Expedited Assessment of Terrestrial Arthropod Diversity by Coupling Malaise Traps with DNA Barcoding

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Abstract

Monitoring changes in terrestrial arthropod communities over space and time requires a dramatic increase in the speed and accuracy of processing samples that cannot be achieved with morphological approaches. The combination of DNA barcoding and Malaise traps allows expedited, comprehensive inventories of species abundance whose cost will rapidly decline as high-throughput sequencing technologies advance. Aside from detailing protocols from specimen sorting to data release, this paper describes their use in a survey of arthropod diversity in a national park that examined 21,194 specimens representing 2,255 species. These protocols can support arthropod monitoring programs at regional, national, and continental scales.

Keywords: malaise trap, DNA barcoding, biological inventory, biomonitoring, barcode index numbers
Introduction

Given unprecedented losses (Lawton and May 1995; Pimm et al. 1995, 2014), improved methods to quantify biodiversity at a massive scale and at low cost are essential, especially for small-bodied organisms such as arthropods. The melding of two technologies – DNA barcoding and passive, large-scale specimen collection – represents a potential solution. DNA barcoding simplifies and accelerates taxonomic identifications (Hebert et al. 2003; Packer et al. 2009; Cristescu 2014; Joly et al. 2014) by employing the 6.12 million reference sequences (July 2018) in the Barcode of Life Datasystems (BOLD; Ratnasingham and Hebert 2007). Coverage of the BOLD reference library varies for geographic regions and taxonomic groups, ranging from nearly complete for some continental faunas, e.g. beetles, spiders, moths and butterflies, (Hebert et al. 2013; Pentinsaari et al. 2014; Hendrich et al. 2014; Huemer et al. 2014; Rougerie et al. 2014; Zahiri et al. 2017; Blagoev et al. 2015; Gwiazdowski et al. 2015) to sparse for many taxa, e.g. nematodes, mites and molluscs (Ferri et al. 2009; Young et al. 2012; Layton et al. 2014). Because the latter groups include many undescribed species, operational taxonomic units (OTUs) must be employed to quantify their diversity. DNA barcoding represents a dramatic advance for such analysis because the Barcode Index Number (BIN) system (Ratnasingham and Hebert 2013) provides an objective approach for OTU delineation of animals that is coupled with a persistent registry. Since BINs correspond well with Linnaean species in many animal groups (Hausmann et al. 2013; Ratnasingham and Hebert 2013; Zahiri et al. 2014; Blagoev et al. 2015), BIN-based biodiversity assessments can be implemented for groups that lack well-developed taxonomy.

The Malaise trap (Malaise 1937) has gained popularity for assessing terrestrial arthropod communities (Karlsson et al. 2005) because it collects large samples with little effort (Marshall et al. 1994). However, the subsequent identification is a substantial challenge as a week-long collection often includes more than 1000 specimens representing several hundred species. Moreover, because many species are only represented by a few specimens, it is important to identify every individual. Conversely, very common species can consume considerable effort, particularly if they
belong to a closely allied group of taxa whose members are difficult to discriminate morphologically. DNA barcoding breaks this taxonomic barrier as it can rapidly assign individuals to OTUs, streamlining the identification process.

While the analysis of bulk samples through DNA metabarcoding (Hajibabaei et al. 2011; Taberlet et al. 2012; Yu et al. 2012; Ji et al. 2013; Gibson et al. 2014; Leray and Knowlton 2015) greatly reduces analytical costs, it has two limitations. It cannot maintain the link between each specimen and its cytochrome c oxidase subunit I (COI) sequence, which inhibits extending the DNA barcode reference library, and cannot determine species abundances.

This study describes a protocol for rapid biodiversity assessments which employs DNA barcoding and passive specimen trapping. Its effectiveness is demonstrated by describing a survey that examined more than 20,000 specimens representing over 2200 BINs from Point Pelee National Park. This protocol has already proven both efficient and effective in several studies (Bukowski et al. 2015; D’Souza et al. 2015; Kohn et al. 2015; Mazumdar et al. 2015; Perez et al. 2015; Zlotnick et al. 2015; Aagaard et al. 2017; Geiger et al. 2016; Hebert et al. 2016; Wirta et al. 2016; Steinke et al. 2017; Ashfaq et al. 2018; D’Souza and Hebert 2018) but is described in detail here for the first time.

