

Contribution of DNA barcoding to the study of the bees (Hymenoptera: Apoidea) of Canada: progress to date

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Abstract—Bees (Hymenoptera: Apoidea, Apiformes) are taxonomically and ecologically diverse, with a wide range of social complexity, nesting preferences, floral associations, and biogeographic restrictions. A Canadian bee checklist, greatly assisted by the gene-assisted approach of DNA barcoding, is nearing completion. Previous evaluation of bee diversity in Canada, assisted by DNA barcoding, was restricted to Nova Scotia, which contains about 25% of the bee species in the country. Here, we summarise efforts to date to build a comprehensive DNA barcode library supporting bee taxonomic studies in Canada, consisting of more than 12 500 barcode-compliant sequences yielding 811 distinct barcode index numbers (BINs). This appears to represent ~95% of the 856 bee species presently recorded from Canada, but comparison with known morphological species in each genus shows that some genera are still under-sampled or may contain cryptic taxa, with much taxonomic work still to be done on bees in Canada. This is particularly true within the taxonomically difficult genera *Andrena* Fabricius (Andrenidae), *Hylaeus* Fabricius (Colletidae), *Melissodes* Latreille (Apidae), *Nomada* Scopoli (Apidae), *Osmia* Panzer (Megachilidae), and *Sphecodes* Latreille (Halictidae). DNA analysis will likely be a key asset in resolving bee taxonomic issues in Canada in the future, and to date has even assisted studies of well-known bee taxa. Here we present summaries of our results, and discuss the use of DNA barcoding to assist future taxonomic work, faunal lists, and ecological studies.

Introduction

Bees (Hymenoptera: Apoidea) are well known for their role as pollinators, and have become the focus of much recent attention as populations are undergoing declines globally (*e.g.*, Committee on the Status of Pollinators in North America 2007; Vamosi *et al.* 2016). Bees are a diverse taxon with estimates of over 20 000 species worldwide (Michener 2007; Ascher and Pickering 2017), with new species being described regularly, including 22 in Canada since 2010 (Gibbs 2010; Rehan and Sheffield 2011; Gibbs *et al.* 2013;

Williams *et al.* 2016). Brown and Paxton (2009) stressed that alpha taxonomy, in particular lack of taxonomic expertise and resources, is one of the major factors limiting ability to conserve bees, since being able to accurately identify species and link them to specific habitats, crops or other plants they pollinate and/or the communities they service is critical for conserving them. However, taxonomic and faunistic knowledge of Canadian bees is incomplete for various reasons (see Sheffield *et al.* 2009, 2014; Droege *et al.* 2010), including the large number of poorly studied taxa, and the large size of the country resulting in many areas

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being poorly sampled. One challenge is underfunding of taxonomic training and research in general, including in Canada (Packer *et al.* 2009b), potentially contributing to slow rates of species discovery and taxonomic revisions (*i.e.*, keys, descriptions and other tools for the identification of all species within a given taxon, including those already described) (see Carvalho *et al.* 2014). This taxonomic impediment ultimately affects many ecological studies and conservation strategies for bees (*e.g.*, Sheffield *et al.* 2016), though is even more of an issue for other taxa (*e.g.*, Maurer 2000) that have not received the same level of attention.

Danks (1979) reviewed the diversity of terrestrial arthropods in Canada as an early initiative of the Biological Survey of Canada, this work considered one of the many outstanding achievements of the Biological Survey of Canada (Danks 2016), this issue celebrating its 40th anniversary. That report (Danks 1979) showed that the arthropod fauna was poorly documented, and pointed out that the 29 975 reported insect species likely represented only about half of the total expected for Canada. Since then, taxonomic advances (including new molecular taxonomy tools) have improved the knowledge base for many groups. For example, Hebert *et al.* (2016) used DNA barcoding to estimate more than 94 000 insect species in Canada, about a 58% increase over Danks (1979) estimate, with Diptera and Hymenoptera (especially Parasitica) being “unexpectedly diverse” and in general poorly known.

In the Hymenoptera superfamily Apoidea, which includes the bees (Apiformes), ~800 species are currently known from Canada (Sheffield *et al.* 2014), an increase of about 50 species since the estimates in Danks (1979; 746 species, though 231 more were predicted). No national checklist of bees for Canada has ever been completed, though regional lists for Nova Scotia (Sheffield *et al.* 2003), Newfoundland (Hicks 2009), and the Prairies Ecozone (Sheffield *et al.* 2014) have. The bee fauna of Nova Scotia was also the first to be subjected to an extensive DNA barcoding effort (Sheffield *et al.* 2009), starting in the early 2000s with ongoing diversity assessments (*i.e.*, Sheffield *et al.* 2003, 2008).

Bee identification is well known to be challenging for several speciose genera in North America (see Packer *et al.* 2009a; Droege *et al.* 2010;

Gibbs 2010) due to lack of keys and taxonomic expertise for some taxa. Historically, even the greatest bee taxonomists of their time had trouble distinguishing species – T.D.A. Cockerell (1866–1948) described more bee species than any other person (with 3132 still considered valid today; Ascher and Pickering 2017), but in several cases he (alone or as a coauthor) described the same species multiple times (sometimes in a single manuscript) that today are considered one. Early taxonomists (and likely many today) had limited ability to accurately link specimens to geography, associate sexes, *etc.*, so often described specimens with slight variations in size and/or colour as new species or subspecies (or varieties). Allometric variation among castes in some eusocial Halictidae species (Gibbs 2010), or linked to body size in *Ceratina* Latreille (Apidae) (Rehan and Sheffield 2011), has also likely contributed to the taxonomic difficulties observed in some bee taxa. Since then, taxonomists have seen the advent of high resolution microscopes, computers and extensive collection databases (*e.g.*, Meier and Dikow 2004), wide and mostly free availability of taxonomic literature and other taxonomic resources (*e.g.*, Biodiversity Heritage Library, university journal databases), and easier exchange of material for study within and among countries, including data sharing (including actual specimens and/or high-quality photographs, and specimen data). Incorporation of high-quality images into web-based interactive taxonomic keys (*e.g.*, the mandate of the Canadian Journal of Arthropod Identification) has also facilitated accurate species level identification for the taxa that have received treatment. Despite these tools, many Canadian bee taxa are in need of revision. The challenge for today’s generation of taxonomists will be overcoming challenges to train the next generation of taxonomists to understand the range of software and procedures to construct and test phylogenies (morphological and molecular), species concepts, predictive models for distribution, and find employment to do so as a career. Ongoing work on bee taxonomy in Canada (primarily at York University, Toronto, Ontario, Canada) will be aided by the recent (since 2009) hiring of three additional full-time bee systematists in collection-based institutions (Royal Saskatchewan Museum, University of Manitoba, Canadian National Collection of Insects,

Arachnids, and Nematodes), which has already resulted in a number of published works on the taxonomy of Canadian bees (Gibbs 2010; Rehan and Sheffield 2011; Sheffield *et al.* 2011b; Dumesh and Sheffield 2012; Onuferko 2017).

