TERRESTRIAL ARTHROPOD MONITORING PROGRAM

METABARCODING REPORT

Collections Unit, Centre for Biodiversity Genomics (CBG), University of Guelph

Field Collection

In 2021, the Terrestrial Arthropod Monitoring Program continued after a hiatus in 2020 due to the COVID-19 pandemic. Malaise traps were deployed in 55 sites in Ontario and Quebec; 52 were in the Boreal Shield ecoregion while 3 were repeated sites in the Mixedwood Plains ecoregion (Supplementary File Tab 1, Figure 1, Table 1). Traps collected samples for 20 weeks from 10-May-21 to 11-Oct-21 with permits and research approvals from over 15 different authorities (Supplementary File Tab 2). Photos were taken of each trap during deployment.

All traps were serviced bi-weekly; traps in northern Ontario were on a 2-week rotating schedule and managed by CBG, while Quebec sites and ON-Red Lake were serviced independently. A grand total of 532 samples were collected over 10 bi-weekly cycles. A summary of all collected samples including collecting dates, weights, and analysis comments is provided in Supplementary File Tab 3.

For more details on field sites and activities, see the following Field Reports.

Ontario Parks (Boreal Shield only)	Field Report	Trap Images
Sépaq	<u>Field Report</u>	Trap Images
Parks Canada	<u>Field Report</u>	
Great Lakes Forestry Centre	<u>Field Report</u>	
Arkell Research Farm	<u>Field Report</u>	
Ojibway Prairie Provincial Nature Reserve	<u>Field Report</u>	
rare Research Reserve	Field Report	

Analysis

Samples were processed through the CBG's standard metabarcoding or "bulk analysis" protocol. In short, the bulk samples were assembled into batches of 30 for tissue lysis at the Canadian Centre for DNA Barcoding (CCDB; http://ccdb.ca/). Three replicates for each sample lysate then underwent bulk DNA extraction. PCR amplification of the DNA barcode region was performed on three pooled replicates, followed by library preparation for high-throughput sequencing. Libraries were then submitted to The Centre for Applied Genomics (TCAG) in Toronto for sequencing on an Illumina NovaSeq platform.

This report outlines the metabarcoding results obtained from 520 samples; 12 samples were excluded from analysis. All sequencing results were uploaded to the online mBrave platform (https://mbrave.net/) and all runs were uploaded to the mBrave project: MBR-TRACE2021, "Tracking Response of Arthropod Communities to Changing Environment (TRACE) - 2021 field season".

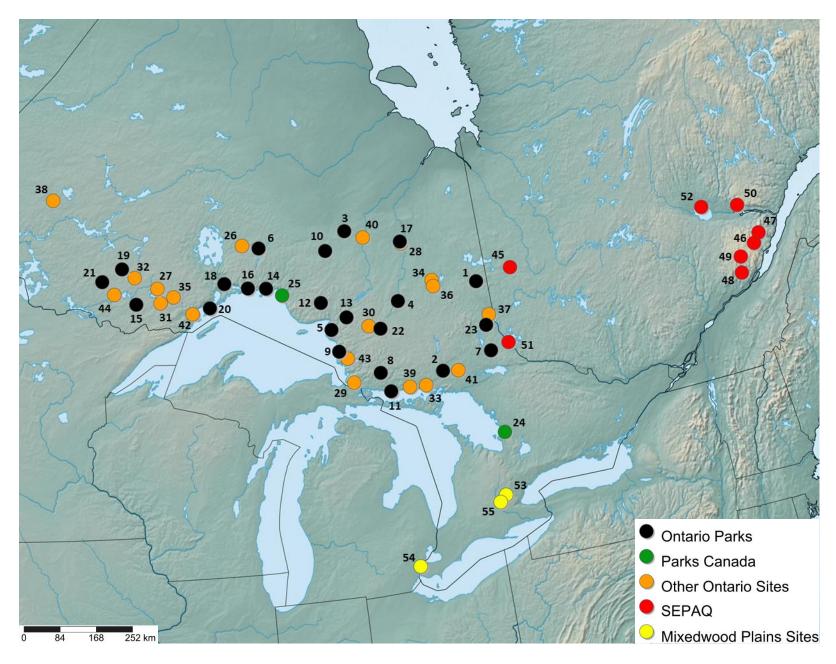


Figure 1. Complete map of trap sites. Colour of circles correspond to locality type and numbers correspond to each site in Table 1.

Table 1. Legend corresponding to site numbers and site type in Figure 1 map.