**Materials and Methods**

*Specimen Collection and Processing*

A Townes-style Malaise trap was deployed for 20 weeks in a cedar-savannah habitat at Point Pelee National Park in southwestern Ontario, from May 2 until September 19, 2012. Each sample was collected in a 500 mL plastic Nalgene bottle that was filled with 375 mL of 95% ethanol and then attached to the trap head (Fig. 1 F2). The catch was harvested weekly and placed in 500 mL of fresh ethanol before storage at -20°C until it was analyzed at the Centre for Biodiversity Genomics (CBG; www.biodiversitygenomics.net).
Each weekly sample was accessioned and its collection data entered into an Access-based Collection Information Management System (CIMS; Fig. 1 P1). To reduce cost, samples collected in odd-numbered weeks (1, 3, 5…) were processed while the others were archived. The first stage in sample processing involved decanting excess ethanol and pouring the specimens into a sorting dish. Specimens were then partitioned by size (small, medium and large) and assigned to a taxonomic order. Most large specimens (>5mm) were pinned, with the exception of those taxa routinely stored in ethanol (e.g., Araneae, Gastropoda); all small and medium specimens were retained in ethanol. After sorting, specimens were arrayed in batches of 95 plus one control (Fig. 1 P2), mirroring the 8 x 12 format of 96 well microplates. Typically, each array included only one order to avoid mixing of taxa requiring different primers (Table 1). Specimens of different orders were only combined when necessary to complete an array. Pinned specimens were placed in Schmitt boxes with an 8 x 12 array grid marked on their foam base, while medium specimens (≈3-5mm) were placed in Matrix storage tubes (Thermo Fisher Scientific; Fig. 1 P2), and small specimens (<3mm) were placed directly in 96-well microplates (Eppendorf; Fig. 1 P2). The sample was also inspected to determine if an excessive number (>300 specimens) of a particular morphospecies was present, and if it could be distinguished morphologically. In these cases, to reduce cost yet still capture the genetic diversity of those morphospecies, at least 24 specimens, which amounted to two rows in a microplate, were selected for barcoding while the others were counted and archived. Each container was given a unique identifier (Root Plate ID, e.g. BIOUGXXXXX) and likewise, each specimen within the container was given a unique identifier reflecting its position in it (Sample ID, e.g. BIOUGXXXXX-A01 to BIOUGXXXXX-H11). The unique identifiers and collection data for each specimen were uploaded to BOLD (Ratnasingham and Hebert 2007; Fig. 1 P3) with records for each sample placed in a separate project to allow easier comparison among weeks. Once this was completed and BOLD Process IDs were generated, labels were printed and affixed to large and medium specimens while small specimens did not require individual labels (Fig. 1 P4). A small fragment of tissue was then removed from each large and medium specimen and placed into a microplate destined for DNA extraction (Fig. 1 P5). Small specimens did not require tissue sampling as they were already in
microplates. Each microplate was then submitted for molecular processing and its progress through the analytical chain was tracked with a Laboratory Information Management System (BOLD-LIMS).

**DNA Barcode Analysis**

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding (CCDB; www.ccdb.ca). An automated, silica membrane-based DNA extraction protocol (Ivanova et al. 2006) was performed in 96-well microplate format using a 3 μm glass fibre over 0.2 μm Bio-Inert membrane filter plate (Pall Corporation). The extraction protocol, however, was modified following Porco et al. (2010; Fig. 1 P7) to allow recovery of vouchers for microplates containing whole specimens. To maximize DNA yield, tissue lysis was performed overnight at 56°C before DNA extraction (Fig. 1 S1 and S2). Subsequent PCR amplification of the COI barcode region was performed in 384-well plate format as this allowed a 50% reduction in reagent volumes from earlier methods (Hajibabaei et al. 2005; deWaard et al. 2008, Wilson 2012). This protocol involved consolidating aliquots of DNA extracts from four 96-well microplates into a 384-well PCR plate containing PCR master mix using a Biomek FX workstation (Beckman-Coulter; Fig. 1 S3) and ensured arthropod orders were processed with the same primer pair. The total PCR reaction volume was 6 µL: 3 µL of 10% D-(+)-trehalose dihydrate for microbiology (≥99.0%; Fluka Analytical), 0.92 µL of ultra-pure water (Hyclone, Thermo Scientific), 0.60 μL of 10× PlatinumTaq buffer (Invitrogen), 0.30 μL of 50 mM MgCl₂ (Invitrogen), 0.06 μL (0.1 uM) of each primer, 0.03 μL of 10 mM dNTP (KAPA Biosystems), 0.03 μL of 5 U/μL PlatinumTaq DNA Polymerase (Invitrogen), and 1 μL of DNA template. Table 1 details the primer pairs used on the first pass. All PCR reactions employed the same thermocycling parameters: 94°C for 1 min, 5 cycles at 94°C for 40 sec, 45°C for 40 sec, 72°C for 1 min, followed by 35 cycles at 94°C for 40 sec, 51°C for 40 sec, 72°C for 1 min, and a final extension at 72°C for 5 min (Fig. 1 S4).

