Expediting Impact Assessments with DNA Barcoding:

A Pilot Study at New Gold's New Afton Site

OVERVIEW

The Biodiversity Institute of Ontario (BIO) has partnered with New Gold Inc. to explore the potential of DNA barcoding for expediting environmental impact assessments. Mining operations must track the impact of their activities on biodiversity and demonstrate the effectiveness of their site restoration programs. This pilot project was conducted in New Gold's New Afton site near Kamloops, BC. It investigates the addition of mass arthropod sampling and DNA barcoding to evaluate the success of site remediation efforts.

Arthropods make up the majority of species in terrestrial habitats and it is critical to assess their responses to environmental changes. However, there is currently no systematic approach to rapidly quantify their baseline diversity or track shifts in response to environmental disturbance. Past efforts to include arthropods in terrestrial assessments have faced two serious barriers: ineffective sampling due to habitat complexities, and unreliable tools for species identification. The latter barrier has now been circumvented by DNA barcoding, a method that utilizes sequence variation in a standardized gene fragment to rapidly sort and objectively differentiate species (Hebert et al., 2003). This approach also makes it possible to carry out large-scale sampling programs and enables a time- and cost-efficient approach for biodiversity assessments.

New Afton's Biodiversity Conservation Management Plan focuses on protecting and improving biodiversity as part of the Mining Association of Canada's Toward Sustainable Mining Initiative. Current biodiversity initiatives at the New Afton site include grassland and wetland recovery projects. With the use of DNA barcoding technology, environmental impact assessments may be expedited comparing previously disturbed remediated sites to undisturbed environments. This will help to provide guidance and planning for biodiversity management at New Afton and other mining sites in the future.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B 270: 313-321.

METHODS

Sample Collection

Malaise traps are tent-like structures that are effective at capturing a variety of arthropod groups (e.g., insects, spiders, springtails) and are easily deployed and cost-effective. The trap design takes advantage of arthropods that once intercepted, fly upward towards light; the tent leads the organisms into a small bottle containing ethanol, which preserves them for DNA analysis. This technique is widely employed by BIO to collect a large number of specimens with minimal effort across Canada and globally in various Malaise Programs (i.e. School Malaise, Global Malaise, and Canadian National Park Malaise Trap Programs).

New Gold staff members maintain these traps by changing the collection bottles weekly. The samples are stored at -20°C and then shipped to BIO for analysis.

Four sites were chosen based on habitat type and proximity to environmental disturbances and restoration. Site 1 (treatment) is positioned at the edge of undisturbed grasslands and reclaimed wetland (2012), while Site 2 (treatment) is characterized by remediated Douglas Fir and Bluebunch/sage grassland (Figure 1). Site 3 (control) is situated adjacent to an open wetland and Site 4 (control) is Douglas Fir and Bluebunch/sage grassland that has recent impact from grazing (Figure 1).

Samples were collected weekly from June to September 2013, each site providing 16 samples, leading to 64 samples collected from the New Afton property in total. Eight bi-weekly samples from each of the four sites were chosen for DNA barcode analysis at BIO. DNA barcode analyses for samples from all four sites were completed in August 2014.