Site Number	Code	Full Site Name
		Boreal Shield - Ontario Parks
1	ESKL	Esker Lakes Provincial Park
2	FAIR	Fairbank Provincial Park
3	FUSL	Fushimi Lake Provincial Park
4	IVAN	Ivanhoe Lake Provincial Park
5	LSUP	Lake Superior Provincial Park
6	MCLD	MacLeod Provincial Park
7	MART	Marten River Provincial Park
8	MSSG	Mississagi River Provincial Nature Reserve
9	MTLR	Montreal River Provincial Park
10	NGGM	Nagagamisis Provincial Park
11	NCHI	North Channel Inshore Provincial Nature Reserve
12	OBAT	Obatanga Provincial Nature Reserve
13	POTH	Potholes Provincial Park
14	PRRM	Prairie River Mouth Provincial Nature Reserve
15	QUET	Quetico Provincial Park
16	RAIN	Rainbow Falls Provincial Park
17	RENB	René Brunelle Provincial Park
18	RUBY	Ruby Lake Provincial Nature Reserve
19	SAND	Sandbar Lake Provincial Park
20	SLPG	Sleeping Giant Provincial Park
21	TURT	Turtle River-White Otter Lake Provincial Nature Reserve
22	WNBG	Wenebegon River Provincial Nature Reserve
23	WJBG	WJB Greenwood Provincial Nature Reserve
		Boreal Shield - Parks Canada
24	GBI	Georgian Bay Islands National Park
25	PUK	Pukaskwa National Park
		Boreal Shield - Other Ontario Sites
26	ATGG	Atigogama Jack Pine Progeny Test
27	CBNL	Cabin Lake
28	EDWB	Edward Bonner Tree Improvement Centre
29	GLFC	Great Lakes Forestry Centre
30	ISLL	Island Lake Research Site
31	KSHB	Kashabowie
32	MRTN	Martin Jack Pine Progeny Test
33	MASS	Massey Private Farm
34	MRCA	Mattagami Region Conservation Authority Office
35	NBTB	NEBIE plot - Thunder Bay
36	NBTM	NEBIE plot - Timmins
37	NLSK	New Liskeard Agricultural Research Station
38	REDL	Red Lake District High School
39	SERP	Serpent River
40	SHNL	Shannon Lake
41	SUDB	Sudbury Ministry of Natural Resources Office
42	TBMN	Thunder Bay Ministry of Natural Resources Office
43	TRKL	Turkey Lakes Research Site
44	WLML	Williamson Lake Black Spruce Family Test
45	04.00	Boreal Shield - SEPAQ
45	QAGB	Parc national d'Aiguebelle
46	QJCA	Parc national des Grands-Jardins
47	QJCB	Parc national de la Jacques Cartier 1
48	QPTL	Parc national de la Jacques-Cartier 1
49 50	QGJD	Parc national de la Jacques-Cartier 2
50 51	QHTG	Parc national des Monts-Valin
51	QMVL	Parc national d'Opémican
52	QOPM	Parc national de la Pointe-Taillon
EO	VDNI	Mixedwood Plains Sites Arkell Poscarch Station
53 54	ARKL OJIB	Arkell Research Station Oilbway Prairie Provincial Nature Reserve
5 4 55	RARE	Ojibway Prairie Provincial Nature Reserve rare Charitable Research Reserve
33	NAME	TUTE CHATTERNIE NESERICH NESERVE

