The Canadian Field-Naturalist

First record of *Crithidia expoeki* (Trypanosomatida: Trypanosomatidae) from native Canadian bumble bees (Hymenoptera: Apidae: *Bombus*)

Kirsten M. Palmier[1,2,*], Amber D. Tripodi[3], Andrew D.S. Cameron[1], James P. Strange[3,4], and Cory S. Sheffield[2]

[1]Department of Biology, University of Regina, 3737 Wascana Parkway, Regina, Saskatchewan S4S 0A2 Canada
[2]Royal Saskatchewan Museum, 2340 Albert Street, Regina, Saskatchewan S4P 2V7 Canada
[3]United States Department of Agriculture, Agricultural Research Service, Pollinating Insect Research Unit, 1410 N 800 East, Logan, Utah 84341 USA
[4]Current address: Department of Entomology, The Ohio State University, Columbus, Ohio 43210 USA
[*]Corresponding author: kir.palmier@gmail.com

Palmier, K.M., A.D. Tripodi, A.D.S. Cameron, J.P. Strange, and C.S. Sheffield. 2020. First record of *Crithidia expoeki* (Trypanosomatida: Trypanosomatidae) from native Canadian bumble bees (Hymenoptera: Apidae: *Bombus*). Canadian Field-Naturalist 134(1): 16–20. https://doi.org/10.22621/cfn.v134i1.2299

Abstract
Bumble bees (*Bombus* Latrielle: Apidae) are important pollinators; however, declines of several species have been documented worldwide. Although pathogens have been linked to some declines, the biology, distribution, and impacts of most pathogens are poorly understood. Here, we report the first record of a recently characterized protozoan pathogen, *Crithidia expoeki* Schmid-Hempel & Tognazzo (Trypanosomatida: Trypanosomatidae), from bumble bees in Canada. This provides further insight on its global distribution and importance as a threat to bumble bees in Canada.

Key words: *Crithidia*; bumble bees; pathogens; Canadian distribution

Introduction
Bumble bees (*Bombus* Latrielle: Apidae) are important pollinators in both agricultural and natural landscapes (Batra 1995; Frier *et al.* 2016; Gibbs *et al.* 2016), but, unfortunately, some native species are experiencing dramatic declines in population size, range, or both. In Canada, six bumble bee species have been assessed as species at risk by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Three of these belong to the subgenus *Bombus* Latrielle *sensu stricto*: Rusty-patched Bumble Bee (*Bombus affinis* Cresson; Endangered; COSEWIC 2010), Western Bumble Bee (*Bombus occidentalis* Greene; Threatened; COSEWIC 2014a), and Yellow-banded Bumble Bee (*Bombus terricola* Kirby; Special Concern; COSEWIC 2015). Two belong to the subgenus *Psithyrus* Lepeletier: Gypsy Cuckoo Bumble Bee (*Bombus bohemicus* (Seidl); Endangered; COSEWIC 2014b) and Suckley's Cuckoo Bumble Bee (*Bombus suckleyi* Greene; Threatened; COSEWIC 2019 *in press*). Both *Psithyrus* species that have been assessed by COSEWIC are nest parasites or cuckoos of the three members of the subgenus *Bombus* indicated above. The sixth species, American Bumble Bee (*Bombus pensylvanicus* (De Geer)), is not closely related to any of the others, but was also recently assessed (Special Concern; COSEWIC 2018). All these species have been assessed based on declines in population abundance, decreases of their former ranges, or both.

Previously, declines in bumble bee populations have been linked to pathogens and parasites (Colla and Packer 2008; Cameron *et al.* 2011; Graystock *et al.* 2013; Tripodi and Strange 2018), but our knowledge is still incomplete with respect to all of the organisms involved and their relative importance. This lack of detailed knowledge of presumed threats has important implications for conservation assessments, such as those of COSEWIC, especially when the causes of declines are not specifically known. Thus, knowing the specific pathogens involved helps to determine the conservation status.