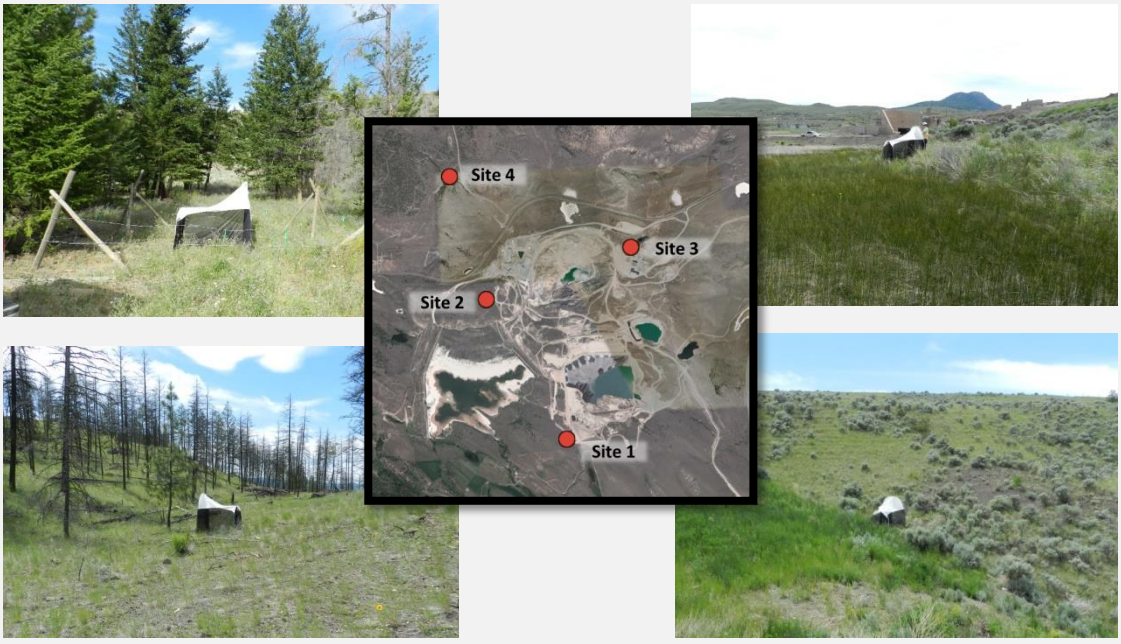

Figure 1. Malaise trap sites at the New Afton property showing the habitat at each site. Top Left: Site 4 disturbed grassland; Top Right: Site 3 disturbed wetland; Bottom Left: Site 2 protected grassland; Bottom Right: Site 1 protected wetland; Inset: Map of Malaise trap sites at New Afton.

Collections Unit

Specimens were initially analyzed with order level taxonomic assignment. Large-bodied specimens were pinned and stored as vouchers after a leg was removed for analysis. Small-bodied specimens were placed directly in 96-well microplates and were recovered after DNA extraction, then stored individually in ethanol. The tissue-filled plates then moved on to the molecular phase of analysis.


Laboratory

Standard barcoding protocols (http://ccdb.ca/resources.php) were followed to recover the barcode region of the cytochrome *c* oxidase I (COI) gene. DNA was first extracted from tissue samples by incubation of tissue plates in a lysis solution overnight. The DNA was then isolated using high throughput robotics, and polymerase chain reaction (PCR) exponentially amplified the target fragment of the isolated DNA. Lastly, using Sanger sequencing technology, the sequencing products were read via laser electrophoresis in a 3730xl DNA Analyzer which ultimately output a DNA sequence that can be interpreted by the human eye. The sequence outputs are termed "DNA barcodes". All collection data and sequence data were compiled into a specimen page and uploaded to the online Barcode of Life Database (BOLD: www.boldsystems.org).


Informatics

Next, the DNA barcodes were compared with existing records on BOLD to obtain species identifications. Barcoded specimens were assigned to an existing or new Barcode Index Number (BIN), a proxy for a formal Linnean species name, as outlined by Ratnasingham & Hebert (2013). Identifications were assigned by the BOLD-ID Engine where possible, allowing preliminary species inventories to be completed for each site. When a DNA barcode matched a species record in BOLD, its source specimen is assigned to that species. In some cases, a barcode was new to the barcode library and was assigned to a taxonomic level above species.

Because all specimens are preserved as vouchers, one additional specimen per species will be imaged. These images, or the specimens themselves, can then be referred to by taxonomic specialists to gain a more refined taxonomic determination. Figure 2 illustrates how the different processes integrate for DNA barcoding.

Ratnasingham, S. and P.D.N. Hebert (2013). A DNA-based registry for all animal species: the Barcode Index Number (BIN) System. Public Library of Science ONE 8: e6621