One important change in taxonomic research since the inception of the Biological Survey of Canada 40 years ago has been the development of molecular taxonomy tools. Genetic-based identification systems such as DNA barcoding (as per Hebert *et al.* 2003a, 2003b) have emerged as powerful and cost-effective tools for accurate identification of biota and assessing and understanding the extent of diversity in groups that have proven difficult using classical taxonomic techniques (Köhler 2007; Packer *et al.* 2009a). For North America, and Canada in particular, several DNA barcoding projects have been undertaken for pollinators (*e.g.*, Sheffield *et al.* 2009; Hebert and Landry 2010; Hebert and Humble 2011; Zahiri *et al.* 2014, 2017), and many other taxa. These DNA barcode libraries can assist traditional morphological taxonomy by allowing identification of life stages for which keys are not available and/or taxonomy is more difficult (*e.g.*, Slowik and Blagoev 2012), associating sexes (*e.g.*, Sheffield *et al.* 2011b), and in studies assessing biological diversity within regional (*e.g.*, Sheffield *et al.* 2009) and poorly studied habitat-specific biotas (*e.g.*, Smith *et al.* 2005, 2009; Stahlhut *et al.* 2013). DNA barcode sequences (*i.e.*, cytochrome *c* oxidase subunit I (COI)) and other genetic sequences themselves also contribute to molecular systematics; combining the DNA barcode gene with a single nuclear gene has proven useful for accurate, node-dated phylogenies for bees (Trunz *et al.* 2016). Ultimately, these efforts to increase taxonomic knowledge can inform conservation efforts (Soltis and Gitzendanner 1999; Hajibabaei *et al.* 2007; Goldstein and DeSalle 2011). Important caveats to relying on DNA methods are that reference material (*i.e.*, identified specimens that have been sequenced) must be accurately identified (Collins and Cruickshank 2013), and that species do not show high levels of intraspecific variation (*e.g.*, Spooner 2009).

The primary objective of this study is to summarise our efforts to date in developing a “Bees of Canada” DNA barcode database to facilitate taxonomic and faunal studies of bees in Canada.

This effort builds on previous DNA-facilitated taxonomic revisions (*e.g.*, Gibbs 2010; Sheffield *et al.* 2011b) and regional data sets (*e.g.*, Sheffield *et al.* 2003, 2009) for Canada, and other regional faunas or taxonomic treatments outside of North America (Carolan *et al.* 2012; Magnacca and Brown 2012; Francoso and Arias 2013; Schmidt *et al.* 2015). Specifically, we will determine how well the accumulated number of unique barcode index numbers, which show high concordance with species (Ratnasingham and Hebert 2013) for sequenced Canadian bees, matches our numerical species tally and the known composition of species from Canada. Barcode index number assignment can be used to verify species identifications as well as document diversity when taxonomic information is lacking (Ratnasingham and Hebert 2013). Comparisons will be done nationally, provincially/territorially for species, and at the genus level. We also highlight how DNA barcoding is contributing to bee faunistic knowledge and traditional taxonomic work in Canada, and provide examples of how DNA barcodes can be, and have been, used to complement morphological methods to increase our taxonomic knowledge, including in phylogenetic studies, and discuss the issues and limitations with the current status of Canadian bee taxonomic knowledge. Thus, the diversity and taxonomy of bees in Canada provides a good illustration of the advances in biodiversity study that have occurred during the time period covered by the Biological Survey of Canada, and will complement the bees of Canada image library (www.yorku.ca/bugsrus/resources/galleries/boc), a checklist of the bees of Canada (in preparation) and a soon to be launched “Bees of Canada” website (www.beesofcanada.com). In addition to a full catalogue treatment, the latter will provide images, distributional information (*i.e.*, jurisdiction, ecozone), floral hosts, and literature for all bee species occurring in Canada.

Methods

Material for DNA barcoding

The development of a DNA barcode library for bees began at a regional level (*i.e.*, Nova Scotia) in the early 2000s, with a summary publication in 2009 (Sheffield *et al.* 2009). Since the onset of the campaign to barcode Canada’s bees in 2006 – a

component of a larger effort to barcode the world's bees (Packer *et al.* 2008, 2009b) – samples have been collected from across North America, with special focus within southern Canada (Fig. 1). These have been combined with museum specimens from the following Canadian and United States of America collections: Packer Collection at York University; Biodiversity Institute of Ontario, University of Guelph (Guelph, Ontario, Canada); J.B. Wallis-R.E. Roughley Museum of Entomology, University of Manitoba (Winnipeg, Manitoba, Canada); Royal Saskatchewan Museum (Regina, Saskatchewan, Canada); Royal British Columbia Museum (Victoria, British Columbia, Canada); Simon Fraser University (Vancouver, British Columbia, Canada); Canadian National Collection of Insects, Arachnids, and Nematodes (Ottawa, Ontario, Canada); American Museum of Natural History (New York, New York, United States of America); Smithsonian National Museum of Natural History (Washington, District of Columbia, United States of America); United States Department of Agriculture, Agricultural Research Service Bee Biology and Systematics Laboratory (Logan, Utah, United States of America); United States Geological Survey Patuxent Wildlife Research Center (Laurel, Maryland, United States of America); and other contributors. The majority of DNA barcoded specimens from Canada are deposited at York University and the Biodiversity Institute of Ontario.

Tissue sampling and molecular protocols

For barcoding bees, tissue samples (*i.e.*, usually a single mesothoracic or metathoracic leg from pinned specimens) were removed and sent to the Biodiversity Institute of Ontario for extraction and sequencing using well-established protocols (*e.g.*, Hajibabaei *et al.* 2005). Barcode index numbers are assigned to sequences in the Barcode of Life Datasystems (Ratnasingham and Hebert 2007) using a sequential process of algorithms using pre-defined distance thresholds with refined clustering of sequences, each of which represents an algorithmically grouped barcode sequence or group of sequences, with constituents of each barcode index number usually showing a high concordance within species boundaries (Ratnasingham and Hebert 2013). To summarise the DNA barcodes of the bee fauna of Canada as a whole, single representatives of each species/barcode index number, regardless

of how many taxon names were associated with that barcode index number, were selected for analysis from the thousands of specimens that are currently barcoded. This differed from studies looking at intraspecific sequence divergence (which use multiple individuals of each species from multiple locations; Bergsten *et al.* 2012). Among this material, sequence data were downloaded from the barcode of life data system and imported in Mega Version 7 (Kumar *et al.* 2016) for sequence alignment and construction of a neighbour-joining tree using the neighbour-joining algorithm with the K2P model, with pairwise deletion of missing data, and the inclusion of all codon positions and substitution types (as used in the barcode of life data system analytical module). The patterns were then visualised using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

A checklist of known Canadian bee species, including those in each province and territory was prepared for a national conservation assessment (Canadian Endangered Species Conservation Council 2016). This species checklist formed the basis for assessing completion of the DNA barcoding campaign for Canada. Genus-level summaries of the number of valid species known to occur in Canada, and comparable number of unique barcode index numbers were prepared. As the species data and barcode index numbers were also partitioned by jurisdiction (*i.e.*, province and territory), tallies of barcode index numbers represented in each province (and associated with taxonomic information) were compared with these data to show diversity of both known species and barcode index numbers per jurisdiction. This included specimens within a single barcode index number, which had no species-level identification. Only one representative of each barcode index number was added to the tally, regardless of the species-level identification associated with the DNA barcoded specimens, as the comparisons were of known species to barcode index numbers within each genus.

