Newsletter

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Alaska Entomological Society

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Upcoming Events

Figure 1: Graphic from Kluane Bioblitz flyer.

Kluane BioBlitz, June 23–25, 2017

Come join us for the Yukon's BioBlitz in the Kluane region. All are welcome to participate for the event which is part of Canada's 150th anniversary celebrations. BioBlitz Canada and the Canadian Wildlife Federation are supporting a series of public BioBlitz's across the nation and the Yukon Conservation Data Centre and Parks Canada in partnership with the Kluane First Nation and Champagne and Aishihik First Nations are hosting the Yukon's 150BioBlitz in the Kluane area, including the Kluane National Park and Reserve. If you'd like more information, contact Bruce Bennett at `bruce.bennett@gov.yk.ca`. Project website: `http://www.inaturalist.org/projects/kluane-bioblitz-2017-bioblitz-de-kluane-2017`.

Eleventh Annual Meeting, January 2018

The eleventh annual meeting of the Alaska Entomological Society will take place in Anchorage in January 2018. Check for updates on our events page as the meeting date approaches.

Western Flower Thrips and Alaska

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by Jacquelyn Schade[1]

Figure 1: Western flower thrips (*Frankliniella occidentalis*) by Jack T. Reed, Mississippi State University (`https://www.invasive.org/browse/detail.cfm?imgnum=5370035`).

Western flower thrips (Pergande, 1895) (WFT, *Frankliniella occidentalis*) is a highly destructive pest of greenhouse crops around the world (Cloyd, 2010). It originated in western North America and spread worldwide via horticultural material in the 1970's and 1980's (Kirk and Terry, 2003). The presence of WFT poses challenges to farmers who wish to export their crops internationally. Some importing countries may restrict or prohibit from entry imported commercial crops which contain WFT. Depending upon the importing country, growers may need to officially certify their crop prior to export and take proper safeguarding measures to ensure commodities remain free from WFT. The first recorded occurrence of WFT in Alaska was in 1956 (Bryan and Smith, 1956). In recent surveys, including Cooperative Agricultural Pest Surveys (CAPS), WFT has been found throughout Alaska. The majority of the surveys that found WFT were conducted in peony fields, but they have also been found in botanical gardens (Curtis Knight, unpublished data and Arctos saved search `http://arctos.database.museum/saved/AlaskaFrankliniellaoccidentalis`).

WFT is considered a highly destructive pest because of its wide host range, damage to crops, ability to vector disease, various environmental and physical adaptations, parthenogenesis, high reproductive potential, lack of obligate diapause, and cryptic habit (Kirk and Terry, 2003). WFT feeds on over 240 host species in over 60 families. Hosts include peony, pea, tomato, pepper, strawberry, rose, cucumber, bean, carrot, onion, and lettuce (Tommasini and Maini, 1995). They cause rasping feeding damage and oviposition scars that permanently mark the plant. In addition to feeding and oviposition damage, WFT is also capable of vectoring tospoviruses, ilarviruses, and carmoviruses. WFT is a major vector for Tomato spotted wilt virus (Jones, 2005). It is believed that they have two ecotypes: one thrives in hot/dry environments and the other thrives in cool/moist environments (Brunner and Frey, 2010). This adaptation allows the WFT to establish in almost any environment. They have a haplo-diploid breeding system which means unfertilized eggs develop into males and fertilized eggs develop into females (Cloyd, 2010).

WFT goes through several developmental stages in its lifecycle. The length of each stage is influenced by the temperature. In ideal conditions (80-85°F), WFT will develop in less than 10 days. Eggs are laid in plant tissue. WFT has two larval stages and two pupal stages. WFT are voracious feeders during the larval stage. The larvae develop beneath terminal foliage or at the petal attachment. In the first larval stage, WFT are small and white. WFT move to the soil for the pupal stages and are fairly inactive. Pupal WFT are soft bodied and develop wings. Adult WFT are generally 1–2 mm long. They have unusual wings that are sparsely covered in long hairs, short, straight antennae, two dark eyes, and are light yellowish to brown in coloring (Swier, 2016). Thrips often overwinter as pupae or adults under leaf litter (Cloyd, 2010).

Little is known about the WFT lifecycle in Alaska. In 2015, Holloway and Gerdeman (2016) conducted research to determine the impact and management of thrips in Alaska peony production. They found that thrips overwintered inside and outside of the fields, concentrating in soil near trees. In Fairbanks, thrips had one full generation and a partial second generation in one season. On the Kenai Peninsula, they observed two full generations and a partial third generation in one season. The number of generations can increase or decrease each year depending on the weather. Longer, warmer summers may result in increased generations per year (Holloway and Gerdeman, 2016).

There are several options for managing WFT in a field and greenhouse. Creating and utilizing an integrated pest management plan will help prevent new WFT introductions to the crops and manage current WFT infestations to keep them at manageable levels. Following are some

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management practices to consider adding to an integrated management plan:

- Buy stock from WFT free locations or nurseries to prevent introducing WFT to your fields (McDougall and Tesoriero, 2011).
- Inspect plants for WFT before bringing them into your greenhouse (Swier, 2016).
- Maintain a high level of farm hygiene (Plant Health Australia, 2012).
- Control weeds (Plant Health Australia, 2012).
- Use mesh and double door entries to greenhouses (Plant Health Australia, 2012).
- Monitor crops routinely for presence of pests. Yellow sticky traps are an excellent monitoring tool for WFT (Plant Health Australia, 2012). Recommended times for setting yellow sticky cards for monitoring thrips in the Fairbanks region are late April/early May (Holloway and Gerdeman, 2016).
- Foliar insecticide spray to control WFT (Plant Health Australia, 2012). WFT develop resistance to pesticides easily, so there are few chemical control options available. Consult a professional to develop a chemical control plan (McDougall and Tesoriero, 2011).

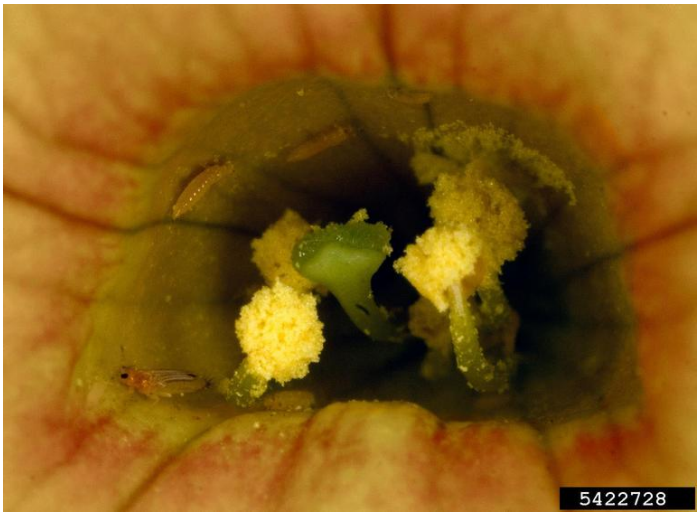

Figure 2: Western flower thrips (*Frankliniella occidentalis*) by David Cappaert (`https://www.invasive.org/browse/detail.cfm?imgnum=5422728`).

The Alaska CAPS program is beginning a three year nursery survey in Alaska in summer 2017. The goal of this survey is to identify what harmful exotic pests are being brought into the state, determine the origin of plant material entering the state, and whether or not imported plant material is acting as a vector to bring in harmful exotic pests. The survey will focus on searching for a variety of pests, including WFT, Asian Longhorned Beetle, European Black Arion Slug, Vineyard Snail, Sudden oak death, stubby root nematode, Tomato spotted wilt virus, and Tobacco rattle virus.

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Orthosia hibisci (Guenée), the speckled green fruitworm: confirmed causing extensive hardwood defoliation in Southcentral and Western Alaska

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by Jason Moan[1]*, Garret Dubois*[2]*, and Steve Swenson*[2]

Introduction

In 2016, the speckled green fruitworm, *Orthosia hibisci*(Guenï£¡e) (Figure 3), was confirmed to be contributing to extensive hardwood defoliation in parts of the Alaska and northern Aleutian Ranges and is suspected as the cause of comparable defoliation in areas of Western Alaska (FS-R10-FHP, 2017). *Orthosia hibisci* was also suspected as having caused widespread defoliation in the aforementioned areas in 2014 and 2015 (Figure 2). This defoliation event was initially documented via aerial surveys, during which the cause of the damage was suspected of being the related species, *Sunira verberata*(Smith), battered sallow moth (FS-R10-FHP, 2016). That moth was thought to be the primary pest in the same area from 2000-2006 (FS-R10-FHP, 2007), but defoliation over those years may have been exacerbated by other species such as *O. hibisci*.

The Defoliation

In 2014, approximately 9,500 acres of willow, alder, and birch defoliation were observed during the forest health aerial detection surveys along the Upper East and West Forks of the Yentna River, as well as on the eastern side of Mystic Pass (Figure 1). At the time, the damage agent was unknown.

