

A Comprehensive DNA Barcode Library for the Looper Moths (Lepidoptera: Geometridae) of British Columbia, Canada

Jeremy R. deWaard^{1,2*}, Paul D. N. Hebert³, Leland M. Humble^{1,4}

1 Department of Forest Sciences, University of British Columbia, Vancouver, British Columbia, Canada, **2** Entomology, Royal British Columbia Museum, Victoria, British Columbia, Canada, **3** Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada, **4** Canadian Forest Service, Natural Resources Canada, Victoria, British Columbia, Canada

Abstract

Background: The construction of comprehensive reference libraries is essential to foster the development of DNA barcoding as a tool for monitoring biodiversity and detecting invasive species. The looper moths of British Columbia (BC), Canada present a challenging case for species discrimination via DNA barcoding due to their considerable diversity and limited taxonomic maturity.

Methodology/Principal Findings: By analyzing specimens held in national and regional natural history collections, we assemble barcode records from representatives of 400 species from BC and surrounding provinces, territories and states. Sequence variation in the barcode region unambiguously discriminates over 93% of these 400 geometrid species. However, a final estimate of resolution success awaits detailed taxonomic analysis of 48 species where patterns of barcode variation suggest cases of cryptic species, unrecognized synonymy as well as young species.

Conclusions/Significance: A catalog of these taxa meriting further taxonomic investigation is presented as well as the supplemental information needed to facilitate these investigations.

Citation: deWaard JR, Hebert PDN, Humble LM (2011) A Comprehensive DNA Barcode Library for the Looper Moths (Lepidoptera: Geometridae) of British Columbia, Canada. PLoS ONE 6(3): e18290. doi:10.1371/journal.pone.0018290

Editor: Sergios-Orestis Kolokotronis, American Museum of Natural History, United States of America

Received: August 31, 2010; **Accepted:** March 2, 2011; **Published:** March 28, 2011

Copyright: © 2011 deWaard et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding was provided by a Forest Investment Account - Forest Science Program Student Grant and an NSERC Graduate Scholarship (to JRD) and by the Canadian Barcode of Life Network through funding from Genome Canada through the Ontario Genomics Institute and from NSERC (to LMH and PDNH). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: jdewaard@interchange.ubc.ca

Introduction

For monitoring biodiversity and detecting invasive species, knowing what species exist in a given location is paramount. However, the subtle morphological characters that separate closely related species often demand expert interpretation (e.g. [1]), forcing studies to either limit their taxonomic scope, or to only identify specimens to a higher taxonomic category (e.g. family, genus). DNA barcoding can circumvent these limits by transforming the often lengthy chore of identifying specimens to a rapid, accurate and unbiased task [2–4]. For the identification of arthropods in particular, where high diversity and low access to taxonomic expertise complicate the job, DNA barcoding has proven capable of the task, in numerous groups including collembolans [5], spiders [6], tephritid fruit flies [2], mosquitoes [7], tachinid flies [8], aphids [9], ants [10], wood wasps [11], black flies [12], and mayflies, stoneflies and caddisflies [13]. Lepidoptera has seen the most studies of barcode performance to date, and results suggest barcodes permit correct identification in >90% of previously recognized taxa [14–17].

To continue the development of DNA barcoding as a tool for biodiversity monitoring and invasive species detection, it is necessary to both construct complete reference libraries and assess

their efficacy for discriminating species. The taxa for which barcoding delivers results that are discordant with current taxonomy are of particular interest — they generally warrant further investigation as they may represent overlooked species [18,19], species that are hybridizing, cases of synonymy or situations that require a secondary barcode marker for species diagnosis. It is also worthwhile to explore the effect of sampling on estimates of genetic variation, both in terms of number [20,21] and geographic coverage [16,17].

The loopers or inchworm moths (Lepidoptera: Geometridae) are one of the largest insect families, composed of nearly 23,000 species worldwide [22] and roughly 1400 in North America [23]. They are an abundant, diverse component of most forest ecosystems — this, along with their weak flight ability and low propensity to migrate [24,25], make them excellent indicators of environmental quality [26]. A large proportion of the species are also important defoliators, including native species such as the fall cankerworm (*Alsophila pometaria* (Harris)) and invasive pests such as the winter moth (*Operophtera brumata* (L.)). Because most larvae and adults possess cryptic coloration, they are a notoriously tough group in which to discriminate species. To further complicate matters, most North American geometrid genera are in need of revision. This latter gap is now being addressed through an

‘integrative approach’ [27] that employs DNA barcodes to accelerate the revisionary process in both North America (e.g., [28,29]) and elsewhere (e.g., [30,31]).