Results and discussion

Much of the sampling to date has been focussed in areas of southern Canada (south of 50°N) (Fig. 1), but this region supports almost all of the species known from Canada (see Sheffield *et al.* 2014),

Fig. 1. Bee barcode sampling intensity in Canada. Darker coloured squares represent areas with more intensive sampling.



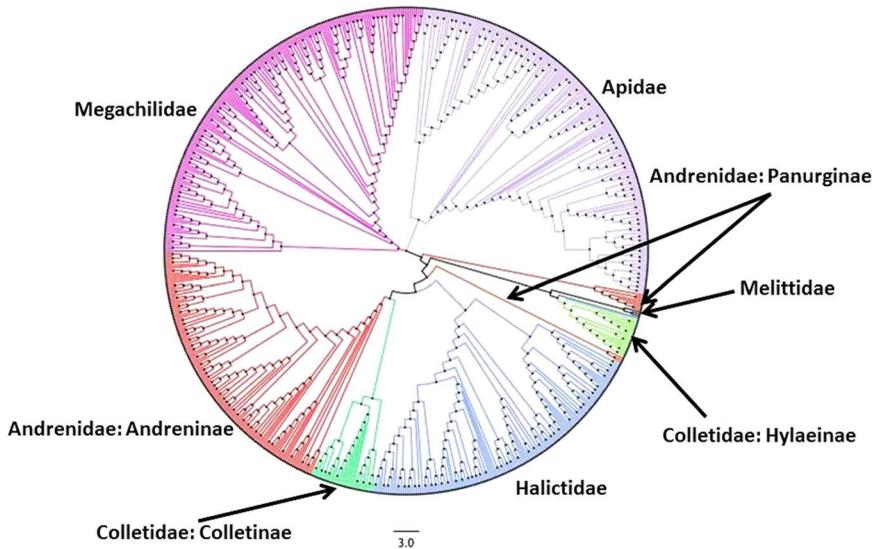
suggesting that the specimens received are largely representative of bee diversity in Canada. Approximately 14 200 bee specimens have been processed for DNA barcoding, and these have yielded 12 600 barcode-compliant sequences, resulting in 811 distinct barcode index numbers from within Canada (Fig. 2). This value represents 95% of the total of 856 species presently known from Canada (Table 1), but comparison of barcode index numbers with known species patterns in different genera shows that many more species may be present. It is likely that the actual number of species, and barcode index numbers assigned to these species, will continue to increase with continued sampling.

Of the 52 genera currently recorded in Canada, only 25 (45%) have all known species barcoded, though most of these are represented by less than five species (Table 1, Figs. 3–4). In most (20) of the remaining genera, fewer barcode index numbers have been recorded than the known morphological species suggesting that these taxa have not been fully sampled. Some of these discrepancies are due to multiple morphologically distinct species sharing a barcode index number (*e.g.*, *Lasioglossum* Curtis (Halictidae); see full discussion under Issue 2, below). By contrast, other

genera have more barcode index numbers recorded for them than the known species in Canada (Table 1, Figs. 3, 4) even though two of these (*Megachile* Latreille (Megachilidae), *Bombus* Latreille (Apidae)) have had recent taxonomic treatment (Sheffield *et al.* 2011b; Williams *et al.* 2014, respectively). The remaining genera in this category are among those needing the most taxonomic work, and are largely responsible for the differences in the number of species and barcode index numbers observed in many parts of Canada (Fig. 5), especially British Columbia, the Prairie Provinces, and Ontario.

At the species level, of the 856 species recorded from Canada, 253 have no clearly associated barcode index number due to lack of material, poor success in obtaining sequences, or taxonomic uncertainty in assigning a name to the specimen associated with a sequence. An additional 28 species share barcode index numbers with other taxa, so the number of barcode index numbers cannot equal the number of species. On the other hand, 237 barcode index numbers are not currently associated to specific taxa (four of these represent cases where subspecific differences in barcode index numbers are observed).

Fig. 2. Neighbour-joining (NJ) tree for single representative COI barcode sequences (BINs) for the bees of Canada. Colours represent the six bee families in Canada. Note that in this NJ tree, the families Andrenidae and Colletidae appear multiple times.



The combined use of species and barcode index numbers alters our understanding of bee diversity in Canada, both nationally and at the provincial/territory level (Fig. 5). This is particularly true in British Columbia where the actual diversity of bees may fall between 500 and 600 species. These data suggest that there is still much taxonomic work to be done on Canadian bees, particularly within the genera *Hylaeus* Fabricius (Colletidae), *Nomada* Scopoli (Apidae), *Sphecodes* Latreille (Halictidae), and *Osmia* Panzer (Megachilidae), though a solid framework supporting taxonomic studies has been built in the barcode of life data system.

One issue to be resolved will be to ensure that all specimens sharing barcode index numbers are examined using traditional morphological taxonomic methods to support accepted taxonomy and nomenclature. Though specimens used to populate the bees of Canada DNA barcode project in barcode of life data system should be identified before being added (*e.g.*, Collins and Cruickshank 2013), some speciose taxa are lacking keys and/or taxonomic specialists (and may have high numbers of undescribed species), making *a priori* identifications unlikely. Another consideration is the fact that many bee species are sexually dimorphic, and one of the sexes for many of these may be

unknown or not associated (*e.g.*, Sheffield and Westby 2007), leading to inaccurate estimates of total species richness. Therefore, accessible DNA-based identification tools can be considered a major innovation in species diversity assessment in the 40 years since the inception of the Biological Survey of Canada. However, even as we should not assume that all taxa can be separated morphologically (*e.g.*, Carman and Packer 1996; Danforth *et al.* 1998; Packer *et al.* 2009a), neither can we assume that barcodes alone will address all biodiversity questions (Gibbs 2010). This concept can be illustrated by examining the discrepancies between the known species and the barcode index numbers found in the bees of Canada data set. We summarise these below, provide a genus-by-genus account of where additional taxonomic work is required, and lastly provide examples of how and where combining DNA barcoding and traditional morphological taxonomy has been successful in studies of Canadian bees.

