Newsletter

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Alaska Entomological Society

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Review of the thirteenth annual meeting

by Alexandria Wenninger[1]

Figure 1: UAF crew at the 2020 AKES meeting. Back row, left to right: Michael Apperson, Giovanni Tundo, Adam Haberski, Kyle Callegari, and Taylor Kane. Front row: Asia Sampson and Derek Sikes. Photo provided by Derek Sikes.

The thirteenth annual meeting of the Alaska Entomological Society Meeting was held at the Alaska Botanical Garden greenhouse in Anchorage on February 15, 2020. We are grateful to Patrick Ryan and Stacey Shriner of the Alaska Botanical Garden for offering us the use of this space.

Presentations

In his talk titled "The Kenelm Philip Lepidoptera Collection no longer exists: A summary of 5 years of curation", **Derek Sikes** guided the audience through the process of curating lepidopterist Ken Philip's incredible personal collection. Most of the pinned specimens have been carefully packed and transported to the Smithsonian, as per an agreement Ken had with the institute. However, as curator of the Entomology Collection at the University of Alaska Museum of the North, Derek was able to select a subset of specimens for integration into the University of Alaska Museum of the North. Ken's hope for his incredible life's work with Lepidoptera was that, one day, his collection would serve as a reference to which future lepidopteran research could be compared.

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Jessie Moan gave an insightful view of the relationships between the public and insects through her talk, "Fun new finds from Extension." As entomologist for the University of Alaska's Cooperative Extension Service, Jessie is often the first line of contact when members of the community have questions about what insects are in their gardens, homes, and soil. Her presentation and photographs showed many exciting, interesting, and unusual insects that she has worked with recently.

Chris Fettig returned this year to update us on his work with spruce beetle in Alaska. Chris is a research entomologist for the USDA Forest Service, currently stationed at the Pacific Southwest Research Station. He has worked extensively with bark beetles throughout North America and has interest in the indirect effects of climate change on invasive bark beetles and their forest habitats. His talk titled "Research for new methods of control of spruce beetle (*Dendroctonus rufipennis*)" gave an overview of his experiments with deterring spruce beetle attack through use of semiochemicals, and how Alaskan and Rocky Mountain populations of spruce beetle compare in their response to the same semiochemicals.

Justin Fulkerson and **Matt Carlson** followed with a joint presentation of the "Seasonal pollinator diversity of rare grasslands in eastern interior Alaska." As botanists with the University of Alaska's Alaska Center for Conservation Science, Justin and Matt have been working to understand the diversity and phenology of Alaska's bee species. They also discussed their use of traditional barcoding as an identification aid when working with groups of bees that are difficult to identify using morphology alone.

We had several fantastic student presentations this year. This year was our first year of awarding one student for best poster presentation in addition to the student award for best oral presentation. Two posters were presented this year. **Kyle Callegari** (UAF) presented his work, "Interior Ecosystem: Wild, Living, Arthropod Divesity in the University of Alaska Museum." We congratulate **Adam Haberski** (UAF), recipient of the 2020 Student Poster Award, for his poster, "Beetle, spider, and bumblebee communities differ across an elevational gradient in Denali National Park, Alaska." Adam conveyed an exceptional understanding of his study organisms and was especially impressive in his ability to answer difficult audience questions about the broader implications of his work. Congratulations, Adam!

In addition to the poster presentations, we also had three student oral presentations at the 2020 meeting. **Giovanni Tundo** (UAF) presented "How aspen tree height influences aspen leaf miner (*Phyllocnistis populiella*) oviposition and performance." **Michael Apperson** (UAF) presented "Mosquito (Diptera: Culicidae) biomass in interior Alaska: No sign of decline 2003–2018." Finally, we congratulate **Asia Sampson** (UAF), recipient of the 2020 Student Presentation Award, for her talk, "A preliminary forensic entomology study in interior Alaska, USA." Asia showed an impressive depth of knowledge and enthusiasm for her work, which was especially remarkable considering all her work was done within a semester capstone project. Congratulations, Asia!

Business items—highlights

- We thank Michael Baldwin for his generous donation of a limited-edition mosquito art print made by Michael Blackstock. This item was available by auction through eBay after the meeting, starting bid $50.
- Starting this year, science fair awards will consist of a physical Bioquip certificate that can be handed to the winner (either by AKES representatives or by science fair personnel), along with a certificate of recognition from AKES with a space for the student's name to be written on. In previous years we have experienced significant difficulty in getting the awards to the students. These changes should streamline that process.
- A new long-term research project subcommittee has been created. This subcommittee will work independently of universities and government agencies. Derek Sikes, Garrett Dubois, Kyle Callegari, and Adam Haberski all volunteered to be a part of this subcommittee.
- Election results: Alexandria Wenninger (president), Robin Andrews (vice president), Taylor Kane (secretary), and Roger Burnside (treasurer).

The minutes from our business meeting are available on our website.

The brown recluse spider (*Loxosceles reclusa*) continues to not occur in Alaska and no specimens from Alaska have ever been archived

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by Derek S. Sikes[1]

A species notably absent from the Alaskan arthropod fauna and worth commenting on is a spider often found indoors in its native range, *Loxosceles reclusa* Gertsch & Mulaik, 1940 (brown recluse spider). No scientific records of this species exist for Alaska (Simpson et al., 2019) despite the belief by some residents of the state that this spider has been present or may even maintain breeding populations in the state. This spider is a well-known species of medical concern and considerable confusion exists in the general public concerning its geographic distribution and habits (Vetter and Bush, 2002).

Over the past decade, many specimens and photographs of spiders have been submitted to staff of the University of Alaska Museum (UAM) Insect Collection by people who suspected they had found this species in Alaska, but none were the brown recluse. This is not surprising, since this spider is rarely found outside of its native range in the midwest and southcentral US states (Figure 1) (Vetter and Bush, 2002; GBIF Secretariat, 2020; iNaturalist.org, 2020).

The spider fauna of Alaska includes over 620 species and the University of Alaska Museum Insect Collection has over 43,000 Alaskan archived spider specimens (Sikes et al., 2017), making spiders one of the more well documented arthropod groups for the state. Although it is sometimes said that, "absence of evidence is not evidence of absence," all evidence to date, combined with the known ecology of this species, indicates it does not occur in Alaska. The University of Alaska Museum Insect Collection is happy to receive specimens or photographs of spiders found in Alaska thought to be the brown recluse. A population must be found in Alaska and specimens archived before this species can be officially considered present in the state. Reports of bites, medical diagnoses, and sightings without specimens are insufficient because many injuries are mistakenly diagnosed as spider bites (Vetter and Bush, 2002) and the spider cannot be identified with confidence without preserved specimens under a microscope or high-quality macrophotographic images.

However, it is possible this species could establish indoors in Alaska. Shipments of goods from within its native range to Alaska and the effects of climate change all increase the chances of it being found in Alaska someday. A close relative from Chile, *Loxosceles laeta* (Nicolet, 1849), has been living non-captive in the Finnish Museum of Natural History in Helsinki since it was introduced in 1963 (Huhta, 1972; Nicholls, 2016). Despite almost 60 years during which this medically important spider has had free reign within the Helsinki museum, only one bite has been recorded, and there was no lasting damage (Nicholls, 2016).

In contrast to the absence of specimens of recluse spiders in Alaska, non-native *Latrodectus* spp. (widow spiders), have been found in Alaska in shipments from lower latitude states. None have ever established a breeding population, however. A summary of known interceptions of these spiders in Alaska can be found in Bowser (2013). An adult female *Latrodectus hesperus* was kept in captivity inside UAM as part of an educational display in 2012 called "Leggy!" (Sikes, 2012) but was killed when the exhibit ended.

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Acknowledgments

I thank Yuri Marusik for telling me about the Helsinki population of *L. laeta.*

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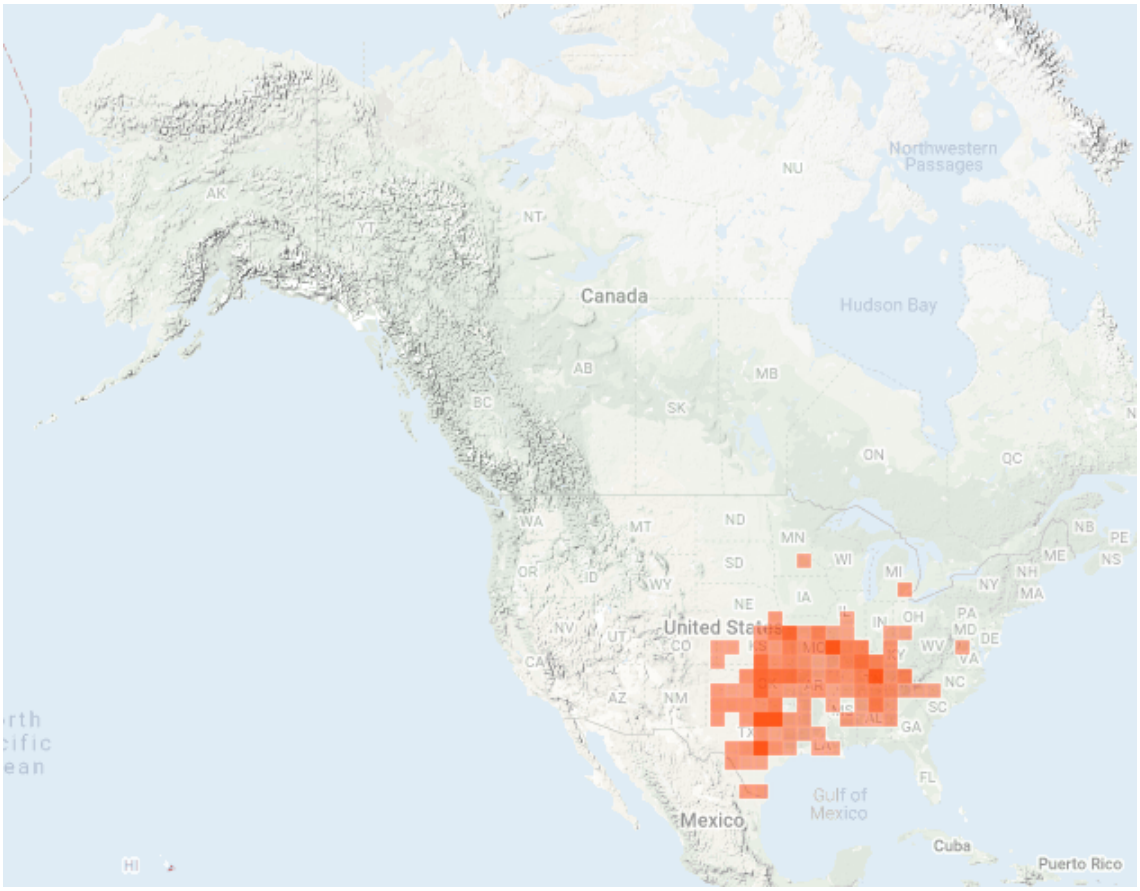

Figure 1: Research-grade iNaturalist observation records ($n = 764$) of *Loxosceles reclusa* as of 26 March 2020 (iNaturalist.org, 2020).

2019 Entomology Highlights from Alaska's Forests

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by Garret Dubois[1], Stephen Burr[1], Elizabeth Graham[1], Jason Moan[2], Jessie Moan[3], Martin Schoofs[2] and Steve Swenson[1]

Alaska's forest health is monitored annually by a multi-agency team with representatives from USDA Forest Service, AKDNR Division of Forestry and UAF Cooperative Extension Service. Our mission is to protect and enhance forest health by providing landowners and managers with information and resources. Part of our core mission is to provide technical assistance and information about forest health conditions in Alaska, including the status of many forest insect pests which is assessed through ground and aerial surveys. The results of this work are published in the annual Forest Health Conditions in Alaska report. The full report can be found at `https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fseprd712413.pdf`.

Spruce beetle

Spruce beetle, *Dendroctonus rufipennis*, is native to Alaska and has a long history of outbreaks particularly in Southcentral and Southwest Alaska. Outbreaks have been recorded in, but are not limited to the 1810s, 1870s, 1910s, and 1970s, usually following one or more years of warmer and drier than average summer conditions. An outbreak in Southcentral Alaska that started with sporadic activity in the 1980's progressed in intensity and severity in the 1990s and continued into the early 2000s. This outbreak affected well over 3 million acres of forest with >90% of the trees killed in many stands. Currently, Southcentral Alaska continues to be in the midst of a new spruce beetle outbreak (Figure 1), estimated to be in its fourth year. Damage has been primarily observed throughout the Matanuska-Susitna Valley areas and the central and northwest Kenai Peninsula. Elevated spruce beetle damage was first observed during annual forest health aerial detection surveys in 2015 with 33,000 acres mapped; double that from the previous year. Forest health surveyors continued to document an increase in spruce beetle damage in 2016, with nearly 200,000 acres of mapped damage, and the acreage again doubled in 2017 to 400,000 acres. In 2018 mapped damage approached 600,000 acres. Damage decreased considerably in 2019, with 139,500 acres mapped statewide during aerial surveys, though, spruce beetle activity continues to intensify in areas that were previously lightly impacted and is expanding in nearly all directions along the periphery of the outbreak area. In some areas white spruce host material is near exhaustion and an increase in spruce beetle attacks on black spruce has been observed.