The Trypanosomatidae (Trypanosomatida) are a diverse group of flagellated protozoan parasites, and many species are of medical and agricultural importance (Dedet and Pratlong 2000; Podlipaev *et al.* 2004). For example, *Crithidia bombi* Lippa & Triggiani is a common, widespread parasite of bumble bees and was the first flagellated protozoan identified from their guts (Gorbunov 1987). Recently, molecular data helped differentiate a second species of *Crithidia*, *Crithidia expoeki* Schmid-Hempel & Tognazzo, from the closely related *Crithidia bombi* (Schmid-Hempel and Tognazzo 2010). Initially, Schmid-Hempel and Tognazzo (2010) found both species of *Crithidia* in Alaska (USA) and Switzerland, and subsequent surveillance has detected *C. expoeki* in the contiguous United States and Mexico (Gallot-Lavallée *et al.* 2016; Tripodi *et al.* 2018). *Crithidia expoeki* is expected to be as widespread as *C. bombi* (Tripodi *et al.* 2018), although additional data are needed to confirm the presence of this recently described pathogen and determine its distribution and host(s).

Crithidia infections in bumble bees have been reported at individual, colony, and population levels. *Crithidia* infections are typically chronic and rarely lead to mortality except under conditions of nutritional limitation (Brown *et al.* 2000; Conroy *et al.* 2016). Workers infected with *Crithidia* exhibit reduced foraging efficiency because of an impaired ability to learn the colour of rewarding flowers (Gegear *et al.* 2006), ultimately resulting in negative impacts on colony (Otterstatter *et al.* 2005) and plant reproductive success (Waser 1983). In spring, bumble bee queens infected with *Crithidia* are less fit than their uninfected counterparts, making them less able to establish colonies successfully; colonies started by infected queens yield fewer workers and reproducing individuals, which lowers the overall genetic variability of populations (Brown *et al.* 2003). When genetic variation within bumble bee populations decreases, it reduces the ability of colonies to overcome the pressures of parasitism (Liersch and Schmid-Hempel 1998) and likely other stressors (Zayed 2009).

Crithidia bombi and *C. expoeki* outbreaks can spread rapidly because these monoxenous (i.e., requiring one host) parasites do not require a vector for transmission between hosts (Maslov *et al.* 2013), unlike the many heteroxenous trypanosomatids that require two hosts and depend on an insect vector for transmission between them. *Crithidia* are transmitted horizontally within colonies via contaminated surfaces and food, whereas transmission between colonies occurs via flower sharing (Durrer and Schmid-Hempel 1994), although the pathogen can only survive outside a living host for short periods (Imhoof and Schmid-Hempel 1999).

Crithidia bombi and *C. expoeki* are microscopic and their appearance varies throughout their life cycles, making it difficult to distinguish between species morphologically. Historically, polymerase chain reaction (PCR) for detecting *Crithidia* in bumble bees did not distinguish below the genus level; thus, all positive results were assumed to be *C. bombi*, as no other taxa were recognized (Tripodi *et al.* 2018). The two species of *Crithidia* were distinguished by DNA sequencing (Schmid-Hempel and Tognazzo 2010). More recently, Tripodi *et al.* (2018) developed a two-step multiplex PCR protocol using species-specific primers that can distinguish *C. bombi* and *C. expoeki* in samples. This multiplex assay can also detect unexpected trypanosomatid relatives that can be identified through subsequent DNA sequencing.

Although *Crithidia* is distributed globally (Durrer and Schmid-Hempel 1995), it is unclear whether individual species follow specific geographic patterns. In the United States, *C. bombi* is more common than *C. expoeki*, but co-infections by both are more common than single *C. expoeki* infections (Tripodi *et al.* 2018). In southern Mexico, bumble bees were more commonly infected by an undescribed *Crithidia* species "*Crithidia mexicana*", followed by *C. expoeki*, with only rare cases of *C. bombi* (Gallot-Lavallée *et al.* 2016). Currently, "*C. mexicana*" has not been detected in North America north of Mexico (Tripodi *et al.* 2018), suggesting that trypanosomatid parasites of bumble bees may follow geographic patterns, although more species-specific studies are needed to interpret distribution patterns. Here, we present the first report of *C. expoeki* in Canada.