What are the issues?

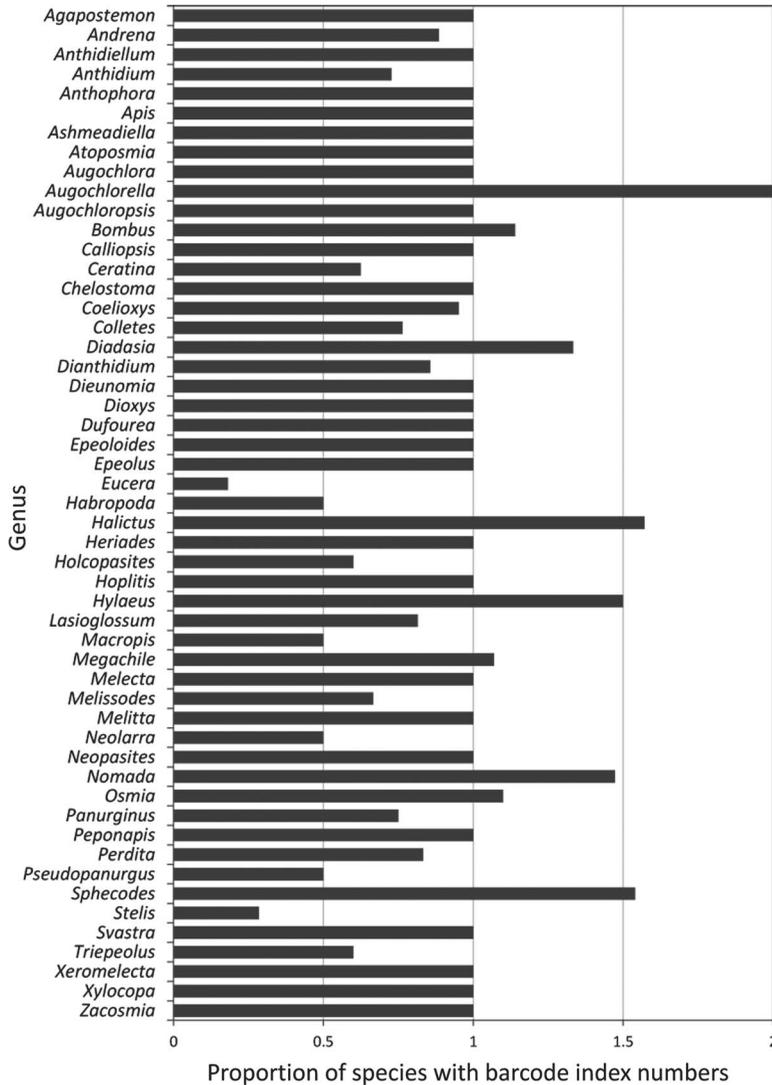
Issue 1. One species: multiple barcode index numbers. Collins and Cruickshank (2013) note that it is desirable to populate barcode of life data system with *a priori* identified specimens to build taxon-specific projects, though multiple

Table 1. Summary of the bee species in Canada with the number of species known and barcoded from each genus.

Family	Genus	Species recorded	Barcode index numbers	Difference	
Colletidae	<i>Colletes</i>	34 [+4 ssp]	26	-8 [-12]	
	<i>Hylaeus</i>	20 [+1 ssp]	30	+10 [+9]	
Andrenidae	<i>Andrena</i>	149 [+1 ssp]	132	-17 [-18]	
	<i>Calliopsis</i>	5	5	*	
	<i>Panurginus</i>	4	3	-1	
	<i>Perdita</i>	12 [+1 ssp]	10	-2 [-3]	
	<i>Pseudopanurgus</i>	12	6	-6	
Halictidae	<i>Agapostemon</i>	7	7		
	<i>Augochlora</i>	1	1		
	<i>Augochlorella</i>	1	2		
	<i>Augochloropsis</i>	1	1		
	<i>Dieunomia</i>	1	1		
	<i>Dufourea</i>	8	8		
	<i>Halictus</i>	7	11	+4	
	<i>Lasioglossum</i>	141	115	-26	
	<i>Sphecodes</i>	33	51	+18	
	Melitidae	<i>Macropis</i>	2	1	-1
<i>Melitta</i>		1	1		
Megachilidae	<i>Anthidiellum</i>	2	2		
	<i>Anthidium</i>	11	8	-3	
	<i>Ashmeadiella</i>	4 [+1 ssp]	4	[-1?]	
	<i>Atoposmia</i>	2	2		
	<i>Chelostoma</i>	5	5		
	<i>Coelioxys</i>	21	20	-1	
	<i>Dianthidium</i>	7	6	-1	
	<i>Dioxys</i>	1	1		
	<i>Heriades</i>	4	4		
	<i>Hoplitis</i>	12 [+3 ssp]	12	[-3]	
	<i>Megachile</i>	40 [+1 ssp]	43	+3 [+2]	
	<i>Osmia</i>	79 [+1 ssp]	87	+8 [+9]	
	<i>Stelis</i>	21	6	-15	
	Apidae	<i>Anthophora</i>	12	12	*
		<i>Apis</i>	1	1	
<i>Bombus</i>		44 [+2 ssp]	51	+7 [+5]	
<i>Ceratina</i>		8	5	-3	
<i>Diadasia</i>		3	4	+1	
<i>Epeoloides</i>		1	1		
<i>Epeolus</i>		12	12	*	
<i>Eucera</i>		11	2	-9	
<i>Habropoda</i>		2	1	-1	
<i>Holcopasites</i>		5	3	-2	
<i>Melecta</i>		3	3		
<i>Melissodes</i>		27 [+1 ssp]	18	-9 [-8]	
<i>Neolarra</i>		2	1	-1	
<i>Neopasites</i>		1	1		
<i>Nomada</i>		55	81	+26	
<i>Peponapis</i>		1	1		
<i>Svastra</i>		1	1		
<i>Triepeolus</i>		20	12	-8	
<i>Xeromelecta</i>		1	1		
<i>Xylocopa</i>		1	1		
<i>Zacosmia</i>	1	1			

"ssp" = multiple subspecies are recognised in Canada. See generic treatment for genera with a "+", "-", or "" in the last column; values in [] represent the difference when subspecies are considered.

Fig. 3. Summary of the proportion of bee species for each genus for which there are barcodes with assigned barcode index numbers. Genera represented by bars less than 1.00 have more species recorded than barcode index numbers; greater than 1 have more barcode index numbers than species recorded in Canada.