The geometrids of British Columbia (BC), Canada present a challenging case for DNA barcoding. There are presently 349 species known from the province — a large fauna with varying levels of taxonomic maturity (JRD unpublished). In this study, we assemble representatives of nearly all these species, from BC and the surrounding region, and from geographically separated individuals, to examine patterns of barcode divergence. We test the hypothesis that the barcode region is able to reliably discriminate geometrid species as demonstrated in other taxa (see [32–35]) and determine which species merit further investigation of their taxonomic status. The result is a reliable identification library with immediate application for monitoring looper moth biodiversity and detecting invasive species in BC.

Materials and Methods

Sampling

We chose the province of British Columbia as the primary scope of our library; its boundary does not correspond with the limits of particular biomes. However, this regional focus was chosen to maximize the development of a barcode library that would have high value for biodiversity monitoring and invasive species detection in the province. Although BC is primarily in the Western Cordillera biome, it includes some plains, maritime and subarctic ecosystems [36] so the fauna has considerable overlap with surrounding provinces, territories and states. Since the ranges of many geometrid moths are poorly known, and many will shift with climate change, we also sampled selected taxa from adjacent regions, including Alaska, Yukon Territory, Alberta, Washington State, and Idaho, but did not attempt to sample their entire faunas. We also included *Callizzia amorata* Packard (Epileminae), the sole BC representative of the Uraniidae, sister group to the Geometridae (e.g., [37]) within the Geometroidea.

We selected specimens from eight regional and national insect collections: Canadian National Collection of Insects and Arachnids (Ottawa, ON), Royal BC Museum (Victoria, BC), Canadian Forest Service (Victoria, BC), University of British Columbia’s Spencer Collection (Vancouver, BC), Washington State University’s James Entomological Collection (Pullman, WA), University of Idaho’s WFBARR Collection (Moscow, ID), Northern Forestry Centre (Edmonton, AB) and University of Alberta’s Strickland Collection (Edmonton, AB). An effort was made to sample at least five geographically distinct specimens for each species, to best appraise the genetic variation across its range. Specimens less than 30 years old were chosen when possible to avoid problems associated with DNA degradation. Some of the specimens may have been misidentified due to the difficulty of the group and lack of an expert curator in most of the collections. Where availability of taxonomic literature and time permitted, species identifications were corrected prior to or following DNA analysis by examining genitalia and external morphology of the vouchered specimens. In addition, a few specimens were freshly collected on targeted collecting trips, or by making requests to entomologists in the region. All specimens were labeled, databased and imaged and made publicly available on the Barcode of Life Data Systems (BOLD) [38] in the project ‘GOBCL – Geometridae of BC Library’. The institution storing each vouchered specimen is listed in the BOLD project and Table S1.

DNA analysis

One or two legs were removed from each dried specimen and stored in an individual tube of a 96-tube sample box (Matrix

Technologies) or an individual well of a microplate. DNA extraction, amplification, and sequencing of the barcode region of the mitochondrial cytochrome *c* oxidase I (COI) gene followed a variety of high-throughput techniques recently developed at the Canadian Centre for DNA Barcoding ([39–41]; www.barcodinglife.ca). The full-length primers LepF1 and LepR1 [42] were attempted first, but amplification and sequencing using the ‘Lep mini primers’ (MLepF1, MLepR1) [14] was necessary for most of the older material. The electropherograms were edited and aligned in Seqscape v. 2.5 (Applied Biosystems), then deposited along with the edited sequences to BOLD and GenBank (accessions are listed in Table S1). In the 61 instances where we were unable to successfully sequence a desired species from BC, sequences were obtained from specimens collected in other regions.