During the 2015 field season, more than 180,000 acres of hardwood defoliation similar to that observed in 2014 were documented in the Alaska and northern Aleutian Ranges and Western Alaska. Also in 2015, numerous reports of damage were received from across the impacted areas. National Park Service (NPS) staff and volunteers forwarded damage reports from Lake Clark National Park and Preserve(LCNP) to Forest Health Protection (FHP) staff and also reported extensive defoliation on the east end of Telaquana Lake. Defoliation was reported in the Shell Hills area of the Susitna River valley and up the Skwentna River by a private citizen. Additional damage was also reported and mapped in the following locations: Lake Chakachamna, upper East and West Forks of the Yentna River, Yenlo Hills, Chakachatna and McArthur Rivers area, Nerka Lake in Wood-Tikchik State Park, and along the Innoko and Yukon Rivers near Holy Cross.

Figure 1: Defoliation caused by *Orthosia hibisci* along the upper Yentna in summer of 2016 J. Moan, AKDOF.

[1]Alaska Department of Natural Resources, Division of Forestry
[2]USDA Forest Service, Forest Health Protection

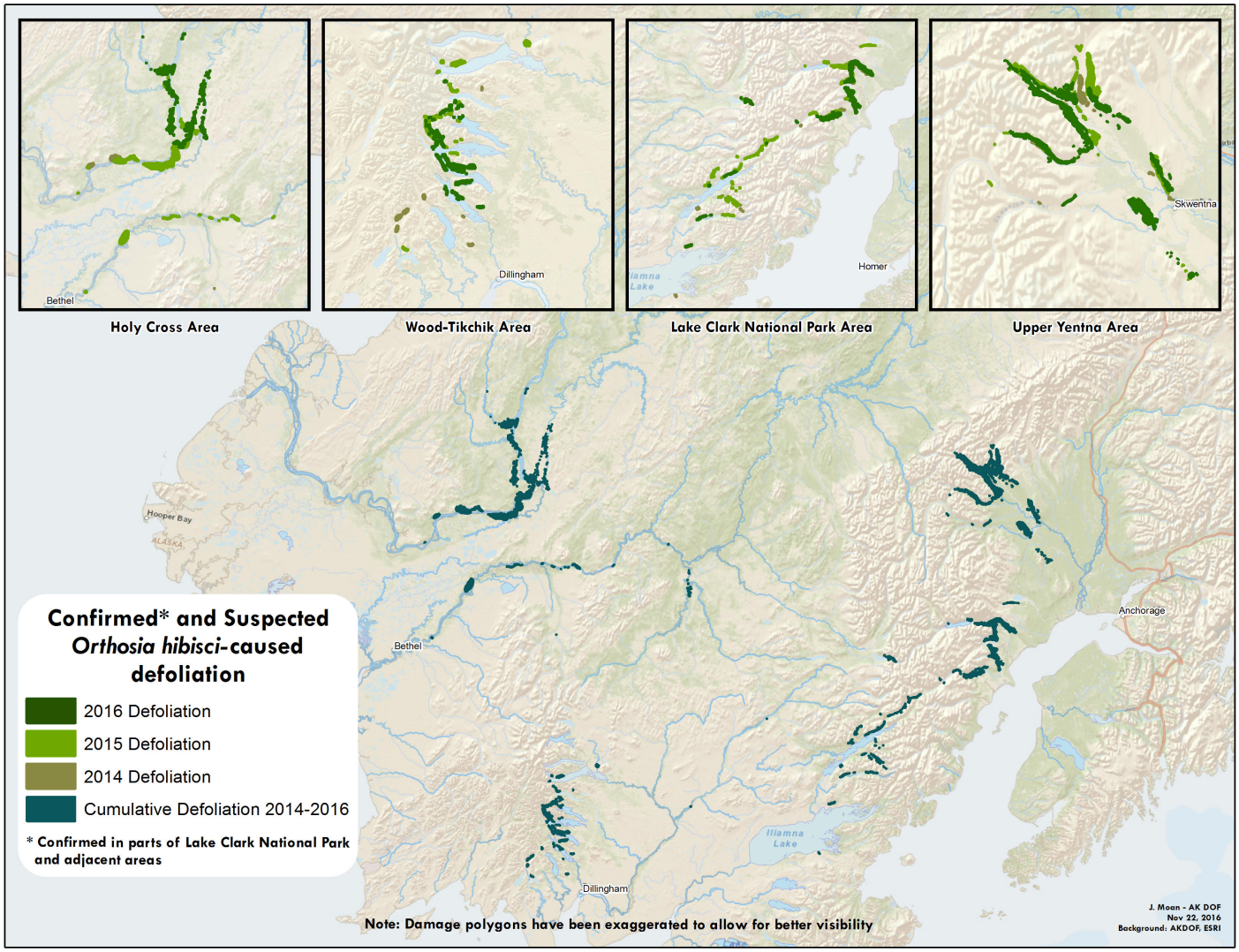

Figure 2: Map showing confirmed and suspected *Orthosia hibisci*-caused defoliation 2014-2016. J. Moan, AKDOF.

Based on the observed symptoms of defoliation, the history of defoliation in parts of the region, and comparison of larval reference images with observed specimens, the defoliation documented in 2014 and 2015 was suspected to have been caused by *Sunira verberata*. Outbreaks of *S. verberata* have been documented during aerial surveys in the past, specifically from 2003 to 2008 in Wood-Tikchik State Park and Katmai National Park. To the best of our knowledge, during previous aerial detection surveys, neither *S. verberata* nor *O. hibisci* had been documented as causing such widespread defoliation as observed during the last few years.

In early summer 2016, damage on the east end of Telaquana Lake was again reported from LCNP staff and volunteers. A private citizen also provided images of larvae and physical moth specimens that were collected along the northeast shore of Lake Clark. Additionally, Bureau of Land Management staff and U.S. Fish and Wildlife Service staff out of the Koyukuk/Nowitna/Innoko National Wildlife Refuges reported heavy defoliation and provided images of larvae and defoliated willows around Holy Cross and along the Innoko River from Shageluk to the Yukon River, up the Yukon River to Anvik, and along the Anvik River. In 2016, defoliation mapped in these areas, as well as the expansion of those defoliated areas mapped in 2015, totaled roughly 160,000 acres.

The Defoliator

A ground survey was conducted by FHP and Alaska Division of Forestry (DOF) staff on June 8, 2016 at

Chakachamna and Telaquana Lakes to collect specimens for rearing and identification (Figure 4). Accurate identification to species using morphological characteristics typically requires adult specimens.

Figure 3: *Orthosia hibisci* moth in spring of 2015. J. Mills and J. Mills, NPS.

Larvae were observed feeding gregariously at both locations. Defoliation and scattered dieback in willow and alder was evident. Defoliation was also observed in cottonwood and birch, though impacts were minor in comparison. Larvae and feeding damage extended onto many non-woody plants in the area, including grasses and other herbaceous groundcover.

Two color variations were observed in the larvae, a green variation and a dark green to almost black variation. Chapman and Lienk (1974) noted that the dark variation can occur when larvae are crowded, such as during high populations (Figure 5). This also occurs in other species such as *Operophtera bruceata*(Hulst) (James Kruse, US Forest Service, personal communication).

Figure 4: *Orthosia hibisci* feeding on willow foliage at Telaquana Lake, June 2016. S. Swenson, USFS FHP.

Figure 5: Dark and light color variations of *Orthosia hibisci* larvae. G. Dubois, USFS FHP.

Larvae were collected at both locations and were then transferred to rearing chambers at both the FHP and DOF labs (Figure 6). The intention was to rear moths for identification. Conditions in the labs were not consistent with natural conditions, staying consistently warmer (60°–70°F) with lighting often brighter and more variable than outdoor conditions. Larvae were fed new host material as needed and by late June, the majority of larvae had pupated (Figure 7). The proportion of larvae that survived to the pupal stage cannot be confirmed across all rearing chambers, but in some cases, survival to prepupa was at or above 50%. Larvae, when possible, buried themselves in the substrate to pupate (Figure 8). By October 2016, the specimens remained as pupae and there was suspicion

that either the rearing conditions had not been ideal or that these specimens were not the suspected *S. verberata*, for which adults are known to emerge in the fall.

Figure 6: Rearing chambers used in the USFS FHP lab. G. Dubois, USFS FHP.

FHP also staff submitted larvae that were collected at lake Telaquana and moths collected in the spring from Lake Clark for DNA identification.

> Samples were identified using LifeScanner (`http://lifescanner.net/`). Lifescanner is a DNA-barcoding service for citizen scientists that consists of a standardized sampling kit, mobile app, and standardized laboratory workflow. It is a project run out of the Centre for Biodiversity Genomics (`http://biodiversitygenomics.net/`) at the University of Guelph that DNA barcodes user-provided samples. Taxonomic identifications are generated through the Barcode of Life Data (BOLD) Systems ID Engine(Sujeevan Ratnasingham, Lifescanner principal Investigator, personal communication, see Ratnasingham and Hebert, 2007).

This DNA analysis identified both the adult moths and larval specimens as *O. hibisci*, a native generalist defoliator in the family Noctuidae. *Orthosia hibisci* has a very wide host preference, but is typically described as a fruit orchard pest feeding on foliage and immature fruits of numerous fruit trees in the family Rosaceae. Upwards of 40 hardwood and coniferous trees and shrubs are also known to be hosts, including alder, white birch, cottonwood, quaking aspen, and willow (Chapman and Lienk, 1974; Rings, 1970).