PCR products were diluted 1:4 with molecular grade water and then unidirectionally sequenced using the appropriate reverse primer (Table 1). Unidirectional sequencing (3' to 5') was also
completed in 384-well format (Fig. 1 S5) to reduce costs. The total sequencing reaction volume was 5.5 µL: 0.14 µL of BigDye terminator v3.1 (Applied Biosystems), 1.04 µL of 5X sequencing buffer [400 mM Tris-HCl pH 9.0 + 10 mM MgCl$_2$ (Invitrogen)], 2.78 µL of 10% D-(+)-trehalose dihydrate from *Saccharomyces cerevisiae* (≥99%; Sigma-Aldrich), 0.48 µL of ultra-pure water (Hyclone, Thermo Scientific), 0.56 µL (0.1 uM) of primer; and 0.5 µL of diluted PCR template was added with a Biomek FX robot. All sequencing reactions employed the same thermocycling protocol: 96°C for 1 min followed by 15 cycles at 96°C for 10 sec, 55°C for 5 sec, 60°C for 1.25 min, followed by 5 cycles at 96°C for 10 sec, 55°C for 5 sec, 60°C for 1.75 min, then 60°C for 15 sec followed by 15 cycles at 96°C for 10 sec, 55°C for 5 sec, 60°C for 2 min and a final extension at 60°C for 1 min (Fig. 1 S6). An automated, magnetic bead-based sequencing cleanup method was employed in 384-well microplates using PureSEQ (ALINE Biosciences) on a separate Biomek FX robot before sequencing on an ABI 3730xL DNA Analyzer (Applied Biosystems; Fig. 1 S7).

Trace files were manually uploaded to BOLD and were automatically assessed for quality based on predefined parameters (Ratnasingham and Hebert 2007). Trace files that received medium and high-quality assessments were automatically trimmed and edited by the BOLD platform. Those deemed low quality or classified as failed reads were ignored. Trimming was performed using a sliding window approach, discarding leading and trailing segments of the sequence that had more than 4 bp with a quality value (QV) score lower than 20 in a window of 20 bp. All sequences with less than 500 bp in the barcode region (the threshold for BIN assignment; see below) were manually edited with CodonCode v. 3.0.1 (CodonCode Corporation) to see if additional sequence information could be recovered (Fig. 1 A1). In cases where multiple trace files were generated for a single individual (see below) they were manually inspected for chimeras.

The initial PCR failed to generate an amplicon from some DNA extracts, likely reflecting DNA degradation or low primer affinity. These failures were hitpicked to assemble new destination 96-well microplates of DNA extracts (Fig. 1 S8), which were subjected to another round of PCR
employing primers that generated two shorter, overlapping COI (307 bp, 407 bp) amplicons (Table 1; Fig. 1 S9). A Biomek NX Span 8 workstation (Beckman-Coulter) was used to hitpick DNA from the failed samples into new plates. This ‘failure tracking’ was supported by data generated by the BOLD-LIMS. The original DNA plates were scanned to identify all specimens that failed to generate a BIN compliant sequence. The well coordinates of these failures in the source and destination microplates were generated for input into a Biomek NX robot. The newly configured microplates were then processed through two PCR reactions followed by bidirectional sequencing and manual assembly as part of the failure tracking protocol (Fig. 1 S10, S11, S12 and A4). Failure-tracking PCR reactions were carried out in 96-well microplates. The total PCR reaction volume was 12.5 µL: 6.25 µL of 10% D-(+)-trehalose dihydrate for microbiology (≥99.0%; Fluka Analytical), 0.125 µL of ultra-pure water (Hyclone, Thermo Scientific), 2.5 µL of 5× KAPA Taq HotStart Buffer (KAPA Biosystems), 1.25 µL of 25 mM MgCl₂ (Invitrogen), 0.125 µL of each primer, 0.0625 µL of 10 mM dNTP (KAPA Biosystems), 0.0625 µL of 5 U/µL KAPA Taq HotStart DNA Polymerase (KAPA Biosystems), and 2 µL of DNA template. Failure-tracking sequencing reactions were also carried out in 96-well microplates. PCR products were diluted 1:5 and bidirectionally sequenced. The total sequencing reaction volume was 11 µL: 0.25 µL of BigDye terminator v3.1 (Applied Biosystems), 1.875 µL of 5X sequencing buffer [400 mM Tris-HCl pH 9.0 + 10 mM MgCl₂ (Invitrogen)], 5 µL of 10% D-(+)-trehalose dihydrate from *Saccharomyces cerevisiae* (≥99%; Sigma-Aldrich), 0.875 µL of ultra-pure water (Hyclone, Thermo Scientific), 1 µL of primer; and 2 µL of diluted PCR template.