Data analysis

To investigate the efficacy of barcodes to differentiate geometrid species, sequence divergence within and between species was calculated using the Kimura 2-parameter model [43] and the neighbour-joining algorithm [44], as implemented in BOLD and MEGA4 [45]. We first tallied the proportion of species that could successfully be distinguished by DNA barcoding to calculate an overall success rate. The successful differentiation of a species required that its barcodes formed monophyletic clusters and were not shared with other species. We also determined which species displayed sequence diversity >3%, an arbitrary threshold that generally falls within the so-called ‘barcode gap’ (i.e. the lack of overlap between intra- and inter-specific divergence, *sensu* [46]). And lastly, to ascertain the potential of sampling bias, we tested the significance of the relationship between mean intra-specific divergence and the number of individuals analyzed by performing a linear regression in SPSS v17 (IBM).

Results and Discussion

A total of 2392 COI sequences were generated in this study, providing coverage for 400 species and 125 genera. Most sequences were derived from specimens from BC (N = 1390) and surrounding provinces, territories and states (N = 966). The remainder was collected in other North American regions (N = 35) and from a single German specimen (of the biological control agent *Minoa murinata* (Scopoli)). Of the 349 species listed for BC (JRD unpublished), only *Hydrelia brunneifasciata* (Packard) was not successfully barcoded. Most species were represented by multiple samples (mean = 6.0 individuals/species; maximum = 46), but 62 species had only a single COI barcode. All but nine sequences were greater than 500 bp (mean = 648 bp, range = 238 to 658 bp) and therefore meet the ‘BARCODE data standard’ (see [20]). The assembly of this comprehensive dataset reveals the important role that natural history collections possess for barcode library construction, both in terms of access to entire regional faunas and to specimens conducive to DNA analysis.

The neighbour-joining analysis resulted in a tree with most species forming distinct, cohesive units displaying minimal sequence variation (Figure S1). We found 27 species (6.8%) with undifferentiated or overlapping barcodes (Table 1), whereas the remaining 373 (93.2%) formed non-overlapping monophyletic clusters. Taxa that have undergone recent taxonomic revision appeared to have a higher proportion of species with diagnostic barcodes e.g. *Eupithecia* spp. (revised in [47]) – 55 of 55 species formed non-overlapping monophyletic clusters; species of Macarini [48] – 53/54; and *Tetraxis* spp. [28] – 6/6. Conversely, taxa known to be in need of revision were often comprised of several species that could not be differentiated by barcodes, such as

Lobophora (noted in [29]) where 3 of 5 species lacked diagnostic barcodes. There was also a single case, the species pair of *Probole alienaria* and *amicaria* Herrich-Schäffer, [1855], where the COI data were unable to differentiate the two taxa, corroborating unpublished revisionary work by Tomon [49] who considers it a single, highly variable species. The rate of species-level identification in the present dataset is slightly lower than in most previous barcoding studies on Lepidoptera [14–17], but it is likely to increase with re-examination of potentially misidentified specimens and further taxonomic investigation of this fauna.

As the mean interspecific divergence between congeneric taxa (9.17%; range = 0 to 17.27%) was 16-fold higher than mean intraspecific variation (0.56%; range = 0 to 8.73%), the distributions of intra- and interspecific divergences showed limited overlap (Figure 1). There was no association between mean intraspecific distance and sample size (Figure 2, linear regression, $R^2 = 0.09$, $P = 0.07$) suggesting our sampling strategy was representative for all taxa. There were 26 instances of high intra-specific divergence ($>3\%$) among the 338 species with multiple samples (Table 2). Of these, 22 cases involved two distinct clusters and 4 involved three clusters. These discrete clusters may

indicate the presence of cryptic species, as barcoding has proven invaluable for flagging species that have gone previously unrecognized (e.g. [19,42,50–52]). Conversely, one or more instances may be attributable to misidentifications. Five of these 26 taxa demonstrating high intraspecific variation are also listed in Table 1 as taxa indistinguishable by barcodes, so the total number of BC geometrid species that require re-examination of specimens and further taxonomic scrutiny is 48.

