Metabarcoding pollinators on Kenai National Wildlife Refuge, Kenai Peninsula, Alaska

by Matt Bowser[16]*, Anya Bronowski, and Dom Watts*[17]

Introduction

Pollinating insects provide important ecosystem services in Alaska (Fulkerson et al. 2021) and the pollinators themselves are wildlife that the Kenai National Wildlife Refuge (KNWR) was established in part to conserve (Kenai National Wildlife Refuge and US Fish & Wildlife Service, Alaska Regional Office, Division of Conservation Planning & Policy 2010). Because many pollinators appear to be generally declining (Potts et al. 2010, Cameron et al. 2011, Koh et al. 2016), our objective was to begin documenting pollinator diversity on KNWR and surrounding lands.

The Alaska Bee Atlas (Fulkerson et al. 2021, https://accs.uaa.alaska.edu/wildlife/ak-bee-atlas) is a sampling program designed to provide information on the biodiveristy of pollinators throughout Alaska. In 2022, KNWR biologists participated in the Alaska Bee Atlas effort.

Methods

Sampling Design

We followed the sampling plan guidance of Fulkerson et al. (2021). Most of KNWR lies within lowest priority areas mapped in Fulkerson et al. (2021), but the southernmost part of the Refuge lies within a medium priority area. We prioritized sampling in this area, but access in this area is difficult. We surveyed only at Emerald Lake in this medium priority area.

We surveyed for insect pollinators at a variety of other sites on the Refuge, trying to sample in diverse habitats (Figure 1). We sampled dry, rocky slopes off of Skilak Lake Road following the advice of Justin Fulkerson (Alaska Center for Conservation Science, Anchorage, Alaska).

We accessed sites by road and floatplane.

Field Methods

We sampled pollinators using bee bowl traps (Figure 2), blue vane traps (Figure 3), and aerial nets (Figure 4), generally following the field methods of Fulkerson et al. (2021) with the exception that we collected specimens into SK picglobal 99.9% pure propylene glycol. Field notes are available from Bowser (2022c) and Bronowski (2022).

Specimen Processing

Samples were stored in a -23°C freezer except when samples were being processed. Invertebrates were separated from debris by hand under a dissecting microscope. Care was taken to reduce possible cross-contamination of DNA among samples.

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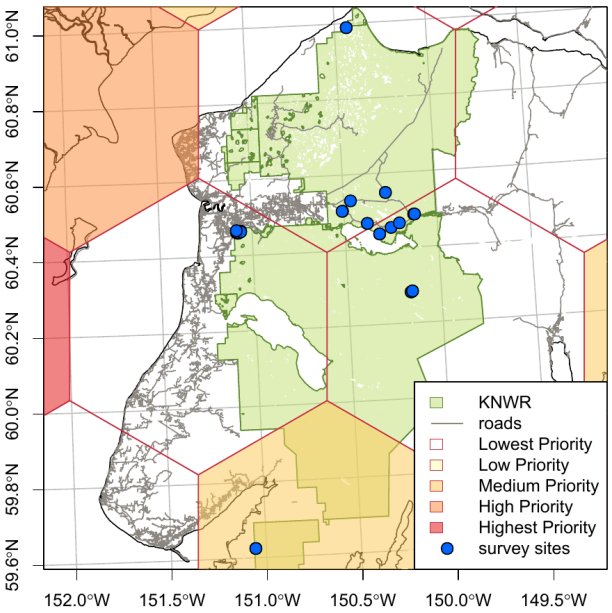

Figure 1: Map of Alaska Bee Atlas sampling priority hexagons as of May 11, 2022 and sites we surveyed for pollinators in 2022. KNWR: Kenai National Wildlife Refuge boundary. The map was generated with R, version 4.2.2 (R Core Team 2022) using the R packages sf, version 1.0-9 (Pebesma 2018) and pdftools, version 3.3.3 (Ooms 2023c).

Figure 2: A bee bowl trap, part of a set bee bowls off of Skilak Lake Road, June 27, 2022 (credit: Matt Bowser/USFWS).

We separated samples that were all or mostly bees from samples that were mostly flies and other invertebrates. We shipped 12 samples of bees to the Alaska Center for Conservation Science[18], University of Alaska Anchorage, Anchorage, Alaska to be processed by methods described by Fulkerson et al. (2021).

We homogenized the remaining 19 samples plus one legacy bulk pollinator sample from a previous project (Bowser 2012) using a blender and cleaning between samples with DIY-DS cleaning solution as described by Buchner et al. (2021). Our sample homogenization protocol is included below.

We homogenized samples using a Nutri Ninja QB3000SS blender.

[18] https://accs.uaa.alaska.edu/

Figure 3: Two blue vane traps near Hidden Lake Campground, June 17, 2022 (credit: Matt Bowser/USFWS).

Figure 4: Dominique Watts collecting pollinators using an aerial net above Twin Lakes, August 3, 2022 (credit: Matt Bowser/USFWS).

DIY-DS recipe

- 20 g NaOH
- 20 g Alconox
- 15.1 g $NaHCO_3$
- 267 ml 4.5% bleach
- deionized water to fill to 2 l

Preparation

1. 120 ml plastic cups should be washed with DIY-DS and rinsed before sampling. Finish by rinsing inside the 120 ml cup with deionized water. Hand dry 120 ml cup with paper towel.

Homogenize samples

1. Before running samples, rinse blender by running 100 ml of deionized water for 20 s.
2. Pre-label a 10 ml plastic vial with the specimen GUID and add a barcode vial label. Also pre-label and add a barcode label to a 120 ml specimen cup.
3. Clean forceps with DIY-DS.
4. Take the label out of the original container with the cleaned forceps and place into the new 120 ml sample container.
5. Add the contents of the sample vial to the blender.
6. Rinse original sample vial with cold, clean propylene glycol and pour rinsate in the blender with the rest of the sample.