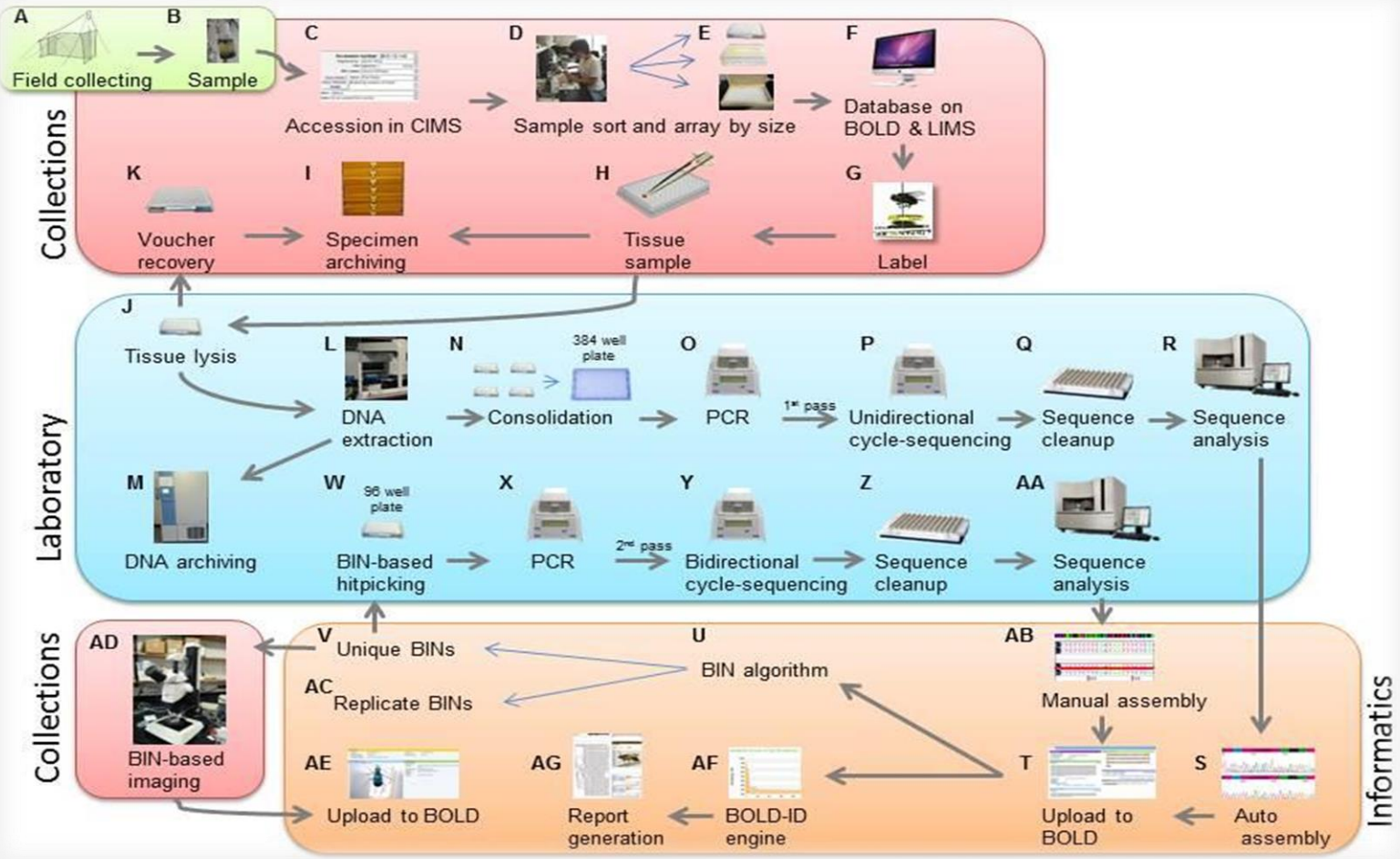

Figure 2. Schematic diagram showing DNA Barcoding workflow. Front end processing begins with field collecting (A) and proceeds through to archiving of specimens (I). Laboratory analysis begins with tissue lysis (J) through to sequence analysis (AA). The informatics workflow includes both manual (AB) and auto sequence assembly (S), and finishes with BIN assignments and subsequent imaging of each BIN (AD).

RESULTS & ANALYSIS

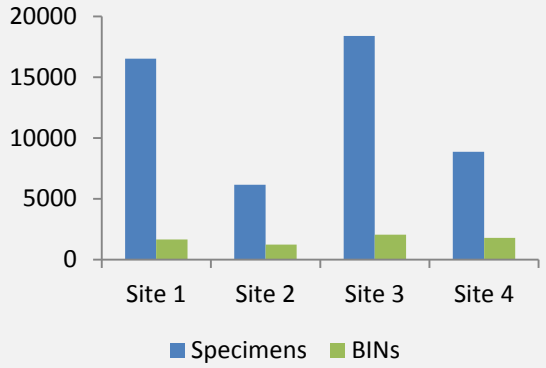

Figure 3. Specimen and BIN counts from the 4 Malaise trap sites in New Afton.