Methods

In July 2016, bumble bees were collected throughout Saskatchewan for a preliminary study to assess their pathogens. Bees were captured using aerial nets and stored individually in 1.5-mL Eppendorf tubes filled with 100% ethanol. The individual bees were frozen until they were ready to be processed. Each bee gut was dissected and screened for additional parasites or abnormalities in the haemocoel, before midgut, fat bodies, Malpighian tubes, and hind gut were removed; voucher specimens used in this study were placed in the invertebrate zoology collection at the Royal Saskatchewan Museum. Sterile techniques were used to prevent cross-contamination among the samples. DNA was extracted from the gut and fat body tissue using a modified protocol 6 from Sambrook and Russel (2001). Trypanosomatids were screened using the two-step multiplex PCR developed by Tripodi *et al.* (2018), which detects and differentiates between *Crithidia* species.

Results

In a subsample of 30 bumble bees, collected from two sites in Saskatchewan (53.2517°N, 104.4757°W; 52.4952°N, 103.5213°W), 44% tested positive for *Crithidia* spp. Of those *Crithidia*-positive individuals, 58% tested positive for *C. bombi*, 25% for *C. expoeki*, and 8% for an uncharacterized typanosomatid (Table 1).The positive-testing individuals occurred in three bumble bee species: Tri-coloured Bumble Bee (*Bombus ternarius* Say), Yellow-banded Bumble Bee, and Half-black Bumble Bee (*Bombus vagans* Smith). One *B. vagans* tested positive for both *Crithidia* species; for three samples, we were unable to diagnose because of failed reactions (Table 1).

Discussion

Historically, *C. bombi* and *C. expoeki* were considered the same species; therefore, little is known about the more recently defined latter species, including its geographic distribution, host specificity, and the specific or differing effects it has on its hosts. Although these effects and host specificity are not considered here, our study does present the first confirmed detection of *C. expoeki* in Canada, which offers some new insight on its distribution. Recommendations for future studies screening for *Crithidia* should distinguish between species and screen for any possibly uncharacterized trypanosomatids. As several species of bumble bee are considered at risk in Canada, including the six assessed by COSEWIC, future screening for *C. expoeki* from recent (and historical) collections would provide valuable information about the importance of trypanosomatids in these declines.

The causes of declines in Canadian bumble bees are poorly understood, but likely include pesticides, competition with introduced/managed species, reductions in flowering plants and other land use practices, climate change, and pathogens (Cameron *et al.* 2011). There is still much to learn about the specific pathogens involved in addition to their mode of transfer and infection, cumulative effect when combined with other threats, and geographic distribution.

Author Contributions

Writing – Original Draft: K.M.P.; Writing – Review & Editing: A.D.S.C., A.D.T., C.S.S., J.P.S., and K.M.P.; Conceptualization: A.D.T. and J.P.S.; Data Curation: C.S.S. and K.M.P.; Funding Acquisition: A.D.S.C. and C.S.S.; Investigation: K.M.P.; Methodology: A.D.T. and J.P.S.; Resources: A.D.T, J.P.S., and K.M.P.; Validation: A.D.T. and J.P.S.; Visualization: K.M.P.

Acknowledgements

We gratefully acknowledge the Saskatchewan Ministry of Agriculture and the Canada–Saskatchewan Growing Forward 2 bi-lateral agreement, delivered by the Agriculture Council of Saskatchewan, for funding and support in this project.