Figure 1: Spruce beetle, *Dendroctonus rufipennis*, activity is still expanding but acres mapped are dropping. Depleted host material may be having an impact.

In 2017, there were a few reports of non-spruce conifers, Scots pine (*Pinus sylvestris*) and Siberian larch (*Laris sibirica*) being attacked by bark beetles in the Susitna River valley. From specimens collected from the attacked trees, Dr. James LaBonte, Insect Taxonomist with the Oregon Department of Agriculture, confirmed that spruce beetle was the species attacking these non-native non-host conifers. Many of the initial attacks on both non-native tree species appeared to have been unsuccessful, though gallery initiation was observed at several attack sites in Scots pine and in at least one attack site in Siberian larch. Lodgepole pine (*Pinus contorta*) are also present in many of the locations where Scots pines have been attacked; no spruce beetle attacks have been observed in lodgepole pine to date. Observed attacks in Scots pine and Siberian larch have thus far been minimal and seemingly unsuccessful, however,

[1]USDA Forest Service R10, Forest Health Protection
[2]AKDNR Division of Forestry
[3]UAF Cooperative Extension Service

heavy beetle attacks were documented in what appear to be jack pines (*Pinus banksiana*), an uncommon ornamental tree in Southcentral Alaska, in 2018. Beetles collected from these affected trees were also confirmed by Dr. LaBonte as spruce beetles. In the spring of 2019, emergence traps were installed on these affected trees, which resulted in the collection of dozens of adult beetles, suggesting the collected beetles likely completed their life cycle in these trees. Additional investigation of the affected trees is needed to confirm this finding.

Figure 2: Aspen leaf miner, *Phyllocnistis populiella*, is found all across the Interior Alaska, this damage was seen out on Manley Hot Springs Road.

Aspen leafminer and willow leafblotch miner

Aspen leafminer, *Phyllocnistis populiella*, and willow leafblotch miner, *Micurapteryx salicifoliella*, continue to be prevalent in the Interior (Figures 2 and 3) and were mapped on 132,000 and 32,000 acres respectively. Mapped acres in 2019 dropped from those recorded in the previous season for both insects, this was likely due to the difficulties encountered during aerial survey in 2019. Wildfires with vast areas of smoke and temporary flight restrictions interrupted, redirected or curtailed many aerial survey missions. Ground surveys, however, confirmed that both insects were widespread, causing heavy damage in many areas. Aspen leafminer is native to Alaska but was first observed as a problem in the 1970s and has been in outbreak in varying portions of the Interior and in the Copper River Valley since the early 2000s. Currently, aspen leafminer seems to be present in nearly every stand of aspen encountered in the Interior and is also present locally in areas around Glennallen and Copper Center.

Figure 3: Willow leafblotch miner, *Micurapteryx salicifoliella*, is in many areas where willow is present. This damage was located along the Tanana River at Manley.

Willow leafblotch miner is native to North America. A single specimen was identified from Bonanza Creek Experimental Forest, south of Fairbanks in the 1980s. There are no other records of willow leafblotch miner in Alaska until the early- and mid-1990s when there were several large outbreaks. Since those initial outbreaks it has been found to be causing minor to substantial damage nearly everywhere in the Interior where its host species are present. Willow leafblotch miner has been known to infest at least 10 of the 30+ species of willow in Alaska. Species like feltleaf willow are rarely attacked because of the dense layer of trichomes on the leaf surfaces that inhibit oviposition and egg attachment (Furniss et al., 2001). Willow leafblotch miner damage is most notable in the Interior especially in the Yukon Flats area.

Figure 4: Hemlock sawfly, *Neodiprion tsugae*, larvae found in Southeast Alaska.

Hemlock sawfly

The hemlock sawfly, *Neodiprion tsugae*, is native to Alaska and population dynamics tend to be linked to entomopathogenic fungi, parasitic wasps and environmental conditions. Because of these interactions, population levels fluctuate from year to year to varying degrees. The current outbreak that began in 2018 is the first since 2013 and has continued throughout Southeast Alaska with over 380,000 acres of damage recorded during the 2019 aerial detection surveys (Figure 4), primarily to western hemlock (*Tsuga heterophylla*). Defoliation is extensive in some areas, especially Prince of Wales, Mitkof, and Kupreanof Islands, and extending north to Juneau. Further details about hemlock sawfly surveys can be found in the hemlock sawfly article (Graham, 2020).

Birch leafminers

In 2019, special late-season aerial surveys were scheduled in both Southcentral and Interior Alaska to better assess the impacts of the non-native and invasive birch leafminers *Profenusa thomsoni* and *Heterarthrus nemoratus* (Figure 5). Late season aerial surveys have allowed us to map damage that was not apparent or had not yet occurred during our standard aerial survey timing. These special survey missions have allowed us to paint a better picture of the severity and extent of the birch leafminer damage, particularly off the road system. Over 280,000 acres of impacted forests were mapped in 2019; 17,000 acres in Interior, over 170,000 acres in the Matanuska-Susitna Borough and more than 80,000 acres on the northern Kenai Peninsula.

Figure 5: Larvae of *Profenusa thomsoni*, the amber marked birch leafminer collected in Fairbanks.

Both birch leafminer species listed above are present in Southcentral and the Interior but the dominant species in each region differs; damage from the two cannot be distinguished to the species responsible from the air. First discovered in the late 1990's, Southcentral populations were heavy to *P. thomsoni*, with some *H. nemoratus* present. Since then, population levels and severity of damage have fluctuated, and in recent years that ratio has flipped. Currently, Southcentral populations are trending toward *H. nemoratus* as the more dominant species. Interior populations, discovered in the early 2000s, have been on the rise more recently and the majority of the population is *P. thomsoni*. In addition to these older established populations, in 2019 a small area of birch leafminer activity was noted in the Western Kenai Peninsula Borough in the Big River Lakes area on the western side of Cook Inlet. Based on the extent of the damage in this area and its geographic isolation and separation from other known infestations of birch leafminers in the region, this appears to be a more recent introduction.

Figure 6: Balsam woolly adelgid, *Adelges piceae*, on the trunk of a subalpine fir in Juneau in 2019. This recent detection is the first known occurrence of this non-native pest of true firs in Alaska.

Balsam woolly adelgid

The non-native balsam woolly adelgid, *Adelges piceae*, was found damaging ornamental subalpine fir (*Picea lasiocarpa*) in June 2019 in Juneau (Figures 6 and 7). This is the first known detection of this invasive species in Alaska. Balsam woolly adelgids are native to Europe and are known to occur in several other parts of the United States where they can be highly damaging to true firs. Balsam woolly adelgids are small sap-sucking insects that feed on true fir trees (*Abies* spp.) and can kill a tree within a few years. Fir trees do not occur naturally in the Juneau area but are a popular ornamental tree. Subalpine fir and Pacific silver fir (*Abies amabilis*) are native in nearby parts of Southeast Alaska, and balsam woolly adelgids can easily be spread over great distances by wind or wildlife. Surveys are underway to determine the extent of the infestation and funding has been made available to try and contain the threat and limit further spread. The majority of the infested trees were located on City and Borough of Juneau property and were removed and destroyed in March 2020. Property owners with fir trees in Juneau have been notified of this invasive species and potential treatment options.

Figure 7: Balsam woolly adelgid, *Adelges piceae*, adult and numerous eggs.

Western Forest Insect Work Conference

The 69th annual Western Forest Insect Work Conference was held in Anchorage, Alaska in April 2019. This marked the first time this conference had ever occurred in Alaska. Entomologists and forest health specialists from universities, state and federal agencies, and private industry across western U.S. and Canada were in attendance for the conference that featured several breakout sessions with an Alaska focus, including two sessions on spruce beetle. A joint fieldtrip with the Alaska Chapter of the Society of American Foresters took place with stops between Anchorage and Portage Lake within the Chugach National Forest. The joint fieldtrip was an excellent opportunity for entomologists and foresters to interact and discuss forest health issues with experts from different disciplines (Figure 8).

Information delivery

USDA Forest Service R10, Forest Health Protection has been working hard to increase timely stakeholder access to for-

est health information and resources: We've created user-friendly ESRI ArcGIS Story Maps as a fast and fun way to learn about Forest Health Highlights in Alaska where users can explore and manipulate maps of our ground and aerial survey data. Along with the Forest Health Conditions of Alaska report, a number of other insect and disease related publications are available for viewing and download on our website: `https://www.fs.usda.gov/main/r10/forest-grasslandhealth`. Additionally, an interagency spruce beetle website was developed as a one-stop shop for Alaska-specific spruce beetle information to provide resources to homeowners and land managers: `https://www.alaskasprucebeetle.org`. This website is maintained by the University of Alaska Fairbanks Cooperative Extension Service, AKDNR Division of Forestry and USDA Forest Service R10, Forest Health Protection.

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Graham, E. 2020. Ground survey to assess hemlock sawfly population during a large-scale outbreak in Southeast Alaska. Newsletter of the Alaska Entomological Society **13**:10–14. doi:10.7299/X70K28X9, URL `http://www.akentsoc.org/doc/AKES_newsletter_2020_n1_a04.pdf`.

Figure 8: Gino Graziano of UAF Cooperative Extension Service addressing the Western Forest Insect Work Conference and the Alaska Chapter of the Society of American Foresters joint fieldtrip at Earthquake Park in Anchorage.

Ground survey to assess hemlock sawfly population during a large-scale outbreak in Southeast Alaska

doi:10.7299/X70K28X9

by Elizabeth Graham[1]

Figure 1: Aggregate of hemlock sawfly feeding on western hemlock.

Introduction

Hemlock sawfly (Hymenoptera: Diprionidae *Neodiprion tsugae*, Figure 1) is an important defoliator of hemlock (*Tsuga* spp.) throughout its range from the panhandle of Southeast Alaska through coastal British Columbia, Washington and Oregon as well as in Interior British Columbia, Idaho and Montana (Hard et al., 1976). Significant outbreaks have been recorded during aerial detection surveys in Southeast Alaska since the mid-1960s (Figure 3) and there were reports of a major outbreak impacting all of the Tongass National Forest during the mid-1950s.

Western hemlock (*Tsuga heterophylla*) is the preferred host. However, they also feed on mountain hemlock (*Tsuga mertensiana*) as well as Sitka spruce (*Picea sitchensis*) and occasionally other conifers. The larvae feed on the older needles, avoiding the new growth, often only eating half of the needle, a feeding pattern called "wasterful feeding" (Figure 2). Because the important new foliage is retained, feeding damage from hemlock sawfly rarely results in mortality, though it can result in topkill and reduction in radial growth. Mortality can occur when outbreaks of hemlock sawfly coincide with an outbreak of western blackheaded budworms (*Acleris gloverana*, Figure 4), which feed in the buds and new foliage resulting in complete defoliation.

Figure 2: Hemlock sawfly larvae preferentially feed on older needles, usually leaving the new growth untouched.