In summary, two tangible products have arisen from the current study. First, a comprehensive reference library was constructed for the Geometridae of British Columbia that can be employed immediately for biodiversity monitoring and invasive species detection. This library provides species-level resolution in over 93% of cases, and resolution to a congeneric species pair or group in the remaining cases. This small proportion of recognized taxa that apparently do not possess diagnostic barcodes, as well as the fraction of species potentially housing cryptic species, constitutes the second product — a catalog of taxa that require taxonomic investigation. Moreover, this catalog includes the materials necessary to facilitate the investigations — a database of specimens vouchered in permanent collections, each linked to publicly

Table 1. Geometrid species not distinguishable by DNA barcodes.

Taxon	Condition	Congener involved
<i>Caripeta divisata</i> Walker	paraphyletic	<i>angustiorata</i> Walker
<i>Eufidonia discospilata</i> (Walker)	paraphyletic	<i>convergaria</i> (Walker)
<i>Hydriomena edenata</i> Swett	paraphyletic	<i>crokeri</i> Swett
<i>Macaria signaria</i> (Hübner)	paraphyletic	<i>oweni</i> (Swett)
<i>Hydriomena furcata</i> (Thunberg)	paraphyletic	<i>quinquefasciata</i> (Packard)
<i>Dysstroma hersiliata</i> (Guenée)	paraphyletic	<i>rutlandia</i> McDunnough
<i>Eustroma semiatrata</i> (Hulst)	paraphyletic	<i>fasciata</i> Barnes & McDunnough
<i>Epirrita autumnata</i> (Borkhausen),	paraphyletic	<i>undulata</i> (Harrison)
<i>Lobophora magnoliatoidata</i> (Dyar)	polyphyletic	<i>nivigerata</i> Walker, <i>simsata</i> Swett
<i>Dysstroma colvillei</i> Blackmore	polyphyletic	<i>formosa</i> (Hulst), <i>hersiliata</i> (Guenée), <i>rutlandia</i> (McDunnough)
<i>Xanthorhoe ramaria</i> Swett & Cassino	polyphyletic	<i>lagganata</i> Swett & Cassino, <i>baffinensis</i> McDunnough
<i>Lobophora simsata</i> Swett	identical barcodes	<i>nivigerata</i> Walker
<i>Lobophora nivigerata</i> Walker	identical barcodes	<i>simsata</i> Swett
<i>Cabera exanthemata</i> (Scopoli)	identical barcodes	<i>erythemaria</i> Guenée
<i>Cabera erythemaria</i> Guenée	identical barcodes	<i>exanthemata</i> (Scopoli)
<i>Drepanulatrix falcata</i> (Packard)	identical barcodes	<i>carnearia</i> (Hulst)
<i>Drepanulatrix carnearia</i> (Hulst)	identical barcodes	<i>falcata</i> (Packard)
<i>Xanthotype urticaria</i> Swett	identical barcodes	<i>sospeta</i> (Drury)
<i>Xanthotype sospeta</i> (Drury)	identical barcodes	<i>urticaria</i> Swett
<i>Orthofidonia exornata</i> (Walker)	identical barcodes	<i>tinctaria</i> (Walker)
<i>Orthofidonia tinctaria</i> (Walker)	identical barcodes	<i>exornata</i> (Walker)
<i>Probole amicaria</i> (Herrich-Schäffer)	overlapping barcodes	<i>alienaria</i> Herrich-Schäffer
<i>Probole alienaria</i> Herrich-Schäffer	overlapping barcodes	<i>amicaria</i> (Herrich-Schäffer)
<i>Chlorosea banksaria</i> Sperry	overlapping barcodes	<i>nevadaria</i> Packard
<i>Chlorosea nevadaria</i> Packard	overlapping barcodes	<i>banksaria</i> Sperry
<i>Rheumaptera subhastata</i> (Nolcken)	identical and overlapping barcodes	<i>hastata</i> (Linnaeus)
<i>Rheumaptera hastata</i> (Linnaeus)	identical and overlapping barcodes	<i>subhastata</i> (Nolcken)

The 27 taxa in the left column cannot be diagnosed by COI based on one of five conditions: paraphyletic with respect to one congener; polyphyletic with two or three congeners; share an identical COI haplotype with a congener; haplotypes of one taxon do not form distinct clusters and overlap with haplotypes of congeners; or a combination of the latter two conditions.

