SCIENTIFIC NOTE

The Interception of *Bombus impatiens* Cresson, 1863 Found in Imported Produce Purchased in Kailua-Kona, Hawaii

Jonathan Berenguer Uhuad Koch[1], Cynthia B.A. King[2], Thuy-Tien Thai Lindsay[1], Janis N. Matsunaga[3], and **Bret Nainoa Mossman[4]**
[1]U.S. Department of Agriculture Agricultural Research Service, Pollinating Insect Biology, Management, Systematics Research Unit, Logan, Utah;
corresponding author, jonathan.koch@usda.gov.
[2,4] Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife, Native Ecosystem Protection and Management Program, [2]Native Invertebrate Program, Honolulu, Hawaii; [4]Native Ecosystem Protection and Management Program, Hilo, Hawaii.
[3]Hawaii Department of Agriculture, Plant Pest Control Branch, Honolulu, Hawaii.

At least 3,220 species of adventive terrestrial arthropods have been documented in Hawaii (Nishida 2002, Matsunaga et al. 2019). The human-mediated pathways in which insects move beyond their native distributions are numerous (Turner et al. 2021), and their impacts range from highly disruptive to seemingly innocuous. Invasive insects can impose significant harm across diverse human and environmental dimensions in Hawaii, with some of the costliest impacts documented in agriculture by insects such as *Hypothenemus hampei* (Ferrari 1867) and Tephritid fruit flies (Diptera) (Vargas et al. 2007, Johnson et al. 2020). Furthermore, direct predation by invasive insects can adversely impact the persistence of endemic species such as the demonstrated impact of invasive ants (Formicidae) on the nesting success of endangered *Hylaeus anthracinus* (Smith 1853) (Hymenoptera: Colletidae) (Plentovich et al. 2021). Introduced Hymenoptera can also act as competitors to native *Hylaeus* bees and serve as reservoirs for pathogens which have the potential to spillover to sensitive wild populations (Santamaria et al. 2018). In this note, we report on the interception of *Bombus impatiens* Cresson, 1863 (Hymenoptera: Apidae) on Hawaii island, which is the first verified quarantine record of a live bumble bee accidentally imported into the Hawaiian Islands.

The movement of pathogens and parasites is an emerging threat across Hymenoptera, including bees (Anthophila) (Figueroa et al. 2019). Several wild bee species, particularly bumble bees are documented to be undergoing extensive range-wide decline (Cameron et al. 2011), with decline linked to the spread of pathogens from commercially managed bumble bee species (Cameron et al. 2011, Cameron et al. 2016). In Hawaii, accidentally introduced bees may be carriers of novel pathogens and parasites which may be vectored/transferred to established bee populations (Martin et al. 2012). At risk are endemic bees such as Hawaiian yellow-faced bees (*Hylaeus* spp.), which includes seven species protected under the U.S. Endangered Species Act. Furthermore, novel pathogens and parasites may also impact non-native *Apis mellifera* Linnaeus, 1758 which are used in agricultural pollination, honey production, and queen bee production in Hawaii (Singh et al. 2010; Santamaria et al. 2018). Finally, pathogens and parasites may also be transferred to at least 21 species of established introduced bees which may then be additional vectors to other bees (Tabor and Koch 2021).

On 8 May 2021, a 32 oz container of Limited Edition™ Fresh Strawberries was purchased by BNM from a grocery retailer in Kailua-Kona (19.684950, -156.016430, 48 m asl). The label on the container stated that it was a "Product of Mexico." Upon return home, BNM opened the container of strawberries where he found a live insect, which he recognized to likely be a species of *Bombus* Latreille, 1802 (Hymenoptera: Apidae). The insect was immediately quarantined and euthanized at –18°C and stored in a freezer until it was shipped on ice to JBUK in the U.S. Department of Agriculture–Agricultural Research Service, Pollinating Insect Biology, Management, Systematics Research Unit (USDA-ARS-PIBMSR) in Logan, Utah. JBUK and TTTL determined the specimen to be a male *Bombus impatiens*. Identification of the specimen to species was made using the taxonomic key available in Williams et al. (2014).