[1]USDA Forest Service R10, Forest Health Protection

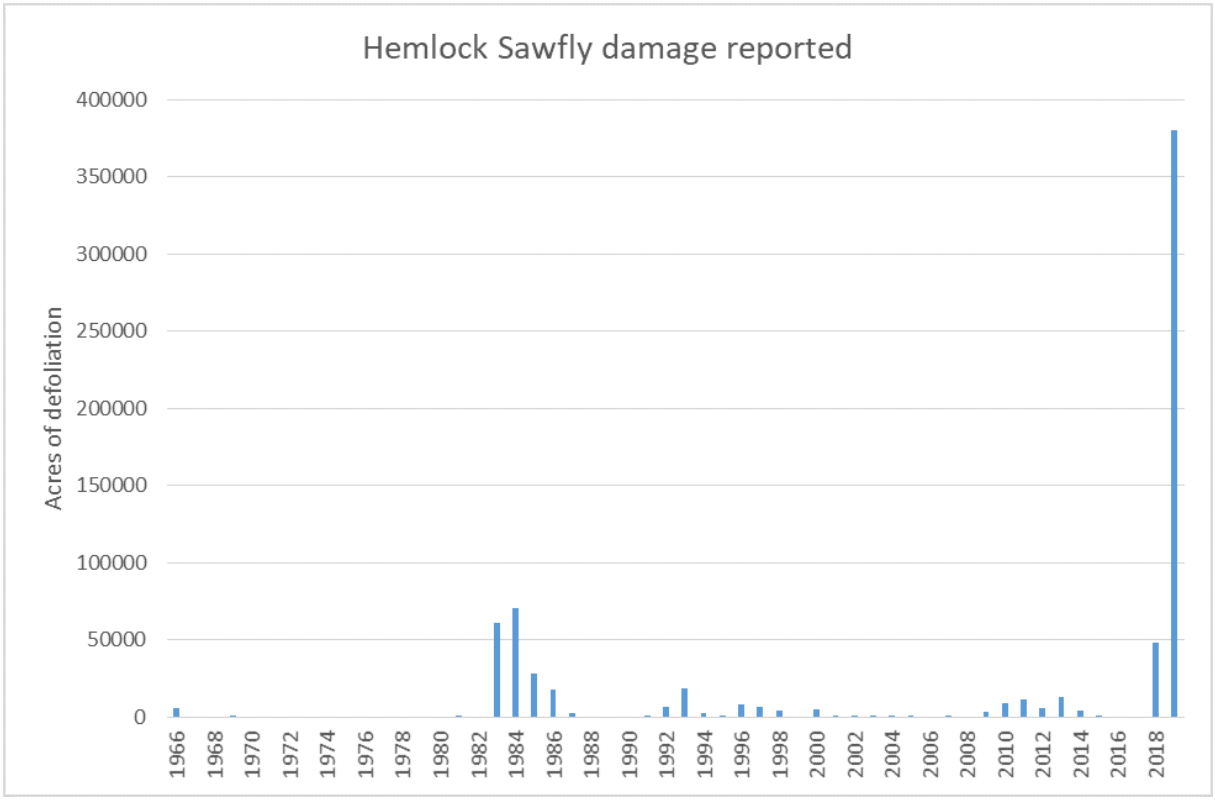

Figure 3: Hemlock sawfly defoliation recorded during annual aerial detection survey since 1966.

Figure 4: Western blackheaded budworm found on a western hemlock in Juneau, Alaska.

The current hemlock sawfly outbreak started in Southeast Alaska in 2018. During our annual aerial detection surveys, defoliation was observed on >48,000 acres of western hemlock. In 2019 the damage rose to >380,000 acres (Figure 5) and was often concentrated on southern to western facing aspects (Figure 6). Outbreaks are closely connected to climate conditions and can be triggered during drier than normal conditions due to a reduction in entomopathogenic fungi, one of the factors that help maintain population levels. Southeast Alaska exhibited warmer and drier than average summer conditions in both 2018 and 2019 which limited entomopathogenic fungal growth, allowing larval populations to build to outbreak densities. In response to the outbreak, a systematic ground survey to sample defoliator populations on hemlock was conducted throughout Southeast Alaska in 2019. The objectives of the survey were to record the species and number of defoliators found on hemlock trees as well as the amount of feeding damage and any other factors that may have an impact on hemlock sawfly populations.

Methods

Ground surveys were conducted between June 26th and July 23rd, 2019, starting in Ketchikan, followed by Prince of Wales, Mikof, Kupreanof, Zarembo, and Wrangell Islands and ending with Sitka and Juneau. Due to time and budget constraints, surveys were limited to areas with accessible road systems. Plot points were randomly created in ArcGIS using the Forest Service road system and a hemlock component as requirements. Actual plot locations were placed

within a 1/4 mile buffer of the randomly generated points, where host tree accessibility was the greatest. Areas with no hemlock or that were inaccessible were eliminated and the next randomized location was used. At each location the plot center was recorded as well as the azimuth from the road. Plot level data consisted of forest canopy class and forest type. For individual trees, DBH and crown ratings were recorded.

Defoliators were sampled using a 28" square canvas beating sheet, onto which the surveyor knocked insects from branches (Figure 7). The number of hemlock sawfly and western blackheaded budworm were recorded by range (<10, 10–20, >20). Any other additional defoliators were also recorded. Hemlock sawflies with fungal infections were recorded separately from healthy sawflies. Hemlock sawfly pupae were also recorded. There were no infected western blackhead budworm or pupae observed.

Results

In total, 76 ground plots were surveyed for hemlock defoliators throughout Southeast Alaska, of which 70 plots had hemlock sawfly larvae present (Figure 8). Defoliation was the greatest on Mitkof and Kupreanof Islands in areas where the outbreak was in its second year (Figure 9). Those two islands also had the highest proportion of trees with sawflies and a high abundance rating. Prince of Wales Island, which is in its first year of an outbreak, had a lower amount of defoliation but a high abundance of sawflies in the plots. Hemlock sawfly activity was lowest on Revillagigedo and Baranof Islands. The results of our ground surveys align closely with the amount of damage mapped during our aerial detection survey in those areas (see Dubois et al., 2020).

Figure 5: Heavily defoliated western hemlock as seen during aerial detection survey.

Figure 6: Defoliation was heaviest on southern and western facing aspects as well as along river drainages.

Figure 7: Forest Service Biotech Isaac Davis uses a beating sheet to sample for defoliating insects on hemlock trees.

Other species of defoliating insects collected on the beating sheets were also recorded during the survey. The most commonly encountered defoliators were western blackheaded budworm and geometrid caterpillers. Western blackheaded budworm was found in 38 of the 76 plots, however, the proportion of trees with budworm was consistently low, as was their abundance rating. No other species of defoliator was found as consistently or in high enough abundance to cause any notable damage.

The highest amounts of hemlock sawfly larvae infected with entomopathogenic fungi were found on Mitkof and Kupreanof Islands, areas in their second year of hemlock sawfly outbreak. Pupal cases collected during the fall from Mitkof Island also had a high parasitism rate.

Conclusion

The hemlock sawfly outbreak of 2019 was extensive throughout Southeast Alaska. It is by far the largest hemlock sawfly outbreak recorded during aerial detection surveys, but it also appears similar to the reported outbreak that occurred in the 1950s. The results of this ground survey indicate the outbreak may be coming to an end in areas like Mitkof, Kupreanof and Wrangell Islands where larvae with fungal infections are more abundant. In some areas defoliation is so great that food sources to support larval population are lacking and starvation is likely to occur. Hillsides of browning hemlock trees are visible to the public, which raises alarms with many in the local communities, but there are some ecological benefits to these large scale outbreaks. The defoliation opens up the forest canopy and provides more light to the forest floor along with a nutrient boost via sawfly frass. Most trees will recover providing they still maintain new foliage, which does not supply the proper nutrients for sawfly development. Unfortunately, top-kill and a reduction of radial growth is likely in areas that are heavily hit. Surveys will continue in future years to determine how well the trees recover.

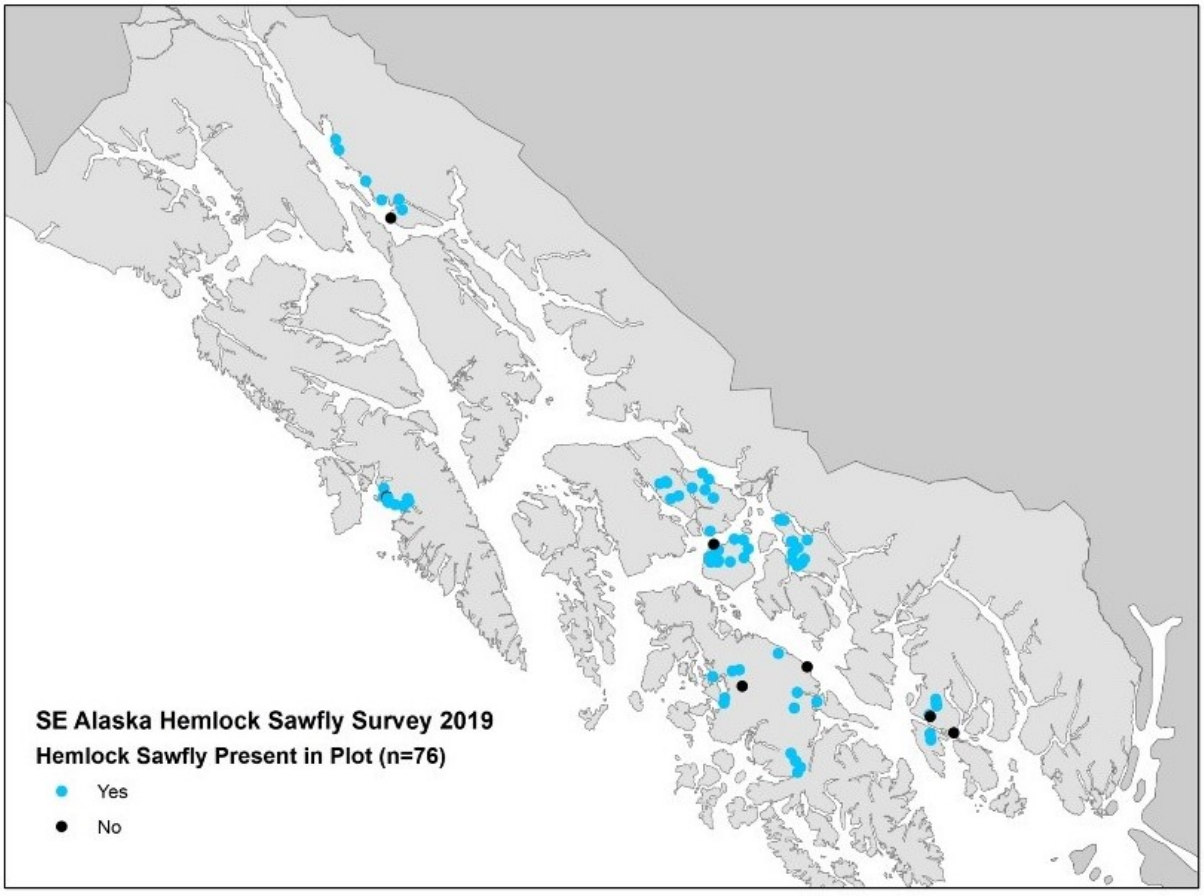

Figure 8: Locations of hemlock defoliator ground plots, plots marked as blue had hemlock sawfly whereas black did not.

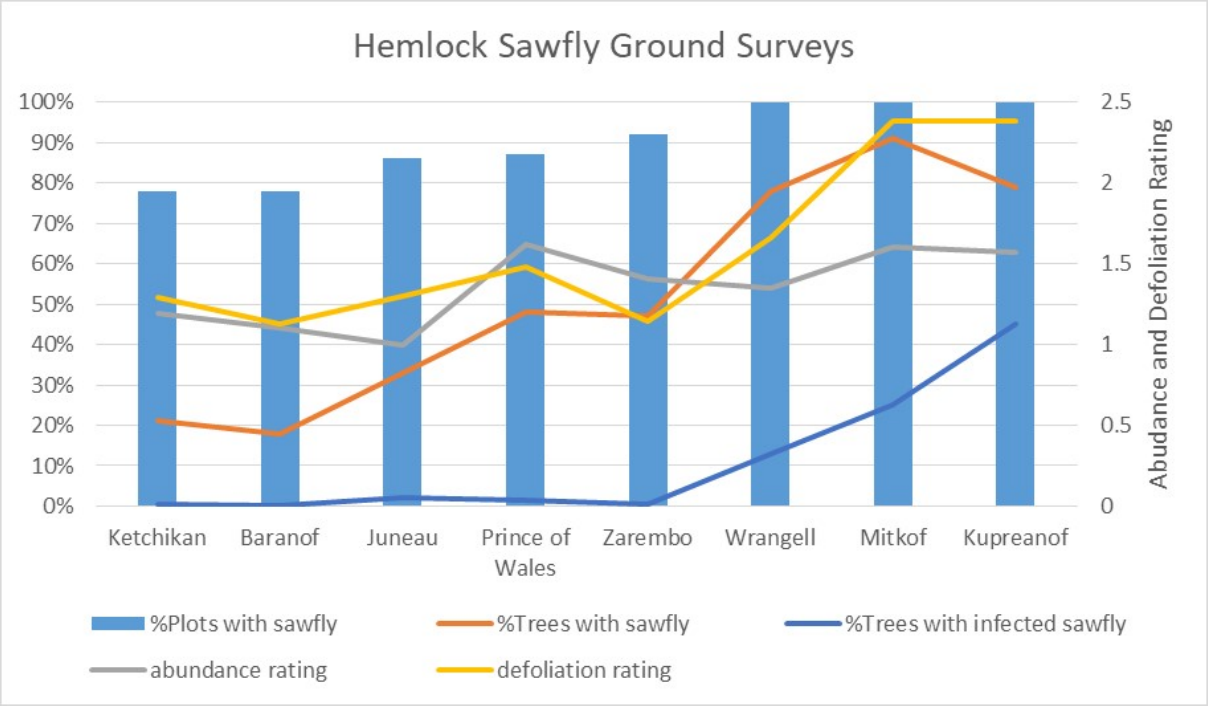

Figure 9: Results from hemlock sawfly ground surveys by island. Shows proportion of plots with hemlock sawfly as well as the proportion of trees within those plots with healthy and infected hemlock sawfly. Also shows the average abundance rating for sawflies (1 = <10 sawflies per branch, 2 = 10–20 sawflies per branch, 3 = >20 sawflies per branch) and the average defoliation rating (1 = 0–20% defoliation, 2 = 21–50% defoliation, 3 = >50% defoliation).