doi:10.1371/journal.pone.0018290.t001

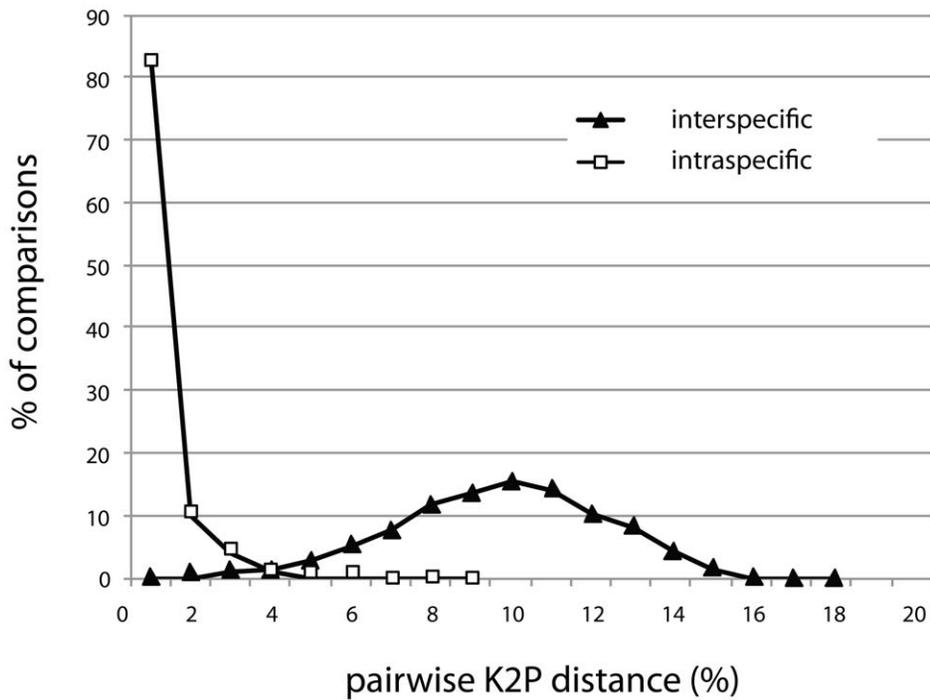


Figure 1. Combined histograms of pairwise Kimura 2-Parameter (K2P) sequence variation. Solid triangles indicate interspecific divergences between 116 congeneric taxa (70,580 comparisons) while the open squares indicate intraspecific divergences in the 339 species with multiple samples (11,949 comparisons).
doi:10.1371/journal.pone.0018290.g001