Figure 7: Various life stages of Orthosia hibisci. G. Dubois, USFS FHP.

Figure 8: Prepupae and pupae buried and partially buried in substrate. Where the substrate was deep enough or enough debris was present, many prepupae would bury themselves completely. G. Dubois, USFS FHP.

In addition to the June collections, in late April and early May of 2016 reports of a mass emergence of unknown moths were received from several locations in Western Alaska, including Bethel and Holy Cross. Numerous reports and photographs were received from these areas. Based on the photos, the moths were initially suspected of being *S. verberata*, however the phenology was not consistent. Acquisition of specimens from this event was facilitated through the Anchorage and Bethel University of Alaska Fairbanks - Cooperative Extension offices. Specimens were obtained by a local Bethel resident and sent to

taxonomists Dr. James J. Kruse and Dr. Clifford D. Ferris for identification and were also confirmed as *O. hibisci.*

Conclusions

Work done throughout the 2016 field season resulted in *O. hibisci* being confirmed as the causal agent of defoliation at several sites. Although *O. hibisci* is currently the only species to be confirmed in the impacted areas, other defoliators may be present and contributing to defoliation in the region. Future efforts will be made to collect and confirm specimens from other similarly impacted areas to determine distribution, defoliator species present, and host plants affected.

Acknowledgements

We would like to thank all the individuals who took the time to report, photograph, collect and provide specimens, or were otherwise involved in helping us determine the when, where, and what of this defoliation event: Amy Miller, National Park Service, LCNP and Jeanette Mills and Jerry Mills LCNP volunteers, provided reports and images from throughout LCNP, including Telaquana Lake. Casey Burns and Bruce Seppi, Bureau of Land Management, provided reports and images from the Holy Cross and Anvik areas. Kenton Moos, Karin Bodony and Kenneth Chase, U.S. Fish and Wildlife Service, Koyukuk/Nowitna/Innoko National Wildlife Refuges, provided reports and images from the Anvik area. Anchorage and Bethel University of Alaska Fairbanks, Cooperative Extension Offices and Local Bethel residents provided moth specimens for identification. Steve Kahn, private citizen, provided reports, images and moth specimens from Lake Clark. Page Spencer, private citizen, provided reports from the Shell Hills and along the Skwentna River. Their contributions, and the contributions of others have been invaluable. We would also like to thank Elizabeth Graham for her review of the article and James Kruse for his comments, insight and review of this article.

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DNA barcoding Alaska's butterflies

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by Derek S. Sikes[1]

Abstract

Ongoing efforts to build a DNA barcode library of the non-marine arthropod fauna of Alaska have, combined with work done in Canada, so far yielded an estimated coverage of ~48.5% of the species in the state. Among these are 71 of the 80 species of butterflies that are known residents of Alaska. This study compares the state's butterfly fauna using the DNA barcode BIN system, which is a proxy for species based on DNA barcodes, and the BOLD identification engine, to the count of species based on traditional taxonomy. The 71 traditional species for which DNA barcodes were obtained correspond to 56 unique BINs. Eleven of these BINs are shared among species of Alaskan butterflies (i.e. more than one Alaskan butterfly species occurs in two or more of these 11 BINs). The greatest BIN sharing was seen in the genus *Colias* which includes 8 traditional species that share two BINs. All the remaining BIN sharing was seen in species of the family Nymphalidae with members of *Boloria*, *Speyeria*, *Polygonia*, *Erebia*, and *Oeneis* sharing BINs. Currently 45 Alaskan butterfly species are in BINs not shared with other Alaskan butterflies, thus allowing a direct match to an Alaskan butterfly species name using the DNA barcode BIN system. Only three species had samples split into more than one BIN (suggestive of high within-species diversity). The test of the BOLD identification engine resulted in 82 of the 108 sequences (76%) being correctly identified. However, sampling of Alaskan butterflies has been sparse, with many species represented by only one or two DNA barcoded specimens, so these conclusions may change as sampling is increased.

Introduction

DNA barcoding (Hebert et al., 2003) has become a relatively common method to investigate biodiversity. Google Scholar returns over 5,000 articles dating between 2016 and March 2017 using the search term "DNA barcoding." DNA barcoding of Lepidoptera in general (e.g. Zahiri et al., 2014) and butterflies in particular (e.g. Huemer et al., 2014; Dincă et al., 2015) has been a strong focus of this large endeavour. The University of Alaska Museum has been collaborating with the Kenai National Wildlife Refuge to build a DNA barcode library of the state's non-marine arthropods (Sikes et al., 2017). To date, these efforts, combined with similar work being performed in Canada, have yielded DNA barcodes associated with ~48.5% of Alaska's 8,277 non-marine arthropod species (Sikes et al., 2017). Discovery of new state records, species new to science, and continued DNA barcoding efforts are ongoing so these values have already changed.

Although adult butterflies are relatively easy to identify via use of available field guides (e.g., Philip and Ferris, 2016) and many can be identified from field photos alone, a DNA barcode library of butterfly species allows identification of all life stages (eggs, larvae, pupae, adults), and identification from partial remains (e.g. gut contents, or fragments in bird nests, or remains on car radiators). As technology improves and prices drop (e.g., Meier et al., 2016; Bowser et al., 2017) identifications of butterflies in trap samples via the use of DNA barcodes could be performed faster and less expensively than traditional methods.

I was interested in seeing how useful this approach would be in obtaining species-level identifications for Alaska's butterfly species. The Barcode Index Number (BIN) system (Ratnasingham and Hebert, 2013) provides a DNA based approach to grouping DNA barcode sequences which often correspond with traditional Linnaean species. I wanted to evaluate the correspondence between Alaska's butterflies that have been DNA barcoded and the BIN system. Perfect correspondence in this case would entail 71 species corresponding with 71 BINs with no species having more than one BIN and no BIN having more than one species.

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Methods

An effort was made during the work described in Sikes et al. (2017) to obtain DNA barcodes from all resident Alaskan butterfly species ($n = 80$). See general methods in Sikes et al. (2017) which describe the process by which specimens were chosen and sequenced in the University of Alaska Museum Insect Collection (UAM) or the Kenelm W. Philip Lepidoptera (KWP) collection. For this focused investigation of Alaska's butterflies, identifications of any UAM or KWP specimens that were involved in BIN-sharing were double-checked. This is a standard quality control measure because one of the likely explanations for specimens that have been identified as different species but share a single BIN is that one has been misidentified. I also performed a test of the BOLD identification engine (Ratnasingham and Hebert, 2007) by querying the identification engine with each DNA barcode sequence of each Alaskan butterfly that I had obtained DNA barcodes for. This was a conservative test because the sequence used to query was present in the DNA barcode library which would increase the likelihood of a correct identification. Therefore any misidentifications from this process would be due to multiple identical sequences existing in the DNA barcode library under different species identifications. I queried the full COI database for these tests (All Barcode Records on BOLD, 4,845,382 Sequences as of 30 March 2017). I used the taxonomic classification of Pohl et al. (2016) which differs slightly from Philip and Ferris (2016).

Table 1: List of all resident Alaskan butterfly species. Count: count of Alaskan records in the online database Arctos. Barcoded: the number of Alaskan specimens successfully DNA barcoded. ID Success: the identification success resulting from query using the DNA barcode sequence obtained for each specimen of the Barcode of Life Datasystems (BOLD) full COI database. BINs: the BIN (Barcode Index Number), or BINs, to which each species was assigned in BOLD. Shared BINs are indicated by shared non-black colors. Species in bold font lack DNA barcodes.

Scientific Name	Count	Barcoded	ID Success	BINs
Hesperiidae				
Hesperiinae				
Hesperia comma (Linnaeus, 1758)	116	1	100%	BOLD:AAA6524
Heteropterinae				
Carterocephalus palaemon (Pallas, 1771)	365	2	100%, 100%	BOLD:AAA6267
Pyrginae				
Erynnis persius (Scudder, 1863)	367	1	100%	BOLD:AAB2800
Pyrgus centaureae (Rambur, 1842)	25	1	100%	BOLD:ACE2929
Papilionidae				
Papilioninae				
Papilio canadensis Rothschild and Jordan, 1906	693	2	100%, 100%	BOLD:ACE3135
Papilio machaon Linnaeus, 1758	913	1	100%	BOLD:AAA5810
Papilio zelicaon Lucas, 1852	1	1	100%	BOLD:AAB0873
Parnassiinae				
Parnassius eversmanni Ménétriés, 1850	461	2	100%, 100%	BOLD:ABZ8243
Parnassius phoebus (Fabricius, 1793)	483	3	100%, 100%, 100%	BOLD:AAB0370
Pieridae				
Coliadinae				
Colias canadensis Ferris, 1982	432	1	misid as *C. tyche*	BOLD:AAA3447
Colias gigantea Strecker, 1900	399	2	100%, 100%	BOLD:AAA3447
Colias hecla Lefèbvre, 1836	1447	2	100%, misid as *C. eurytheme* (but also *C. hecla* at 100%)	BOLD:AAA3447, BOLD:ACE5358
Colias christina kluanensis Ferris, 1981	119	2	100% (as syn. *C. christina*), ×2	BOLD:AAA3447
Colias nastes Boisduval, 1832	622	2	100%, 2nd misid as *C. palaeno* but *C. nastes* also at 100%	BOLD:AAA3447, BOLD:ACE5358
Colias palaeno (Linnaeus, 1761)	1473	2	100%, 100%	BOLD:AAA3447

Continued on next page...