Of the 64 weekly Malaise trap samples collected from the New Afton site, 32 samples were analyzed and a total of 51 264 specimens were collected. A range of 294 to 5560 individuals were captured per sample (week), with Site 3 collecting the most specimens (18 390) and Site 2 collecting the least specimens (6155) (Figure 3). However, species richness, measured by the number of BINs collected, was highest in Site 3 with 2049 distinct species (Figure 3).

A barcode recovery rate of 86.9% led to 44 528 specimens being successfully DNA barcoded. A total of 42 063 specimens acquired BINs (Appendix 1) with over half of the specimens being flies (Diptera), followed in abundance by bees, ants and wasps (Hymenoptera) (Figure 4).

A total of 4015 distinct species were observed (Appendix 2), and rarefaction analysis suggests that approximately 6659 species may be present in this location and could be collected with this method if sampling effort was extended (Figure 5). 28% (N = 1120) of the BINs documented were new to BOLD as of August 2014. Using the BOLD-ID Engine, BINs received taxonomic identifications based on their matches to BOLD (Table 1, Appendix 3).

Table 1. Number of BINs identified to taxonomic level using reference library.

Number of BINs	Taxonomic Level
3841	Family
1781	Genus
716	Species

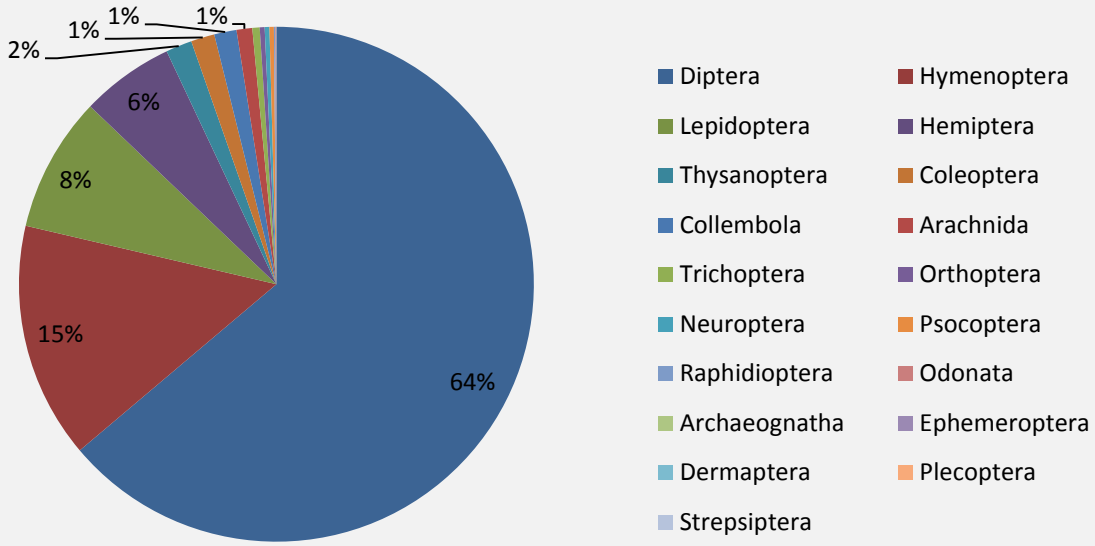

Figure 4. Percentage relative abundance of taxonomic orders collected at New Afton location.

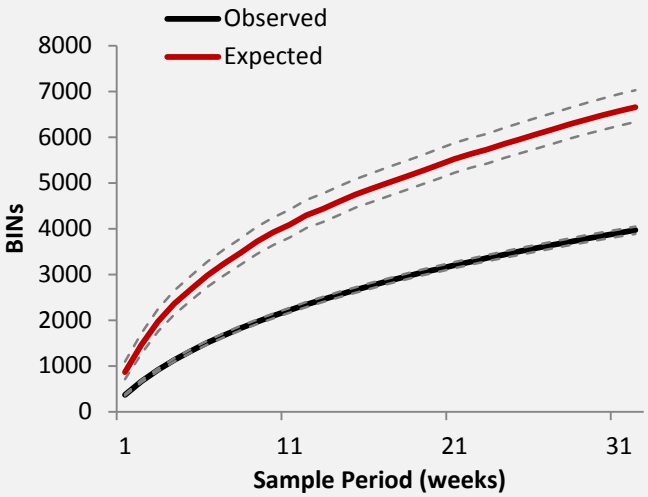

Figure 5. Accumulation curve for 32 Malaise trap samples collected from the 4 sites in New Afton.