The final step in barcode analysis involved a second round of ‘BIN hitpicking’ to ensure that each BIN was represented, whenever possible, by five individuals with bidirectional sequence coverage. BIN information on BOLD was utilized in conjunction with the BOLD-LIMS to select representatives of each BIN with <5 individuals with bidirectional coverage (Fig. 1 A5) and instructions were automatically generated for the Biomek NX Span 8 workstation. The hitpicked destination DNA microplates were then processed through the PCR to bidirectional sequencing steps (Fig. 1 S8 to S12), manually edited (Fig. 1 A4) and uploaded to BOLD (Fig. 1 A2).
Data Release and Barcode Index Numbers

Specimen and sequence data are available on BOLD (Fig. 1 A2) in the public dataset DS-PPNP12 entitled "Point Pelee National Park Malaise Trap Program 2012" (http://dx.doi.org/10.5883/DS-PPNP12). The record for each specimen includes its date and locality of collection, its taxonomic assignment (see Taxonomic Assignment and Data Analysis), and voucher specimen details. If its barcode was recovered, the specimen record also includes trace files, quality scores, its sequence, and corresponding GenBank accession. After final validation, the specimen data were also uploaded to the Global Biodiversity Information Facility (GBIF) as a Darwin Core Archive (Wieczorek et al. 2012) via the University of Guelph’s Integrated Publishing Toolkit (Robertson et al. 2014) installation and are available at http://dx.doi.org/10.15468/mbwnw9. A condensed version of the data is available in Table S1.

The source specimen for each sequence that met quality checks was automatically designated a BIN by the Refined Single Linkage (RESL) algorithm implemented on BOLD (Ratnasingham and Hebert 2013; Fig. 1 A3). The requirements for BIN membership are >=500 bp coverage of the barcode region between positions 70 and 700 of the BOLD alignment (Ratnasingham and Hebert 2013), <1% ambiguous bases, and the absence of a stop codon or contamination flag. Alternatively, specimens can gain BIN assignment without formal membership if the sequence is 300–500 bp and unambiguously matches an existing BIN member (i.e. no conflicts among top matches at any hierarchy level), but will not create or split BINS. RESL runs monthly on all qualifying barcode sequences (see above) in BOLD which currently totals 6.12 million specimens and 0.56 million BINS (July 2018). The BIN designations generated through this approach are transparent, reproducible, and globally accessible through DOI-designated ‘BIN pages’ that collate the specimen and sequence information of its members (e.g., Danaus plexippus http://dx.doi.org/10.5883/BOLD:AAA9566).

Archiving and Imaging
All voucher specimens are archived in the natural history collection (institution code = BIOUG) at the CBG, University of Guelph, where they are available for taxonomic study (Fig. 1 P6). Large pinned specimens were assigned to an archive location using BIOUG’s CIMS and transferred to a drawer in the dry collection. Each medium-sized specimen was retained in its storage tube in the Matrix box, assigned an archive location, and stored in BIOUG’s fluid collection. Small specimens were returned from the CCDB after voucher recovery (Porco et al. 2010; Fig. 1 P7), retained in their microplates, and archived in BIOUG’s fluid collection. All residual DNA extracts are stored in the DNA Archive at the CBG (Fig. 1 S13), where they are available for further sequence characterization.

Once sequence analysis was complete and specimens were designated BINs, up to three representatives of each BIN were photographed to aid taxonomic validation and build a digital image library (Fig. 1 I1) by employing a database query to recognize BINs lacking an image. Specimens were photographed at high resolution and the images were made accessible through both specimen and BIN pages under Creative Commons (BY-NC-CA) license.

**Taxonomic Assignment and Data Analysis**

Following BIN designation, every specimen received a taxonomic assignment based upon querying BOLD (Fig. 1 A6). If the specimen’s BIN contained other specimens identified to a single family, genus or species by a taxonomic expert (i.e. denoted by the identifier and/or identification method field on BOLD), it received this identification. However, if a BIN contained specimens with multiple, conflicting identifications, the specimens gained the lowest level of taxonomy without discordance. Specimens assigned to a BIN lacking expert identification were queried through the BOLD Identification Engine (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) If the result was a close match (<10% divergence for family, <5% for genus, e.g. Coddington et al 2016) and the query sequence fell within a cluster of BINs assigned to a particular genus or family in the taxon ID tree (see below), the record was assigned to this taxon. All assignments were further validated using the
taxon ID tree (Fig. S1)\textsuperscript{3} along with matching specimen images (Fig. S2)\textsuperscript{3}. Any anomalies in tree topology were investigated by retrieving the vouchered specimen and ensuring that all ancillary data on BOLD were correct (including the specimen image and preliminary identification). If the sequence was revealed as representing a contamination event, it was flagged, tagged on BOLD as a contamination, and removed from the analysis and its BIN page.