Scientific Name	Count	Barcoded	ID Success	BINs
Colias philodice Godart, 1819	699	2	misid ×2 as *C. christina* (but more hits to *C. philodice* at 100% than *C. christina*)	BOLD:AAA3447
Colias tyche Böber, 1812	452	2	100%, 100%	BOLD:AAA3447
Pierinae				
Anthocharis sara Lucas, 1852	128	1	100%	BOLD:AAE4180
Euchloe ausonides (Lucas, 1852)	493	2	100%, 100%	BOLD:AAB5508
***Euchloe creusa* (E. Doubleday, 1847)**	**257**	**0**		
Euchloe naina Kozhanchikov, 1923	10	1	100%	BOLD:AAA5532
Pontia occidentalis (Reakirt, 1866)	782	1	100%	BOLD:AAB1348
Pieris marginalis complex	2726	4	*P. oleracea* + *P. angelika* ×4	BOLD:AAA2226
Lycaenidae				
Lycaeninae				
Lycaena dorcas (W. Kirby, 1837)	437	2	misid as *L. helloides* (*L. dorcas* also at 100%) ×2	BOLD:AAA7619
***Lycaena mariposa* (Reakirt, 1866)**	**6**	**0**		
***Lycaena phlaeas* (Linnaeus, 1761)**	**136**	**0**		
Polyommatinae				
Agriades glandon (de Prunner, 1798)	499	1	100%	BOLD:AAA5321
Agriades optilete (Knoch, 1781)	606	1	100%	BOLD:AAB5172
Celastrina lucia (W. Kirby, 1837)	541	1	misid as *C. neglecta* (+ *C. ladon* + *C. lucia*)	BOLD:ACF0806
Cupido amyntula (Boisduval, 1852)	403	1	100%	BOLD:AAA4838
Glaucopsyche lygdamus (E. Doubleday, 1841)	980	1	100%	BOLD:AAA5424
Icaricia saepiolus (Boisduval, 1852)	447	2	100%, 100%	BOLD:AAA4621
Plebejus idas (Linnaeus, 1761)	900	2	100%, 100%	BOLD:AAA3628
Theclinae				
***Callophrys augustinus* (Westwood, 1852)**	**366**	**0**		
Callophrys polios (Cook & F. Watson, 1907)	331	1	100%	BOLD:ACE6026
Nymphalidae				
Heliconiinae				
Boloria alaskensis (W. Holland, 1900)	1103	2	100%, 100%	BOLD:AAA9406
Boloria astarte (E. Doubleday, 1847)	220	1	100%	BOLD:AAB2859
Boloria chariclea (Schneider, 1794)	2822	4	100%, 100%, 100%, 100%	BOLD:AAA2067, BOLD:ACS2432
Boloria cf. *chariclea*	541	4	misid? as *B. chariclea* ×4	BOLD:AAA2067
***Boloria epithore* (W. H. Edwards, 1864)**	**2**	**0**		
Boloria eunomia (Esper, 1800)	374	1	100%	BOLD:AAA3397
Boloria freija (Thunberg, 1791)	2674	1	100% (also *B. natazhati* shares same DNA barcode)	BOLD:AAA6974
Boloria frigga (Thunberg, 1791)	925	1	100%	BOLD:ACE3748
Boloria improba (Butler, 1877)	706	1	100%	BOLD:AAC2010
***Boloria natazhati* (Gibson, 1920)**	**3**	**0**		
Boloria polaris (Boisduval, 1828)	1123	1	100%	BOLD:AAA3398
Boloria selene (Schiffermüller, 1775)	81	2	100%, 100%	BOLD:AAA5114
Speyeria atlantis (W. H. Edwards, 1862)	199	2	misid as *S. callippe* (but also *S. atlantis* & *S. hesperis* mixed at 100%) ×2	BOLD:AAA6312
Speyeria mormonia (Boisduval, 1869)	138	2	100%, 100%	BOLD:AAA4400

Continued on next page...

Scientific Name	Count	Barcoded	ID Success	BINs
Speyeria zerene (Boisduval, 1852)	12	1	100%	BOLD:AAA4400
Limenitidinae				
Limenitis arthemis (Drury, 1773)	395	1	100%	BOLD:ABZ6037
Nymphalinae				
Aglais milberti (Godart, 1819)	232	2	100%, 100%	BOLD:AAC2128
***Euphydryas anicia* (E. Doubleday, 1847)**	**7**	**0**		
Nymphalis antiopa (Linnaeus, 1758)	477	1	100%	BOLD:AAA7166
Nymphalis l-album (Esper, 1781)	293	1	100%	BOLD:ACE3441
Phyciodes pratensis Behr 1863	488	1	100%	BOLD:AAA2812
Polygonia faunus (W. H. Edwards, 1862)	504	1	100%	BOLD:AAA6982
Polygonia gracilis (Grote and Robinson, 1867)	204	1	100%	BOLD:ABY8345
Polygonia satyrus (W. H. Edwards, 1869)	128	1	100%	BOLD:ABY8345
Satyrinae				
Coenonympha tullia Müller 1764	756	2	100%, 100%	BOLD:AAA3561
Erebia disa (Thunberg, 1791)	936	2	100%, 100%	BOLD:AAB9133
Erebia discoidalis (W. Kirby, 1837)	647	1	100%	BOLD:AAB9132
Erebia epipsodea Butler, 1868	241	1	100%	BOLD:AAC6702
Erebia fasciata Butler, 1868	1057	1	100%	BOLD:ABZ6050
Erebia lafontainei Troubridge and Philip, 1983	127	1	misid as *E. youngi* (also *E. lafontainei* in 100% mix)	BOLD:ABZ1487
Erebia mackinleyensis Gunder, 1932	117	1	100%	BOLD:ABZ6050
Erebia mancinus E. Doubleday, 1849	373	1	misid as *E. disa*	BOLD:AAB9133
Erebia occulta Roos and Kimmich, 1983	365	1	100% (but lots of *E. youngi* mixed at 100%)	BOLD:AAB9739
Erebia pawloskii Ménétriés, 1859	438	2	100% but barely—no other 100% match & lots of spp. at 98%+	BOLD:AAB2107
Erebia rossii (J. Curtis, 1835)	1202	2	100%, 100%	BOLD:AAB9785
Erebia youngi W. Holland, 1900	339	1	misid as *E. occulta* (lots of *E. youngi* mixed in 100%)	BOLD:AAB9739
Oeneis alpina Kurentsov, 1970	94	1	100%	BOLD:AAD0556
Oeneis bore (Schneider, 1792)	1057	2	100%, 100%	BOLD:AAA8029
***Oeneis chryxus* (E. Doubleday, 1849)**	**11**	**0**		
Oeneis jutta (Hübner, 1806)	422	2	100%, 100%	BOLD:AAA3562
Oeneis melissa (Fabricius, 1775)	692	2	misid as *O. jutta* (but lots of *O. melissa* in 100% mix) ×2	BOLD:AAA3562
Oeneis philipi Troubridge, 1988	105	1	misid as *O. polixenes* (but lots of *O. philipi* in 100% mix)	BOLD:ACE5691
Oeneis polixenes (Fabricius, 1775)	652	1	100%	BOLD:ACE5691
Oeneis tanana Warren and Nakahara, 2016	156	1	misid as *O. chryxus* (*O. bore* in mix)	BOLD:AAA8029
***Oeneis uhleri* (Reakirt, 1866)**	**6**	**0**		

Results and Discussion

The results are shown in Table 1. DNA barcodes or BIN assignments were not obtained for nine of Alaska's 80 species of resident butterflies. These are indicated in Table 1 by their lack of a BIN code. These nine species are generally rare, such as *Lycaena mariposa*, which occurs in Alaska only in the Southeast and is represented in the combined UAM and KWP collections by only three specimens: two from 1947 and one from 1996. None of the specimens belonging to species with more than one BIN were found to be misidentified during post-sequencing identification efforts.