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Misidentifications in science: An example based on *Scathophaga impudicum* (Diptera: Scathophagidae)

doi:10.7299/X7VT1SF1

by Derek S. Sikes[1]

> "*nomina si nescis perit cognitio rerum.*"
> ("If you know not the names of things, the knowledge of things themselves perishes.")
>
> —Coke (1628)

I speak on behalf of many entomologists, naturalists, and hopefully most scientists, that organism misidentifications are an often overlooked but important source of error in scientific practice.

It is not difficult to find examples of misidentification in scientific articles. Such errors often share a number of scientific and editorial failings, notably, an absence of statements on how the identifications were made or who made the identifications, a citation of the source of the taxonomic names, and a statement indicating which public collection the voucher specimen(s) have been, or will be, deposited in (if any museum-quality voucher specimens were prepared at all), which would allow re-examination of the specimens and correction of misidentifications.

Without the above elements, the use of scientific names approaches the status of unverifiable anecdote at the opposite extreme of rigorous science on which one can base solid conclusions. These lapses are reflective of an all too widespread disregard for whole-organism biology and well-established protocols to maintain high quality science.

In the article by Croll et al. (2005), published in the peer reviewed journal *Science* and cited 485 times according to Google Scholar, there is a fly species documented from the Aleutian islands of Alaska where this study took place. This fly species, *Scathophaga impudicum*, is mysterious. First, a simple Google search on the name (in quotes, to ensure an exact match to the binomen) finds only hits pointing to the original article and to lecture notes explaining the problems associated with this name. The name with this spelling exists in no online database of scientific names indexed by Google although it can also be found in the related work by the same team, Maron et al. (2006), on which the *Science* article was based. Scientific names are supposed to act a little like passwords—they're a unique string of letters that should provide access to a wealth of information about that organism. In the case of "*Scathophaga impudicum*" this name is a dead-end.

This name seems to be an alternate spelling of *Scathophaga impudica* (Reiche, 1857) which is a junior synonym of *Scathophaga litorea* (Fallen, 1819) according to Vockeroth (1965) and Šifner (2008), and thus invalid. *Scathophaga litorea* is a species which occurs on beaches in Europe, Greenland, and eastern North America (Vockeroth, 1965; Šifner, 2008; GBIF Secretariat, 2020), but not, as far as any reliable sources indicate, beaches of the Pacific or the Bering Sea. Thus, its use in Croll et al. (2005) and Maron et al. (2006) is almost certainly based on a misidentification.

Additionally, this research found an effect due to fox presence/absence on the marine isotope signatures in this fly species. *Scathophaga* in the Aleutians are predatory shore flies that prey on marine-detritivore shore flies such as *Thoracochaeta*, which are common on beach detritus in the Aleutians (e.g., Walker et al., 2013). Marine detritus is relatively common on the beaches of all islands regardless of fox presence, which makes the finding of Croll et al. (2005) seem highly implausible. This makes it even more important to understand which fly species (singular or plural) was/were actually sampled. Given the isotopic results it is much more likely to be a fly species that lives inland and is less involved in such a primarily marine-based food web.

Notably missing from the above work is any indication of who did the fly identification (probably not a taxonomist of Diptera), how it was done (morphological or DNA or ?), where the voucher specimens are deposited, or even if any voucher specimens were saved and deposited, and from what publication the name *Scathophaga impudicum* came. Ideally, taxonomic names should come from a recent taxonomic revision, catalog, or taxonomic name server indexed by Google.

The loss in training and funding of traditional taxonomic skills is often justified in part on the incorrect notion that identification of organisms is easy enough to accomplish that no special mention of the process is required. Those taxonomists who curate names and specimens, and can provide reliable identifications, are professional biologists and should be credited with their contribution to a study.

As the above example illustrates, proper identification of organisms is not trivial and when errors arise the results can range from embarrassment to irreproducible science. If one's science is to be rigorous, readers must be given enough information to judge the quality of the identifications and access to voucher specimens to verify any identifications that might be questionable. This is, of course,

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even more critical when cryptic species are discovered after a study has been completed. Without voucher specimens it may be impossible to know which of two or more sympatric cryptic species was actually studied.

As Packer et al. (2018) pointed out, who performed an excellent review of these problems in the entomological literature, there is vast room for improvement in proper documentation supporting scientific identifications. We all must do better.

Acknowledgements

Thanks to the author of the blog post which corrected my misunderstanding of who wrote the Latin quote at the start of this paper—not Linnaeus but Edward Coke (http://languagehat.com/three-years-of-languagehat/).

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A harebrained attempt to collect during peak snowshoe hare

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by Adam Haberski

My study site looked like the aftermath of a hurricane. Pitfall traps and bee bowls had been torn up from the ground and thrown far from where I had carefully set them two weeks before. Any specimens they might have contained were long gone. I dropped my heavy backpack, sat down in the center of the wreckage, and made an entry into my field notebook. "19 July 2018: Total destruction. Again."

I was well into the third field season of my thesis work studying alpine arthropods in Denali National Park & Preserve, Alaska. Working in Denali had been a dream come true. The scenery and wildlife viewing are unparalleled. I was joined by my advisor, Derek Sikes, the park entomologist Jessica Rykken, and her interns Felix and Amber. The work wasn't always easy, and we had overcome challenges—incessant rain, curious bears, indignant tourists, and navigating bus traffic along the precipitous Polychrome Pass to name a few—but we were a crack team and it was the kind of "type two fun" that I enjoy. But with each destroyed sample, my excitement was fading into frustration. The culprits, of all things, were snowshoe hares (Figure 1).

Snowshoe hares (*Lepus americanus*) are normally innocuous herbivores, but something about the site of plastic pitfall traps excites them into a frenzy. Their usual modus operandi is to grip the rim of the trap between their teeth and toss it disdainfully over their heads. Other times, they will dig under a bee bowl and chew a small hole in the bottom to drain the liquid without disturbing the bowl. A family of hares can reduce a study site to plastic splinters in a matter of hours. I set up a motion sensing camera to record them in the act. The mischief began only 20 minutes after I left the area and didn't stop until the camera's memory card was full. I combined the images into a time-lapse video (`https://youtu.be/d1hfV4lYR7E`). It resembles a scene from *Night of the Lepus* or *Monty Python and the Holy Grail*.

Open any ecology textbook and you will find a graph of the hare population cycle. Hare populations rise and fall every ten years or so, followed closely by their predator, the Canada Lynx (*Lynx canadensis*) (Krebs et al., 2001). In 2018, the Denali population was approaching its peak. It was evident in the evenings when they gathered along the Park Road in the hundreds. At that density, they become non-demonic intrusion incarnate. I feared for my thesis.

Figure 1: An audacious snowshoe hare (*Lepus americanus)* caught in the act of vandalizing precious entomological equipment.

We naïvely believed that, as resourceful scientists, we could outsmart the hares. Our first attempt was to add a bittering agent to the traps' liquid preservative. Denatonium benzoate is the most bitter chemical compound known, with a bitterness threshold of only 0.01 parts per million. A single grain will leave a bad taste in your mouth for days (I can speak from experience). We mixed a teaspoon into every gallon of preservative. Jessica went as far as to spray denatonium solution on the outside of her bee bowls. One taste should have sent the hares running. Unfortunately, we were unaware that animals perceive bitterness differently than we do. Rodents, the sister order to hares, are 100,000 times less sensitive to denatonium than humans (Frank et al., 2004).

The next step was mechanic exclusion. I built chicken wire cages and secured them over the traps with 8-inch steel spikes (Figure 2). The hares dug below the spikes and flipped the cages aside with the same contempt they showed my pitfall traps. By that time, the season was nearly over, and our spirits were broken.

Figure 2: This chicken wire cage proved ineffective at excluding hares and preserving my pride.

In the end, the only solution was the natural ebb of the hare population cycle. I drove the Park Road one evening in 2019 and the throngs of hare were gone. The park was once again safe for entomologists. The final toll was 30% of my traps tipped, chewed, lost, or otherwise destroyed, but it was enough to finish my thesis. Still, I fear for the future. This project was envisioned as the beginning of a long-term research project to monitor arthropods' response to climate change. The original schedule called for re-sampling every ten years, perfectly coinciding with the next peak in snowshoe hare.

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A pilot study examining the diet of introduced Alaska blackfish (*Dallia pectoralis* T. H. Bean, 1880) in Kenai, Alaska, by metabarcoding

doi:10.7299/X78052XK

by Matt Bowser[1] *and Apphia Bowser*

Figure 1: Panoramic montage of a pond off of the Kenai Spur Highway and Candlelight Drive, the locality from which blackfish specimens were collected. A full resolution image is available on Arctos (doi:10.7299/X7ZP46FP).

Introduction

Last year we wrote about some food items of the Alaska blackfish, *Dallia pectoralis* T. H. Bean, 1880 (Bowser et al., 2019), a fish species that is native to most of Alaska, but not the Kenai Peninsula (Eidam et al., 2016; Bowser, 2018). We wanted to learn more about how these introduced fish may alter the ecology of Kenai Peninsula waters, especially how blackfish may affect native fish species through competition for invertebrate prey.

Methods

We collected blackfish under Alaska Department of Fish & Game permit Number SF2019-111.

On 23 August 2019 we collected blackfish from a small, shallow pond in Kenai, Alaska (60.5681 °N, -151.1901 °W ± 40 m) (Bowser, 2019), the same pond from which we had obtained blackfish the previous year (Bowser et al., 2019). This pond (Figure 1) is fed by a small inlet stream and its level is maintained by a dam at the outlet, from which the stream flows through the Kenai Golf Course and into the Kenai River. There is little open water; most of the pond is thickly filled with *Potamogeton* and flocculent iron bacterial scum. Only one other fish species, a single specimen of a nine-spined stickleback (*Pungitius pungitius* (Linnaeus, 1758), `https://www.inaturalist.org/observations/31561030`), was observed in this pond.

We attempted to collect blackfish from other reaches of the stream below this pond where there would have been more potential for interaction between blackfish and other fish species, but found only small, juvenile blackfish downstream.

The collected blackfish were placed on ice in a cooler, transported to the lab, and frozen. Later we thawed five adult blackfish (Arctos records KNWRObs:Fish:12–KNWRObs:Fish:16), measured their lengths, dissected out their entire guts, and squeezed gut contents into vials of UniGard -100 propylene glycol antifreeze.

Vials of gut contents were shipped to RTL Genomics in Lubbock, Texas (`https://rtlgenomics.com/`) for RTL Genomics' standard microbial diversity assay using the *mlCOIint/jgHCO2198* (GGWACWGGWTGAACWGTWTAYCCYCC/TAIACYTCIGGRTGICCRAARAAYCA) primer set.

Extraction methods, sequencing methods, and resulting raw sequence data are provided in Bowser and Bowser (2020).