7. Fill blender to 100 ml with cold, clean propylene glycol.
8. Blend for 90 s.
9. Using a new disposable pipette, fill the pre-labelled 10 ml plastic vial with about 9.5 ml of homogenate.
10. Pour the rest of the sample into the pre-labeled 120 ml specimen cup.
11. Rinse blender by running 100 ml tap water for 10 s.
12. Wash blender by running 100 ml of DIY-DS for 10 s.
13. Rinse this out in the lab sink with tap water.
14. Rinse blender by running 100 ml deionized water for 10 s.

We shipped 9 ml of homogenate from each of the 20 homogenized samples to Molecular Research Laboratory[19], Shallowater, Texas for metabarcoding.

Molecular Methods

We chose to use the *mlCOIintF/jgHCO2198* (GGWACWGGWT GAACWGTWTA YCCYCC / TAIACYTCIG GRTGICCRAA RAAYCA) primer set of Leray et al. (2013) for PCR, targeting a 313 bp region of the COI DNA barcoding region.

The *mlCOIintF/jgHCO2198* primer pair was used with barcodes on the forward primer in 30–35 PCR cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 30–35 cycles of 94°C for 30s, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. The pooled and purified PCR product was used to prepare an illumina DNA library. Sequencing was performed at MR DNA on a MiSeq following the manufacturer's guidelines.

Bioinformatics

The bioinformatics pipeline was run on the Yeti supercomputer (USGS Advanced Research Computing 2021). We used the MetaWorks pipeline, version 1.11.3 (Porter and Hajibabaei 2022) with the RDP classifier (Wang et al. 2007) and the Eukaryote CO1 reference set for the RDP Classifier, version 4.0.1 (Porter and Hajibabaei 2018). We processed data in R, version 4.2.2 and 4.2.3 (R Core Team 2022, 2023) using the R packages ape, version 5.7-1 (Paradis and Schliep 2019); Biostrings, version 2.66.0 (Pagès et al. 2022); bold, version 1.2.0 (Chamberlain 2021a); curl, version 5.0.0 (Ooms 2023a); ips, version 0.0.11 (Heibl 2008); msa, version 1.30.1 (Bodenhofer et al. 2015); openssl, version 2.0.6 (Ooms 2023b); reshape2, version 1.4.4 (Wickham 2007); ritis, version 1.0.0 (Chamberlain 2021b); and uuid, version 1.1-0 (Urbanek and Ts'o 2022).

We compared our sequences to sequences from a local reference library (Bowser 2022a) using the `vsearch --usearch_global` command of vsearch, version 2.21.1 (Rognes et al. 2016).

In order to exclude potential false positive detections as defined by MacKenzie et al. (2006) due to demultiplexing errors (see Deiner et al. 2017), we conservatively removed from the Exact Sequence Variant (ESV) table all occurrences that represented less than 0.4% of the total number of reads for any ESV, based the experience of (Bowser 2023b), where an apparent rate of mis-assignment of up to 0.36% was found. We also removed all occurrences represented by only one or two reads.

Complete methods including all configurations, commands, and scripts used for processing data are available from Bowser (2023a).

Identfications of *Bombus* species were conformed to the names provided by Sikes and Rykken (2020).

[19] https://www.mrdnalab.com/

Results and Discussion

Data Availability

Project data and project photos are available on Arctos via an Arctos project record (https://arctos.database.museum/project/10003917) and specimen records can be viewed via an Arctos search[20]. Project information is also available from a project record on ServCat (https://ecos.fws.gov/ServCat/Reference/Profile/148742). Raw sequence data from this project are available from Bowser (2022b). Resulting occurrence data have been published as an occurrence dataset (Bowser et al. 2023). Results from specimens sent to the Alaska Center for Conservation Science are available from the 2018 to 2022 Results Map at https://arcg.is/1myveP.

Results Summary

The single legacy sample from 2011 yielded 71 species and 18 BINs (Table 4). The 17 samples collected in 2022 yielded 206 species and 85 BINs (Table 5).

Table 4: Numbers of species and BINs observed in the sample from 2011 by orders.

Phylum	Class	Order	Species	BINs
Arthropoda	Arachnida	Araneae	1	0
Arthropoda	Insecta	Coleoptera	1	0
Arthropoda	Insecta	Diptera	61	14
Arthropoda	Insecta	Hemiptera	1	2
Arthropoda	Insecta	Hymenoptera	2	2
Arthropoda	Insecta	Lepidoptera	3	0
Arthropoda	Insecta	Odonata	1	0
Ascomycota	Dothideomycetes	Capnodiales	1	0

Table 5: Numbers of species and BINs observed in the sample from 2022 by orders.

Phylum	Class	Order	Species	BINs
Annelida	Clitellata	Crassiclitellata	1	0
Arthropoda	Arachnida	Araneae	11	0
Arthropoda	Arachnida	Sarcoptiformes	2	0
Arthropoda	Arachnida	Trombidiformes	0	1
Arthropoda	Collembola	Collembola	3	0
Arthropoda	Collembola	Entomobryomorpha	1	1
Arthropoda	Collembola	Symphypleona	0	1
Arthropoda	Insecta	Coleoptera	22	3
Arthropoda	Insecta	Diptera	99	60
Arthropoda	Insecta	Ephemeroptera	1	0
Arthropoda	Insecta	Hemiptera	16	9
Arthropoda	Insecta	Hymenoptera	28	10
Arthropoda	Insecta	Lepidoptera	9	0
Arthropoda	Insecta	Orthoptera	1	0
Arthropoda	Insecta	Psocodea	1	0
Arthropoda	Insecta	Thysanoptera	2	0
Arthropoda	Insecta	Trichoptera	1	0