Most specimens have a species-level identification in some taxonomic groups (e.g., Lepidoptera, Araneae), but the taxonomic framework required to provide names is lacking for many BINs in other groups. The order Lepidoptera has the largest barcode coverage and representative images for most BINs are provided in Appendix 4. The BOLD reference library recently gained increased species coverage for spiders and syrphid flies. All 28 Syrphidae BINs are identified to genus, and only 5 lack species names. Of the spiders, only 1 of the 41 BINs does not have a species name. Image libraries for Syrphidae and Araneae BINs are provided in Appendix 5 and 6 respectively.

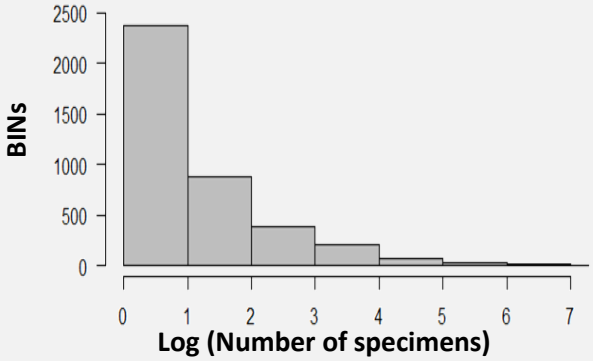

Figure 6. Relative species abundance for samples collected at the New Afton location.

The pattern of relative species abundance is quite typical, with a few species represented by many individuals (67 species with >100 individuals) – including 1047 individuals of one non-biting midge species of the Chironomidae family – and a large number of species with few individuals (1810 singletons) (Figure 6).

Table 2. Species overlap between the 4 New Gold sites.

Chao-Sorensen-Raw Abundance-based				
	Site 1	**Site 2**	**Site 3**	**Site 4**
Site 1				
Site 2	0.671			
Site 3	0.692	0.573		
Site 4	0.609	0.677	0.574	

Species turnover between sites was moderately high, ranging from 57% (between Site 2 and 3 and between Site 3 and 4) to 69% (Site 1 and 3) (Table 2). This indicates relatively high connectivity between the four sites.

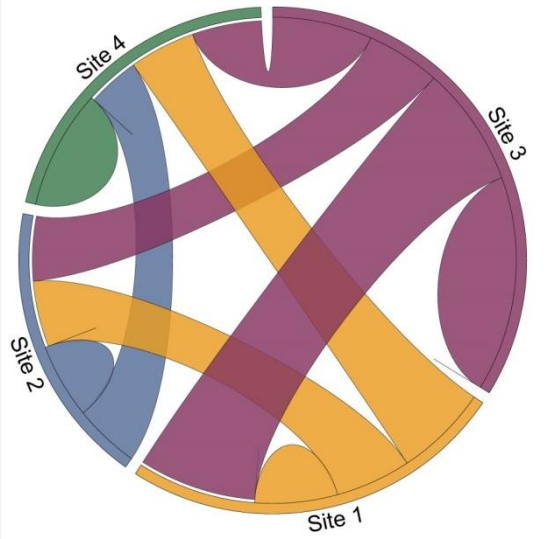

Figure 7. Circos diagram of shared species between the 4 New Gold sites. The width of each slice represents relative species richness of each site. The width of each ribbon represents the proportion of species shared between two sites, or the proportion of species unique to a site.