Given the implications of novel pathogen transfer to endemic, managed, and invasive bee species to Hawaii, the specimen was subject to gross microscopy and a molecular diagnostic test to determine the presence of some known macroparasites and pathogens, respectively. We did not test for the presence of significant bee viruses as the specimen was not preserved appropriately for such molecular diagnostics. Gross microscopy of the external and internal features of the specimen found no evidence for the following known bumble bee macroparasites: Nematoda spp. Diesing, 1861 (including Mermethidae Braun 1883), Acari spp. Leach, 1817, Conopidae spp. Latreille, 1802 (Diptera), and Phoridae spp. Curtis, 1833 (Diptera) (Macfarlane et al. 1995, Tripodi and Strange 2018). Upon dissection, the midgut, the hindgut, and the male internal reproductive apparatus tissues appeared normal.

We tested for the presence of Microsporidia Balbiani, 1882 (including *Nosema* spp.), Neogregarinorida Grassé & Schrével 1953 (including *Apicystis* spp.), and Trypanosomatidae Doflein 1901 (including *Crithidia* spp.) DNA in the specimen using the molecular diagnostic PCR multiplex presented in Table 1 of Mullins et al. (2019) (Tognazzo et al. 2012, Tripodi et al. 2018). In brief, the specific primer sets we used include the following: MSporF2 and MSporDegR (expected product size ~ 270 –316 nucleotides [nts]), ApicysITSF and ApicysITSR (expected product size ~ 357 nts), and CB-SSUrRNA-F2 and CB18SR2 (expected product size ~ 584 nts). Total DNA was extracted from the gut tissue with *Quick*-DNA MiniPrep Kit (Zymo Research) following manufacturer's protocols. The three pathogen primer sets were multiplexed with 2 uL of extracted template DNA under the following PCR conditions described in Mullins et al. (2019). The PCR products were visualized on a 2% agarose gel stained with 2.5x GelRed (Biotium) at 120V for 40 minutes along with positive and negative controls for the three pathogens, bumble bee reaction control (Mullins et al. 2019), and a 100 bp ladder (GeneRuler DNA Ladder, Thermofisher Scientific). Based on this diagnostic method, we found no evidence for Microsporidia, Neogregarinorida, or Trypanosomatidae infection in the *Bombus impatiens* specimen. Voucher tissues of the specimen are housed at –80°C at the USDA-ARS-PIBMSR under the accession number JBK-071.

In summary, we report on the first verified quarantine record of a non-native bumble bee, *Bombus impatiens*, in the Hawaiian archipelago, specifically Hawaii island. Given the species identity, documentation of a live individual in a container of strawberries, and Mexican origin of the product, we hypothesize that the *Bombus impatiens* specimen originates from a commercial colony that was likely used as a pollinator of the Limited Edition™ strawberry crop.

Additionally, *Bombus impatiens* is not native to Mexico, demonstrating further evidence of the specimen's commercial origin. We postulate that the specimen may have accidentally found its way into the strawberry container during harvesting and was trapped when the container was sealed and was not discovered during routine inspection by quarantine inspectors. This *Bombus impatiens* interception record is one example of the various pathways in which alien bees may be accidentally introduced outside of their native ranges and into vulnerable ecosystems. While this individual did not carry external and internal parasites and tested negative for targeted pathogens, we did not test for common Hymenoptera viruses as viable RNA was not available for assessment (Alger et al. 2019). However, this incident underscores the importance of monitoring pathways for invasive species and provides a framework on how to investigate bee parasites and pathogens in surveillance programs.

Acknowledgments

We thank Dr. Scott Geib for facilitating the shipment of the specimen from Hilo, HI to Logan, UT. The United States Department of Agriculture–Agricultural Research Service (USDA-ARS) is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. The mention of commercial products and organizations in this manuscript is solely to provide specific information. It does not constitute an endorsement by USDA-ARS over other products and organizations not mentioned.

Literature Cited

Alger, S.A., P.A. Burnham, H.F. Boncristiani, and **A.K. Brody.** 2019. RNA virus spillover from managed honeybees (*Apis mellifera*) to wild bumblebees (*Bombus* spp.). PloS one 14:e0217822.