[20] https://arctos.database.museum/search.cfm?project_id=10003917

Phylum	Class	Order	Species	BINs
Chordata	Aves	Galliformes	1	0
Chordata	Mammalia	Rodentia	1	0
Mollusca	Gastropoda	Stylommatophora	3	0
Ascomycota	Dothideomycetes	Capnodiales	1	0
Ascomycota	Sordariomycetes	Hypocreales	2	0

In the single sample from 2011, the most abundant species in terms of read abundances was 16,848 reads of *Ctenicera angusticollis* (Figure 5). Other abundant identifications were flies in the families Muscidae, Anthomyiidae, and Fanniidae. In 2022, the highest read abundance was for *Speyeria mormonia* (Boisduval, 1869) (Lepidoptera: Nymphalidae), which we detected in three samples (Figure 6). Reads of *Helina* species (Diptera: Muscidae), *Rhadiurgus variabilis* (Zetterstedt, 1838) (Diptera: Asilidae), and *Xylota subfasciata* Loew, 1866 were also abundant.

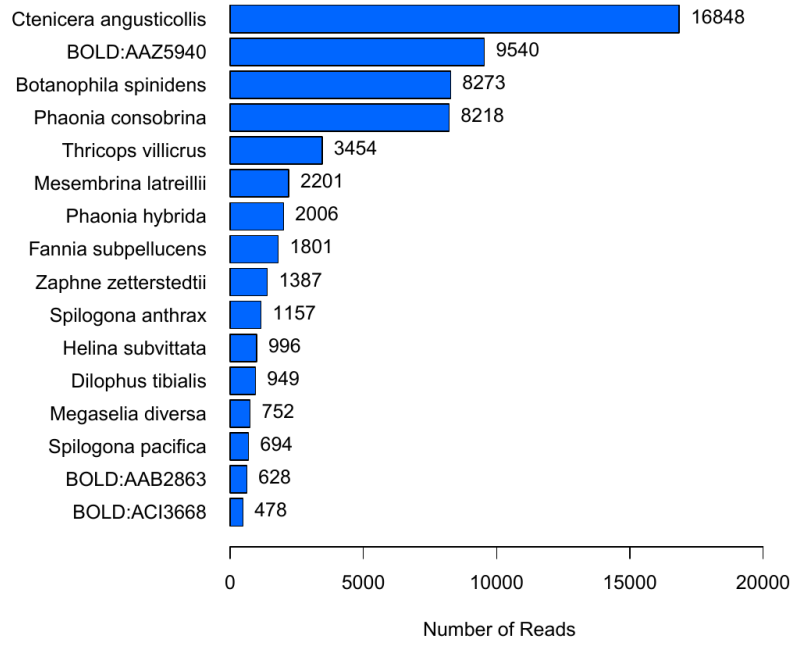

Figure 5: Top 16 most abundant identifications in terms of DNA read abundances from the single 2011 pollinator sample. BOLD:AAZ5940: *Hiatomyia* sp. BOLD:AAZ5940 (Diptera: Syrphidae). BOLD:AAB2863: *Dasysyrphus* sp. BOLD:AAB2863 (Diptera: Syrphidae). BOLD:ACI3668: *Delia* sp. BOLD:ACI3668 (Diptera: Anthomyiidae).

The most frequently observed identifications were four *Helina* species that were detected in 5–10 out of the 17 samples (Figure 7). The fungus *Cladosporium allicinum* (Fr.) Bensch, U.Braun & Crous (Capnodiales: Cladosporiaceae) was detected in 6 samples. The bee parasite *Apocephalus borealis* Brues, 1924 (Diptera: Phoridae) was detected in four samples.

Bees

We detected no bees in the sample from 2011 and 12 species of bees in 2022 (Table 6). All of these bee species are widespread in Alaska based on occurrence records available through the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/), but we are not aware of other records of *Andrena*

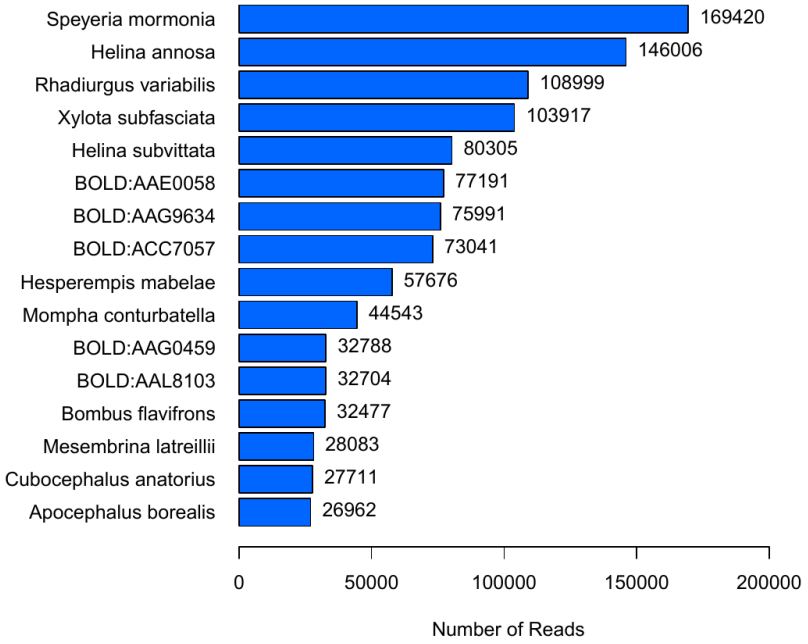

Figure 6: Top 16 most abundant identifications in terms of DNA read abundances from the 2022 pollinator samples. BOLD:AAE0058: *Mydaea* sp. BOLD:AAE0058 (Diptera: Muscidae). BOLD:AAG9634: *Dolichopus* sp. BOLD:AAG9634 (Diptera: Dolichopodidae). BOLD:ACC7057: *Phaonia* sp. BOLD:ACC7057 (Diptera: Muscidae). BOLD:AAG0459: *Suillia* sp. BOLD:AAG0459 (Diptera: Heleomyzidae). BOLD:AAL8103: Anthomyiidae sp. BOLD:AAL8103.

milwaukeensis or *Halictus rubicundus* from the Kenai Peninsula. All of the *Bombus* species we found are known to be abundant or common in our area (Rykken 2022).