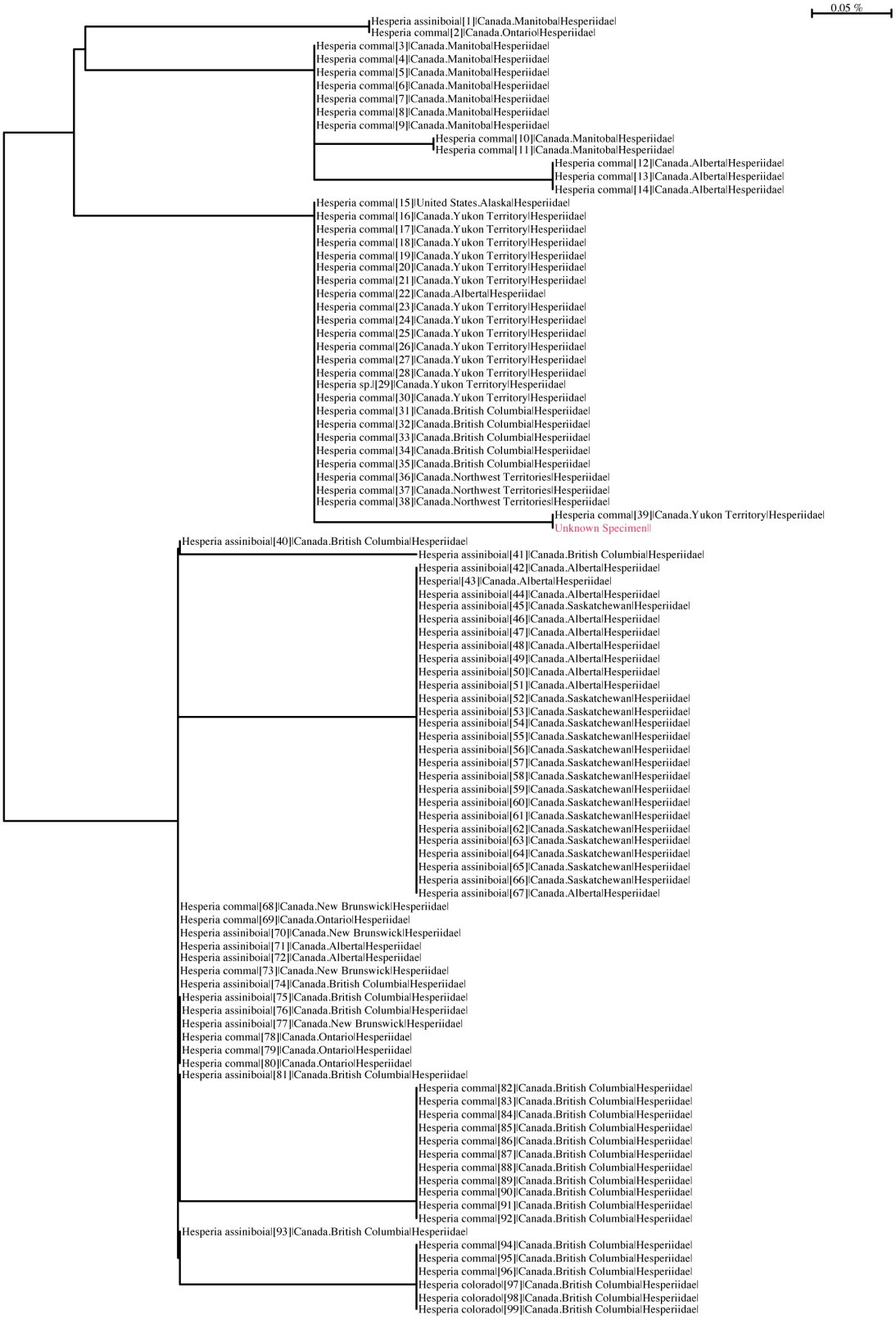

Figure 1: BOLD neighbor-joining tree for tree-based identification of KWP:Ento:36504 (indicated by purple "Unknown Specimen" in tree), *Hesperia comma*. Prepared using BOLD's full COI database, limited to sequences >200 base pairs in length, which includes records without species designation, using a K2P model for sequence correction and BOLD's amino-acid based alignment.

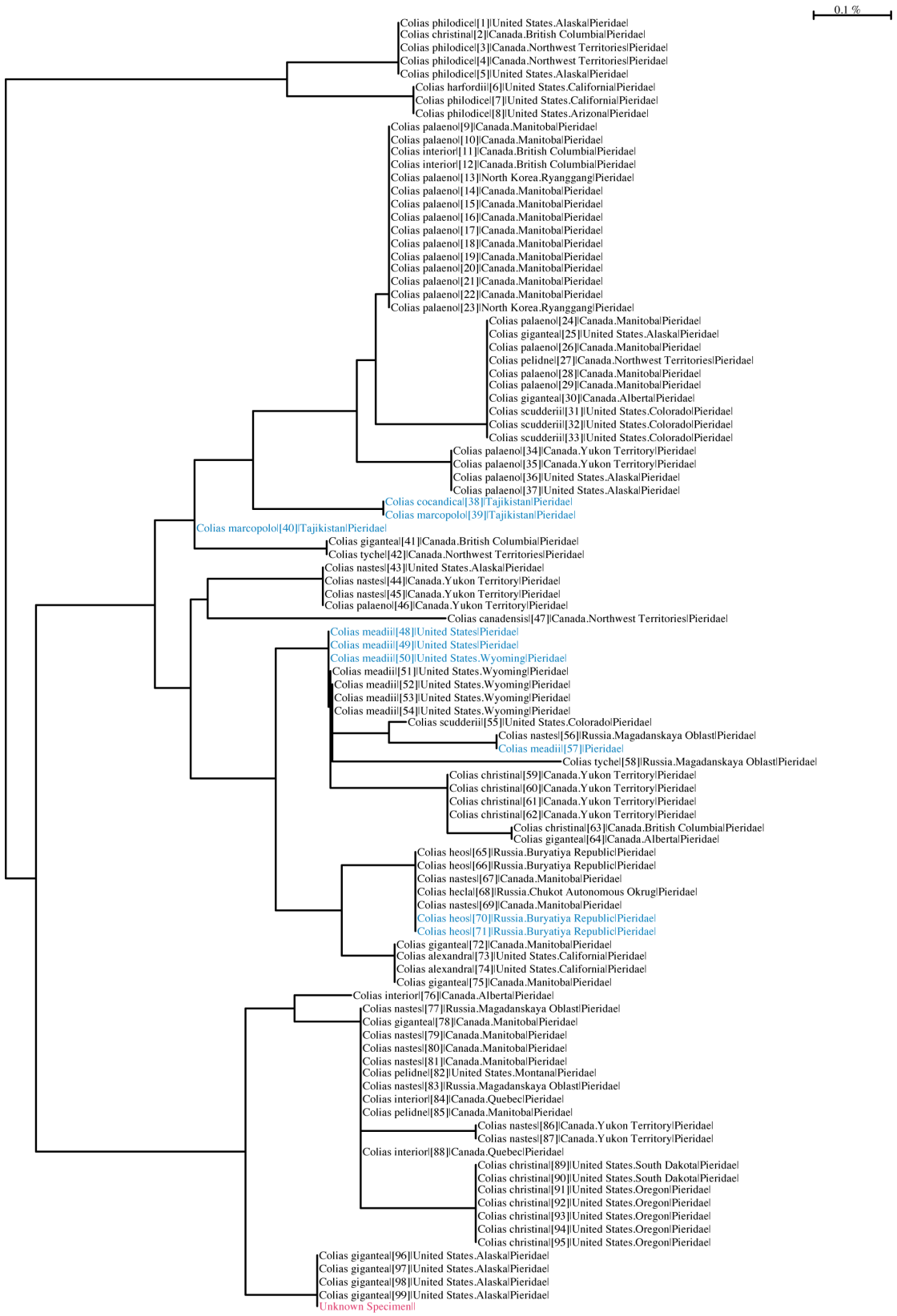

Figure 2: BOLD neighbor-joining tree for tree-based identification of UAM:Ento:19009 (indicated by purple "Unknown Specimen" in tree), *Colias gigantea*. Prepared using BOLD's full COI database, limited to sequences >200 base pairs in length, which includes records without species designation, using a K2P model for sequence correction and BOLD's amino-acid based alignment.

The 71 traditional species (a total of 108 DNA barcodes) correspond to 56 unique BINs (Table 1). Eleven of these BINs are shared among species of Alaskan butterflies (i.e. more than one Alaskan butterfly species occurs in two or more of these 11 BINs). The greatest BIN sharing was seen in the genus *Colias* which includes all eight traditional species in Alaska sharing two BINs. However, only two specimens of two species fell into the second BIN but in both cases their conspecific specimens fell into the first BIN that is shared with all eight species (Table 1).

The second BIN in *Colias hecla* (BOLD:ACE5358) was one of just three cases encountered in which an Alaskan butterfly species was sorted into two BINs. The other two cases were *Colias nastes* and *Boloria chariclea*. Such cases are often indicative of possible cryptic species (two species under one name) or misidentification, or high within-species genetic variation. I ruled out misidentification. It is also likely that the number of such cases would increase if sampling were increased. Most (57.7%) of the species sampled were based on single specimens which, by definition, cannot be sorted into more than one BIN. Only 30 of these 71 species were represented by more than one specimen.

All the remaining BIN sharing was seen in species of the family Nymphalidae with members of *Boloria*, *Speyeria*, *Polygonia*, *Erebia*, and *Oeneis* sharing BINs. Currently 45 of these 71 Alaskan butterfly species (63.4%) are in BINs not shared with other Alaskan butterflies, thus allowing a direct match to an Alaskan butterfly species name using the DNA barcode BIN system. Note that in most cases the BINs in which these 45 species fall also contain other species of butterflies but these other species do not occur in Alaska.