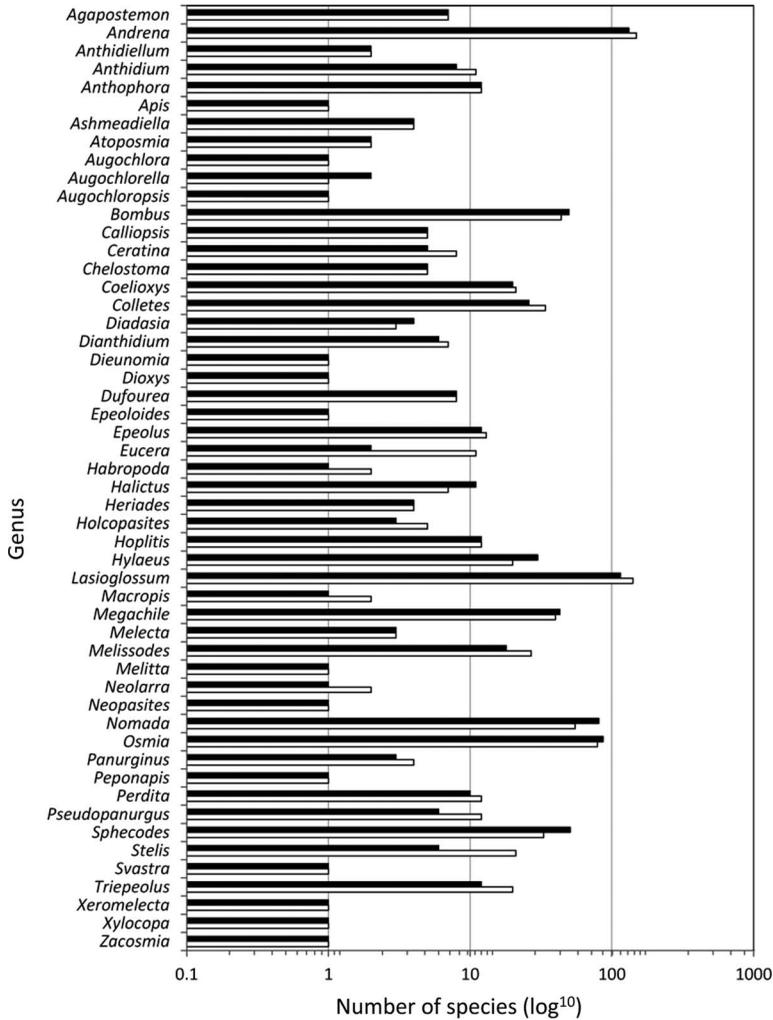


barcode index numbers and/or high levels of sequence variation can be recognised after the fact. There are a few cases in which bee specimens recognised as good morphologically identified species have multiple barcode index numbers. For instance, among the well-studied bumble bees (Apidae: Apinae: Bombini), *Bombus impatiens* Cresson and *B. ternarius* Say each have three barcode index numbers represented by Canadian material in barcode of life data system. The fact that the representatives

of each barcode index number cluster together suggests that this is not the result of mis-identification but rather slight, but consistent variation in COI, without any pattern associated with morphology or geography.

Other discrepancies are likely due to cryptic species within poorly studied groups, or groups needing further study (see Discussions below). Another possibility is heteroplasmy, in which a single specimen can yield two different DNA barcodes (and possibly barcode index numbers),

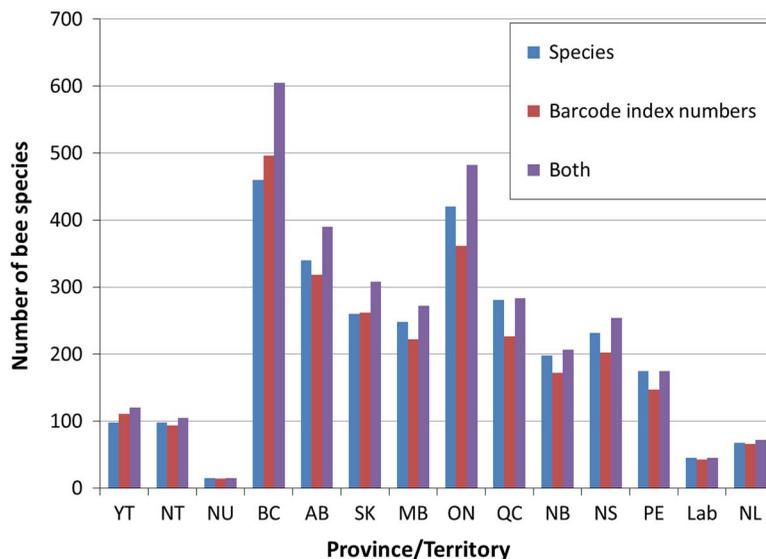
Fig. 4. The number of species (white bars) and number of barcode index numbers (black bars) for each genus of bee within Canada; x-axis \log^{10} scale.



as reported for the genus *Hylaeus* by Magnacca and Brown (2009, 2010), though this may largely go undetected as usually only a single tissue sample (e.g., a leg) is used for each specimen. Currently, studies have begun to address these types of patterns within the genus *Hylaeus* in Canada. At least three species, *Hylaeus coloradensis* (Cockerell), *H. mesillae* (Cockerell), and *H. modestus* Say show multiple barcode index numbers, though this group is rather difficult taxonomically, and much morphological variation within species has resulted in many synonymies. For example, *H. modestus* has two recognised

subspecies and eight additional junior synonyms (Hurd 1979). These examples may also illustrate that for widespread species, it is important to sample multiple specimens of each species across its range; Bergsten *et al.* (2012) suggest that at least 70 individuals from throughout the range (of a wide ranging species) would be required to account for 95% of the variation within a species. This may be even more important for Holarctic species; *Andrena barbilibris* (Kirby) (Andrenidae) is an example of a Holarctic species with three barcode index numbers in North America alone. *Halictus confusus* Smith (Halictidae) is an even

Fig. 5. The number of recorded bee species (blue bars) and barcode index numbers (red bars) for each jurisdiction in Canada. The combined data for both (purple bar) includes species and barcode index numbers, including barcode index numbers not associated with species names, so likely represents an overestimation of total species. YT, Yukon; NT, Northwest Territories; NU, Nunavut; BC, British Columbia; AB, Alberta; SK, Saskatchewan; MB, Manitoba; ON, Ontario; QC, Québec; NB, New Brunswick; NS, Nova Scotia; PE, Prince Edward Island; Lab, Labrador; NL, Newfoundland.



more pronounced example, with five barcode index numbers in North America, though two subspecies are recognised on the continent, which may explain this partially, though Rosenmeier and Packer (1993) found no species-level differences among *H. confusus* populations from Alberta, Ontario, and Nova Scotia using electrophoretic methods for 40 loci. Sheffield and Perron (2014) offered some discussion of the implications for nomenclature for this species. There are other recent examples of Holarctic species being recognised as multiple taxa (e.g., Gibbs *et al.* 2013), including in the genus *Bombus* subgenus *Alpinobombus* Skorikov, in which multiple Holarctic species were recognised as separate Old and New World taxa (Williams *et al.* 2015); Williams *et al.* (2016) later described one of these as a new species, *B. kluanensis* Williams and Cannings.