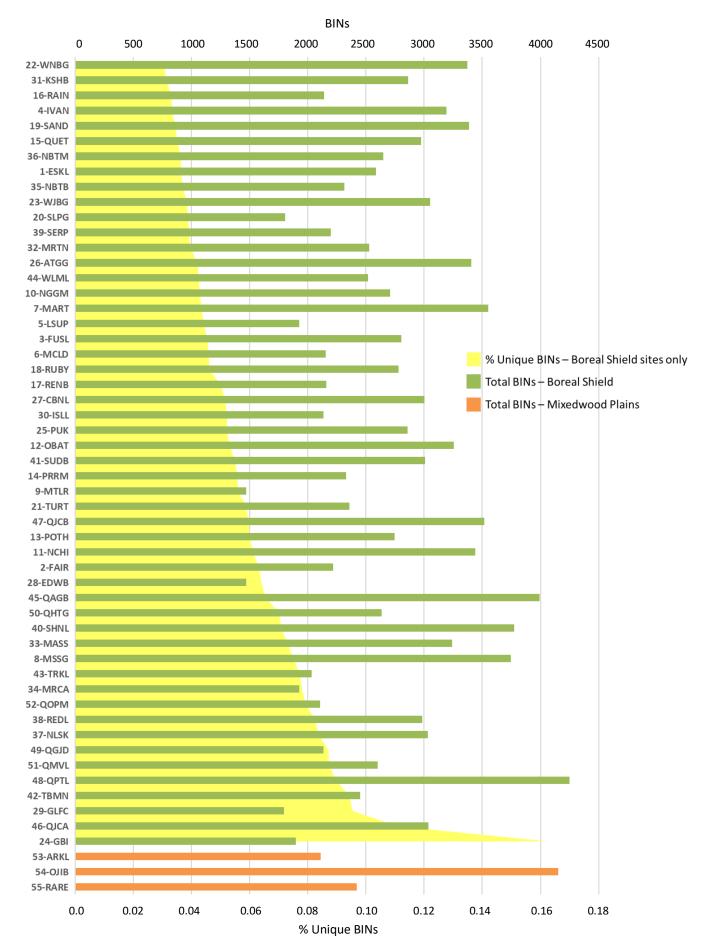


Figure 2. Total BINs of all sites and proportion of BINs unique to each Boreal Shield site.

Results

Approximately 1M specimens were analyzed, from an estimate of 2,000 per sample. This resulted in 278,055 occurrence records and a total of 27,167 distinct BINs (Barcode Index Numbers, a proxy for species; Supplementary File Tab 4). The breakdown between both ecoregions is outlined in the table below.

Ecoregion	Samples	Occurrence records	BINs
Boreal Shield	490	259061	24815
Mixedwood Plains	30	18994	6566

ON-Montreal River PP had the lowest total BIN count with 1467 BINs, while QC - Jacques-Cartier Site 1 obtained the highest total BIN count of 4251. The average number of BINs captured per trap throughout the whole season was 2712 BINs (Figure 2, Supplementary File Tab 5). Looking at only the Boreal Shield sites, 33.3% of BINs were only captured in one trap, approximately 159 BINs per trap on average or 5.9% of its BINs. Jacques-Cartier 1 had the highest number of unique BINs (382) while Georgian Bay Islands NP had the highest proportion of unique BINs (16%).

Biomass ranged from a low of 2.02g (ON-MacLeod PP|I) to a high of 138.75g (ON-Island Lake RS|D). One sample (ON-MRCA Office|C) appears to be a major outlier with a wet weight of 81.6g but only 9 BINs retrieved (Figure 3).

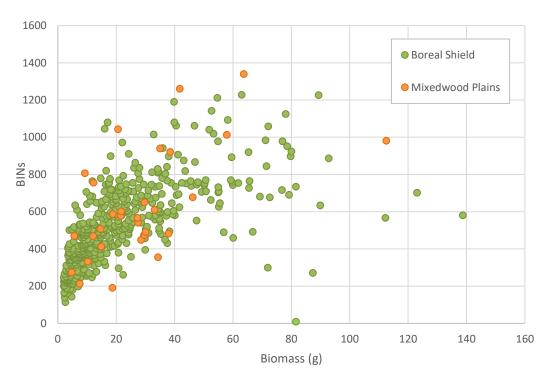


Figure 3. Total distinct BINs captured in each sample compared against its biomass measurement (wet weight, g). One Mixedwood Plains sample appears to be an outlier weighing 81.6g but only 9 BINs were recovered.

Excluding the outlier, BIN counts per cycle ranged from a low of 114 (ON-Edward Bonner TIC|I) to a high of 1340 (Ojibway|D), with a mean of 521. The sample with the highest BIN count in the Boreal Shield ecoregion was QC-Jacques-Cartier 1|C (N=1228).

Half of BINs collected were Diptera (50.6%), followed by Hymenoptera (22.9%), Lepidoptera (8.4%), Coleoptera (5.6%), and Hemiptera (4.5%; Figure 4). A taxonomy reference for all BINs captured is provided in Supplementary File Tab 6 and a species inventory list can be found in Tab 7.

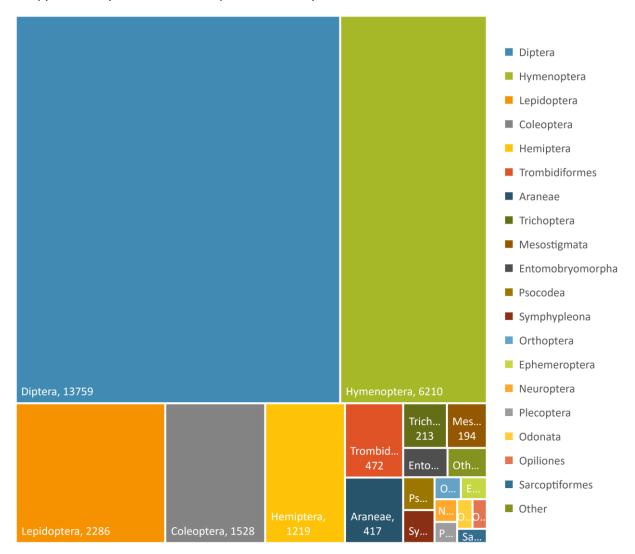


Figure 4. Taxonomic breakdown of BINs collected from all 55 traps.