Literature Cited

Batra, S.W.T. 1995. Bees and pollination in our changing environment. Apidologie 26: 361–370. https://doi.org/10.1051/apido:19950501

Brown, M.J.F., R. Loosli, and P. Schmid-Hempel. 2000. Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91: 421–427. https://doi.org/10.1034/j.1600-0706.2000.910302.x

Brown, M.J.F., R. Schmid-Hempel, and P. Schmid-Hempel. 2003. Strong context-dependent virulence in a host–parasite system: reconciling genetic evidence with theory. Journal of Animal Ecology 72: 994–1002. https://doi.org/10.1046/j.1365-2656.2003.00770.x

Cameron, S.A., J.D. Lozier, J.P. Strange, J.B. Koch, N. Cordes, L.F. Solter, and T.L. Griswold. 2011. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences of the United States of America 108: 662–667. https://doi.org/10.1073/pnas.1014743108

Colla, S.R., and L. Packer. 2008. Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. Biodiversity and Conservation 17: 1379. https://doi.org/10.1007/s10531-008-9340-5

TABLE 1. *Bombus* species in Saskatchewan, Canada, that tested positive through a multiplex polymerase chain reaction for *Crithidia bombi*, *Crithidia expoeki*, or an uncharacterized trypanosomatid.

Bombus species	No. positive tests for a trypanosomatid			No. *Crithidia* co-infections	No. negatives for *Crithidia* spp.
	C. bombi	*C. expoeki*	Un-characterized		
Bombus ternarius (*n* = 13)	1	1	2	0	7
Bombus terricola (*n* = 7)	2	0	0	0	5
Bombus vagans (*n* = 10)	4	2	1	1	3

Note: Deviations from total sample sizes are a result of failure of the positive control in two specimens of *B. ternarius* and one of *B. vagans*.

Conroy, T.J., E.C. Palmer-Young, R.E. Irwin, and L.S. Adler. 2016. Food limitation affects parasite load and survival of *Bombus impatiens* (Hymenoptera: Apidae) infected with Crithidia (Trypanosomatida: Trypanosomatidae). Environmental Entomology 45: 1212–1219. https://doi.org/10.1093/ee/nvw099

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2010. COSEWIC assessment and status report on the Rusty-patched Bumble Bee, *Bombus affinis*, in Canada. COSEWIC, Ottawa, Ontario, Canada.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2014a. COSEWIC assessment and status report on the Western Bumble Bee, *Bombus occidentalis* subspecies (*Bombus occidentali occidentalis*) and the *mckayi* subspecies (*Bombus occidentalis mckayi*), in Canada. COSEWIC, Ottawa, Ontario, Canada.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2014b. COSEWIC assessment and status report on the Gypsy Cuckoo Bumble Bee, *Bombus bohemicus*, in Canada. COSEWIC, Ottawa, Ontario, Canada.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2015. COSEWIC assessment and status report on the Yellow-banded Bumble Bee, *Bombus terricola*, in Canada. COSEWIC, Ottawa, Ontario, Canada.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2018. COSEWIC assessment and status report on the American Bumble Bee, *Bombus pensylvanicus*, in Canada. COSEWIC, Ottawa, Ontario, Canada.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2019. *in press*. COSEWIC assessment and status report on Suckley's Cuckoo Bumble Bee, *Bombus suckleyi*, in Canada. COSEWIC, Ottawa, Ontario, Canada.

Dedet, J.P., and F. Pratlong. 2000. *Leishmania*, *Trypanosoma* and monoxenous trypanosomatids as emerging opportunistic agents. Journal of Eukaryotic Microbiology 47: 37–39. https://doi.org/10.1111/j.1550-7408.2000.tb00008.x

Durrer, S., and P. Schmid-Hempel. 1994. Shared use of flowers leads to horizontal pathogen transmission. Proceedings of the Royal Society B: Biological Sciences 258: 299–302. https://doi.org/10.1098/rspb.1994.0176

Durrer, S., and P. Schmid-Hempel. 1995. Parasites and the regional distribution of bumblebee species. Ecography 18: 114–122. https://doi.org/10.1111/j.1600-0587.1995.tb00331.x

Frier, S.D., C.M. Somers, and C.S. Sheffield. 2016. Comparing the performance of native and managed pollinators of Haskap (*Lonicera caerulea*: Caprifoliaceae), an emerging fruit crop. Agriculture, Ecosystems & Environment 219: 42–48. https://doi.org/10.1016/j.agee.2015.12.011