Although BINs generally correspond to Linnaean species, when they do not there are a variety of possible explanations including misidentifications, lab errors, young or incipient species (incomplete lineage sorting, haplotype sharing, introgression / hybridization), taxonomic oversplitting (Linnaean species names that should be synonymized), taxonomic undersplitting (cryptic species), or species with unusually high within-species genetic diversity. DNA barcodes do not make it clear which of these situations is present but they do at least help draw attention to the problems for future investigation.

The nine species which lacked DNA barcodes or BIN assignments are generally rare (at least in recent times, e.g. *Lycaena phlaeas*). I therefore expect that the 71 species with BIN assignments represent the vast majority of butterfly species one is likely to encounter in Alaska.

In addition to the BIN system one can explore the ability of BOLD's tree-based identification methods. I chose as examples one sequence of *Colias gigantea* and one of *Hesperia comma*. The *Hesperia comma* DNA barcode samples obtained were not involved in BIN sharing with other taxa but across the full BOLD dataset this species appears in two BINs. The tree-based identification (Figure 1) shows deep splits separating different samples of this species, although the Alaskan samples occur within one clade and BIN. This suggests that exploration of possible cryptic species within this species may be worthwhile. However, as indicated by the great amount of BIN sharing among Alaskan *Colias* samples (Table 1), the tree-based identification of *Colias gigantea* (Figure 2) shows great discordance between traditional identifications and tree structure. All four samples of *Colias gigantea* from Alaska share the same clade, shared with no other samples in the tree. However, this species also occurs in many other clades in the tree, along with various other *Colias* species. Despite this, the BOLD identification engine, when queried with the DNA sequence for this sample, returns an identification of *Colias gigantea* with 100% probability due to its perfect match to five sequences in BOLD (one of which is the same sequence used in the search). This indicates that despite BIN sharing among *Colias* species in Alaska, DNA barcodes can still be used to identify these species.

The test of the BOLD identification engine resulted in 82 of the 108 sequences (76%) being correctly identified. These 82 sequences belonged to 58 of the 71 species with DNA barcodes. One of the species that could not be identified is the newly described *Oeneis tanana* (Warren et al., 2016). The BOLD identification engine does not consider the haplotype of *O. tanana* to be distinct enough from five other species, primarily *O. chryxus* and *O. bore*, to separate these species. It is possible, however, that some or all of the *O. chryxus* that have identical DNA barcodes to *O. tanana* are actually misidentified *O. tanana*. Currently there is only one *O. tanana* record in BOLD, as more records are added these identification results might change. Interestingly, the results of these tests were not perfectly predictable from the assessment of BIN sharing. Some species that shared BINs were nevertheless correctly identified by the BOLD identification engine (e.g. the *Colias gigantea* above), and some species that did not share BINs were misidentified.

Some of these species, after additional study, may prove to be oversplit and if synonymized, the success rate of DNA barcode-based identification should increase. DNA barcoding has been criticized because it could lead to oversplitting of species. For example, some taxonomists find some of the results of Hebert et al. (2016) hard to believe. If all the molecular taxonomic units (BINs) identified in that study were to be formally named, one family of Diptera, the Cecidomyiidae, would see an increase from 100 species in Canada to ~16,000 species. However, with butterflies there is the opposite pattern—that if any oversplitting has occurred it has resulted from traditional taxonomic practice. Where recent traditional field and lab work have suggested new species might exist (e.g. *Boloria chariclea* vs. *Boloria* cf. *chariclea* (Philip and Ferris, 2016) these DNA barcoding results disagree. However, DNA barcodes are known to disagree with young species for various reasons. Most tax-

onomists would agree that most of the species in Table 1 that share BINs are well established on morphological and/or ecological grounds.

Future Directions

I hope to increase sampling to obtain at least two sequences from different populations of each species and hope to add in the nine species that are missing from the current dataset. I also hope this study spurs additional focus on the discordance between the traditional taxonomy and the DNA barcode results.

Acknowledgements

I thank the Alaska Department of Fish and Game and the United States Fish and Wildlife Alaska Region NWRS Inventory and Monitoring Initiative who funded the building of the DNA barcode library. I am also thankful for the taxonomists who performed the traditional identifications, especially Kathryn Daly, Clifford Ferris, Zdenek Fric, Kenelm Philip, and Andrew Warren.

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Notes on using LifeScanner for DNA-based identification of non-marine macroinvertebrates

doi:10.7299/X7DN4578

by Matt Bowser[1]

Introduction

We at the Kenai National Wildlife Refuge (KNWR) have been intent on inventorying and monitoring invertebrates for some time in fulfillment of our Congressional mandate to "to conserve fish and wildlife populations and habitats in their natural diversity..."[2] Recently, the US Fish & Wildlife Service, Alaska Region has collaborated with the University of Alaska Museum to build a DNA barcode library of Alaskan non-marine arthropods to better enable identifications of Alaskan material by DNA barcoding (Sikes et al., 2017). In this project arthropods were submitted for DNA barcoding in 95-well plates to the Canadian Center for DNA Barcoding (CCDB) and resulting sequences were uploaded to BOLD (Ratnasingham and Hebert, 2007).

At KNWR, DNA barcoding has enabled us to add to KNWR's checklist (Kenai National Wildlife Refuge biology staff, 2017) species that could not have been identified by morphological methods. We have also added to our list molecular operating taxonomic units (MOTUs, Blaxter et al., 2005) not necessarily associated with any accepted name, especially those recognized by BOLD's Barcode Index Number (BIN) algorithm (Ratnasingham and Hebert, 2013).

Beginning in fall 2015 KNWR obtained a number of LifeScanner kits (`http://lifescanner.net/`) for identification of animal specimens. I also purchased kits for use in a homeschool science project on willow rose gall midges and to identify pest insects around my family's small farming operation in Kasilof. In this article I will present a summary of the results, highlight some of the more noteworthy identifications obtained, and discuss the pros and cons of the LifeScanner service based on my experience.

Methods

From fall 2015 through March 2, 2017 I submitted 172 specimens from six invertebrate phyla to be DNA barcoded via LifeScanner kits.

Most specimens were collected opportunistically for various reasons. Two tissue samples from preserved specimens in the KNWR's entomology collection were sent in for identification. I submitted two specimens from gut contents that may have had degraded DNA: a snail that had been collected from the stomach of an arctic char in 2005, dried, and then left in a jar until February 2017 (KNWR:Ento:11200); and an insect leg fragment from the stomach of a shrew that had been caught in a mousetrap (KNWR:Ento:11204, Figure 1).

Figure 1: An insect leg (KNWR:Ento:11204) dissected from the stomach of a shrew (`http://www.inaturalist.org/observations/4999968`)

Five specimens were submitted by participants in a youth archaeology camp on the Kenai Refuge (Eskelin, 2016).

My children and I sampled willow gall midges and their associates as a homeschool science project, focusing special effort on *Rabdophaga rosaria* group midges, the galls of which were illustrated by Collet (2002).

One minute annelid worm was collected as part of a study of Kenai Peninsula grasslands (Bowser et al., 2017). Most of the annelids were collected in 2016 as part of a biotic inventory of the upper Slikok Creek watershed using the mustard powder extraction method (Lawrence and Bowers, 2002).

Results summary

Of the 172 specimens submitted, 144 sequences were obtained excluding sequences that were believed to have been contaminated, an overall success rate of 84% (Table 1). I included in the contaminated category all sequences that did not match the identification of what went in the vial. For example, I counted as contamination a nematode sequence obtained from a fly larva (KNWR:Ento:11110), even though the nematode may have been present as a parasite or other associate. I did not attempt to ascertain here the reasons for contaminations or sequencing failures. These could have happened anywhere beginning with potential

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[2]Alaska National Interest Lands Conservation Act of 1980

contamination or mix-ups out in the field through the wet lab work to publishing data at the end. In two cases where Trichoptera larva were submitted, workers at the lab interpreted these as plant specimens. Plant primers were used, yielding plant DNA barcode sequences from the caddisflies' casings. Of the 143 specimens for which we have received sequences, 110 (77%) were over 600 bp (Figure 2).

For the two specimens mentioned above having potentially degraded DNA, we received reads of 583–609 bp.

Our records can be viewed via BOLD dataset "DS-BOWSER"(doi:10.5883/DS-BOWSER) and on Arctos via a saved search (`http://arctos.database.museum/saved/KNWREntoLifeScanner`).