When combined with the Mixedwood Plains 2018 and Boreal Shield 2020 metabarcoding results, a grand total of 35,361 BINs was obtained with 40% being captured from both ecoregions (Figure 5). There was a total of 259K BIN occurrence records and 23,281 distinct BINs from the Mixedwood Plains. And 317K BIN occurrence records and 26,404 distinct BINs from the Boreal Shield (Figure 6). The 2021 sampling added 1073 BINs to the Mixedwood Plains ecoregion and 13,404 BINs to the Boreal Shield ecoregion (Figure 7).

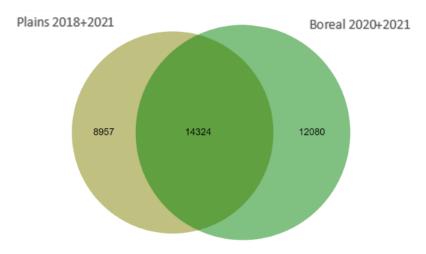


Figure 5. Total BIN count from terrestrial arthropod metabarcoding projects from 2018-2021 showing overlap between the Mixedwood Plains ecoregion and the Boreal Shield ecoregion.

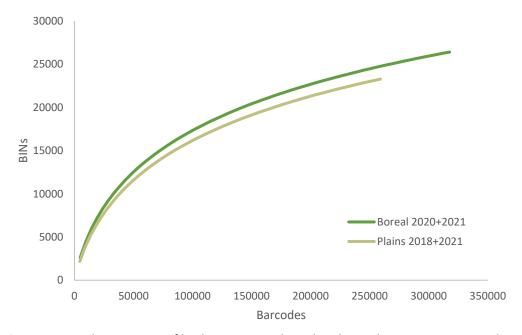


Figure 6. BIN accumulation curve of both ecoregions based on barcode occurrence records.

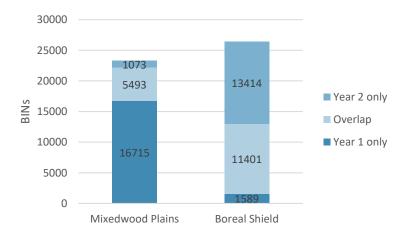


Figure 7. Overlap of BINs collected in two years of sampling the two separate ecoregions.

Supplementary File

- Tab 1. Trap site details including locality, habitat, and GPS coordinates
- Tab 2. Permit authority details for all collection sites
- Tab 3. Complete sample summary including collection dates and weight of analyzed sampled
- Tab 4. BIN Occurrence Records per Sample (Trap, Sample, BINs)
- **Tab 5.** Trap-Cycle summary (BINs per sample, total BINs and min, mean, max BINs per cycle)
- **Tab 6.** BIN Taxonomy Reference
- Tab 7. Taxonomy Report

Acknowledgements

CBG would like to thank the various staff of Ontario Parks (area supervisors, park superintendents, park ecologists), the Ontario Ministry of Natural Resources and Forestry, and Parks Canada for their support in this project – including logistical consultation and organizing appropriate accommodations for CBG field crew. We also appreciate the support and independent work conducted over the past few years by the Sépaq team, in Quebec, led by Benoit Dubeau. The Great Lakes Forestry Centre, and especially Amanda Roe, were also critical in this project as they served as CBG's remote base of operations. Finally, we'd like to thank our field technicians, Brenden Bertrand, Emily Kyle, and Lauren Janke, for their great work ethic and reliability despite many COVID-19 considerations.

LAND ACKNOWLEDGEMENT

All of Canada resides on traditional and current unceded and treaty lands of First Nations, Inuit, and Métis. We recognize that all our research occurs on Indigenous land. We are grateful to the Indigenous peoples for their care for and teachings about our earth and wish to honour their ongoing legacy.

The CBG and University of Guelph are situated on the treaty lands and territory of the Mississaugas of the Credit. The sampling work conducted for this project, mostly in Northern Ontario, occurred in 13 Indigenous territories that we would like to acknowledge: (in alphabetical order) the Abitibiwinni Aki, Anishinabewaki, Anishininiimowin (Oji-Cree), Attiwonderonk (Neutral), Cree, Haudenosaunee, Ililiwaskiy (Moose Cree), Mississauga, Myaamia, Nitassinan (Innu), Omàmìwininìwag (Algonquin), Peoria, and Wendake-Nionwentsïo.

Our acknowledgement of the land is our declaration of our collective responsibility to this place and its peoples' histories, rights, and presence. It is important to understand the longstanding history that has brought us to and across Turtle Island, and to seek to understand our place within that history.

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FOOD FROM THOUGHT