Table 6: Bee species observed in 2022.

Family	Species
Andrenidae	*Andrena milwaukeensis* Graenicher, 1903
Apidae	*Apis mellifera* Linnaeus, 1758
Apidae	*Bombus flavifrons* Cresson, 1863
Apidae	*Bombus frigidus* Smith, 1854
Apidae	*Bombus insularis* (Smith, 1861)
Apidae	*Bombus lapponicus sylvicola* Kirby, 1837
Apidae	*Bombus melanopygus* Nylander, 1848
Apidae	*Bombus mixtus* Cresson, 1879
Apidae	*Bombus sitkensis* Nylander, 1848
Halictidae	*Halictus rubicundus* (Christ, 1791)
Megachilidae	*Megachile melanophaea* Smith, 1853
Megachilidae	*Megachile relativa* Cresson, 1878

Flies

Flies were by far the most speciose group collected by our sampling effort. In the single sample from 2011 we documented 61 species and 14 BINs representing 24 families (Table 7). In 2022 we found 99 species and

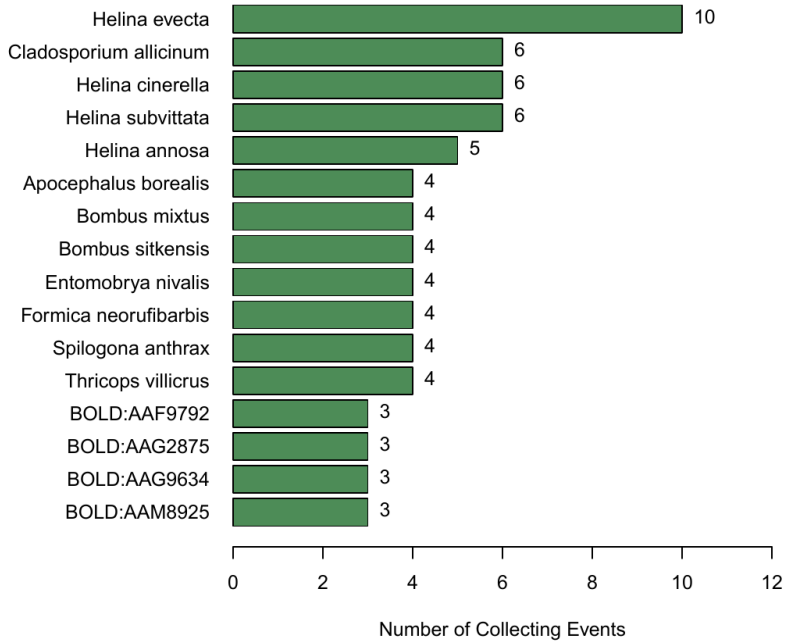

Figure 7: Top 16 most frequently observed identifications. BOLD:AAF9792: Empididae sp. BOLD:AAF9792. BOLD:AAG2875: *Ceratagallia* sp. BOLD:AAG2875 (Hemiptera: Cicadellidae). BOLD:AAG9634: *Dolichopus* sp. BOLD:AAG9634 (Diptera: Dolichopodidae). BOLD:AAM8925: *Lygus* sp. BOLD:AAM8925 (Hemiptera: Miridae).

60 BINs of flies in 32 families (Table 8). In both years the most diverse families observed were Muscidae and Anthomyiidae.

Table 7: Numbers of species and BINs of flies observed in the sample from 2011 by families.

Family	Species	BINs
Anisopodidae	1	0
Anthomyiidae	12	1
Bibionidae	1	0
Calliphoridae	2	0
Chloropidae	0	1
Empididae	1	2
Fanniidae	1	0
Lauxaniidae	1	0
Limoniidae	1	1
Lonchaeidae	0	2
Muscidae	20	1
Mycetophilidae	0	1
Phoridae	1	0
Pipunculidae	2	0
Psilidae	1	0
Sarcophagidae	1	0
Scathophagidae	3	1
Sciaridae	2	0
Sciomyzidae	1	0

Family	Species	BINs
Sepsidae	1	0
Stratiomyidae	1	0
Syrphidae	6	3
Therevidae	0	1
Tipulidae	2	0

Table 8: Numbers of species and BINs of flies observed in the sample from 2022 by families.

Family	Species	BINs
Agromyzidae	1	1
Anthomyiidae	10	6
Anthomyzidae	1	0
Asilidae	1	0
Bibionidae	1	0
Calliphoridae	2	0
Ceratopogonidae	0	1
Chironomidae	4	4
Chloropidae	4	3
Culicidae	1	1
Dolichopodidae	6	5
Empididae	2	2
Ephydridae	2	0
Fanniidae	2	2
Heleomyzidae	2	1
Hybotidae	2	4
Lauxaniidae	1	0
Muscidae	27	6
Mycetophilidae	6	3
Phoridae	8	8
Pipunculidae	1	0
Rhagionidae	0	1
Sarcophagidae	2	1
Scathophagidae	1	0
Scatopsidae	1	0
Sciaridae	2	3
Sepsidae	1	0
Simuliidae	1	4
Sphaeroceridae	1	1
Stratiomyidae	2	0
Syrphidae	3	2
Tachinidae	1	1

Pollinator Associates

Apocephalus borealis, a parasitoid of bees and vespine wasps (Tihelka et al. 2021) was both frequently observed in our samples from 2022 and abundant in terms of read counts. Adult *A. borealis* might have been collected, but it is more likely that these internal parasites were within their hosts at the time they were collected.