The final stage of the workflow involved report generation (Fig. 1 A7) aided by the varied functions on BOLD for calculating summary statistics. As well, supplementary analyses were performed to demonstrate the utility of the protocol for rapid biodiversity assessment. To explore the completeness of the inventory, sample- (with each weekly catch considered a sample) and individual-based BIN accumulation curves were computed using the software product R, version 3.1.1 (R Development Core Team) and the vegan package (Oksanen et al. 2013). The curves were computed as the mean of 1000 randomized BIN accumulation curves without replacement. As another measure of completeness, log-normal abundance plots were calculated using R and the package vegan. These software programs were also used to estimate total BIN richness for both sample- and individual-based data using the nonparametric incidence-based species richness estimator Chao 2 (Chao 1987). We summarized the number of specimens and BINs captured for each order and in each weekly sample, along with relative abundance, the incidence of unique and rare BINs, and the turnover of BINs among samples and across time. Finally, we compared our DNA barcode-based inventory to a 40-year (1970 – 2009) morphological inventory from Point Pelee National Park (Marshall et al. 2009), and combined these two inventories to generate a more comprehensive checklist for the park.

**Results**

**DNA Barcode Analysis**

\textsuperscript{3} Supplementary data are available with the article through the journal Web site
All specimens in the ten weekly samples were processed except for three abundant morphospecies, each from a different sample (week 3: 8,595 specimens of a chironomid; week 5: 313 specimens of a chironomid; week 9: 334 specimens of a trombidiform mite), which were excluded from the analysis. In total, 21,194 specimens were processed from the ten samples with first pass analysis generating successful sequences (i.e. > 0 bp) from 81.6% of them (17,300; Fig. 2). The second pass analysis recovered another 1885 sequences, bringing the success rate to 90.5% (19,185; Fig. 2). Aside from these records, 144 sequences were found to be contaminants and another eight possessed stop codons (Fig. 2). Sequence recovery varied among taxa with Acari displaying the lowest success (chi-square test, p< 0.0001) with just 48.0% of specimens generating a BIN compliant sequence. There was also evidence of a taxonomic bias (chi-square test, p<0.0001) in the 309 (1.6%) specimens that were either destroyed or unrecoverable after analysis, with most being small, soft-bodied Hemiptera (104 specimens, 33.7%), Diptera (75 specimens, 24.3%) and Acari (67 specimens, 21.7%).

**Specimen and BIN Analyses**

Among the specimens that generated a sequence, most (99.4%) received a BIN designation (n = 19,071) (Fig. 2). From these specimens with BINs, 2,043 specimens represented new BINs on BOLD (at the time of analysis) and were ‘BIN hitpicked’ to acquire a bidirectional sequence and 3,662 specimens were imaged (mean = 1.6 images/BIN).The 114 sequences that failed to meet the criteria for BIN designation were run through the stand-alone version of the RESL algorithm (using the function ‘Cluster sequences’ on BOLD) to estimate the number of additional OTUs (or species) represented; this analysis revealed 65 OTUs. One representative of each OTU was queried against the BOLD ID Engine: 49 were highly similar (p-distance > 97.8%) and matched to known BINs while 16 appeared to be new to BOLD, as they were less similar to known BINs (p-distance < 97.8%).

All subsequent analyses considered the 19,071 specimens with a BIN designation. They included taxa belonging to four classes and 25 orders (Fig. 3, Table S2). Diptera were dominant comprising...
57.0% of the specimens (Fig. 3a) and 49.7% of the BINs (Fig. 3b). Hymenoptera was also very
diverse with the third highest percentage of specimens (11.3%) and the second highest proportion
of BINs (25.3%).

In total, 2,255 BINs were present in the ten samples with an average of 458 BINs and 1,907
specimens per sample (BIN range = 253–640, specimen range: 814–3,795) (Fig. 4, Table S3). Most
BINs were uncommon; 47.6% (1,074) were represented by a single specimen while only 36 (1.6%)
had >100 specimens (Fig. 5). There was a positive correlation between the number of individuals in
a sample and the number of BINs unique to it \( (R^2 = 0.69, p = 0.003, \text{Fig. 6}) \), reinforcing the prevalence
of rare BINs and the effort required to discover them.