Table 1: Summary of LifeScanner sequencing results by taxonomic groups

Phylum	**Class**	**Order**	**# sequences/# samples**	**success rate (%)**
Annelida	Clitellata	Haplotaxida	22/28	79
Annelida	Clitellata	Lumbriculida	2/2	100
Annelida	Clitellata	Rhynchobdellida	1/1	100
Annelida	Clitellata	unknown	0/1	0
Arthropoda	Arachnida	Araneae	1/2	50
Arthropoda	Arachnida	Opiliones	1/1	100
Arthropoda	Arachnida	Pseudoscorpiones	1/1	100
Arthropoda	Arachnida	Trombidiformes	2/2	100
Arthropoda	Branchiopoda	Anostraca	0/1	0
Arthropoda	Chilopda	Geophilomorpha	1/1	100
Arthropoda	Chilopda	Lithobiomorpha	2/2	100
Arthropoda	Collembola	Poduromorpha	0/1	0
Arthropoda	Collembola	Symphypleona	1/1	100
Arthropoda	Insecta	Coleoptera	5/6	83
Arthropoda	Insecta	Diptera	59/64	92
Arthropoda	Insecta	Ephemeroptera	2/2	100
Arthropoda	Insecta	Hemiptera	6/7	86
Arthropoda	Insecta	Hymenoptera	18/20	90
Arthropoda	Insecta	Lepidoptera	12/12	100
Arthropoda	Insecta	Psocodea	1/1	100
Arthropoda	Insecta	Trichoptera	0/2	0
Arthropoda	Insecta	unknown	0/4	0
Arthropoda	Maxillopoda	Siphonostomatoida	1/1	100
Cnidaria	Hydrozoa	Anthoathecata	1/1	100
Mollusca	Bivalvia	Veneroida	1/1	100
Mollusca	Gastropoda	Hygrophila	3/3	100
Mollusca	Gastropoda	Stylommatophora	1/2	50
Nematomorpha	unknown	unknown	0/1	0
Platyhelminthes	Cestoda	Pseudophyllidea	0/1	0
Total			**144/172**	**84**

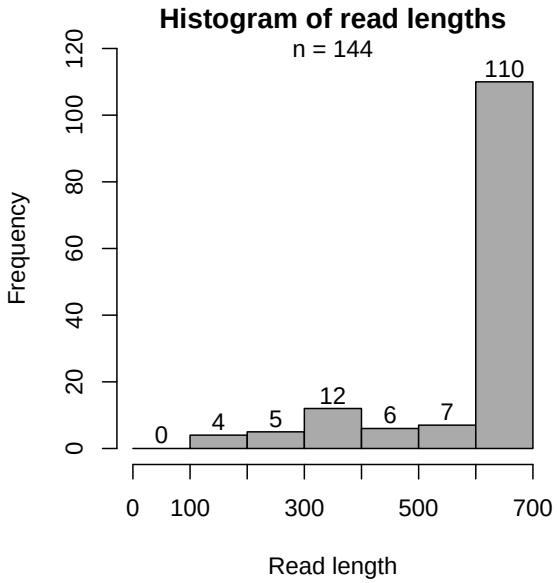

Figure 2: Histogram of read lengths of COI sequences from 144 sequences obtained via LifeScanner.

Identifications worthy of mention

I will not have time to include all that I would like to here. I have selected some examples that are new geographic records, newly documented biological relationships, corrections to my past erroneous conclusions, and findings that point toward more work to be done.

Radix auricularia

Radix auricularia (Linnaeus, 1758) (Hygrophila: Lymnaeidae) had been known from Alaska, but it was believed to have been introduced in North America, including Canada and Alaska (Clarke, 1981; Baxter, 1987; Bowser, 2017). I submitted tissues from two Kenai Peninsula specimens. Collection data: USA: Alaska, Kenai Peninsula, Stormy Lake, 60.769°N, 151.057°W, ± 20 m, 12.July.2016, ML Bowser, JM Morton, J Stone (KNWR:Ento:11102); Kenai Peninsula, Fish Lake, 60.7242°N, 150.7272°W ± 490 m, 1.June.2005, Arctic char collected by D France, snail extracted from stomach contents by RD Reger (KNWR:Ento:11200).

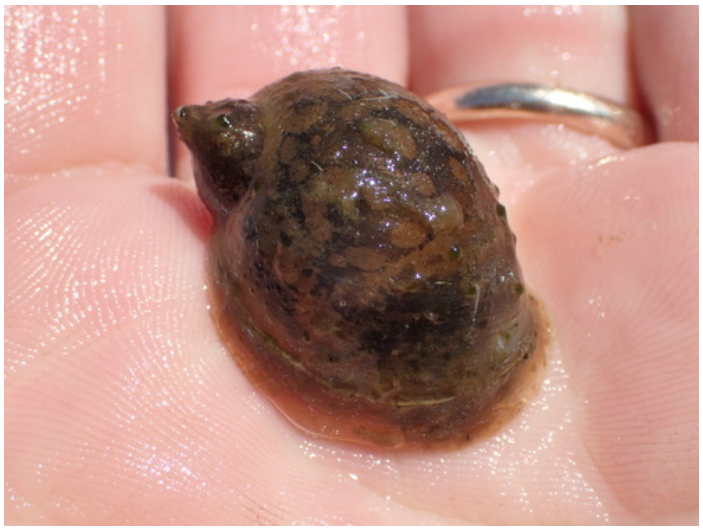

Figure 3: *Radix auricularia* at Stormy Lake, July 12, 2016 (KNWR:Ento:11102).

The first of these specimens became the founder of a new BIN (BOLD:ADE9904); the second specimen's sequence has not yet been run through the BIN algorithm, but the two specimens had a p-dist similarity of 99.34%. The next closest sequences in BINs BOLD:ACS5563 and BOLD:ACS5564 were about 98% similar to ours[3]. These sequences from the Kuril Islands and Kamchatka had been obtained by Bolotov et al. (2014). In contrast, specimens from Ontario fell within BIN BOLD:AAD6712 from Europe. This observed pattern suggests that, while *R. auricularia* was introduced to eastern North America, the Alaskan specimens may be Beringian relicts, contrary to what I had previously thought.

Leptobunus borealis

For some time I had believed that specimens of *Leptobunus* (Opiliones: Phalangiidae) from the Mystery Hills, Kenai Peninsula, Alaska represented a new species of *Leptobunus* distinct from *Leptobunus borealis* Banks, 1899. Examination of more Alaska specimens borrowed from the University of Alaska Museum and the Harvard University Museum of Comparative Zoology led me to doubt this. A DNA barcode from one of our specimens (KNWR:Ento:10851) via LifeScanner was 98.9–99.84% similar to specimens of *L. borealis* from Kasatochi Island and Adak Island in the Aleutians, further indicating that that the Mystery Hills *Leptobunus* is *L. borealis*.

Microbisium sp. BOLD:ACI8632

In 2013 I had collected a series of pseudoscorpions from a hummock in Headquarters Lake wetland, Sol-

[3]See BOLD TaxonID Trees at doi:10.7299/X74B31H7 and doi:10.7299/X7833S64.

dotna, Alaska, a black spruce muskeg. Collection data: USA, Alaska, Soldotna, Headquarters Lake, 60.4652°N, 151.0656°W ± 6 m, 8.Aug.2013, ML Bowser and S Bailey, berlese sample of moss, duff, and roots from black spruce muskeg (KNWR:Ento:10324, KNWR:Ento:11206).

Figure 4: *Microbisium* specimen (KNWR:Ento:10324).

I had attempted to identify them by using available keys, by posting a photo to BugGuide.Net, and by asking experts if I could loan out a specimen, but I initially failed. Recently, I submitted immature specimens from the series for COI sequencing via LifeScanner. These were identified as *Microbisium* (Pseudoscorpiones: Neobisiidae), a genus not previously reported from Alaska. The length of the chelae of the adult specimen was about 0.79 mm, making this specimen *Microbisium brunneum* (Hagen, 1868) based on the key of Buddle (2010) and conclusions of Nelson (1984).

The COI sequence from the Kenai specimen was also quite similar (99.38%) to specimens identified on BOLD only as *Microbisium* from Germany[4], so I compared my specimen with the key of Christophoryová et al. (2011), in which this specimen keyed to *Microbisium brevifemoratum* (Ellingsen, 1903).

Both *M. brunneum* and *M. brevifemoratum* are obligates of bog-like habitats (Nelson, 1984; Glime, 2013).

The generally parthenogenetic genus *Microbisium* has been problematic both in the Palearctic (see Schawaller, 1989; Dashdamirov and Golovatch, 2005) and the Nearctic (Nelson, 1984). From Canada there are currently 14 BINs on BOLD identified as genus *Microbisium* in contrast with the two species known from Canada.

Rabdophaga cf. *saliciscornu*

At the outlet of Nordic Lake I found horn-like galls (Figure 5) on *Salix pulchra* Cham. (Malpighiales: Salicaceae) that were identical in form to those caused by *Rabdophaga saliciscornu* (Osten Sacken, 1878) on *Salix humilis* Marshall in Illinois (Gagné, 1989) and by *Rabdophaga repenticornua* Bland, 2001 on *Salix repens* L. in Scotland (Bland, 2001). Collection data: USA: Alaska, Soldotna, outlet stream of Nordic Lake, 60.4444°N, 151.0818°W ± 200 m, 21.Dec.2016, ML Bowser, in horn-like bud gall on *Salix pulchra* (KNWR:Ento:11183, KNWR:Ento:11184).

Figure 5: Horn-like galls on *Salix pulchra* caused by *Rabdophaga* cf. *saliciscornu* (KNWR:Ento:11183 and KNWR:Ento:11184).

The closest match on BOLD for the single specimen I submitted was 88.23% similar. The sequence has not been assigned to a BIN.

Cecidomyiidae sp. BOLD:ADF0280 on *Sambucus racemosa*

On October 26, 2016 I collected tiny Diptera larvae from within a lateral bud gall of *Sambucus racemosa* L. (Dipsacales: Adoxaceae). Collection data: USA: Alaska, Soldotna, Ski Hill Road, floatplane dock access road, 60.4631°N, 151.0782°W ± 15 m, 26.Oct.2016, ML Bowser (KNWR:Ento:11166).