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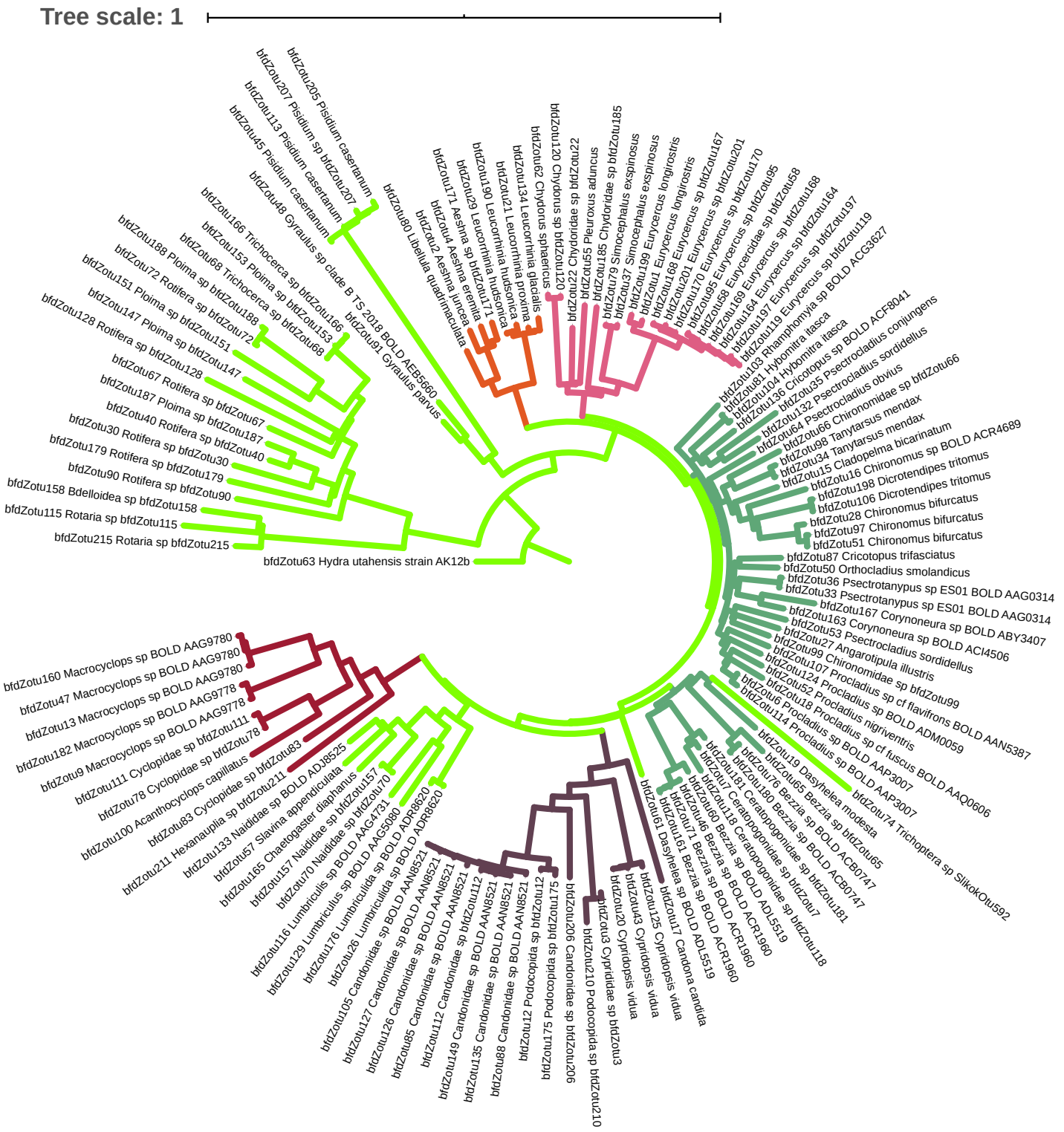

Figure 2: Phylogram of retained ESV sequences. Colors representing major groups are the same as in Figure 3. The tree can be viewed interactively or downloaded from `https://itol.embl.de/tree/164159612768115852377 67`.

Raw reads were processed using the SCVUC COI metabarcode pipeline version 4.3.0 (https://github.com/Hajibabaei-Lab/SCVUC_COI_metabarcode_pipeline). This pipline runs SeqPrep (St. John, 2016), CUTADAPT (Martin, 2011), VSEARCH (Rognes et al., 2016), UNOISE (Edgar, 2016), and the RDP classifier (Wang et al., 2007) using the COI Classifier v4 reference dataset (Porter and Hajibabaei, 2018). Processing steps were run via Snakemake (Köster and Rahmann, 2012). Our SCVUC configuration file (Bowser, 2020*b*) and snakefile (Bowser, 2020*c*) are available on Arctos.

The resulting exact sequence variants (ESVs) were also compared to ESVs obtained by Bowser et al. (2020) (dataset: Bowser, 2020*d*), sequences from an Alaska terrestrial arthropod DNA barcode COI reference library (https://github.com/mlbowser/AKTerrInvCOILib), and a FASTA file of sequences from the authors' LifeScanner (http://lifescanner.net/) records (http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-BOWSER) using `vsearch --usearch_global`. We also submitted our ESVs to NCBI BLAST (Johnson et al., 2008) and the BOLD ID Engine (Ratnasingham and Hebert, 2007) searches and scrutinized the results. We followed the guidlines of Sigovini et al. (2016) when assigning provisional names.

We removed all reads identified as *Dallia pectoralis*; *Bos taurus* Linnaeus, 1758; and all non-animals. The small numbers of *Bos taurus* reads likely came from bovine serum albumin added during DNA amplification. As a final check of identifications, we generated a phylogeny of the filtered ESVs using NGPhylogeny.fr, "NGPhylogeny Analyse - FastME/OneClick" option (Desper and Gascuel, 2002; Criscuolo and Gribaldo, 2010; Junier and Zdobnov, 2010; Katoh and Standley, 2013; Lefort et al., 2015; Lemoine et al., 2019) and examined the tree using iTOL (Letunic and Bork, 2019) (Figure 2). The FASTA file of retained ESV sequences is available from Arctos (Bowser, 2020*a*).

To prevent reporting false postive occurrences, we removed occurrences represented by $\leq$ 0.05% of the total number of reads of an ESV. Complete analysis details are provided in Bowser (2020*e*).

We tried to follow the guidelines of Penev et al. (2017) by publishing occurrence data on Arctos, which supplies occurrence data to GBIF. Specimen records, images, and other related files have been made available via an Arctos project at http://arctos.database.museum/project/10003367.

Results

The retained 131 Exact Sequence Variants (Figure 2) were represented by 63,172 reads. The ESVs were assigned to 103 uniquely identified food items and 137 occurrence records of these food items (Arctos records UAMObs:Ento:244406–UAMObs:Ento:244542). Arthropods represented by 62,166 (98%) of the reads, followed by rotifers (431 reads, 0.7%), annelid worms (384 reads, 0.6%), molluscs (160 reads, 0.3%), and one species of hydra (*Hydra utahensis* Hyman, 1931, strain AK12b *sensu* Martínez et al. (2010), 31 reads, 0.05%). The most abundant groups in terms of read abundances were odonates (32%), dipterans (24%), cladocerans (20%), ostracods (16%), and copepods (7%) (Figure 3).

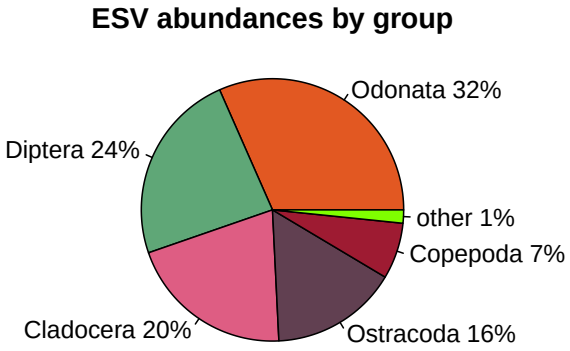

Figure 3: Percentages of ESV abundances in blackfish diet by taxonomic group.

Of the 103 unique identifications, 13 were comparatively abundunt, each representing $\geq$ 1% of the total number of reads (Figure 4). All of the reads of *Aeshna eremita* Scudder, 1866 (Odonata: Aeshnidae), the most abundant species identified, came from a single blackfish. We detected *Aeshna juncea* Linnaeus, 1758, the second most abundant species in our samples, from three fish. Ceratopogonidae sp. bfdZotu7 was both abundant and frequent in our samples, detected in gut contents of four out of five blackfish.

The relative abundance of each food item in terms of read abundances varied widely among the five blackfish individuals. For each fish, a different prey species was the most abundant food item.

Three of the most abundant ESVs could be associated with niether described species nor BOLD Barcode Index Numbers (Ratnasingham and Hebert, 2013). The ESV identified as Ceratopogonidae sp. bfdZotu7 was 98.71% similar (p-dist) to a private record on BOLD. The ESV tentatively identified as Cyprididae sp. bfZotu3 had no close matches in BOLD or BASTn search results, but the closest matches (83.99% similarity) were Cyprididae. The ESV identified as Podocopida sp. bfdZotu12 was closest (95.44% similar) to a sequence from an ostracod specimen identified as Podocopida (BOLD processid: OZFWC245-11).

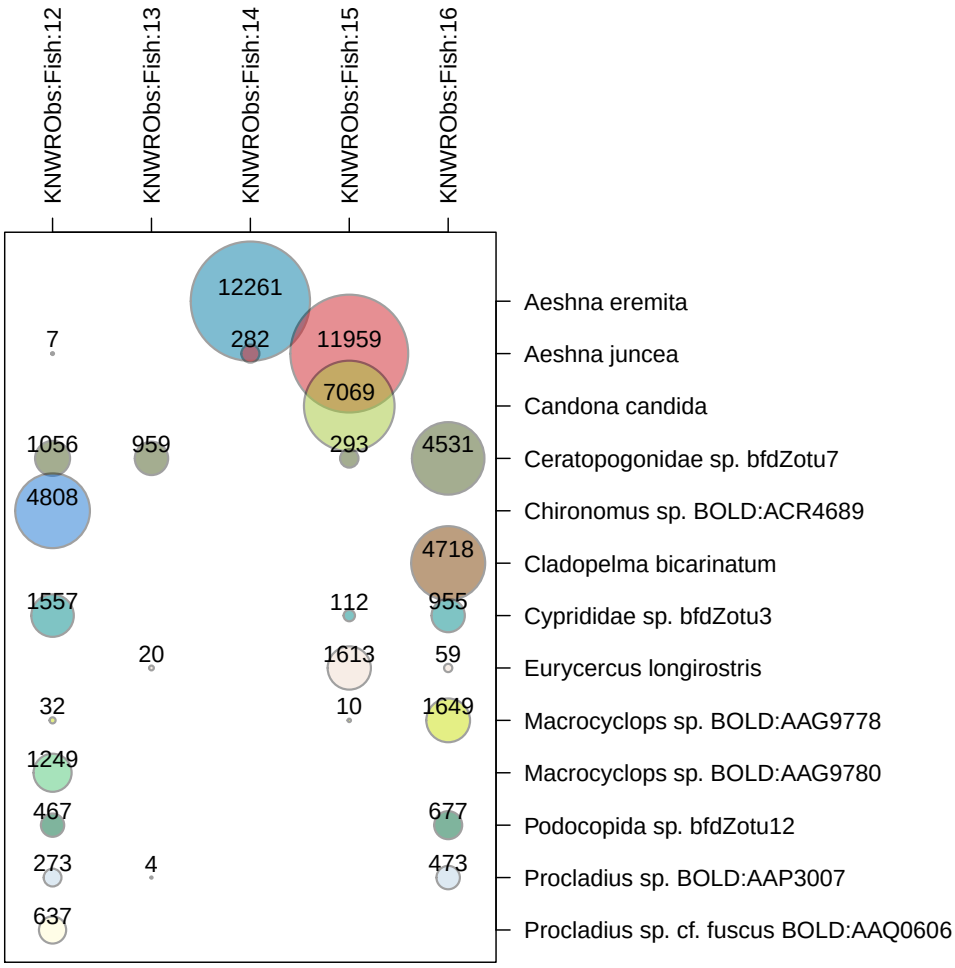

Figure 4: Read abundances of identified food items from each of five blackfish specimens. Only food items that represented $\geq$ 1% of the total number of reads were included. The area of each circle is proportional to read abundances.

Some of the ESVs matched DNA barcode sequences from locally collected entities that had not been associated with a formally described species. These included Lumbriculida sp. BOLD:ADR8620, a lumbriculid worm collected previously from near Nordic Lake, Soldotna (BOLD processid: MOBIL6661-18); *Lumbriculus* sp. BOLD:AAG4731, another lumbriculid worm documented from a temporary pool in Soldotna (BOLD processid: MOBIL1270-16); and Trichoptera sp. SlikokOtu592, an ESV from near Headquarters Lake documented by Bowser et al. (2020) (Arctos GUID: UAMObs:Ento:239239).

Seven chironomid species identified from our samples appeared to be new distribution records for Alaska. These were *Chaetocladius conjugens* Brundin, 1947; *Chironomus bifurcatus* Wuelker, Martin, Kiknadze, Sublette & Michiels, 2009; *Cladopelma bicarinata* (Brundin, 1947); *Cricotopus trifasciatus* (Meigen, 1813); *Dicrotendipes tritomus* (Thienemann & Kieffer, 1916); *Orthocladius smolandicus* Brundin, 1947; and *Procladius nigriventris* (Kieffer, 1924).

Discussion

It appeared that the adult blackfish that we collected had recently consumed exclusively invertebrates, mostly arthropods. No DNA from other fish species was detected. It should be noted, however, that other fish were comparatively rare in this pond. A single nine-spined stickleback was the only other fish documented. It may have been possible that juvenile blackfish were consumed by adult blackfish. These would not have been detected because all blackfish reads were removed from the analysis.

Overall, our results are consistent with other studies of blackfish diet (Ostdiek and Nardone, 1959; Chlupach, 1975; Gudkov, 1998; Eidam, 2015; Eidam et al., 2016; Bowser et al., 2019) which collectively show that the most important prey groups include cladocerans, ostracods, flies, dragonflies, snails, caddisflies, and copepods. What separates our results from previous studies is that metabarcoding methods yielded much finer identifications, allowing us to document which species were consumed by blackfish.

In previous studies, almost all identifications were coarse, with identifications lumped by orders or even higher-level groupings.

