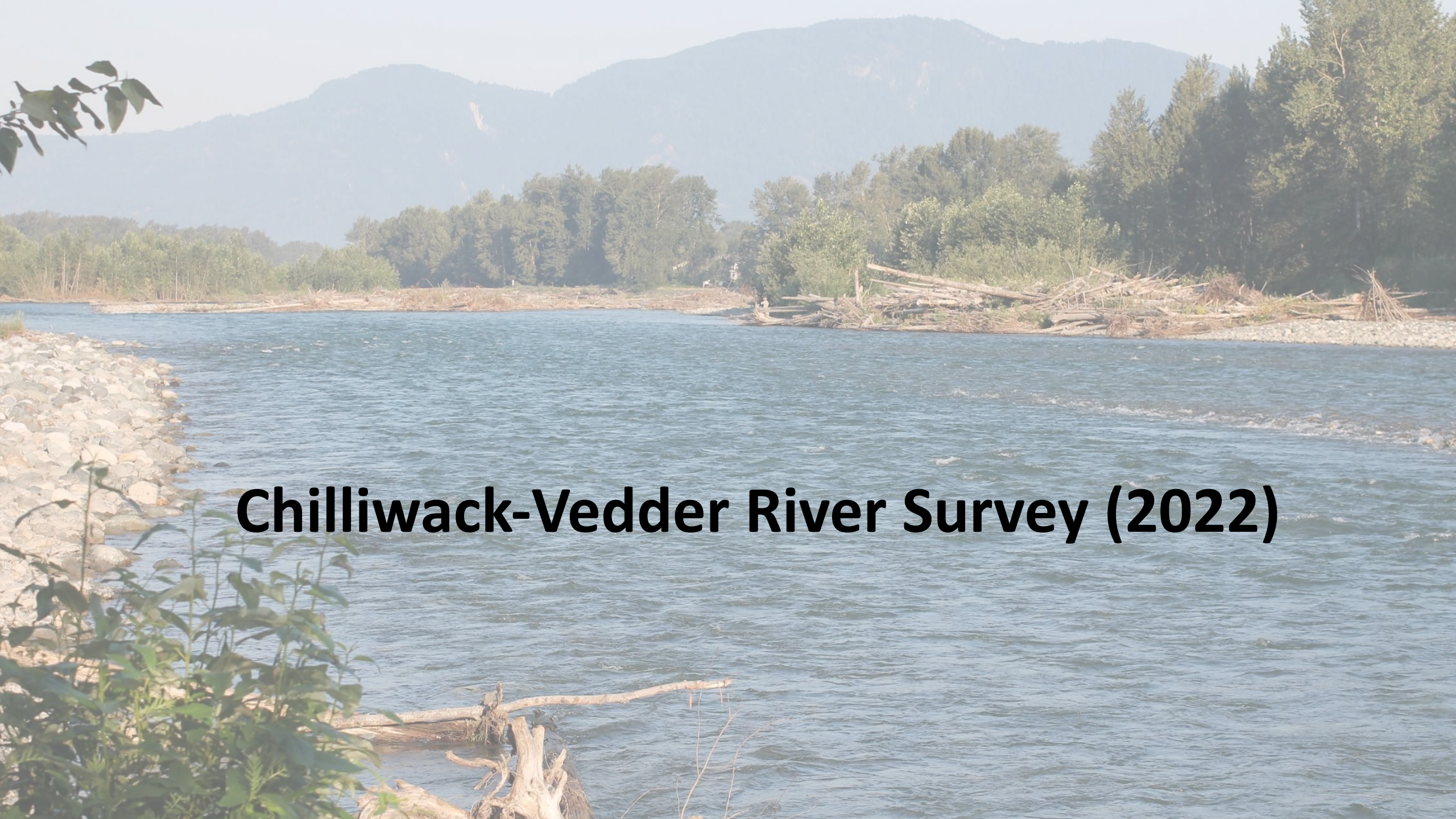
X

Giant knotweed

Reynoutria sachalinensis

Less invasive than Japanese