**Species Richness and Turnover**

Species richness extrapolation based on the (Preston) log-normal species distribution indicated that
complete sampling of the Malaise-trappable arthropod fauna at this site in Point Pelee would reveal
about 5,700 BINs, roughly double the observed number (Fig. 7). A similar result (6,161 BINs) was
obtained when the analysis was repeated with the specimen totals for the three excluded BINs (see
above). BIN accumulation curves based on Chao 2 suggested a lower count with an estimate of
3,836 (SE ± 133) BINs based on specimens (Fig. 8a) and 3,889 (SE ± 125) based on samples (Fig.
8b). These three estimators suggest the site inventory is roughly 36.6–58.8% complete.

Individual samples contained an average of 458 BINs, but their similarity was low (mean shared
BINs = 0.33; mean Jaccard index = 0.16) (Table S4). The proportion of shared BINs (for adjacent
and non-adjacent weekly samples) increased as the season progressed (Fig. 9a) and decreased
with the interval between samples \( (R^2 = 0.52, p << 0.001, \text{Fig. 9b}) \) with similarity values (Jaccard
index) halved in 81.1 days. For example, only 99 BINs were shared between weeks 1 and 19,
samples that contained 461 and 486 BINs, respectively. By comparison, samples from weeks 7 and
9 (containing 641 and 619 BINs respectively) shared 266 BINs.
**Taxon Diversity and Abundance**

The Cecidomyiidae (351), Ichneumonidae (127) and Chironomidae (113) included the most BINs while the Chironomidae (10,827), Cicadellidae (3,070), and Cecidomyiidae (1,919) were represented by the most specimens. The most abundant BINs were BOLD:AAG2868 (Cicadellidae: *Empoasca fabae*), BOLD:AAB7030 (Chironomidae: *Chironomus* sp.) and BOLD:AAV0161 (Cicadellidae: *Erythroneura bakeri*) with 555, 446 and 431 specimens respectively. Each of these species and many of the abundant taxa had closely-related allies, often morphologically indistinguishable and in low frequency, making oversampling unavoidable without risking the oversight of some species.

**New and Existing Inventories**

Three quarters of the specimens (n = 14,313/19,071) with a sequence gained a genus- or species-level taxonomic assignment following their comparison with records on BOLD. They represented 58.6% of all BINs (n = 1320); the other BINs were assigned to a subfamily or family. A few species were represented by more than one BIN [e.g., Araneae: Thomisidae: *Xysticus pellax* was represented by BINs BOLD:ACE4932 and BOLD:ACE4935], but most species (95.5%) showed perfect correspondence between a single taxon name and a single BIN.

By comparison, a 40-year (1970 – 2009) inventory using morphology (Marshall et al. 2009) revealed 2,423 taxa identified to a genus- or species-level among 30,000 specimens collected from Point Pelee and vicinity. After merging the two inventories, there were 3,217 genera/species combinations in the checklist with just 7.8% overlap (Table S5, doi:10.5883/DS-PPNP12). The overall taxonomic coverage includes 343 families, 597 subfamilies, 1,783 genera, 2,290 species and another 118 interim or uncertain species. While the study by Marshall et al. (2009) only examined insects, the present study examined four classes of arthropods. Only considering insects, the present inventory revealed more species of Trichoptera, Thysanoptera, and Psocodea. When all BINs are considered, the present inventory was biased toward Diptera and Hymenoptera where it collected 19.8% and
13.0% more respectively. By contrast, the diverse collecting methods employed by Marshall et al. (2009) yielded more Coleoptera, Hemiptera and Lepidoptera (64.6%, 31.6%, 22.2%). In total, the present effort added 780 taxonomic records to the checklist (Table S5; doi:10.5883/DS-PPNP12) which included 523 new species, 396 new genera, 91 new subfamilies, and 86 new families.

**Discussion**

This paper describes the steps involved in moving from specimen collection through DNA barcode analysis to a summary of species, their abundances and associated diversity metrics. Aside from enabling a rapid, inexpensive assessment of terrestrial arthropod diversity, this approach aids extension of the DNA barcode reference library.