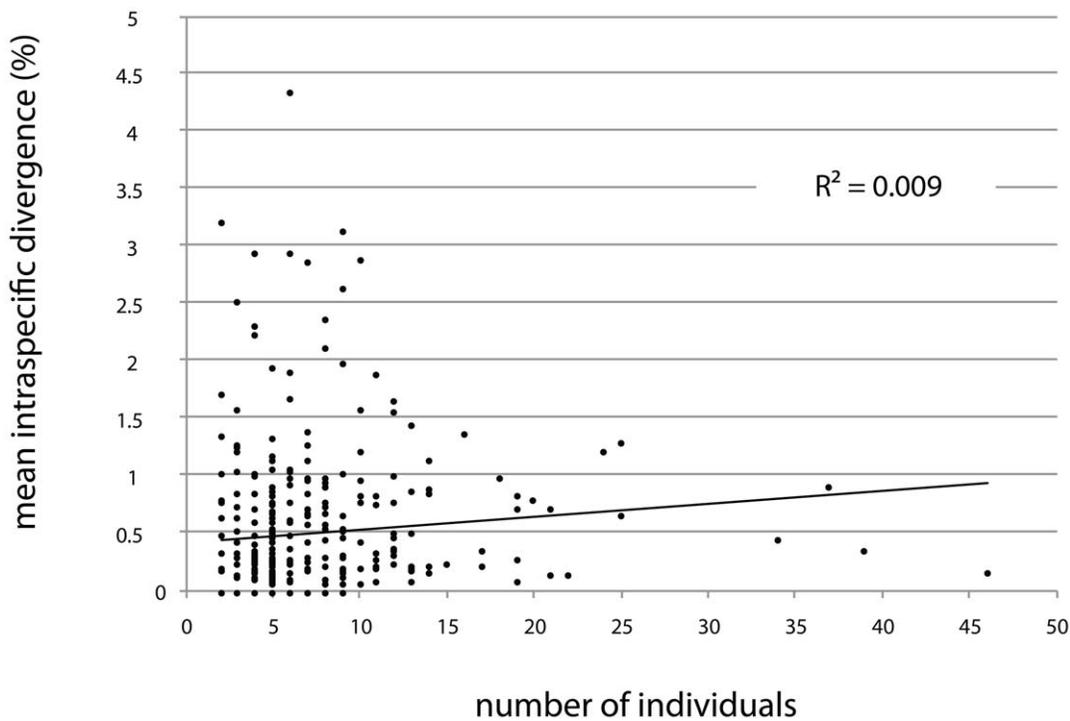


Figure 2. The relationship between mean intra-specific divergence and the number of individuals analyzed. The linear regression is not significant ($P = 0.07$).
doi:10.1371/journal.pone.0018290.g002

Table 2. Geometrid species with high intraspecific COI variation.

Taxon	Individuals per lineage	Mean sequence divergence (%)
<i>Aethalura intertexta</i> (Walker)	1/9	5.31
<i>Dichorda rectaria</i> (Grote)	1/2	3.37
<i>Digrammia irrorata</i> (Packard)	4/3	4.87
<i>Dysstroma colvillei</i> (Guenée)	2/2	3.37
<i>Ectropis crepuscularia</i> (Denis & Schiffmüller)	1/20	4.61
<i>Eupithecia annulata</i> (Hulst)	2/12	3.13
<i>Eupithecia lachrymosa</i> (Hulst)	3/9	3.71
<i>Eupithecia longipalpata</i> Packard	1/2	4.08
<i>Eustroma atrifasciata</i> (Hulst)	1/1	3.20
<i>Eustroma semiatrata</i> (Hulst)	2/4/3	4.28
<i>Hydriomena perfracta</i> (Swett)	1/3	4.43
<i>Macaria colata</i> (Grote)	1/7/1	5.04
<i>Macaria decorata</i> (Hulst)	2/8	7.56
<i>Mesoleuca gratulata</i> (Walker)	8/1	4.39
<i>Nemoria unitaria</i> (Packard)	1/1/7	4.45
<i>Plataea trilinearia</i> (Packard)	4/4	3.59
<i>Plemyria georgii</i> Hulst	1/5	7.31
<i>Probole alienaria</i> Herrich-Schäffer	1/5	3.63
<i>Rheumaptera hastata</i> (Linnaeus)	1/2/3	5.73
<i>Rheumaptera subhastata</i> (Nolcken)	1/7	5.05
<i>Sicya macularia</i> (Harris)	5/6	3.25
<i>Spodolepis danbyi</i> (Hulst)	1/11	3.94
<i>Synchlora aerata</i> (Fabricius)	2/8	3.64
<i>Synchlora bistrifaria</i> (Packard)	1/36	5.75
<i>Triphosa haesitata</i> (Guenée)	1/9	3.64
<i>Xanthorhoe lacustrata</i> (Guenée)	1/6	4.58

The number of specimens per cluster is separated by a forward slash (/) with two numbers indicating cases with two distinct clusters and three numbers indicating three clusters. The mean sequence divergence calculated for each species was calculated using the Kimura 2-parameter distance model.
doi:10.1371/journal.pone.0018290.t002

available genetic and collateral data. Used in combination, these components can accelerate integrative taxonomic studies [51,53] and define the ‘taxonomy of the future’ [54].

Supporting Information

Figure S1 Neighbour-joining tree for 400 species of Geometridae and Uraniidae from British Columbia, Canada and surrounding provinces, territories and states. BOLD process IDs and collection localities are provided for each sequence. (PDF)

Table S1 List of specimens analyzed in the present study. Specimen accessions, BOLD process IDs, GenBank accessions, collection localities, and the storing institution are provided for each specimen. (PDF)

References

- Packer L, Gibbs J, Sheffield CS, Hanner R (2009) DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources* 9: 42–50.
- Armstrong KF, Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B* 360: 1813–1823.