The fungus *Cladosporium allicinum* is common from environmental samples worldwide, collected from living and dead plants, air, water, and humans (Schubert et al. 2007, Bensch et al. 2012). We found no

literature records of *Cladosporium allicinum* taken from insects, but *Cladosporium* species can function as symbionts or pathogens of insects (Liu et al. 2022).

We detected *Wolbachia* (Rickettsiales: Anaplasmataceae) sequences in the 2011 sample and in 15 out of the 17 samples from 2022. *Wolbachia* bacteria infect many insect species and alter their hosts' reproductive systems (Werren 1997). *Wolbachia* can be present in high proportions of pollinator populations (Evison et al. 2012).

We also detected *Steinernema* (Rhabditida: Steinernematidae) in one sample[21], a sample where most reads were of *Bombus* species. The *Steinernema* sequence was 97.34% identical to a sequence identified as *Steinernema kraussei* (Steiner, 1923) Travassos, 1927, a species known to be pathogenic to *Bombus terrestris* (Linnaeus, 1758) (Dutka et al. 2015).

New Distribution Records

Ero canionis Chamberlin & Ivie, 1935 (Araneae: Mimetidae); *Atomaria testacea* Stephens, 1830 (Coleoptera: Cryptophagidae); *Liriomyza baptisiae* (Frost, 1931) (Diptera: Agromyzidae); *Fannia neopolychaeta* Chillcott, 1961 (Diptera: Fanniidae); *Tachypeza fenestrata* (Say, 1823) (Diptera: Hybotidae); *Phaonia protuberans* Malloch, 1923 (Diptera: Muscidae); *Phaonia serva* (Meigen, 1826); *Megaselia hirticrus* (Schmitz, 1918) (Diptera: Phoridae); *Megaselia lucifrons* (Schmitz, 1918); *Agria housei* Shewell, 1971 (Diptera: Sarcophagidae); *Boettcheria litorosa* (Reinhard, 1947) (Diptera: Sarcophagidae); *Olethreutes bipunctana* (Fabricius, 1794) (Lepidoptera: Tortricidae); *Coleophora quadruplex* McDunnough, 1940 (Lepidoptera: Coleophoridae); *Peristenus howardi* Shaw, 1999 (Hymenoptera: Braconidae) and *Cubocephalus anatorius* (Gravenhorst, 1829) (Hymenoptera: Ichneumonidae) appear to be new records for Alaska. The the non-native species *Odontothrips loti* (Haliday, 1852) and *Uroleucon taraxaci* (Kaltenbach, 1843) also appear to be new for the state.

Coleophora quadruplex was known from multiple Canadian provinces (Pohl et al. 2018, GBIF.Org 2023e), but we found no previous records from Alaska. *Olethreutes bipunctana* had also been reported from Canada (Pohl et al. 2018, GBIF.Org 2023f), but apparently not from Alaska.

Fannia neopolychaeta had been known from as close to Alaska as British Columbia (Chillcott 1960) and Yukon Territory (GBIF.Org 2023a), but our record appears to be new for Alaska. *Eudasyphora canadiana* Cuny, 1980 had been reported from Alaska by Cuny (1980), but there were no georeferenced Aslakan records in GBIF. *Phaonia protuberans* was known from the Northwest Territories and Yukon Territory (Huckett 1965, GBIF.Org 2023b), but it had not been reported from Alaska. *Phaonia serva* (Meigen, 1826) occurs in the Northwest Territories (Huckett 1965), but ours appears to be the first record from Alaska. *Tachypeza fenestrata* appears to be a new record for Alaska, but this species is present nearby in Yukon Territory. *Aspistes spathis* had been reported from Alaska by Cook (1965), but we found no georeferenced Alaskan occurrences (GBIF.Org 2023d). We found no Alaska records of *Megaselia lucifrons*, but this species is known from Yukon Territory. *Agria housei* and *Boettcheria litorosa* appear to be new records for Alaska, but there are records of these species from Yukon Territory.

Peristenus howardi (Hymenoptera: Braconidae) had been reported from Idaho and Washington by Day et al. (1999) and it has since been found in Alberta (Zhang 2018, GBIF.Org 2023c), but this species had not been reported from Alaska.

Non-native Species

We documented occurrences of seven non-native species. The European honey bee, *Apis mellifera* Linnaeus, 1758, was detected in bee bowls set out at Headquarters lake wetland near Soldotna[22]. The thrips *Odontothrips loti* (Haliday, 1852) (Thysanoptera: Thripidae) was detected in bee bowls deployed in a meadow off of Skilak Lake Road[23]. We found *Uroleucon taraxaci* in a disturbed clearing off of Ski Hill Road[24]. *Pol-*

[21] https://www.gbif.org/dataset/86875091-d166-4986-802a-343b341424c6/event/12127687
[22] https://www.gbif.org/occurrence/4093719140
[23] https://www.gbif.org/occurrence/4093715897
[24] https://www.gbif.org/occurrence/4093719079

lenia vagabunda (Meigen, 1826) (Diptera: Polleniidae) was collected in bee bowls at Kenai National Wildlife Refuge headquarters in Soldotna[25], where this species had been found previously by Bowser (2015). We detected the earthworm *Dendrobaena octaedra* (Savigny, 1826) (Crassiclitellata: Lumbricidae) in bee bowls set at Picnic Lake[26]. Sequences we obtained of the slug *Deroceras agreste* (Linnaeus, 1758) (Stylommatophora: Agriolimacidae) from bee bowls set at multiple locations[27] were 99.35–99.68% identical to sequences identified by Zając and Stec (2020) as *Deroceras agreste*. We found the slug *Arion fuscus* (O.F.Müller, 1774) (Stylommatophora: Arionidae) off of the Vista Trail[28].