**Capturing Presence and Abundance**

The current pipeline overcomes several barriers that usually constrain Malaise trap surveys of arthropod diversity. Most importantly, DNA barcoding minimizes the time demand on taxonomic experts by automating the identification of specimens that belong to species in the reference library (deWaard et al. 2009, Telfer et al. 2015). As a consequence, taxonomic advice is only required when a new BIN is encountered or when a BIN contains conflicting information. The use of BINS also streamlines barcode workflows. For example, imaging representatives of each BIN facilitates the detection of contamination and mis-identification, but also the assignment of taxonomy at higher levels (e.g. Order, Family). Similarly, a carefully edited bidirectional sequence is required for each new BIN, but a unidirectional sequence is perfectly adequate for BIN assignment since intraspecific variation within a population is low (Bergsten et al. 2012). Sequencing error rates are also expected to be lower than intraspecific variability, making the unidirectional BIN assignment a great option in the vast majority of cases. For instance, two BINS of Empoasca (Hemiptera: Cicadellidae) were represented in the Point Pelee collection by 555 (BOLD:AAG2868) and one (BOLD:ACZ4093) specimens respectively. Just a few representatives of the abundant BIN were imaged and bidirectionally sequenced, but every specimen could be identified by unidirectional analysis. Aside
from allowing the strategic deployment of analytical effort, the key advantage of DNA barcoding lies in its capacity to allow technicians with no taxonomic training to generate the species abundance data needed for most diversity indices (Magurran 2004). As well, abundance data are valuable to employ functional traits to quantify ecosystem processes and services (e.g., Devictor et al. 2010). In addition, abundance data coupled with sequence information on each specimen allows genetic diversity to be quantified (Miraldo et al. 2016), which enables follow-up examinations such as probing the correlation between species richness and genetic diversity (Vellend 2005).

**Assembly of Resources**

As evidenced by our study at Point Pelee, this approach generates a taxonomic inventory, an image library, a DNA archive, sequence data and specimens with associated collection data; information that can be shared through diverse online portals (e.g. Telfer et al. 2015). It also expands the DNA barcode reference library with an alternate approach that complements the analysis of legacy specimens that is complicated by degraded DNA (Hebert et al. 2013; Prosser et al. 2016). As well, the analysis of newly collected specimens permits supplemental investigations, such as genome size determination (Hanner and Gregory 2007) and stable isotope analysis (Dittrich et al. 2017). The barcode library has utility beyond species identification, including the reconstruction of community phylogenies (e.g. Boyle and Adamowicz 2015) for studying the structure and assembly of biological communities, as well as for flagging new species (e.g. van Nieukerken et al. 2015) and new occurrence records (Fernandez-Triana et al. 2014).

**Protocol Use and Refinements**

The present method has gained wide adoption (Perez et al. 2015, www.globalMalaise.org; Zlotnick et al. 2015; Steinke et al. 2017) and has been employed in several studies (Bukowski et al. 2015; D'Souza et al. 2015; Kohn et al. 2015; Mazumdar et al. 2015; Aagaard et al. 2017; Geiger et al. 2016; Hebert et al. 2016; Wirta et al. 2016; Ashfaq et al. 2018; D'Souza and Hebert 2018). As of July 2018, 3.1 million specimens have now been processed using this method. Large core facilities are
best-suited for the high-throughput execution of this method — where the front-end processing, laboratory analysis and informatics workflows are supported under one roof. However, this detailed protocol can also guide smaller-scale projects and facilities that can partition the workflow into sections that can be done ‘in-house’, and those contracted out, such as the sequencing component.

This work has led to one important modification — a standard primer cocktail, C_LepFolF and C_LepFolR (Folmer et al. 1994; Hebert et al. 2004) that can be used for all arthropods, simplifying consolidation and sequencing. The present protocol generates high quality barcode records for approximately $5 a specimen with about two thirds of the cost derived from Sanger sequencing. A substantial reduction in analytical costs can be achieved by shifting to a high-throughput sequencing (HTS) platform that allows samples to be individually tagged and subsequently multiplexed; the CBG has recently integrated the PacBio Sequel System for this purpose (Hebert et al. 2018). The Illumina MiSeq and Ion S5 platforms reduce sequencing costs four-fold (e.g. Shokralla et al. 2014, 2015, Meier et al. 2016, Morinière et al. 2016) while the Sequel System reduces them 40-fold (Hebert et al. 2018). Although HTS platforms are frequently associated with increased error rates compared to Sanger technology (Kircher and Kelson 2010), these rates can be reduced to a comparable level given sufficient read depth per specimen (see Hebert et al. 2018).

A Global Terrestrial Arthropod Monitoring Network?

The deployment of an extensive network of Malaise traps is relatively inexpensive, as evidenced by past deployments in national parks (Perez et al. 2015), schoolyards (Steinke et al. 2017), and backyards (Zlotnick et al. 2015). Once the present approach has been integrated with HTS, the mass samples resulting from a broad trap network will deliver accurate occurrence data while extending the barcode reference library. By monitoring biodiversity on a massive scale, this activity would advance each country’s capacity to deliver factually-based reports on the status of biodiversity as required to meet the Convention on Biological Diversity’s Aichi Targets of the Strategic Plan for Biodiversity 2011–2020 (https://www.cbd.int/sp/targets/).
Acknowledgements

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References


Figure Captions

**Figure 1.** Workflow for biodiversity monitoring through DNA barcoding.

**Figure 2.** Flowchart showing the success in sequence recovery from 21,194 specimens of arthropods in ten Malaise trap samples.