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. Proc. Nat. Acad. Sci. 108:662–667.

Cameron, S.A., H.C. Lim, J.D. Lozier, M.A. Duennes, and **Thorp, R.** 2016. Test of the invasive pathogen hypothesis of bumble bee decline in North America. Proc. Nat. Acad. Sci. 113: 4386–4391.

Johnson, M.A., C.P. Ruiz-Diaz, N.C. Manoukis, and **J.C. Verle Rodrigues.** 2020. Coffee berry borer (*Hypothenemus hampei*), a global pest of coffee: perspectives from historical and recent invasions, and future priorities. Insects 11:882.

Macfarlane, R.P., J.J. Lipa, and **H.J. Liu.** 1995. Bumble bee pathogens and internal enemies. Bee World 76:130–148.

Martin, S.J., A.C. Highfield, L. Brettell, E.M. Villalobos, G.E. Budge, M. Powell, S. Nikaido, and **D.C. Schroeder**. 2012. Global honey bee viral landscape altered by a parasitic mite. Science 336:1304–1306.

Matsunaga, J.N., F.G. Howarth, and **B.R. Kumashiro.** 2019. New state records and additions to the alien terrestrial arthropod fauna in the Hawaiian Islands. P. Hawaii. Entomol. Soc. 51:1–71.

Mullins, J.L., J.P. Strange, and **A.D. Tripodi.** 2019. Why are queens broodless? Failed nest initiation not linked to parasites, mating status, or ovary development in two bumble bee species of *Pyrobombus* (Hymenoptera: Apidae: *Bombus*). J Econ. Entomol. 113:575–581.

Nishida, G.M., 2002. Hawaiian Terrestrial Arthropod Checklist. Bishop Museum Technical Report 22:313.

Plentovich, S., J.R. Graham, W.P. Haines, and **C.B.A. King.** 2021. Invasive ants reduce nesting success of an endangered Hawaiian yellow-faced bee, *Hylaeus anthracinus*. NeoBiota 64:137–154.

Santamaria, J., E.M. Villalobos, L.E. Brettell, S. Nikaido, J.R. Graham, and **S. Martin.** 2018. Evidence of *Varroa*-mediated deformed wing virus spillover in Hawaii. J. Invert. Pathol. 151:126–130.

Singh, R., A.L. Levitt, E.G. Rajotte, E.C. Holmes, N. Ostiguy, D. Vanengelsdorp, W.I. Lipkin, C.W. Depamphilis, A.L. Toth, and **D.L. Cox-Foster.** 2010. RNA viruses in hymenopteran pollinators: evidence of inter-Taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. PloS one 5:e14357.

Tabor, J.A., and **J.B. Koch.** 2021. Ensemble models predict invasive bee habitat suitability will expand under future climate scenarios in Hawaii. Insects. 12: 443.

Tognazzo, M., R. Schmid-Hempel, and **P. Schmid-Hempel.** 2012. Probing mixed-genotype infections II: high multiplicity in natural infections of the trypanosomatid, *Crithidia bombi*, in its host, *Bombus* spp. PloS one 7:e49137.

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.

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*). J. Invert. Pathol. 153:147–155.

Turner, R.M., E.G. Brockerhoff, C. Bertelsmeier, R.E. Blake, B. Caton, A. James, A. MacLeod, H.F. Nahrung, S.M. Pawson, M.J. Plank, and **D.S. Pureswaran.** 2021. Worldwide border interceptions provide a window into humanmediated global insect movement. Ecol. App. 31:e02412.

Vargas, R.I., R.L. Mau, E.B. Jang, R.M. Faust, and **L. Wong.** 2009. The Hawaii fruit fly areawide pest management program. *In* O. Koul, G. Cupres (eds.), Areawide Pest Management: Theory and Implementation. London, UK: CABI Books.

Williams, P.H., R.W. Thorp, L.L. Richardson, and **S.R. Colla.** 2014. Bumble bees of North America: an identification guide. Princeton Field Guides. Princeton, NJ: Princeton University Press.