Issue 2. Multiple species: one barcode index number. In contrast to species with multiple barcode index numbers discussed above, there are also several cases in this data set in which multiple species share a single barcode index numbers. Examples are found in the genera *Ceratina*,

Lasioglossum, and *Bombus*. Rehan and Sheffield (2011) recently described a new species of *Ceratina* (subgenus *Zadontomerus* Ashmead) in eastern North America, one of four species sharing a single barcode index numbers. However, phylogenetic analysis of the COI data combined with morphological (Rehan and Sheffield 2011) and ecological data (Vickruck *et al.* 2011) supported the recognition of all four species as valid. Similarly, some clearly recognised species of bumble bees share barcode index numbers, though additional molecular analysis has facilitated the recognition of valid Nearctic/Palaearctic forms (Williams *et al.* 2015) and new species (Williams *et al.* 2016). In other cases (discussed below), incidences of multiple species sharing barcode index numbers have resulted in synonymies, largely supporting that many bumble bees are colour variable (Williams *et al.* 2014). Lastly, there are many species of *Lasioglossum* subgenus *Dialictus* Robertson that share a barcode index number. In one example, at least 16 species share one barcode index number and would not be readily separated by DNA barcoding alone (i.e., use of barcode index numbers),

though they can be separated by morphology and geography (Gibbs 2010).

Issue 3. Multiple specimens, no barcode index numbers. Despite our attempts to obtain full DNA barcodes, a number of species have not yielded fully compliant sequences with barcode index numbers assigned. These include *Andrena carlini* Cockerell, *Calliopsis chlorops* Cockerell, *Lasioglossum athabascense* (Sandhouse), *L. coeruleum* (Robertson), *L. colatum* (Vachal), *L. pallidellum* Ellis, and *L. reasbeckae* Gibbs. Similar issues were found for some species in other bee-barcode campaigns, often associated with the presence and coamplification of the bacterial endosymbiont *Wolbachia* Hertig (Rickettsiaceae) (Magnacca and Brown 2012; Schmidt *et al.* 2015).

Some Canadian species are very rare, some known only from type material collected decades previously (*e.g.*, holotype and two paratypes of *Andrena fulgida* LaBerge), so it is likely that a DNA barcode library will never be complete unless these species are deemed synonymies of other taxa. It is important to note that there has been success in getting sequences from old material (Hajibabaei *et al.* 2006; Shokralla *et al.* 2011), though this is also influenced by methods used to collect and/or store specimens (Sheffield *et al.* 2009). The willingness of institutions to have historic and rare type material subject to tissue removal for DNA barcoding is also likely an issue. Further, the rarity and/or taxonomic ambiguity of some of these species increases the probability that they will not be routinely sampled. As discussed by Lim *et al.* (2012), the sampling effort required to obtain some rare species is enormous.

Despite some of the difficulties mentioned above, many of which should be resolved with further molecular work and analyses (*e.g.*, Downton *et al.* 2014; Williams *et al.* 2015, 2016) and increased sampling (Bergsten *et al.* 2012), the value of DNA barcoding to support traditional taxonomic work has been illustrated in several works (see above and Collins and Cruickshank 2014). Analysis of the Canadian bee fauna provides a good example of this for some groups, and also illustrates where more taxonomic work needs to be done. We discuss the genera with discrepancies between the number of species and the number of barcode index numbers below

(arranged by family and alphabetically by genus within each family) (Table 1).

Family Colletidae

Colletes Latreille. At present, 34 species are known from Canada, four of these with recognised subspecies. Specimens have not been collected and/or COI sequences have not yet been obtained for eight of these. However, *Colletes kincaidii* Cockerell is represented by two unique, yet closely associated barcode index numbers.

Hylaeus Fabricius. There are 30 unique barcode index numbers recorded from Canada, potentially representing ten more species than the 20 presently recorded from Canada. The taxonomy and identification of *Hylaeus* has traditionally been based on the presence and extent of colour markings, and variations within species have resulted in multiple junior synonyms for some (*e.g.*, *H. mesillae*). In other species (*e.g.*, *H. affinis* (Smith), *H. modestus*), females are very difficult to reliably distinguish. Members of this genus are also easily introduced outside of their native range, with several non-native species now established in North America, with many recent arrivals (Sheffield *et al.* 2011a; Gibbs and Dathe 2017; Martins *et al.* 2017). Thus, undocumented non-native species are also a possibility. Magnacca and Brown (2009, 2010) indicated that heteroplasmy within some *Hylaeus* (species outside of North America) creates some issues for successful specimen identification using DNA barcodes (*i.e.*, potentially multiple barcode index numbers for a single specimen), though it is not presently known if this is an issue within the Canadian fauna as typically only a single tissue sample is used for each specimen.

Family Andrenidae

Andrena Fabricius. There are likely many more *Andrena* species yet to be recorded in Canada, in addition to the 149 species confirmed, 132 barcode index numbers have been recorded, 19 of which have not yet been examined for identification to species level. Several species have multiple barcode index numbers, including *Andrena barbilabris* (a Holarctic species represented by three barcode index numbers in North America alone). *Andrena* still requires much attention, as many misidentifications within

barcode of life data system have resulted in multiple names associated with barcode index numbers.

***Calliopsis* Smith.** Five species and five barcode index numbers have been recorded from Canada, though two of the barcode index numbers are from a single species, *Calliopsis andreniformis* Smith. *Calliopsis chlorops* has not yet been successfully barcoded, despite numerous attempts.

***Panurginus* Nylander.** Four species are known from Canada, and only two have been barcoded with barcode index numbers. One species, *P. ineptus* Cockerell has two barcode index numbers. An additional specimen with a non-barcode-compliant sequence may represent a fifth species.

***Perdita* Smith.** A total of 12 species have been recorded from Canada, with one represented by two subspecies. There are 10 barcode index numbers, two of these not yet associated with a named species.

***Pseudopanurgus* Cockerell.** Of the 12 species recorded from Canada, two have associated barcode index numbers, with five additional barcode index numbers not yet assigned to a species.

Family Halictidae

***Augochlorella* Sandhouse.** Coelho (2004) synonymised *A. striata* (Provancher) under *A. aurata* (Smith) resulting in one species recorded from Canada, but there are two barcode index numbers. Ordway (1966) originally suggested much morphological variation in *A. striata*, so it is likely that more detailed analysis of morphology and barcoding is required.

***Halictus* Latreille.** At present, seven species are known from Canada, represented by 11 barcode index numbers. As discussed above, the Holarctic species *H. confusus* is responsible for much of the discrepancy (four barcode index numbers) (also see discussion in Sheffield and Perron (2014)), with *H. tripartitus* Cockerell accounting for two barcode index numbers.