The variation in abundances of food items across the five blackfish individuals suggests that these fish are opportunistic, consuming whatever invertebrates they find and not seeking out any particular kind of prey item. It was surprising that we found none of the food items documented by Bowser et al. (2019) from blackfish from the same pond. Some differences in diet might have been expected due to season variation. Bowser et al. (2019) had collected blackfish on 18–19 October, two months later than our 23 August collecting date. Some of the food items documented by Bowser et al. (2019) were terrestrial wetland inhabitants that had likely become available to the blackfish due to flooding at the time. The water level of the pond was much lower when we sampled in August 2019 due to a warm, dry summer. Even with these differences in sampling date and water levels, we had expected to document at least some of the same species. The observed lack of overlap of observed prey items between the two studies supports our conclusion that blackfish are highly opportunistic.

The rotifer ESVs and other small-bodied invertebrates we observed may have been prey items of the blackfish or they may have been eaten by arthropods that were then eaten by blackfish.

It should be noted that, due to potential biases related to metabarcoding methods, the relative read abundances that we report may not be directly related to the relative proportions of food items in the diets of the blackfish that we collected (see Deagle et al., 2019, for an overview). Regardless of potential metabarcoding biases due to differences in recovery and amplification of target DNA across taxonomic groups, we believe that some of the differences in the wide range of read abundances that we observed had to do with how recently prey items had been consumed. Recent meals in blackfish stomachs would be expected to have more intact DNA than the remains of food items further along in the intestines, where much of the DNA would have been broken down.

In conclusion, we documented trophic relationships between Alaska blackfish and their prey at a particular time and place. To learn more about how blackfish interact with other fish species, we would like to see similar work done in waterbodies where there may be more interactions among fish species. It would also be good to examine diets from a wider range of sizes of blackfish as was done by Chlupach (1975) and to compare blackfish diets with diets of other fish species in the same systems to learn more about potential competition and predation among fish species.

Acknowledgments

We thank Mike Baldwin for reviewing our list of potential new distribution records and pointing out that *Angarotipula illustris* (Doane, 1901) was already known to occur in Alaska (Brodo, 2018). Rob Massengill, Kristine Dunker, and Derek Sikes provided comments that substantially improved the article.

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University of Alaska Museum Insect Collection specimen count verification

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by Voss Whitmore[1]*, Derek S. Sikes*[2,3] *and Adam Haberski*[2]

Abstract

One of our best tools for understanding the natural world are museum collections. However, there are challenges in understanding collections themselves. Questions as simple as how many specimens are in a collection can quickly become complicated. Some collection objects may represent a single specimen while others, often referred to as 'lots', represent multiple specimens. The contents of lots are often estimated to save time, creating ambiguity. We wanted to see how accurate prior guesses were in determining the number of specimens in the University of Alaska Museum Insect Collection (UAM) by either calculating or exactly counting the specimens in vials that had previously had their counts "guesstimated." We re-counted 27 vials and found that 70% of the original counts were too low and 30% were too high. The means and medians of the original counts were significantly different than the means and medians of the new counts. The sum of the original counts of the 1,099 vials in our sample was 272,033 specimens. Assuming our subsample was representative, we estimate these 1,099 vials probably hold closer to 421,749 specimens. This indicates our prior specimen count estimation methods systematically under-count vial contents.

Introduction

Museum collections are invaluable to the scientific community as vast repositories of information. Natural History collections contain thousands of databased and even more undatabased collection objects, which could include anything from ethanol vials of specimens, envelopes of invertebrates, study skins and skeletons of vertebrates, pinned insects, and many more. Many museums are currently in the process of digitizing their collections and very few are thoroughly digitized, particularly among insect collections (Sikes et al., 2016). Some objects may represent one specimen while others, often referred to as 'lots' represent multiple specimens (Sikes, 2015). Most specimens are sorted by taxonomy, ideally to genus or species, but there are also unsorted bulk objects which may represent many mixed higher taxa. These are less valuable for research purposes, which can include studies on anything from evolution to long term ecological changes (Muñoz and Price, 2019) to analysis of DNA (van der Valk et al., 2017). Collections hold dozens of samples from hundreds of species both extant and extinct, which provide important records for research.

To make such collections maximally useful it is important to know the basic information about what is contained in the collection: How many specimens there are versus how many collection objects, and how those specimens are stored (on pins, in envelopes, in vials, etc.). Many specimens are individually databased but some may be stored as multiple parts, with each part (e.g. genitalia on slide, DNA in frozen tissue collection, etc.) having its own barcode. There could also be any number of parasites and other hangers-on in what is cataloged as a single specimen (Welicky et al., 2019; Sikes, 2015). It is difficult to determine the number of specimens in a large insect collection.

In the UAM Insect wet collection, there is a substantial number of vials that hold an imprecisely counted number of small and numerous specimens. Only a small number of projects completed by the UAM Insect Collection required precise enumeration of every specimen collected, particularly among what are often non-target taxa such as mites and Collembola, that can number in the thousands per vial. In such cases, because it is time consuming and costly to count every specimen, UAM Insect Collection preparators estimate how many specimens are in each vial by "guessti-

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mation" (similar to the well-known challenge of trying to guess how many jellybeans are in a jar). Some have a few dozen specimens while some have thousands. We know these estimates are imprecise but we don't know to what degree they are imprecise. In order to determine the precision of these estimates, we did an exact, or in some cases, a carefully calculated count of the specimens in randomly selected vials. This allowed us to better estimate how many specimens are in the museum collection as a whole. In particular, we were curious to discover if the current estimation procedure was significantly under- or over-counting the contents of these vials, or if the estimation procedure was generating counts that are non-significantly different from more carefully made, precise counts.

Methods

Our first step was to find which vials needed to be precisely counted. We searched the collections database used by the University of Alaska Museum Insect Collection, Arctos, for all objects stored in ethanol that have the part remarks "estimated" or "approximate." We excluded all results that contained fewer than 30 specimens, as well as vials of mixed taxa with counts per taxon that required taxon identification skills to recount. This left us with 1,099 vials.

Vials to be re-counted were selected at random from our list of 1,099 vials, using the `RANDBETWEEN` formula of Google Sheets (`https://www.google.com/sheets/about/`). We used the object tracking system in Arctos to find which shelf and unit tray in the UAM wet collections a vial was in, retrieved the vial, counted its contents and returned the vial to its unit tray when done. The vial's contents were poured into a sorting dish and then counted under a Leica MZ16 dissecting scope with the help of a handheld tally counter. Vials with original counts fewer than 200 specimens were counted exactly (every specimen counted). Those with original counts of more than 200 specimens were counted by evenly distributing the vial's contents on a gridded sorting dish with 36 squares, randomly counting four of the grid squares, and using that total to calculate an average which was then multiplied to estimate the total in the dish. The counting method (exact vs. calculated) was recorded along with the counts. We intended to count approximately 10% of the 1099 vials, but due to the 2020 coronavirus pandemic we had to halt work and were only able to count 2.5% of the vials ($n = 27$).

Examination of the data and their residuals in R version 3.3.0 (R Core Team, 2016) using the Shapiro-Wilk normality test showed the data and their residuals had a non-normal distribution. We therefore log transformed the data and ran a paired, two-tailed t-test in R. Because the residuals were not normally distributed we also ran a paired, two-tailed Mann-Whitney U test in R on the non-log transformed data, using the following command:

```
wilcox.test(A, C, paired = TRUE, alternative =
↪  "two.sided", mu = 0.0, exact = TRUE, correct
↪  = TRUE, conf.int = TRUE, conf.level = 0.95)
```

To determine if the original count, 27-vial subsample was representative of the original counts for the full 1,099 set, we ran an unpaired, two-tailed Mann-Whitney U test in R.

To estimate a corrected total from our subsample, we fit a simple linear regression to the log-transformed data in R. We then used the regression formula to predict new counts for each of the 1,099 vials and summed these to estimate the total of the 1,099 vials.

Results

The results are in Table 1. In eight of the 27 vials the new counts were lower than the original estimates (29.6%) and in the remaining 19 vials the new counts were higher than the original estimates (70.4%). The medians of our randomly selected 27 vial subsample and the full 1,099 vial set were not significantly different (unpaired Mann-Whitney U-test, p-value = 0.5735). The means (paired t-test, p-value = 0.001478) and medians (paired Mann-Whitney U-test, p-value = 0.000009835) of the two methods of counting the subsampled 27 vials were significantly different. The relationship between the original and new counts in x-y space is illustrated in Figure 1. The sum of the original counts for the 1,099 vials was 272,033 specimens. Our regression analysis was used to estimate more precise specimen count values for all 1,099 vials. The sum of these estimated values indicates these 1,099 vials likely hold closer to 421,749 specimens.

Discussion

It was unknown whether the specimen counts in these vials were under-counts, over-counts, or "close enough" to the actual specimen counts. Our analysis suggests these were significant under-counts. Thus, the UAM Insect Collection total specimen count is probably larger than currently reported by its database. Our sample size was smaller than we had intended it to be because our work was halted by the 2020 coronavirus pandemic. However, the 27 vials chosen at random appear to be a good representation of the full set of 1,099 vials, based on the median counts of these two sets being not significantly different. Thus, we feel the current analysis is worth reporting despite our smaller than ideal sample size.

Table 1: Original, imprecisely estimated specimen counts of 27 randomly selected vials from the University of Alaska Museum Insect Collection, with new counts and counting method indicated. The GUID corresponds to the record of each vial in the database. Instances in which the new count was lower than the original are in bold.

GUID	Original Count	New Count	Method
UAM:Ento:245277	**30**	**18**	exact
UAM:Ento:371563	**100**	**33**	exact
UAM:Ento:261183	**50**	**39**	exact
UAM:Ento:245614	30	42	exact
UAM:Ento:355237	30	51	exact
UAM:Ento:261291	35	64	exact
UAM:Ento:261186	**80**	**72**	exact
UAM:Ento:287403	30	79	exact
UAM:Ento:245251	50	87	exact
UAM:Ento:287418	40	102	exact
UAM:Ento:287585	40	130	exact
UAM:Ento:370825	100	192	exact
UAM:Ento:351713	**400**	**198**	calculated
UAM:Ento:251320	60	211	exact
UAM:Ento:305972	**500**	**324**	calculated
UAM:Ento:376150	60	330	exact
UAM:Ento:251333	90	340	exact
UAM:Ento:351300	**500**	**351**	calculated
UAM:Ento:370711	70	357	exact
UAM:Ento:350555	500	405	calculated
UAM:Ento:307413	250	585	calculated
UAM:Ento:307823	200	675	calculated
UAM:Ento:307122	200	711	calculated
UAM:Ento:350840	750	873	calculated
UAM:Ento:350625	900	1,170	calculated
UAM:Ento:307573	700	2,322	calculated
UAM:Ento:341669	500	3,510	calculated
Totals	**6,295**	**13,271**	
Averages	**233**	**492**	
Medians	**90**	**211**	

Prior to our use of regression to estimate the total specimen count of the 1,099 vials we made this estimate using a simple ratio of new to original specimen counts from our set of 27 vials. This value was 2.108181, which indicated that, on average, each vial actually had ∼2.11 times as many specimens as originally estimated. We then used this to multiply the original total of the 1,099 vials (272,033 specimens) to predict the total specimen count for all 1,099 vials. This produced an estimate of 573,494 specimens which is roughly 75% larger than the more statistically sound estimate from our regression analysis (421,749 specimens).

Some error in our improved counts was likely introduced during our gridded-petri dish-based extrapolation process but we felt the benefits of increasing our sample size due to the time saved was worth this additional error. Improvements to our calculated count process could include counting more of the 36 grids (e.g. 6 or 8) to estimate the total, although this would increase the time spent per vial and consequently reduce the final sample size. Alternatively, we could have tried using ImageJ image processing software to do the counts automatically based on photos (Parker et al., 2020). However, ImageJ probably has a higher error rate with heterogeneously sized specimens.

Typically, large natural history entomology collections report the size of their collection as a count of specimens plus 'lots' and ignore the number of specimens inside each lot. Sikes (2015) discussed this practice and some of its implications. With more insect collections becoming more thoroughly digitized it is becoming easier to count and report actual specimen numbers for large collections, although as described herein, challenges remain.