Pollenia vagabunda had been found at the Kenai National Wildlife Refuge headquarters area previously (Bowser 2015). *Uroleucon taraxaci* (Kaltenbach, 1843) is believed to be introduced in North America (Foottit et al. 2006) and has been recorded from as close to Alaska as Yukon Territory (Maw et al. 2000). We detected this aphid in in bee bowls set in the back lawn of KNWR headquarters, where its host, *Taraxacum officinale* Weber ex Wiggins, is abundant. The epigeic earthworm *Dendrobaena octaedra* is almost ubiquitous near roads on KNWR (Saltmarsh et al. 2016) and this worm does climb (Römbke et al. 2017), so its presence in a bee bowl was not surprising. *Deroceras agreste* had previously been found in the Ski Hill Road area by Bowser et al. (2020); its occurrence at Picnic Lake in the Mystery Creek area was new. Our finding of *Arion fuscus* was the first record of an arionid slug on KNWR. *Arion fuscus* had previously been identified in Alaska from Sitka (Schade 2018).

Intersting Non-insect Records

We detected two vertebrate species: a single record of Willow Ptarmigan (*Lagopus lagopus* (Linnaeus, 1758)) at an alpine meadow above Twin Lakes[29] and three records of northern red-backed voles (*Myodes rutilus* (Pallas, 1779)) in the vicinity of the Kenai National Wildlife Refuge's headquarters in Soldotna[30]. At Twin Lakes we had seen and heard a family of Willow Ptarmigan within about 100 m of the area where we had sampled pollinators using aerial nets. In the sample from which the Willow Ptarmigan DNA was detected, no biting flies were detected, so the ptarmagin record was not from a blood meal of a fly. There might have been ptarmigan DNA in or on muscid or phorid flies in the sample. There were also no biting flies detected in any of the three bee bowl samples where vole DNA was found. Voles may have tasted the propylene glycol or otherwise explored the bee bowls.

Identification Notes

Some of our reads were 100% similar to sequences both identified as *Spilogona alticola* (Malloch, 1920) (Diptera: Muscidae) and *Spilogona contractifrons* (Zetterstedt, 1837) in BOLD BIN BOLD:AAB5278[31]. Huckett (1965) expressed his doubt that these two species were distinct. We assigned these to *Spilogona contractifrons* as we have done in previous work (Bowser et al. 2020). Other reads were 100% similar to sequences of *Spilogona* sp. 12AKR *sensu* Renaud (2012), which had also been documented locally by Bowser et al. (2020).

Conclusions

Complementing morphological identifications by metabarcoding enabled us to efficiently identify many more non-bee species than we would have been able to process and identify in a timely way and it also

[25] https://www.gbif.org/occurrence/4093716650
[26] https://www.gbif.org/occurrence/4093717859
[27] https://www.gbif.org/occurrence/4093716677, https://www.gbif.org/occurrence/4093716136, https://www.gbif.org/occurrence/4093717282
[28] https://www.gbif.org/occurrence/4093715392
[29] https://www.gbif.org/occurrence/4093716617
[30] https://www.gbif.org/occurrence/4093718979, https://www.gbif.org/occurrence/4093718205, https://www.gbif.org/occurrence/4093716762
[31] https://doi.org/10.5883/BOLD:AAB5278

provided detections of some bee parasites. We believe that using multiple methods is an expedient way to improve our understanding of insect pollinators in Alaska.

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References

Bensch K, Braun U, Groenewald JZ, Crous PW (2012) The genus *Cladosporium*. Studies in Mycology 72: 1–401. https://doi.org/10.3114/sim0003

Bodenhofer U, Bonatesta E, Horejs-Kainrath C, Hochreiter S (2015) msa: An R package for multiple sequence alignment. Bioinformatics 31: 3997–3999. https://doi.org/10.1093/bioinformatics/btv494

Bowser ML (2012) Update on the 2011 Kenai Arthropod Rapid Ecological Assessment. Newsletter of the Alaska Entomological Society 5: 15–20. Available from: http://www.akentsoc.org/doc/AKES_newsletter_2012_I.pdf.

Bowser ML (2015) First record of a cluster fly (Calliphoridae: *Pollenia*) in Alaska. Newsletter of the Alaska Entomological Society 8: 1–2. Available from: http://www.akentsoc.org/doc/AKES_newsletter_2015_I.pdf.

Bowser ML (2022a) Kenai COI reference library. Available from: https://ecos.fws.gov/ServCat/Reference/Profile/148827 (March 22, 2023).

Bowser ML (2022b) Raw metagenomic data from the Kenai National Wildlife Refuge's 2022 pollinator project. Available from: https://ecos.fws.gov/ServCat/Reference/Profile/148741.

Bowser ML (2022c) Work journal 2022. U.S. Fish; Wildlife Service, Kenai National Wildlife Refuge, Soldotna, Alaska. Available from: https://ecos.fws.gov/ServCat/Reference/Profile/143245 (May 2, 2023).

Bowser ML (2023a) 2022 Kenai National Wildlife Refuge pollinator surveys data analysis. USFWS Kenai National Wildlife Refuge, Soldotna, Alaska Available from: https://ecos.fws.gov/ServCat/Reference/Profile/154138 (May 3, 2023).

Bowser ML (2023b) Report on invertebrate inventories in the Miller Creek watershed, Kenai Peninsula, Alaska, 2021–2022. USFWS Kenai National Wildlife Refuge, Soldotna, Alaska Available from: https://ecos.fws.gov/ServCat/Reference/Profile/154047 (April 24, 2023).