**Figure 3.** Taxonomic breakdown of the Malaise trap samples by (a) specimens and (b) BINs.

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**Figure 9.** Species overlap between the ten Malaise trap samples, shown (a) in chronological order with the size of each node proportional to the number of BINs in a sample while the width of each
arcs reflects BIN overlap between samples. (b) as a comparison of BIN overlap with time between samples ($R^2 = 0.52$, $p << 0.001$).
### Tables

**Table 1.** Primers used for DNA barcode analysis. For each taxonomic group, there was a single first pass primer pair (listed first) used to amplify the 658 bp barcode region of COI and two second pass PCR primer pairs (list second and third) used to amplify two smaller, overlapping COI fragments (307 bp and 407 bp). The listed primer was used for sequencing unless indicated by a symbol.

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>PCR Primer Pair</th>
<th>Forward Primer(s)</th>
<th>Sequence (5'→3')</th>
<th>Reverse Primer(s)</th>
<th>Sequence (5'→3')</th>
<th>Fragment Length (bp)</th>
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<tbody>
<tr>
<td>Diptera,</td>
<td>C_LepFolR/C_LepFolR</td>
<td>LepF1</td>
<td>ATTCAACCAATCATAAGATATTGG</td>
<td>LepR1</td>
<td>TAAACTTCTGGATGTCCAAATACTCA</td>
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<td>Coleoptera,</td>
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<td></td>
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<td>HCO2198</td>
<td>TAAACTTCAGGGTGACCAAAAAATCA</td>
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<td>Arachnida,</td>
<td>C_LepFolR/MLepR2</td>
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<td>ATTCAACCAATCATAAGATATTGG</td>
<td>MLepR2</td>
<td>GTTCAWCCWGTWCCWGCYCCATTTC</td>
<td>307</td>
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<tr>
<td>Collombola and small Orders</td>
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<td>MLepF1</td>
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<td>LepR1</td>
<td>TAAACTTCTGGATGTCCAAATACTCA</td>
<td>407</td>
</tr>
<tr>
<td>Lepidoptera</td>
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<td>LepF1</td>
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<td>LepR1</td>
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<tr>
<td></td>
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<td>ATTCAACCAATCATAAGATATTGG</td>
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<td>LepR1</td>
<td>TAAACTTCTGGATGTCCAAATACTCA</td>
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<tr>
<td>Hymenoptera</td>
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<td>TAAACTTCTGGATGTCCAAATACTCA</td>
<td>658</td>
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<tr>
<td></td>
<td>LepF1/C_ANTMR1D†</td>
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<td>N/A</td>
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<tr>
<td></td>
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<td>RonMWASPdeg_t1†</td>
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<td>LepR1</td>
<td>TAAACTTCTGGATGTCCAAATACTCA</td>
<td>407</td>
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<tr>
<td>Hemiptera</td>
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<tr>
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<td>LepF2_t1/MHemR</td>
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<td>LepR1</td>
<td>TAAACTTCTGGATGTCCAAATACTCA</td>
<td>407</td>
</tr>
</tbody>
</table>

* M13 tailed forward primers sequenced with M13F
† C_ANTMR1D cocktail not used in sequencing reaction
Supplementary material

**Fig. S1** Neighbor-Joining tree based on sequence divergences at COI (K2P distance model) for one representative of all 2,255 BINs.

**Fig. S2** Image library matching the COI Neighbor-Joining tree of BIN representatives. In a few instances, an image for the BIN representative was unavailable because the specimen was not recovered after DNA extraction. In these cases, an image of a different representative of the same BIN from another site was chosen, or in rare cases, from the nearest neighbor BIN (as marked below the image).

**Table S1** BOLD and GenBank accessions, as well as BIN assignments and collection details for the 19,185 arthropods from Point Pelee National Park.

**Table S2** Summary of specimens and BINs by taxonomic order.

**Table S3** Summary of specimens, BINs, and BINs unique to each weekly sample.

**Table S4** Jaccard similarity index and temporal distance in days between each pair of weekly samples.

**Table S5** Combined checklist of genera and species recorded at Point Pelee National Park by morphological (Marshall et al. 2009) and DNA barcode inventories.
Figure 1. Workflow for biodiversity monitoring through DNA barcoding.

250x199mm (300 x 300 DPI)
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280x184mm (300 x 300 DPI)
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187x262mm (300 x 300 DPI)
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197x275mm (300 x 300 DPI)