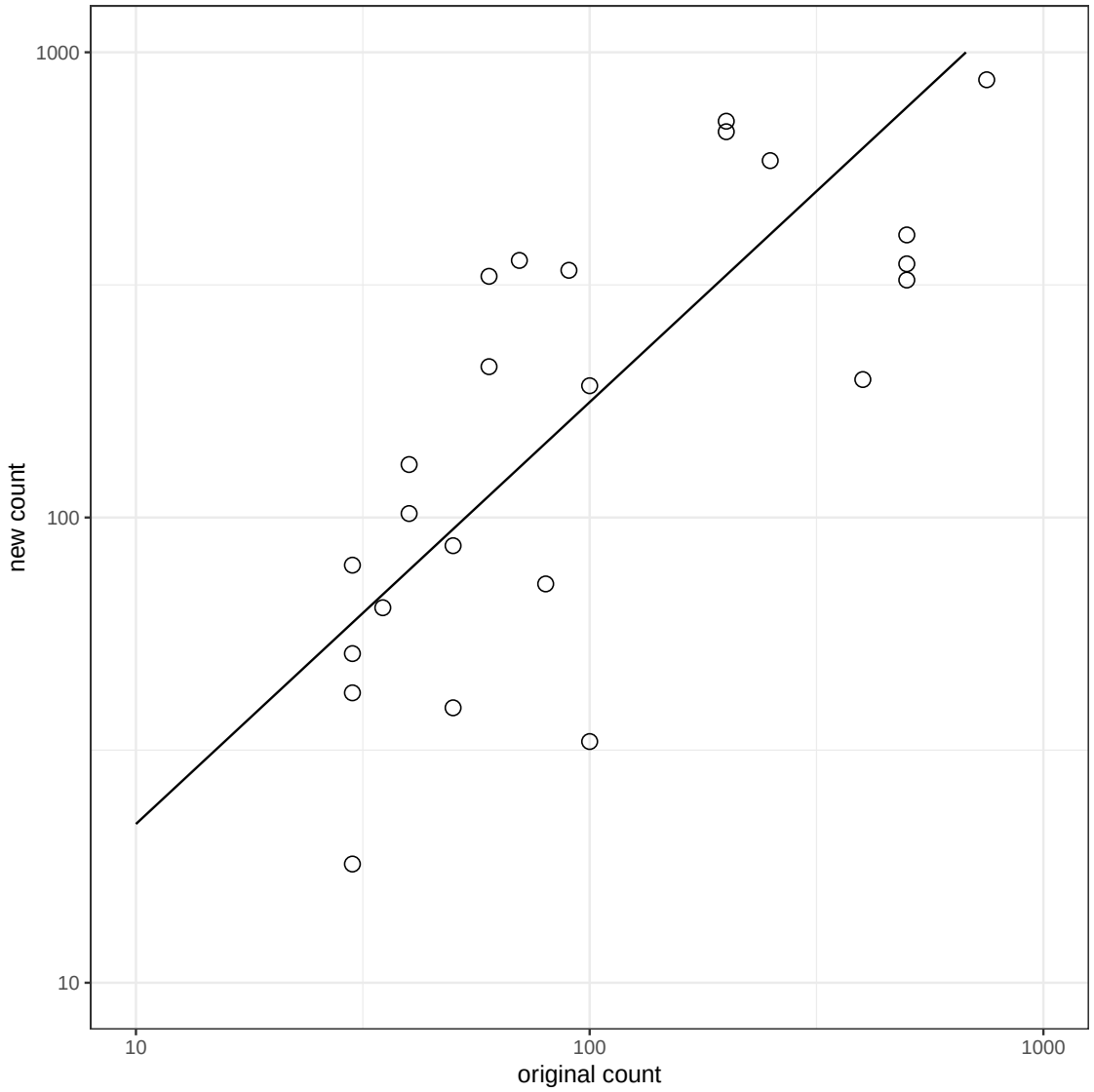

Figure 1: Original specimen counts vs new specimen counts for 27 randomly selected vials in the University of Alaska Museum Insect Collection and plotted in x-y space using base 10 logarithmic scale axes. Line indicates the predicted values from our linear regression of the log-transformed data, $y = 0.4346 + 0.9067x$, $R^2 = 0.64$.

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Author Contributions

The first author did the lab work and drafted a report, the second author designed the project and helped with writing and analysis. The third author helped with analysis and writing.

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Update to the identification guide to female Alaskan bumble bees and a summary of recent changes to the Alaskan bumble bee fauna

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by Derek S. Sikes[1] *and Jessica J. Rykken*[2]

Summary

We summarize numerous recent changes to the taxonomy of the bumble bee fauna of Alaska since Pampell (2010, 2013), Koch and Strange (2012) and Pampell et al. (2012, 2015). Nine species are now referred to using different names and two new species were described.

1. Williams et al. (2014) resulted in the names *Bombus bohemicus*, *Bombus flavidus*, and *Bombus cryptarum* replacing the previous names of *Bombus ashtoni*, *Bombus fernaldae*, and *Bombus moderatus*, respectively. The former three names replace the latter three for all records in Alaska.

2. Williams et al. (2015) elevated *Bombus natvigi* and *Bombus kirbiellus* from invalid as synonyms under *Bombus hyperboreus* and *Bombus balteatus*, respectively, to valid species status. All former records of *B. hyperboreus* in Alaska are now *B. natvigi*. All former records of *B. balteatus* in Alaska are now *B. kirbiellus*.

3. A new species, *Bombus kluanensis*, was described by Williams et al. (2016) from Yukon, Canada and Denali National Park and Preserve, Alaska.

4. Since 2017 we consider *Bombus centralis* to be a doubtful member of the Alaskan fauna with all prior records of this species most likely being *Bombus flavifrons*.

5. Martinet et al. (2019) concluded *Bombus sylvicola* is conspecific with *Bombus lapponicus* and established it as a subspecies, thus all Alaskan *Bombus sylvicola* are now *Bombus lapponicus sylvicola*.

6. A new apparently rare species was described from the Alaskan Arctic: *Bombus interacti* by Martinet et al. (2019).

7. Since December 2019 we consider *Bombus suckleyi* to be a doubtful member of the Alaskan fauna with all prior records of this species most likely being *Bombus bohemicus*.

8. Ghisbain et al. (2020) split *Bombus bifarius* into two species and *B. bifarius* does not occur in Alaska. All Alaskan *B. bifarius* records should be considered *Bombus vancouverensis*.

9. The Alaskan bumble bee fauna now has 22 confirmed species and 1 doubtful species for a possible total of 23 species.

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Introduction

There has been a considerable increase in interest in pollinators, and specifically bumble bees, across North America. In part, this interest stems from concerns about observed bumble bee declines, especially in relation to effects from climate change, introduced pathogens, and habitat fragmentation (Cameron et al., 2011; Kerr et al., 2015; Soroye et al., 2020). In Alaska, there has also been interest in basic questions about species diversity, biology, and distribution Koch and Strange (2012); Koch et al. (2012); Hatten et al. (2015); Pampell et al. (2015); Rykken (2015, 2017). Since these published works there have been some taxonomic name changes, misidentifications discovered, new species described, and synonymies published which we summarize and comment on here to provide a handy reference for ongoing and future work on the Alaskan bee fauna. Numerous records in GBIF.org of Alaskan *Bombus* exist under old taxonomic concepts or are misidentifications so such public data should be corrected or subsampled before use.

We have updated the color guide of Pampell (2013) with these corrections and have made changes to the colors of some species figured in the guide to better match Alaskan populations. Note that identification of many species cannot be confirmed by color alone; there are other microscopic characters that may need examination (like malar length), so we strongly recommend this guide be used in conjunction with the Williams et al. (2014) field guide. We also recommend the free PDF guide to bumble bees of the western states by Koch et al. (2012). Color can vary within species (especially on tergites) and our updated guide shows primarily the most common conditions. Color is best observed on clean and dry specimens; it is very difficult to identify bumble bees with matted, dirty hairs. We have marked rarely collected species, those with fewer than 40 Alaskan specimens known to us, with an asterisk in the guide. A review of Alaskan *Bombus* species with state conservation status assessments was recently completed by the Alaska Center for Conservation Science and can be found online (`https://accs.uaa.alaska.edu/wildlife/pollinator-diversity/`).

This guide is for female bumble bees. The guide can help for male identification, but males can show a higher degree of variability than females. Females have six visible abdominal (metasomal) segments called tergites, stingers, antennae with 10 flagellomeres, and their mandibles are wide apically and scoop-like. Males have seven visible tergites with the tip of their abdomen blunt and lacking a stinger, have antennae with 11 flagellomeres, and male mandibles are narrow and notably bearded.

Results

1) Replacement of the names *Bombus ashtoni*, *Bombus fernaldae*, and *Bombus moderatus* by *Bombus bohemicus*, *Bombus flavidus*, and *Bombus cryptarum*

Williams et al. (2014) resulted in the replacement of the names for three Alaskan species, *Bombus ashtoni*, *Bombus fernaldae*, and *Bombus moderatus* by *Bombus bohemicus*, *Bombus flavidus*, and *Bombus cryptarum*, respectively. The latter three names replace the former three for all records in Alaska. Details on the justifications for these changes can be found in Williams et al. (2014) and the online catalog of Williams (2020), which cites Cameron et al. (2007) as justification for the first two. We have updated the names in the color guide accordingly (Figure 1).

2) Replacement of the names *Bombus hyperboreus* and *Bombus balteatus* by *Bombus natvigi* and *Bombus kirbiellus*

Williams et al. (2015) elevated *Bombus natvigi* and *Bombus kirbiellus* from invalid as synonyms under *Bombus hyperboreus* and *Bombus balteatus*, respectively, to valid species status. This conclusion was justified based on genetic data that split the former Holarctic species into separate Palearctic and Nearctic species. All former records of *B. hyperboreus* in Alaska are now *B. natvigi*. All former records of *B. balteatus* in Alaska are now *B. kirbiellus*. We have updated the names in the color guide accordingly (Figure 1).

3) Discovery of new species in Denali National Park, Alaska—*Bombus kluanensis*

Bombus kluanensis, recently described by Williams et al. (2016), is currently known in Alaska only from Denali National Park and Preserve. In the park, it has been collected in several alpine tundra sites. Many more specimens have also been collected in the Kluane region and St. Elias Range in Yukon, Canada. It is very likely that *B. kluanensis* occurs in Wrangell-St. Elias National Park and Preserve, which encompasses the eastern end of the Alaska Range and the western end of the St. Elias Range. However, a brief survey of alpine tundra sites off the Nabesna Road in Wrangell-St. Elias in 2018 yielded no *B. kluanensis* specimens. Further surveys are needed for this species to fully document its range in Alaska.