Bowser ML, Brassfield R, Dziergowski A, Eskelin T, Hester J, Magness DR, McInnis M, Melvin T, Morton JM, Stone J (2020) Towards conserving natural diversity: A biotic inventory by observations, specimens, DNA barcoding and high-throughput sequencing methods. Biodiversity Data Journal 8: e50124. https://doi.org/10.3897/BDJ.8.e50124

Bowser ML, Bronowski A, Watts D (2023) Kenai National Wildlife Refuge pollinator surveys. Version 1.5. https://doi.org/10.15468/h7p7gm

Bronowski A (2022) Field notes 2022. U.S. Fish; Wildlife Service, Kenai National Wildlife Refuge, Soldotna, Alaska. Available from: https://ecos.fws.gov/ServCat/Reference/Profile/147197 (May 2, 2023).

Buchner D, Haase P, Leese F (2021) Wet grinding of invertebrate bulk samples – a scalable and cost-efficient protocol for metabarcoding and metagenomics. Metabarcoding and Metagenomics 5: e67533. https://doi.org/10.3897/mbmg.5.67533

Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL (2011) Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences 108: 662–667. https://doi.org/10.1073/pnas.1014743108

Chamberlain S (2021a) bold: Interface to Bold Systems API. Available from: https://CRAN.R-project.org/package=bold.

Chamberlain S (2021b) ritis: Integrated Taxonomic Information System client. Available from: https://CRAN.R-project.org/package=ritis.

Chillcott JG (1960) A revision of the Nearctic species of Fanniinae (Diptera: Muscidae). The Memoirs of the Entomological Society of Canada 92: 5–295. https://doi.org/10.4039/entm9214fv

Cook EF (1965) A Contribution toward a Monograph of the Scatopsidae. Part IX. The Genera *Aspistes* and *Arthria* (Diptera: Scatopsidae: Aspistinae). Annals of the Entomological Society of America 58: 713–721. https://doi.org/10.1093/aesa/58.5.713

Cuny R (1980) Revision of the genus *Eudasyphora* Townsend (Diptera: Muscidae), and reflections on its evolution. The Canadian Entomologist 112: 345–373. https://doi.org/10.4039/Ent112345-4

Day WH, Baird CR, Shaw SR (1999) New, native species of *Peristenus* (Hymenoptera: Braconidae) Parasitizing *Lygus hesperus* (Hemiptera: Miridae) in Idaho: Biology, importance, and description. Annals of the Entomological Society of America 92: 370–375. https://doi.org/10.1093/aesa/92.3.370

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L (2017) Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology 26: 5872–5895. https://doi.org/10.1111/mec.14350

Dutka A, McNulty A, Williamson SM (2015) A new threat to bees? Entomopathogenic nematodes used in biological pest control cause rapid mortality in *Bombus terrestris*. Mikheyev A (Ed.). PeerJ 3: e1413. https://doi.org/10.7717/peerj.1413

Evison SEF, Roberts KE, Laurenson L, Pietravalle S, Hui J, Biesmeijer JC, Smith JE, Budge G, Hughes WOH (2012) Pervasiveness of parasites in pollinators. PLOS ONE 7: e30641. https://doi.org/10.1371/journal.pone.0030641

Foottit RG, Halbert SE, Miller GL, Maw E, Russell LM (2006) Adventive aphids (Hemiptera: Aphididae) of America North of Mexico. Proceedings of the Entomological Society of Washington 108: 583–610. Available from: https://handle.nal.usda.gov/10113/8716 (April 5, 2023).

Fulkerson JR, Carlson ML, Burns CT (2021) Bee Survey and Monitoring Plan for Alaska. Alaska Center for Conservation Science, University of Alaska Anchorage, Anchorage, Alaska, 33pp. Available from: https://accs.uaa.alaska.edu/wp-content/uploads/Alaska_Bee_Atlas_2021.pdf (March 22, 2023).

GBIF.Org (2023a) Occurrence download. https://doi.org/10.15468/dl.9663nb

GBIF.Org (2023b) Occurrence download. https://doi.org/10.15468/dl.9ka3wy

GBIF.Org (2023c) Occurrence download. https://doi.org/10.15468/dl.a9ymn2

GBIF.Org (2023d) Occurrence download. https://doi.org/10.15468/dl.eqmasn

GBIF.Org (2023e) Occurrence download. https://doi.org/10.15468/dl.hb5dn7

GBIF.Org (2023f) Occurrence download. https://doi.org/10.15468/dl.krb9h7

Heibl C (2008) PHYLOCH: R language tree plotting tools and interfaces to diverse phylogenetic software packages. Http://www.christophheibl.de/rpackages.html.

Huckett HC (1965) The Muscidae of northern Canada, Alaska and Greenland (Diptera). Memoirs of the Entomological Society of Canada 42: 1–369. https://doi.org/10.4039/entm9742fv

Kenai National Wildlife Refuge, US Fish & Wildlife Service, Alaska Regional Office, Division of Conservation Planning & Policy (2010) Comprehensive Conservation Plan: Kenai National Wildlife Refuge. U.S. Fish & Wildlife Service, Anchorage, Alaska. Available from: https://ecos.fws.gov/ServCat/Reference/Profile/149784 (December 13, 2022).

Koh I, Lonsdorf EV, Williams NM, Brittain C, Isaacs R, Gibbs J, Ricketts TH (2016) Modeling the status, trends, and impacts of wild bee abundance in the United States. Proceedings of the National Academy of Sciences 113: 140–145. https://doi.org/10.1073/pnas.1517685113

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. Frontiers in Zoology 10: 34. https://doi.org/10.1186/1742-9994-10-34

Liu W, Yu S-H, Zhang H-P, Fu Z-Y, An J-Q, Zhang J-Y, Yang P (2022) Two *Cladosporium* fungi with opposite functions to the Chinese white wax scale insect have different genome characters. Journal of Fungi 8. https://doi.org/10.3390/jof8030286

MacKenzie DI, Nichols JD, Royle JA, Pollock KH, Bailey LL, Hines JE (2006) Occupancy Estimation and Modeling. Elsevier, New York.