3. Janzen DH, Hajibabaei M, Burns JM, Hallwachs W, Remigio E, et al. (2005) Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1835–1845.
4. Smith MA, Fisher BL, Hebert PDN (2005) DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions of the Royal Society B* 360: 1828–1834.
5. Hogg ID, Hebert PDN (2004) Biological identification of springtails (Hexapoda, Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology* 82: 749–754.
6. Barrett RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology* 83: 481–491.
7. Cywinska A, Hunter FF, Hebert, PDN (2006) Identifying Canadian mosquito species through DNA barcodes. *Medical and Veterinary Entomology* 20: 413–424.
8. Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America* 103: 3657–3662.
9. Footitt RG, Maw HEL, von Dohlen CD, Hebert PDN (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources* 8: 1189–1201.
10. Smith MA, Fisher BL (2009) Invasions, DNA barcodes, and rapid biodiversity assessment using ants of Mauritius. *Frontiers in Zoology* 6: 31.
11. Wilson AD, Schiff NM (2010) Identification of *Sirex noctilio* and native North American woodwasp larvae using DNA barcodes. *Journal of Entomology* 7: 60–79.
12. Rivera J, Currie DC (2009) Identification of Nearctic black flies using DNA Barcodes (Diptera: Simuliidae). *Molecular Ecology Resources* 9(Suppl. 1): 224–236.
13. Zhou X, Adamowicz SJ, Jacobus LM, Dewalt RE, Hebert PDN (2009) Towards a comprehensive barcode library for arctic life—Ephemeroptera, Plecoptera, and Trichoptera of Churchill, Manitoba, Canada. *Frontiers in Zoology* 6: 30.
14. Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* 103: 968–971.
15. deWaard JR, Landry J-F, Schmidt BC, Derhousoff J, McLean JA, et al. (2009) In the dark in a large urban park: DNA barcodes illuminate cryptic and introduced moth species. *Biodiversity and Conservation* 18: 3825–3839.
16. Hebert PDN, deWaard JR, Landry J-F (2010) DNA barcodes for 1/1000 of the animal kingdom. *Biology Letters* 6: 359–362.
17. Lukhtanov V, Sourakov A, Zakharov EV, Hebert PDN (2009) DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. *Molecular Ecology Resources* 9(Suppl. 1): 1302–1310.
18. Elias-Gutierrez M, Valdez-Moreno M (2008) A now cryptic species of *Leberis* Smirnov, 1989 (Crustacea, Cladocera, Chydoridae) from the Mexican semi-desert region, highlighted by DNA barcoding. *Hidrobiologica* 18: 63–74.
19. Gibbs J (2009) Integrative taxonomy identifies new (and old) species in the *Lasioglossum* (*Dialictus*) *tegulare* (Robertson) species group (Hymenoptera, Halictidae). *Zootaxa* 2032: 1–38.
20. Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, et al. (2008) Identifying Canadian freshwater fishes through DNA barcodes. *PLoS ONE* 3: e2490.
21. Kerr KCR, Lijtmaer DA, Barreira AS, Hebert PDN, Tubaro PL (2009) Probing evolutionary patterns in Neotropical birds through DNA barcodes. *PLoS ONE* 4: e4379.
22. Scoble MJ, Hausmann A (updated 2007): Online list of valid and available names of the Geometridae of the World [http://www.lepbarcoding.org/geometridae/species_checklists.php].
23. Ferguson DC (1983) Geometridae. In Checklist of the Lepidoptera of America North of Mexico. Hodges RW, Dominick T, Davis DR, Ferguson DC, Franclemont JG, et al. eds. E.W. Classey Ltd. and Wedge Entomological Research Foundation. London, UK. pp 88–107.
24. Nieminen M (1986) Migration of a moth species in a network of small islands. *Oecologia* 108: 643–651.
25. Doak P (2000) Population consequences of restricted dispersal for an insect herbivore in a subdivided habitat. *Ecology* 81: 1828–1841.
26. Scoble MJ (1992) *The Lepidoptera. Form, Function and Diversity*. Oxford, UK: Oxford University Press.
27. Padiál PM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16.
28. Ferris CD, Schmidt BC (2010) Revision of the North American genera *Tetracis* Guenée and synonymization of *Synaxis* Hulst with descriptions of three new species (Lepidoptera: Geometridae: Ennominae). *Zootaxa* 2347: 1–36.