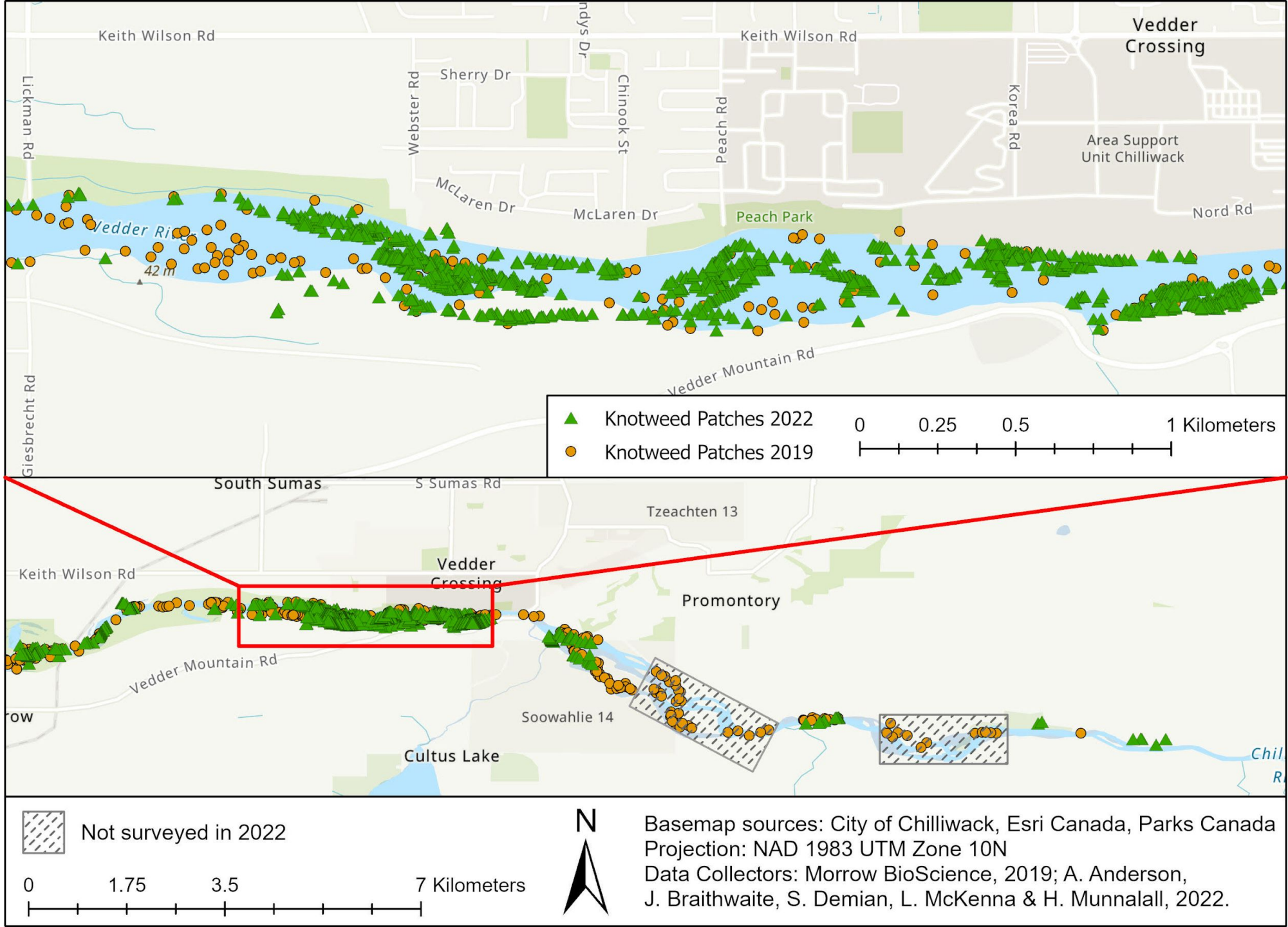
Bohemian knotweed through hybridization *Reynoutria ×Bohemica*