Maw HEL, Foottit RG, Hamilton KGA (2000) Checklist of the Hemiptera of Canada and Alaska. NRC Research Press, Ottowa, Ontario, Canada.

Ooms J (2023a) curl: A modern and flexible web client for R. Available from: https://CRAN.R-project.org/package=curl.

Ooms J (2023b) openssl: Toolkit for encryption, signatures and certificates based on OpenSSL. Available from: https://CRAN.R-project.org/package=openssl.
Ooms J (2023c) pdftools: Text extraction, rendering and converting of PDF documents. Available from: https://CRAN.R-project.org/package=pdftools.
Pagès H, Aboyoun P, Gentleman R, DebRoy S (2022) Biostrings: Efficient manipulation of biological strings. Available from: https://bioconductor.org/packages/Biostrings.
Paradis E, Schliep K (2019) ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526–528. https://doi.org/10.1093/bioinformatics/bty633
Pebesma E (2018) Simple features for R: Standardized support for spatial vector data. The R Journal 10: 439–446. https://doi.org/10.32614/RJ-2018-009
Pohl GR, Landry J-F, Schmidt BC, Lafontaine JD, Troubridge JT, Macaulay AD, Nieukerken E van, deWaard JR, Dombroskie JJ, Klymko J, Nazari V, Stead K (2018) Annotated checklist of the moths and butterflies (Lepidoptera) of Canada and Alaska. Pensoft Publishers, Sofia, Bulgaria. Available from: https://ebooks.pensoft.net/books/13080 (January 17, 2024).
Porter TM, Hajibabaei M (2018) Automated high throughput animal CO1 metabarcode classification. Scientific Reports 8: 4226. https://doi.org/10.1038/s41598-018-22505-4
Porter TM, Hajibabaei M (2022) MetaWorks: A flexible, scalable bioinformatic pipeline for high-throughput multi-marker biodiversity assessments. PLOS ONE 17: 1–11. https://doi.org/10.1371/journal.pone.0274260
Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: Trends, impacts and drivers. Trends in Ecology & Evolution 25: 345–353. https://doi.org/10.1016/j.tree.2010.01.007
R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.
R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.
Renaud AK (2012) Biodiversity of the Muscidae (Diptera) from Churchill, Manitoba, Canada, with taxonomic issues revealed or resolved by DNA barcoding. Master's thesis. University of Manitoba Available from: http://hdl.handle.net/1993/5029 (April 4, 2023).
Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 4: e2584. https://doi.org/10.7717/peerj.2584
Römbke J, Blick T, Dorow WHO (2017) *Allolobophoridella eiseni* (Lumbricidae), a truly arboreal earthworm in the temperate region of Central Europe. Soil Organisms 89: 75–84. Available from: https://soil-organisms.org/index.php/SO/article/view/79 (March 27, 2023).
Rykken J (2022) Bumble bees of Alaska: A field guide to identification & natural history. National Park Service. Available from: https://www.nps.gov/articles/000/alaska-bees.htm (May 2, 2023).
Saltmarsh DM, Bowser ML, Morton JM, Lang S, Shain D, Dial R (2016) Distribution and abundance of exotic earthworms within a boreal forest system in southcentral Alaska. NeoBiota 28: 67–86. https://doi.org/10.3897/neobiota.28.5503
Schade J (2018) Slugs and snails in Alaska. Alaska Department of Natural Resources
Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, Hoog GS de, Crous PW (2007) Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Studies in Mycology 58: 105–156. https://doi.org/10.3114/sim.2007.58.05
Sikes DS, Rykken JJ (2020) Update to the identification guide to female Alaskan bumble bees and a summary of recent changes to the Alaskan bumble bee fauna. Newsletter of the Alaska Entomological Society 13: 31–38. Available from: http://www.akentsoc.org/doc/AKES_newsletter_2020_n1_a09.pdf.
Tihelka E, Hafernik J, Brown BV, Quock C, Zink AG, Croppi S, Cai C-Y, Zhu C-D (2021) Global invasion risk of *Apocephalus borealis*, a honey bee parasitoid. Apidologie 52: 1128–1140. https://doi.org/10.1007/s13592-021-00892-4
Urbanek S, Ts'o T (2022) uuid: Tools for generating and handling of UUIDs. Available from: https://CRAN.R-project.org/package=uuid.
USGS Advanced Research Computing (2021) USGS Yeti Supercomputer. U.S. Geological Survey, Denver, Colorado Available from: https://doi.org/10.5066/F7D798MJ (February 22, 2021).

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73: 5261. https://doi.org/10.1128/AEM.00062-07

Werren JH (1997) Biology of *Wolbachia*. Annual Review of Entomology 42: 587–609. https://doi.org/10.1146/annurev.ento.42.1.587

Wickham H (2007) Reshaping data with the reshape package. Journal of Statistical Software 21: 1–20. Available from: http://www.jstatsoft.org/v21/i12/.

Zając KS, Stec D (2020) Molecular approach to the identification of three closely related slugs *Deroceras* species (Gastropoda: Eupulmonata: Agriolimacidae). Zoological Studies 59: 55. https://doi.org/10.6620/ZS.2020.59-55

Zhang Y (2018) The evolution of *Peristenus* (Hymenoptera: Braconidae): Taxonomy, phylogenetics and ecological speciation. Master's thesis. University of Central Florida Available from: https://stars.library.ucf.edu/etd/5967 (April 28, 2023).