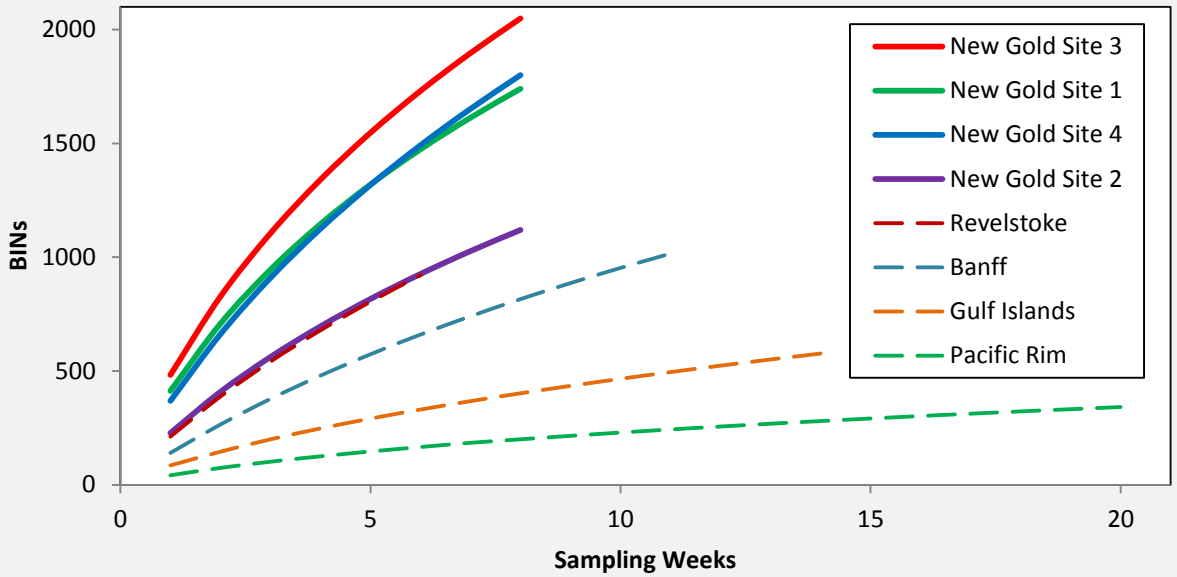

Figure 8. Comparison of BIN accumulation curves for 85 Malaise samples collected from 8 different sampling sites.

The number of BINs found at a site is correlated to the number of specimens collected (R^2 = 0.60, $p < 0.001$). Species richness was found to be significantly greater in wetland than grassland sites (paired t-test, $p < 0.01$). Species richness was found to be higher in Site 4 in comparison with Site 2 (paired t-test, $p< 0.01$). This was not observed between Site 1 and Site 3 (paired t-test, $p> 0.1$).

Although Site 2 and 4 are considered Douglar fir and sage grassland ecosystems, these two sites vary in vegetation cover and composition. This variation could account for the difference in species richness that was observed. In addition, the impact of grazing at Site 4 may facilitate the co-existence of more species based on the intermediate disturbance hypothesis. The equal species richness in Site 1 compared to Site 3 may indicate that remediation efforts have been successful in recovering local arthropod diversity in this wetland site.

Malaise traps in the New Gold mine collected a markedly greater number of specimens in comparison to other sampling sites in British Columbia (Table 3). Generally higher BIN diversities were also found in the New Gold sites despite longer sampling periods in National Parks (Figure 8). So far, BIO has only analyzed samples collected from mountain habitats (Banff and Revelstoke) and temperate rain forest habitats (Gulf Islands and Pacific Rim) which are very different from the habitats at the New Gold sites. It will be important to analyze the New Gold results against similar low elevation sites in the Montane Cordillera ecozone in the future for a better comparison.

Table 3. Average specimens collected per week at different sampling locations in British Columbia/Alberta.

Location	Mean Specimens/Week
Pacific Rim NP	106
Banff NP	317
Gulf Islands NP	356
New Gold Site 2	664
Revelstoke NP	690
New Gold Site 4	1054
New Gold Site 1	1612
New Gold Site 3	1929

APPENDICES

Appendix 1. Complete data spreadsheet of specimens with BINs collected by Malaise trap at the four New Afton Mine sites.

Appendix 2. Neighbour-joining tree of one representative per BIN from the Malaise traps deployed at the New Afton Mine sites.

Appendix 3. Taxonomy report for the New Gold New Afton site.

Appendix 4. Images for 342 of the 348 Lepidopteran BINs collected at the New Gold New Afton site.

Appendix 5. Images for 28 Syrphidae BINs collected at the New Gold New Afton site.

Appendix 6. Images for 41 Araneae BINs collected at the New Gold New Afton site.

FURTHER INFORMATION

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LINKS

International Barcode of Life - http://ibol.org/
Biodiversity Institute of Ontario - http://www.biodiversity.ca
Barcode of Life Datasystems - http://www.boldsystems.org
Canadian Centre for DNA Barcoding - http://www.ccdb.ca
School Malaise Trap Program - http://www.malaiseprogram.ca

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