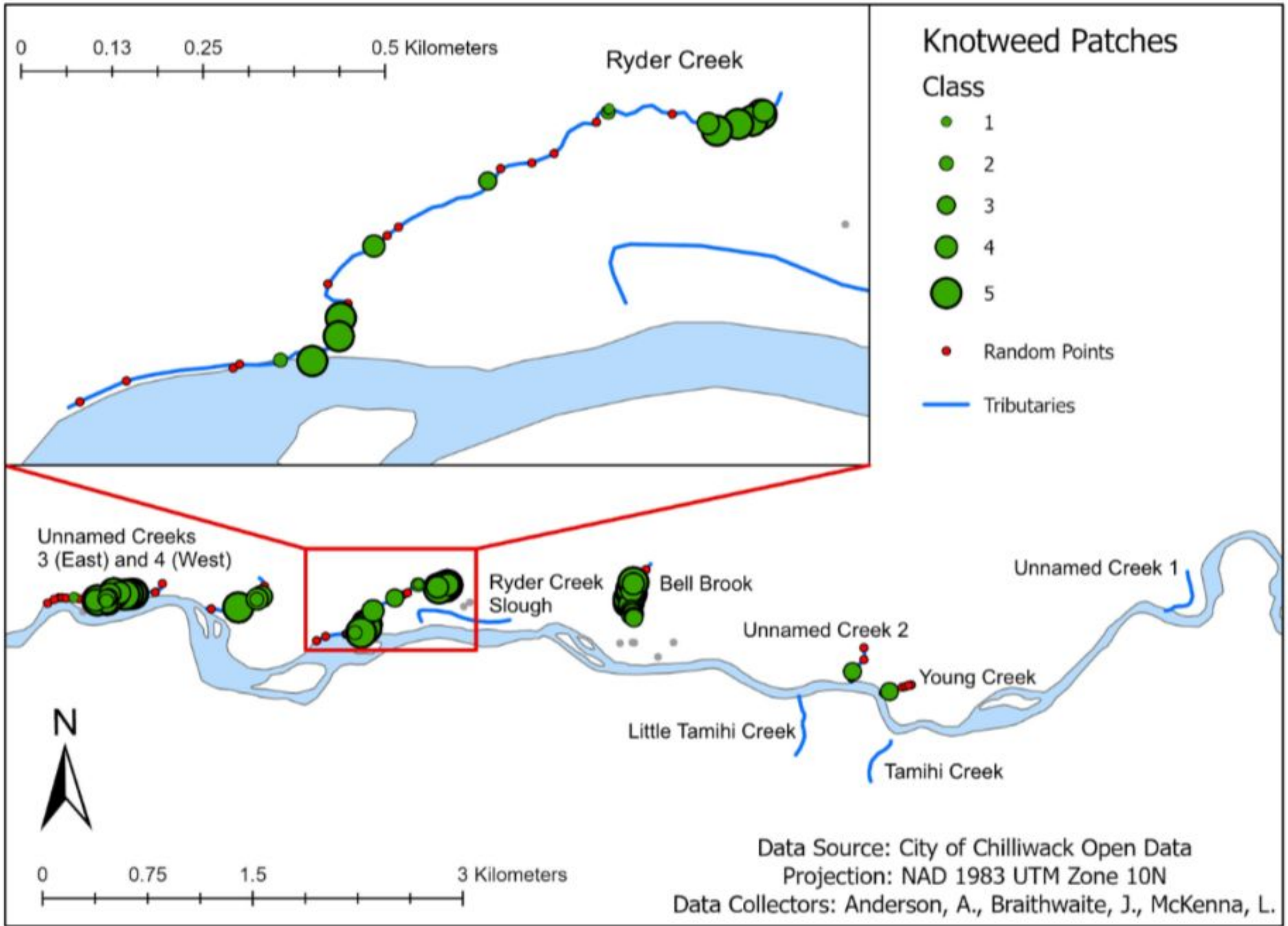
Chilliwack-Vedder River Survey (2022)