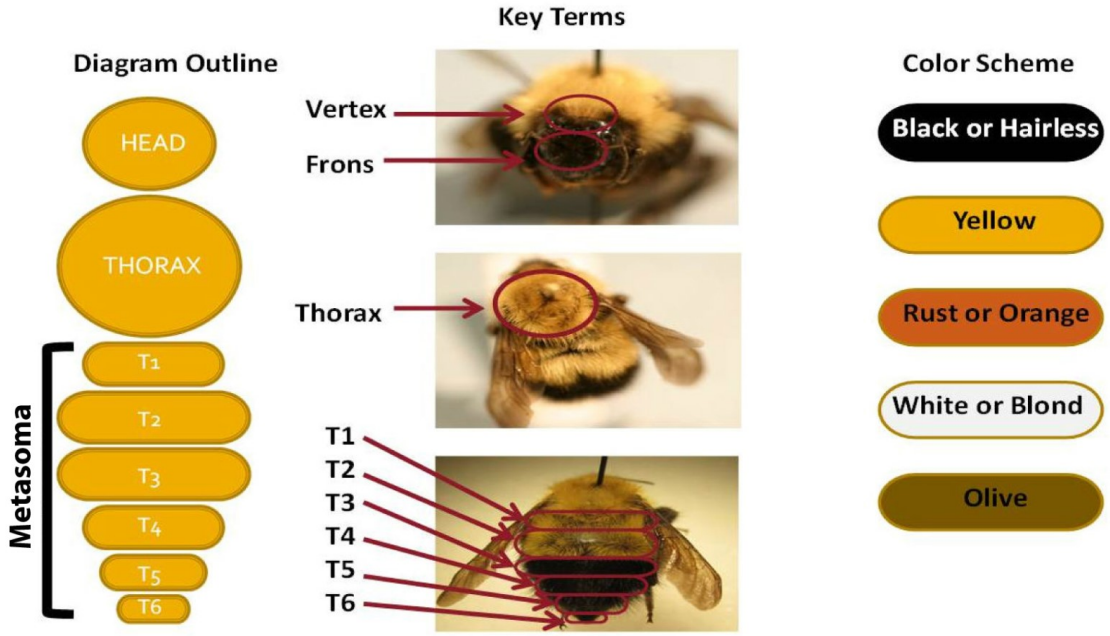
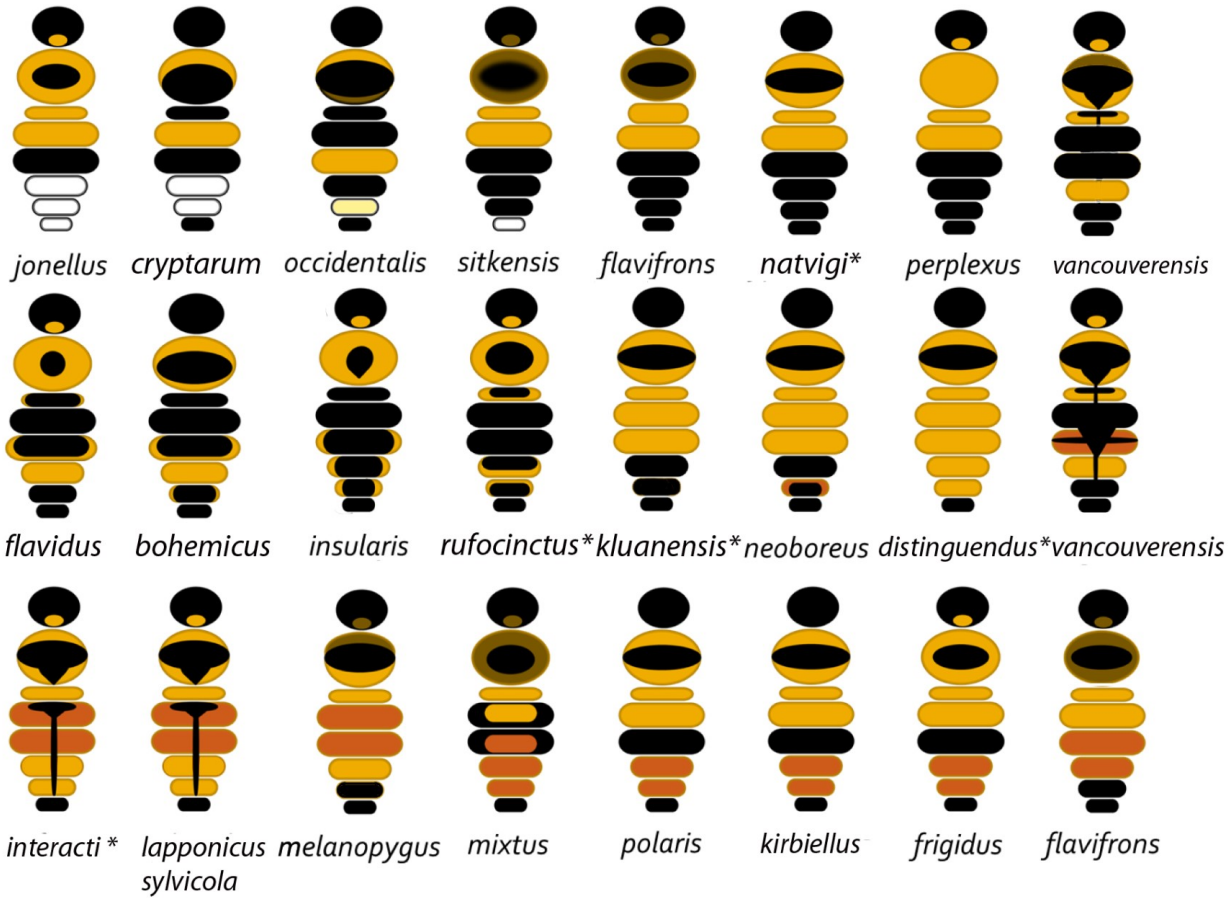


Figure 4: Diagram on how to use the Color Guide.



4) *Bombus centralis* and *Bombus flavifrons* misidentification

In 2017 we discovered and corrected a large misidentification problem that had occurred between the species *Bombus centralis* and *Bombus flavifrons*. This involved 3,313 specimens in the University of Alaska Museum that had been identified as *B. centralis* and cited as such in Pampell (2010) and Pampell et al. (2015), but subsequent examination by the first author determined them to be *B. flavifrons*. The cause of this problem stemmed from the Alaska color guide (Pampell, 2013). In that guide there was only one color form (the non-orange) for *B. flavifrons*. However, *B. flavifrons* has an orange color form that is almost identical to *B. centralis* (Williams et al., 2014). In Alaska, we have found both color forms occurring in the same area. We have updated the guide with this other form added and removed *B. centralis* (Figure 1). This case demonstrates the importance of not relying only on single color patterns for bumble bee identification and stresses the importance of archiving specimens that allow verification of identifications.

Figure 2: Image of *B. flavifrons* showing the black hairs intermixed with yellow in the anterior band. *Bombus centralis* has only yellow hairs with no black hairs intermixed.

The anterior band of yellow hairs on the thorax differs in color between these species. *Bombus flavifrons* has black hairs mixed among the yellow hairs (Figure 2) while *B. centralis* has only yellow hairs (although we have no authoritatively identified specimens of *B. centralis* in the University of Alaska Museum Insect Collection (UAM)). Males are harder to distinguish because they have far fewer black hairs in the anterior region and some appear to lack black hairs entirely in that region.

Sikes et al. (2017) DNA barcoded two UAM *B. centralis* specimens which fell into a BIN (Barcode Index Numbers (Ratnasingham and Hebert, 2013)) with over 110 *B. flavifrons* specimens (BOLD:ACE3465). After having confirmed the specimens had the diagnostic black hairs of *B. flavifrons* we edited the records of these two specimens in BOLD (Barcode of Life Data System (Ratnasingham and Hebert, 2007)) to correct their identifications to *B. flavifrons*. These two species are each other's nearest neighbors in BOLD with a genetic distance of only 1.12%.

Williams et al. (2014) includes a small number of records of *B. centralis* in Alaska. Presumably these are records that were confirmed by the authors so this species may belong on the Alaskan list—but if so, it is apparently quite rare. We consider its presence in Alaska doubtful and have not included it on our list (Table 1).

5) *Bombus sylvicola* in Alaska is now *Bombus lapponicus sylvicola*

Martinet et al. (2019) concluded *Bombus sylvicola* is conspecific with *Bombus lapponicus* and established it as a subspecies. Thus, all *Bombus sylvicola* are now *Bombus lapponicus sylvicola*. The integrative taxonomic research described below for *B. interacti* led to this seemingly well-founded conclusion.

6) New, apparently rare, Arctic species—*Bombus interacti* Martinet, Brasero & Rasmont, 2019

A new, currently rare, and potentially difficult to identify *Bombus* species was described from the Toolik Field Station in the Alaskan Arctic: *Bombus interacti* by Martinet et al. (2019). Martinet et al. (2019) wrote regarding morphological diagnosis of this new species:

> *Bombus interacti* males differed from *B. sylvicola* in the pubescence of the tibia, which is hairier in *B. sylvicola*. No difference in the structures of the genitalia was detected. Females of *B. interacti* differed from *B. sylvicola* in the face clypeus coloration: black with intermixed dark yellow hair in *B. interacti* and yellow in *B. sylvicola*. Besides, the density of pubescence of tergite 5 is higher in *B. interacti* and the yellow coloration of the collar does not reach the bases of the legs.

Table 1: Twenty-three species of *Bombus* recorded from Alaska based on 33,794 catalog records with the number of records indicated, with links to those records provided through the numbers of records. Records are a mix of specimen and literature records.

Record Count	Species
5,310	*Bombus flavifrons* Cresson, 1863
4,252	*Bombus jonellus* (Kirby, 1802)
4,194	*Bombus lapponicus sylvicola* Kirby, 1837
3,960	*Bombus frigidus* Smith, 1854
3,632	*Bombus vancouverensis* Cresson, 1878
3,442	*Bombus mixtus* Cresson, 1878
2,744	*Bombus occidentalis* Greene, 1858
1,572	*Bombus melanopygus* Nylander, 1848
1,553	*Bombus perplexus* Cresson, 1863
997	*Bombus cryptarum* (Fabricius, 1775)
470	*Bombus insularis* (Smith, 1861)
439	*Bombus kirbiellus* Curtis, 1835
389	*Bombus flavidus* Eversmann, 1852
322	*Bombus polaris* Curtis, 1835
296	*Bombus bohemicus* Seidl, 1838
83	*Bombus sitkensis* Nylander, 1848
70	*Bombus neoboreus* Sladen, 1919
36	*Bombus natvigi* Richards, 1931
14	*Bombus rufocinctus* Cresson, 1863
10	*Bombus distinguendus* Morawitz, 1869
4	*Bombus kluanensis* Williams & Cannings, 2016
2	*Bombus nevadensis* Cresson, 1874
1	*Bombus interacti* Martinet, Brasero, & Rasmont 2019

Martinet et al. (2019) used two genetic markers, one nuclear and one mitochondrial, cephalic labial gland secretions, morphometrics, and qualitative characters, all of which supported their new species and synonymy decision.

Despite what appears to be a thorough investigation, Martinet et al. (2019) has a few important shortcomings. The authors ignored relevant publicly available genetic data, chose to sequence genetic markers that are not widely used (their COI sequences only partially overlap the widely used DNA barcode region), thus making their work hard to compare to more standardized efforts, and they overlooked museum specimens relevant to their study. Despite having written, "all easily available material has been evaluated, including specimens from the Aleutian Islands," (Martinet et al., 2019) they did not contact the University of Alaska Museum Insect Collection, which holds over 4,500 *Bombus lapponicus sylvicola* Alaskan specimens (among over 30,000 other Alaskan *Bombus specimens*—all digitized and easily found, open access records shared with GBIF.org) and *B. lapponicus sylvicola* is one of the presumed closest relatives to their new species. This new species, *B. interacti*, is known from only a single site in Alaska making its distribution smaller than any other *Bombus* in Alaska and possibly North America. If this is indeed a good species, it likely occurs over a much wider region, but the authors did not determine if this was the case.

To estimate the distribution of *B. interacti* in Alaska, all Alaskan *B. lapponicus sylvicola* specimens need to be reexamined and targeted surveys conducted. Also, DNA barcode sequences need to be obtained and added to BOLD for confirmed *B. interacti*. Because this species is currently known only from Alaska (although it may also occur in nearby Canada), and from only one site in Alaska, it should be a high priority for conservation efforts such as those focused on the more widespread and declining species *Bombus occidentalis*.

Using the genbank COI sequence (MG280603.1) from the holotype male of *B. interacti* for an identification match on BOLD, using BOLD's full-length sequence database that provides maximum overlap with the DNA barcode region, returns no confident species-level match and the nearest species is >2% divergent: *Bombus monticola* (at 97.59% similar)—a European species. Other close matches

at greater divergences include another European species: *Bombus glacialis* and a Nearctic species: *Bombus bimaculatus*, which occurs primarily in the lower 48 US states in the eastern half of the US. No Alaskan species in BOLD is within the top most similar species to *B. interacti*.

The four DNA barcoded UAM Alaskan *B. lapponicus sylvicola* in BOLD are in two BINs. One BIN (BOLD:AAA8078) has many specimens which are a mix of species, primarily *B. sylvicola* and *B. lapponicus* (which supports the syonymization of these two names by Martinet et al. (2019)—see below). The other BIN (BOLD:ACN5269) has only 1 specimen (Alaskan *B. l. sylvicola*, Arctos: UAM:Ento:193437, BOLD: UAMIC758-13, GenBank: KU874450). We compared the COI sequence of this record to that of the holotype of *B. interacti* and they are 9.61% divergent. Thus, this unusual *B. l. sylvicola* is not *B. interacti*. This confirms there are no *B. interacti* sequences currently in BOLD. This rare Alaskan species has thus so far evaded detection by the various other efforts to document the bumble bee fauna of Alaska. We look forward to seeing if any specimens are among the over 4,500 *Bombus lapponicus sylvicola* specimens in UAM.

7) *Bombus suckleyi* appears to not be part of the Alaskan fauna

In December 2019 the second author studied the 13 *B. suckleyi* specimens in UAM and changed their identifications to *Bombus bohemicus*—a cuckoo bumble bee that is a designated endangered species in Canada (Colla, 2017). We subsequently edited all the Alaskan *B. suckleyi* literature records in the UAM Alaskan arthropod checklist to *Bombus* sp. with a note about the identification of *B. suckleyi* being doubtful. Pampell et al. (2015) also reported doubt about the presence of this species in Alaska. We plan to DNA barcode many of the UAM specimens to see what their DNA barcodes can tell us. We removed *B. suckleyi* from the color guide (Figure 1) and our species list (Table 1).

8) All *Bombus bifarius* records in Alaska are now *Bombus vancouverensis*

Ghisbain et al. (2020) split *B. bifarius* into two species based in part on them being ~6.9% divergent in their COI and concluded *B. bifarius* does not occur in Alaska. All Alaskan *B. bifarius* records are now *B. vancouverensis* and we have updated the color guide accordingly (Figure 1).

9) Alaska has 22 confirmed and 1 doubtful Bombus species

Table 1 lists the 23 species of *Bombus* recorded from Alaska based on 33,794 UAM catalog records, with the number of records, and links to those records provided. *Bombus nevadensis* is listed from Alaska in Krombein et al. (1979) but we consider it a doubtful member of the Alaskan fauna; leaving only 22 confirmed species. It is possible that *Bombus nevadensis* rarely occurs in Alaska because it is well documented from western regions of North America and Canada.

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