29. Pohl GR, Anweiler GG, Schmidt BC, Kondla NG (2010) An annotated list of the Lepidoptera of Alberta, Canada. *ZooKeys* 38: 1–549.
30. Hausmann A, Hebert PDN, Mitchell A, Rougerie R, Sommerer M, et al. (2009) Revision of the Australian *Oenochroma vinaria* Guenée, 1858 species-complex (Lepidoptera: Geometridae, Oenochrominae): DNA barcoding reveals cryptic diversity and assesses status of type specimen without dissection. *Zootaxa* 2239: 1–21.
31. Huemer P, Hausmann A (2009) A new expanded revision of the European high mountain *Sciadia tenebraria* species group (Lepidoptera, Geometridae). *Zootaxa* 2117: 1–30.
32. Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1805–1811.
33. Vogler AP, Monaghan MT (2007) Recent advances in DNA taxonomy. *Journal of Zoological Systematics and Evolutionary Research* 45: 1–10.
34. Waugh J (2007) DNA barcoding in animal species: progress, potential and pitfalls. *Bioessays* 29: 188–197.
35. Mitchell A (2008) DNA barcoding demystified. *Australian Journal of Entomology* 47: 169–173.
36. Marshall IB, Schut PH (1999) A national ecological framework for Canada. Ottawa, ON: Environment Canada, Ecosystems Science Directorate, and Agriculture and Agri-Food Canada, Research Branch.
37. Regier JC, Zwick A, Cummings MP, Kawahara AY, Cho S, et al. (2009) Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BMC Evolutionary Biology* 9: 280.
38. Ratnasingham S, Hebert PDN (2007) The Barcode of Life Data System. *Molecular Ecology Notes*, (http://www.barcodinglife.org) 7: 355–364.
39. Hajibabaei M, deWaard JR, Ivanova NV, Ratnasingham S, Dooh R, et al. (2005) Critical factors for the high volume assembly of DNA barcodes. *Philosophical Transactions of the Royal Society B* 360: 1959–1967.
40. Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998–1002.
41. deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes: Analytical Protocols. In *Methods in Molecular Biology: Environmental Genetics* Cristofre Martin, ed. Totowa, USA: Humana Press Inc.. pp 275–293.
42. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America* 101: 14812–14817.
43. Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.
44. Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution* 4: 406–425.
45. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.
46. Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology* 3: 2229–2238.
47. Bolte KB (1990) Guide to the Geometridae of Canada (Lepidoptera). VI. Subfamily Larentiinae. 1. Revision of the genus *Eupithecia*. *Memoirs of the Entomological Society of Canada* 151: 1–253.
48. Ferguson DC (2008) Geometroidae: Geometridae (part): Ennominae (part) — Abraxini, Cassymini, Macariini. Fasc. 17.2. In *The Moths of America North of Mexico* Dominick RB, Ferguson DC, Franclemont JG, Hodges RW, Munroe EG, eds. Washington, D.C.: Wedge Entomological Research Foundation. 576 p.
49. Tomon TT (2007) A revision of the genus *Probole* Herrich-Schäffer (Lepidoptera: Geometridae) [abstract]. *Entomological Society of America, 2007 Annual Meeting, 9–12 December 2007, San Diego, CA. Entomological Society of America, Lanham, MD.* (http://esa.confex.com/esa/2007/techprogram/paper_31679.htm).
50. Witt JDS, Threlloff DL, Hebert PDN (2006) DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology* 15: 3073–3082.
51. Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, et al. (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences of the United States of America* 105: 12359–12364.
52. Locke SA, McLaughlin JD, Dayanandan S, Marcogliese DJ (2010) Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome *c* oxidase I and internal transcriber spacer sequences. *International Journal of Parasitology* 40: 333–343.
53. Miller SE (2007) DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences of the United States of America* 104: 4775–4776.
54. Penev L, Erwin T, Thompson FC, Sues H-D, Engel MS, et al. (2008) ZooKeys, unlocking Earth's incredible biodiversity and building a sustainable bridge into the public domain: From “print-based” to “web-based” taxonomy, systematics, and natural history. *ZooKeys* 1: 1–7.