Knotweed patches surveyed along the Chilliwack-Vedder River

1690 patches in 2022 vs.
341 patches in 2019



Knotweed patches surveyed along the Chilliwack-Vedder River Tributaires



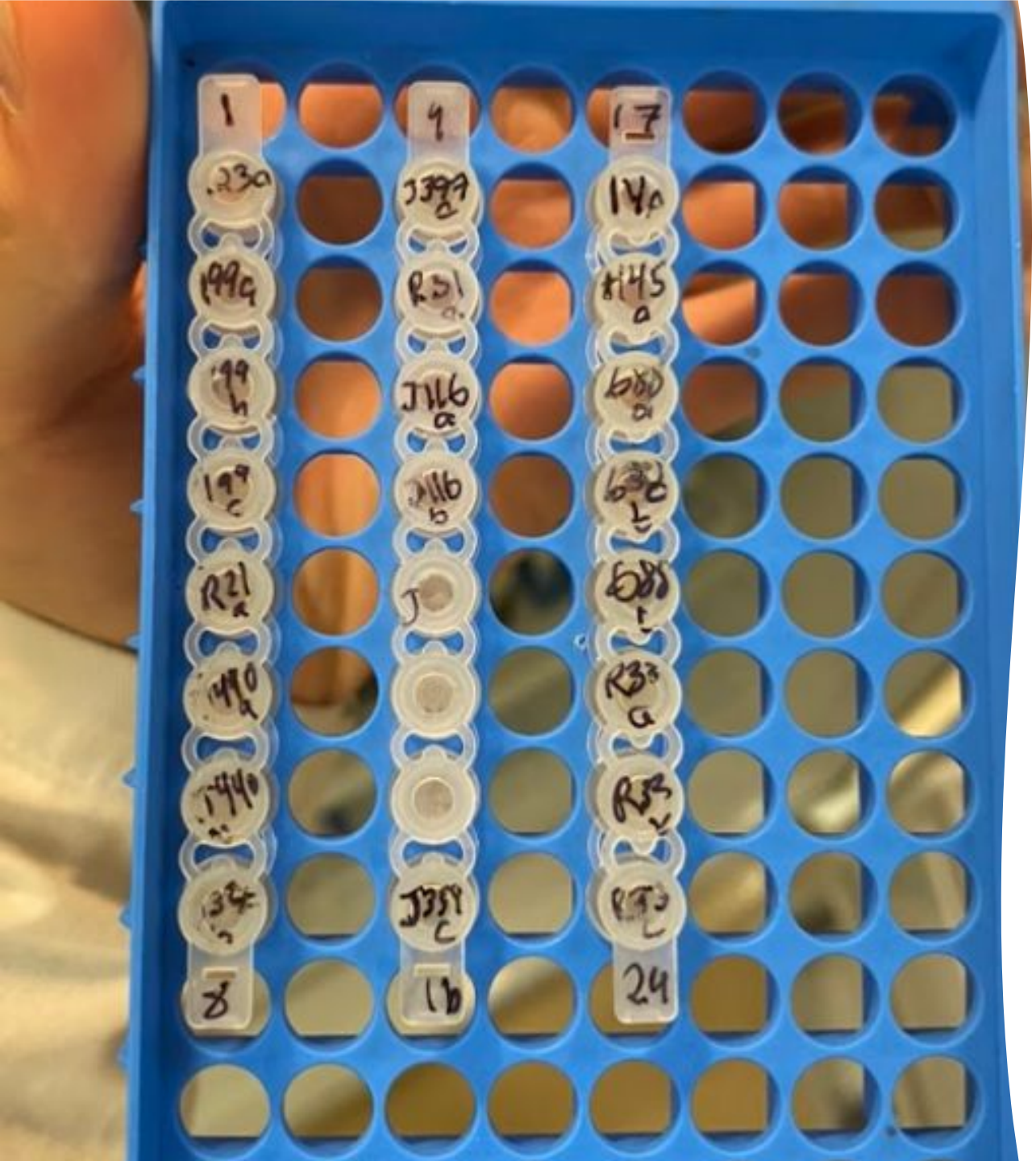
Leave Collection + Storage

For collection, all clones among the river were sampled and a few will be selected for further processing. Leaves will be selected as close to the middle of the clone as possible at equidistant locations. Samples will be stored in zip-lock bags with Activa silica gel until further DNA analysis. All samples collected will be stored in the fridge between the collection date and the analysis

DNA Analysis

Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit 2016.

DreamTaq Hot Start PCR MasterMix for RbcL gene



Why use cpDNA RbcL ?

The rbcl gene, ribulose-1,5-bisphosphate carboxylase/ oxygenase large subunit, was chosen due to its popularity for sequencing plant taxa. The rbcl gene has been found to be successful with inferring at a lower taxonomic level, such as intra- and inter-generically (Gielly & Taberlet, 1994).

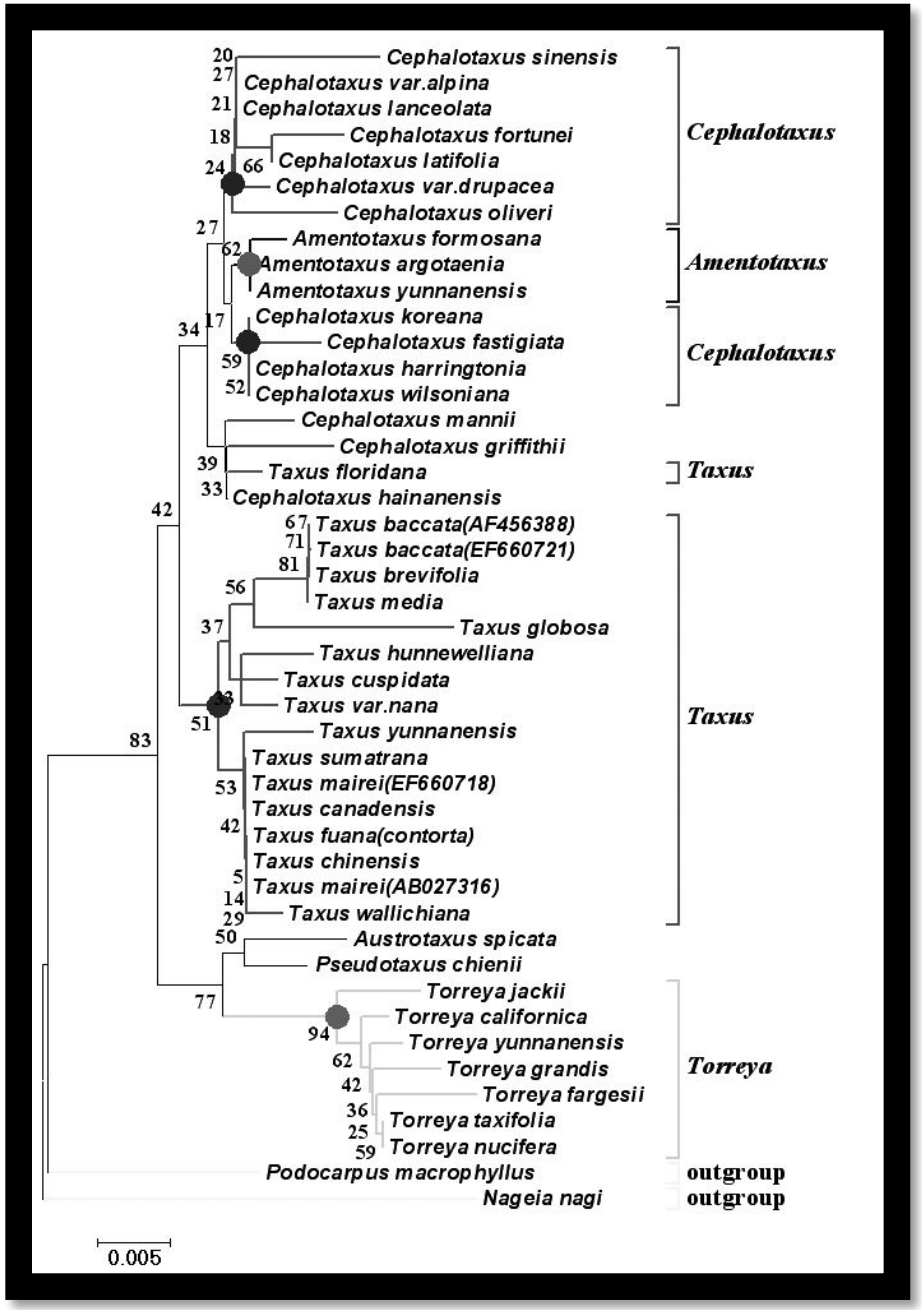
Cpdna is used to determine the species as cpdna is inherited from the female in the majority of angiosperms, all hybrids should contain the cpdna haplotype of the female parent.