***Lasioglossum* Curtis.** The genus *Lasioglossum* has 141 known Canadian species and is represented by 115 barcode index numbers. Many of the species currently recognised morphologically (see Gibbs 2010) share barcode index numbers and account for much of the discrepancy between barcode index numbers and species, with most of the remaining discrepancy due to the number of species not yet barcoded. There are cases, such

as *L. cressonii* (Robertson) and *L. ruidosense* (Cockerell), where morphologically and geographically defined species have multiple barcode index numbers (Gibbs 2010). The recent works of Gibbs (2009, 2010, 2011) and Gibbs *et al.* (2013) are examples of the benefit of including DNA barcodes as part of revisionary taxonomy to resolve issues in difficult bee taxa.

***Sphecodes* Latreille.** *Sphecodes* is one of the genera in Canada in most need of revision. At present, 33 species have been recorded from Canada, though there are 51 barcode index numbers. Currently, no key to species exists for western North America, and that for the east (Mitchell 1960) is not representative of all the species in the area.

Family Melittidae

***Macropis* Panzer.** Two species occur in Canada, both oligoleges of *Lysimachia* Linnaeus (Primulaceae) flowers, though one (*M. ciliata* Patton) is very rare, and no material has been barcoded.

Family Megachilidae

***Anthidium* Fabricius.** At present, 11 species of *Anthidium* (three are non-native), are known from Canada, and eight of these have barcode index numbers.

***Ashmeadiella* Cockerell.** There are four species recorded from Canada, one with two subspecies, and four barcode index numbers. It is likely that the DNA barcodes do not differ in the two subspecies.

***Coelioxys* Latreille.** There are 21 species confirmed from Canada, and 20 barcode index numbers, though two species have no barcode index numbers associated with them, and two other species have multiple barcode index numbers. There may be misidentifications in barcode of life data system, but this group needs revision.

***Dianthidium* Cockerell.** There are seven species recorded from Canada, and six barcode index numbers; two species (*D. curvatum* (Smith) from British Columbia, and *D. simile* (Cresson) from Ontario) share a barcode index number.

***Hoplitis* Klug.** For the 12 species recorded in Canada, there are 12 barcode index numbers, but one species has not yet been barcoded. Though two species occurring in Canada have subspecies,

no variation in barcodes is apparent within these species. Two taxa have multiple barcode index numbers.

Megachile Latreille. Since Sheffield *et al.* (2011b) revised the 38 species in Canada, Bzdyk (2012) and Sheffield and Genaro (2013) raised two subspecies to species (*M. snowi* Mitchell and *M. cleomis* Cockerell, respectively), resulting in 40 species in Canada. In all, 43 barcode index numbers have been recorded, but one species, *M. umatillensis* (Mitchell) has no barcode index number associated with it, *M. gemula* Cresson and *M. relativa* Cresson each have two, and *M. pugnata* Say has three (supporting subspecies).

Osmia Panzer. Due to the species richness of this genus, and the difficulty in identifying species, *Osmia* is one of the genera most in need of revision. Currently, there are 79 species recorded from Canada, and 87 barcode index numbers. Only 66 species have associated barcode index numbers, and there are 21 barcode index numbers not yet assigned to specific taxa and/or representing multiple barcode index numbers of a single named taxon. Likely many of these are the result of misidentification.

Stelis Panzer. These cleptoparasites are in need of revision, with 21 species recorded from Canada but only six with associated barcode index numbers.

Family Apidae

Anthophora Latreille. There are 12 species recorded from Canada, and 12 barcode index numbers, though three species are without barcode index numbers, and three have two barcode index numbers each. One from the latter group likely represents a misidentification.

Bombus Latreille. There are 44 bumble bee species recorded from Canada, including one new species described (Williams *et al.* 2016) since the last taxonomic treatment of the genus (Williams *et al.* 2014). The 51 barcode index numbers from Canada include some species with multiple barcode index numbers (see *B. impatiens* and *B. ternarius* discussion above), species with recognised subspecies (*B. bifarius* Cresson and *B. occidentalis* Greene), and two others currently undergoing further taxonomic study.

Ceratina Latreille. Eight species of *Ceratina* are known from Canada, represented by five barcode index numbers. The shared barcode

index number of four eastern species of *Ceratina* (*Zadontomerus*) is discussed above.

Diadasia Patton. The three species in Canada are represented by four barcode index numbers. One species, *Diadasia australis* (Cresson), is represented by two closely associated barcode index numbers, one on the eastern side of the Rockies, one on the west.

Epeolus Latreille. There are 12 unique barcode index numbers for the 12 species recorded from Canada, although a 13th species (yet to be barcoded) has been also been recorded in the country (Romankova 2004). The Canadian species of *Epeolus* were recently revised by Onuferko (2017).

Eucera Scopoli. Of the 11 species thought to be in Canada, only six have been barcoded and have yielded barcode index numbers. It is therefore likely that some species of *Eucera* are rare in Canada, but material in the barcode of life data system identified as five different species share a single barcode index number. This genus requires revision.

Habropoda Smith. Only one of the two species known from Canada, *Habropoda cineraria* (Smith), has been barcoded.

Holcospites Ashmead. Five species are known from Canada, and three of these have barcode index numbers.

Melissodes Latreille. *Melissodes* is one of the taxa in need of attention; there are 27 species reported from Canada (one with two subspecies), and 18 barcode index numbers, though six of these are not yet associated with valid species.

Neolarra Ashmead. Only one of the two species known from Canada, *Neolarra vigilans* (Cockerell) has been barcoded.

Nomada Scopoli. *Nomada* is one of the most taxonomically difficult groups of bees in North America, with few recent keys, and many species known from one sex. This genus is perhaps most in need of taxonomic revision. There are 81 barcode index numbers in barcode of life data system from Canada, representing 55 known species. Only 15 species have been associated with barcode index numbers (and some of these are likely misidentified), and 66 barcode index numbers are without names.

Triepeolus Robertson. There are 20 species recorded from Canada, represented by 12 barcode index numbers, two of these not associated with a species name.

Future prospects for DNA barcoding and bee taxonomic studies

Associating sexes

Among the bees, many speciose genera have high proportions of species known from one sex; Sheffield and Westby (2007) indicated that ~37% of North American leafcutter bees were known from one sex. In a recent revision of the genus *Megachile* for Canada, Sheffield *et al.* (2011b) associated the sexes for all 38 species known at that time, resulting in several synonymies. For instance, *Megachile alamosana* Mitchell, previously known only from the male, was synonymised with *Megachile anograe* Cockerell, which was known only from the female (Sheffield *et al.* 2011b) (Fig. 6). In addition, the male of *M. sublaurita* Mitchell was described for the first time (Sheffield *et al.* 2011b). Similarly, in the genus *Sphecodes*, Sheffield *et al.* (2009) associated *Sphecodes carolinus* Mitchell to *S. coronus* Mitchell, known from the female and male, respectively. Gibbs (2010) also made 14 sex associations for *Lasioglossum* (*Dialictus*) in Canada, in a study that combined both morphological and molecular techniques.