Gallot-Lavallée, M., R. Schmid-Hempel, R. Vandame, C.H. Vergara, and P. Schmid-Hempel. 2016. Large scale patterns of abundance and distribution of parasites in Mexican bumblebees. Journal of Invertebrate Pathology 133: 73–82. https://doi.org/10.1016/j.jip.2015.12.004

Gegear, R.J., M.C. Otterstatter, and J.D. Thomson. 2006. Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. Proceedings of the Royal Society B: Biological Sciences 273: 1073–1078. https://doi.org/10.1098/rspb.2005.3423

Gibbs, J., E. Elle, K. Bobiwash, T. Haapalainen, and R. Isaacs. 2016. Contrasting pollinators and pollination in native and non-native regions of highbush blueberry production. PLoS ONE 11: 1–24. https://doi.org/10.1371/journal.pone.0158937

Gorbunov, P.S. 1987. Endoparasitic flagellates of the genus *Crithidia* (Trypanosomatidae, Zoomastigphora) from the alimentary canal of bumblebees. Zoologicheskii Zhurnal 66: 1775–1780.

Graystock, P., K. Yates, B. Darvill, D. Goulson, and W.O.H. Hughes. 2013. Emerging dangers: deadly effects of an emergent parasite in a new pollinator host. Journal of Invertebrate Pathology 114: 114–119. https://doi.org/10.1016/j.jip.2013.06.005

Imhoof, B., and P. Schmid-Hempel. 1999. Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*. Insectes Sociaux 46: 233–238. https://doi.org/10.1007/s000400050139

Liersch, S., and P. Schmid-Hempel. 1998. Genetic variation within social insect colonies reduces parasite load. Proceedings of the Royal Society B: Biological Sciences 265: 221–225. https://doi.org/10.1098/rspb.1998.0285

Maslov, D.A., J. Votýpka, V. Yurchenko, and J. Lukeš. 2013. Diversity and phylogeny of insect trypanosomatids: all that is hidden shall be revealed. Trends in Parasitology 29: 43–52. https://doi.org/10.1016/j.pt.2012.11.001

Otterstatter, M.C., R.J. Gegear, S.R. Colla, and J.D. Thomson. 2005. Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. Behavioral Ecology and Sociobiology 58: 383–389. https://doi.org/10.1007/s00265-005-0945-3

Podlipaev, S.A., N.R. Sturm, I. Fiala, O. Fernandes, S.J. Westenberger, M. Dollet, D.A. Campbell, and J. Lukeš. 2004. Diversity of insect trypanosomatids assessed from the spliced leader RNA and 5S rRNA genes and intergenic regions. Journal of Eukaryotic Microbiology 51: 283–290. https://doi.org/10.1111/j.1550-7408.2004.tb00568.x

Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Schmid-Hempel, R., and M. Tognazzo. 2010. Molecular divergence defines two distinct lineages of *Crithidia bombi* (Trypanosomatidae), parasites of bumblebees. Journal of Eukaryotic Microbiology 57: 337–345. https://doi.org/10.1111/j.1550-7408.2010.00480.x

Tripodi, A.D., and J.P. Strange. 2018. Rarely reported, widely distributed, and unexpectedly diverse: molecular characterization of mermithid nematodes (Nematoda: Mermithidae) infecting bumble bees (Hymenoptera: Apidae: *Bombus*) in the USA. Parasitology 145: 1558–1563. https://doi.org/10.1017/S0031182018000410

Tripodi, A.D., A.L. Szalanski, and J.P. Strange. 2018. Novel multiplex PCR reveals multiple trypanosomatid species infecting North American bumble bees (Hymenoptera: Apidae: *Bombus*). Journal of Invertebrate Pathology 153: 147–155. https://doi.org/10.1016/j.jip.2018.03.009

Waser, N.M. 1983. The adaptive nature of floral traits: ideas and evidence. Pages 241–285 *in* Pollination Biology. *Edited by* L.A. Real. Academic Press, Orlando, Florida, USA.

Zayed, A. 2009. Bee genetics and conservation. Apidologie 40: 237–262. https://doi.org/10.1051/apido/2009026

Received 4 July 2019
Accepted 28 February 2020