DNA Analysis

PCR’ed product will be sent to UBC to be genetically sequenced. To confirm that PCR was successful, gel electrophoresis is done to confirm that the RbcL sequence was successfully cut.

Results

Results will be displayed using a phylogenetic tree to show the relatedness of certain stands along the river.



Example of a Phylogenetic tree

Hao, Da-Cheng & Mu, Jun & Xiao, G.P.. (2010). Molecular evolution and positive Darwinian selection of the gymnosperm photosynthetic Rubisco enzyme. Botanical Studies. 51. 491-510.

Objective 1

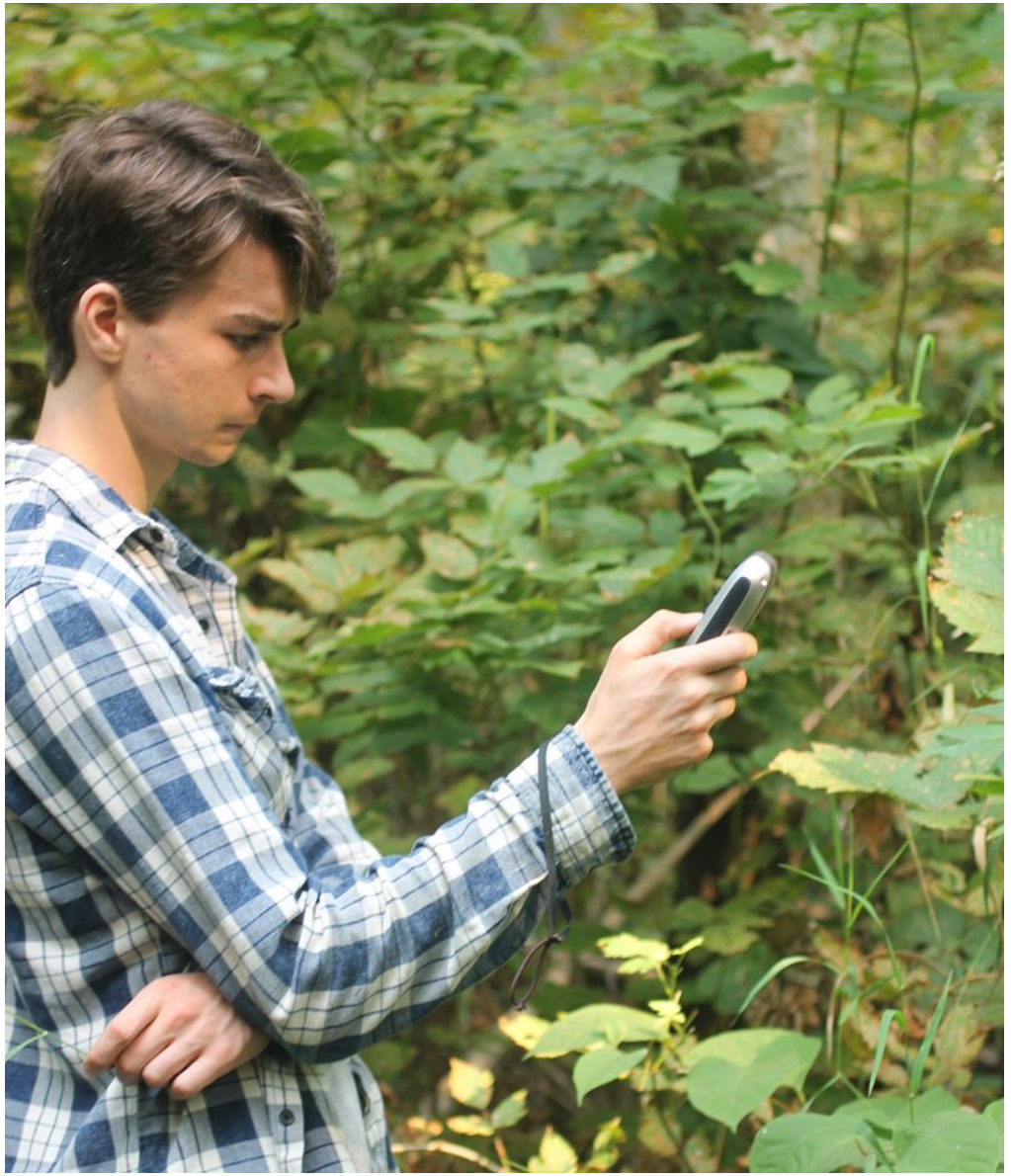
To determine the percentage of Japanese knotweed (Reynoutria japonica), Giant knotweed (R. sachalinesis, Bohemian knotweed (R.x bohemica) and backcrossed bohemian knotweed and Japanese knotweed. Using DNA analysis, we can determine the ratio of species found among the Chilliwack River and identify the relative success of various genetic forms.

Objective 2

To determine which knotweed clones are related in order to determine the methods of dispersal. Using DNA analysis, we can determine the relatedness of clones among the knotweed forms present to determine whether they travelled downriver. The analysis would also help us see whether the November 2021 flooding in Chilliwack increased the dispersal rate of individual clones and whether the mode of distribution was asexual or sexual.

Objective 3

To determine whether tributaries which flow into the Chilliwack river are a major source of spreading knotweed. By taking DNA samples of all knotweed stands in tributaries and comparing the gene sequence from the rcbL gene to stands downriver we can determine if any are clones of the tributary stands.



Timeline

June 29th 2022	6.5 inch x 5.9 inch zip-lock bags filled with 50ml of Activa silica gel and labelled with appropriate clone numbers (1-a, 1-b, 1-c, 2-a, 2-b …)
September 2022 – June 2024	DNA will be extracted using DNeasy plant kit in Trinity Western University lab. DNA extracted will be stored in the fridges until used for PCR tests. PCR will be completed by June 2024
June 2024-December 2025	All PCR products will be sent to be analyzed and data analysis will be done.

Significance

The significance of this study is that by using DNA analysis we can determine how far individual clones have travelled along the Chilliwack River. This will allow us to show the negative impacts of the 2021 Chilliwack flooding and the severity of needing to act against this invasive species.

Acknowledgements

The Knotweed Lab

TRINITY WESTERN UNIVERSITY

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FRASER VALLEY INVASIVE SPECIES SOCIETY


Questions?

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