Morphological variation

In addition to being sexually dimorphic, some bee species also exhibit high morphological variability, especially colour. In bumble bees (genus *Bombus*) this is particularly common, though for some highly variable species (*e.g.*, *B. rufocinctus* Cresson), no variation in COI has been reported, nor are there any obvious corresponding geographical patterns associated with these colour variants. In another example, Williams *et al.* (2014) considered *B. californicus* Smith a synonym of *B. fervidus* (Fabricius) due to little to no variation in COI (*i.e.*, same barcode index number), though the darker form (*i.e.*, *B. californicus*) is largely distributed in the west (including British Columbia and Alberta), though with intergrades occurring with the eastern yellow form (*B. fervidus*). However, despite the molecular evidence and synonymy of Williams *et al.* (2014), Dolan *et al.* (2017) still recognised both species in Montana. In another series of studies with bumble bees, Williams *et al.* (2012) and Sheffield *et al.* (2016) used geographic patterns of COI to recognise two subspecies of

B. occidentalis, the latter study also including an analysis of geography-based colour variation to help define the distribution of each designatable unit, and assist in the Committee On the Status of Endangered Wildlife In Canada conservation assessment for each (Committee on the Status of Endangered Wildlife in Canada 2014).

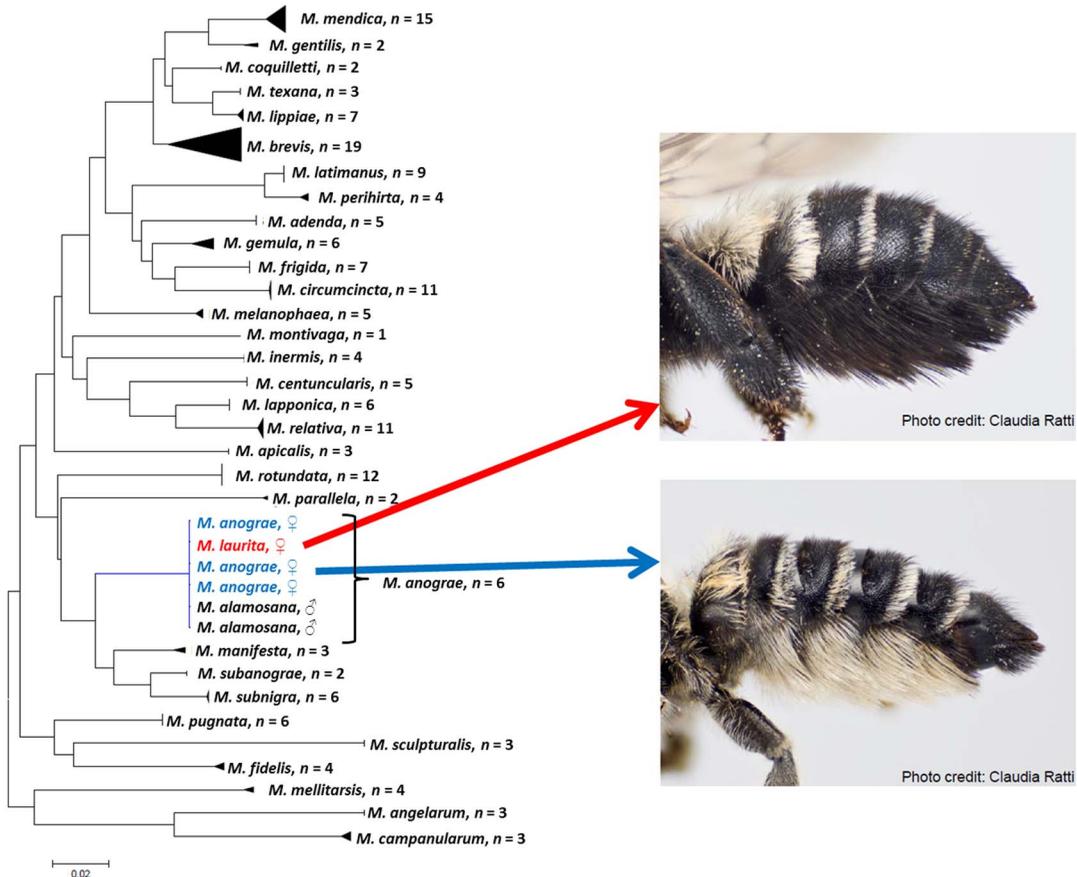
Holarctic distribution and introduced species

Global DNA barcode initiatives, such as Bee-BOL (Packer *et al.* 2008, 2009b) can help detect introduced species and clarify distributions. Introduced bees are commonly detected in Canada (Sheffield *et al.* 2010, 2011a; Gibbs and Dathe 2017; Martins *et al.* 2017). More recently, DNA barcoding has helped clarify the distributional status of several bee species, including the confirmation of a Holarctic distribution in *Bombus distinguendus* Morawitz (Sheffield and Williams 2011), and the recognition of separate Nearctic/Palaearctic distributions of bumble bees of the subgenus *Alpinobombus* (Williams *et al.* 2015).

Pollination studies

In addition to the benefits of incorporating DNA barcoding into traditional bee taxonomic studies, the opportunities for incorporating this technique into bee/pollinator ecological studies are many (Valentini *et al.* 2009; and see Vamosi *et al.* 2016 for a discussion related to pollinators and pollination). For instance, the development of techniques for DNA barcoding land plants (*e.g.*, Kress *et al.* 2005, 2015; Kress and Erickson 2007; Hollingsworth *et al.* 2011; Li *et al.* 2015), and bee-collected pollen (*e.g.*, Galimberti *et al.* 2014) and/or honey (Valentini *et al.* 2010) allow pollinating bees to be linked to their floral hosts in a range of ecosystems. However, pollen is not the only plant tissue used by bees – recently, MacIvor (2016) demonstrated how DNA barcoding could be used to identify the plant species that leafcutter bees (Megachilidae) cut leaf pieces from to build their nests. Lastly, with the development of a DNA barcode library for bees, opportunities for associating cleptoparasitic bees to their host(s) using larvae excavated from nests would provide valuable information on bee communities (*e.g.*, Sheffield *et al.* 2013).

Fig. 6. A typical neighbour-joining tree for specimens of *Megachile* (Megachilidae) species in Canada. In this example, one clade is expanded to illustrate that DNA barcoding revealed that *M. laurita* and *M. anograe* (previously considered valid species known from the female only) were actually just colour variants of the same species. Also illustrated is the association of the male of *M. anograe* (*M. alamosana*, also previously considered a valid species). See Sheffield *et al.* (2011a).



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