The closest match on BOLD was only 88.44% similar and was identified only as Cecidomyiidae. My specimen founded a new BIN, BOLD:ADF0280.

A search through Gagné (1989), Gagné (2010), and Ellis (2017) yielded ten species of cecidomyiids from genus *Sambucus*: *Arnoldiola sambuci* (Kieffer, 1901); *Asphondylia sambuci* Felt, 1908; *Contarinia sambuci* (Kaltenbach, 1873); *Contarinia sambucifoliae* Felt, 1907; *Lasioptera koreana* Kovalev, 1967; *Neolasioptera pierrei* Gagné, 1972; *Placochela nigripes* (Löw, 1877); *Schizomyia umbellicola* (Osten Sacken, 1878); *Trotteria ligustri* Barnes, 1954; and *Youngomyia podophyltae* (Felt, 1907). Of these cecidomyiids, only *Placochela nigripes* is represented on BOLD (BOLD:ACD8760). I have so far failed to find more biological information about *Contarinia sambucifoliae*, but, of the rest, only *Asphondylia sambuci* makes lateral bud galls on *Sambucus* similar to what I had found.

[4]BOLD TaxonID Tree: doi:10.7299/X73B609Z

Cecidomyiidae sp. BOLD:ADF0805 on *Alnus viridis*

Figure 6: Two minute cecidomyiid larvae in winter bud of *Alnus viridis*.

On October 31, 2016 I collected minute Diptera larvae from a winter bud of *Alnus viridis* (Chaix.) D.C. (Fagales: Betulaceae, Figure 6). This specimen became the basis for a new BIN (BOLD:ADF0805). The closest available match, a specimen identified only as Cecidomyiidae from Glacier National Park, British Columbia (SSGLA6706-15) had a COI sequence that was 96.11% similar.

This may have been *Dasineura serrulatae* (Osten Sacken, 1862), which galls buds of *Alnus* species in eastern North America (Osten Sacken, 1862; Gagné, 1989), including *Alnus viridis* (listed by Gagné, 1989, as *Alnus crispa*). I found no other cecidomyiid species documented from bud galls of alders in Gagné (1989), Gagné (2010), or Ellis (2017).

Chirosia similata on *Dryopteris expansa*

For years these fly-induced galls (Figure 7) on fronds of *Dryopteris expansa* (C. Presl) Fraser-Jenk. & Jermy (Polypodiales: Dryopteridaceae) have been a conspicuous feature of the forest around the KNWR headquarters building. Several times I have tried and failed to rear out these anthomyiids. I expected that these might be *Chirosia* because all larvae of this genus feed on fronds of ferns while no other anthomyiids eat ferns (Griffiths, 2004). The observed galls looked very much like those caused by *Chirosia betuleti* (Ringdahl, 1935) on various ferns.

Figure 7: Terminal gall on frond of *Dryopteris expansa*.

Figure 8: Two *Chirosia* larvae in terminal leaf gall on *D. expansa*.

On June 10, 2016 I collect a larva from one of these galls (Figure 8) into a LifeScanner vial for sequencing. Collection data: USA: Alaska, Soldotna, Ski Hill Road, near Keen Eye Trail, 60.466°N, 151.072°W ± 20 m, 10.June.2016, ML Bowser (KNWR:Ento:11152).

We obtained only a short, 265 bp sequence, but this had 99.61–100% similarity (p-dist) with other specimens identified as *Chirosia similata* (Tiensuu, 1939) (Diptera: Anthomyiidae) on BOLD. Although this holarctic species was reported from as close as British Columbia by Griffiths (2004), I found no previous record from Alaska. *Chirosia betuleti* is known from Alaska (Griffiths, 2004), so I checked

available records on BOLD. Both *C. betuleti* and *C. similata* are represented by sequenced specimens from the Palearctic and Nearctic, forming two distinct BINs: BOLD:ACG4337 for *C. betuleti* and BOLD:ACG3973 for *C. similata*. For both of these BINs, within-BIN distance is less than 2% while the distance between members of the two BINs is a little more than 5%.

The biology of *C. similata* was previously unknown (Griffiths, 2004; Pitkin et al., 2017), but now it appears to be similar to that of *C. betuleti*.

Mompha sp. BOLD:AAY9167 from *Chamerion angustifolium*

On June 16, 2017 I collected a larva from a stem gall of *Chamerion angustifolium* (Myrtales: Onagraceae). Collection data: USA: Alaska, Soldotna, Ski Hill Road, Cheechako Trail, 60.4605°N, 151.0805°W ± 10 m, 16.June.2016, ML Bowser (KNWR:Ento:11147).

Figure 9: Larvae in dissected stem gall of *Chamerion angustifolium* (KNWR:Ento:11147).

The larva was identified as *Mompha* (Lepidoptera: Momphidae) and placed in BIN BOLD:AAY9167. This BIN is widely distributed across northern North America based on records in BOLD, but it is not presently associated with a Linnaean species name. Members of this BIN likely belong in the *Mompha divisella* group, all which form galls on the closely-related genera *Chamerion* and *Epilobium* (Koster and Sinev, 1996). Among sequence data available on BOLD, BIN BOLD:AAY9167 falls within a clade[5] including *Mompha divisella* Herrich-Schäffer, 1854 and *Mompha unifasciella* (Chambers, 1876), both stem gallers of *Chamerion* or *Epilobium* (Braun, 1921; Koster and Sinev, 1996). *Mompha unifasciella* is currently represented on BOLD by three BINs and 58 public records, none of them Alaskan, but Ferris et al. (2012) did report this species from Alaska.

[5]BOLD TaxonID Tree: doi:10.7299/X7CV4HWP

Phycitodes mucidellus from *Senecio psuedoarnica*

On August 2, 2016 while out on a family outing I found a larva in a capitulum of *Senecio psuedoarnica* Lessing (Asterales: Asteraceae). Collection data: USA: Alaska, Kenai Peninsula, Homer, Bishop's Beach, 59.637°N, 151.539°W ± 20 m, 2.Aug.2017, ML Bowser (KNWR:Ento:11160).

This species was identified by the LifeScanner app as *Phycitodes reliquellum* (Dyar, 1904) (Lepidoptera: Pyralidae). There is some uncertainty about the identity of this specimen based on its DNA barcode because there appears to be no gap between sequences from *P. reliquellum* and *Phycitodes mucidellus* (Ragonot, 1887), (see BIN BOLD:AAA7815). These two species have been variously synonymized or considered to be distinct species, but Pohl et al. (2015) considered that western records of *P. reliquellum* referred to *P. mucidella*.

This genus appears to be a new record for Alaska as there are Alaska records neither in Arctos nor in Ferris et al. (2012). Species of *Phycitodes* are known to feed on Asteraceae (see Robinson et al., 2017), but *Senecio psuedoarnica* appears to be a new host record for this genus.

Endothenia hebesana

On April 12, 2016 I collected a larva in a seed pod of *Iris setosa* Pall. ex Link (Asparagales: Iridaceae) that was identified by the LifeScanner app as *Endothenia hebesana* (Walker, 1863) (Lepidoptera: Tortricidae). Collection data: USA: Alaska, Kenai Peninsula, Nordic Lake, 60.4459°N, 151.082°W, ± 40 m, 12.Apr.2016, ML Bowser (KNWR:Ento:10861). This appears to be a new record for Alaska. The genus *Iris* is known to be a host of *E. hebesana* (Robinson et al., 2017).

Torymidae sp. BOLD:ACR4259

On November 16, 2016 I collected a larva from horn-like galls on *Salix pulchra* presumably caused by *Rabdophaga* cf. *saliciscornu* as described above. Collection data: USA: Alaska, Soldotna, outlet stream of Nordic Lake, 60.4451°N, 151.0818°W ± 15 m, 16.Nov.2016, ML Bowser (KNWR:Ento:11173).

This specimen's COI sequence identified it as a torymid in BIN BOLD:ACR4259, which has also been collected in the

Northwest Territories. This wasp is likely a parasitoid of *R.* cf. *saliciscornu*.

Discussion

In listing the pros and cons from my experience with LifeScanner, I will give the bad news first.

Cons

By far the greatest problem with the LifeScanner workflow was when sequences and associated data were mixed up. In one case two sequences were swapped. These had been collected at different times and places, with the vial barcodes recorded at the time of collection, so I am confident that I had not swapped them before mailing them in for processing. There were other cases of apparent lab contamination that were not as easy to untangle.

Two specimens (MOBIL1271-16, a dytiscid larva; MOBIL1325-16, a fairy shrimp), collected at the same collecting event and submitted in the same shipment, both yielded *Sialis* (Megaloptera: Sialidae) sequences. The nearest *Sialis* record I could find geographically was UBCZ catalog number SEM-UBC MEG-0103 (`http://www.gbif.org/occurrence/813343006`), over 1,500 km away on the Petitot River near the intersection of the Yukon, Northwest Territories, and British Columbia (60°N, 122.98°W). These sequences were almost certainly contaminated or swapped with other samples submitted to the LifeScanner processing pipeline.

In another case (MOBIL1641-16) I sent in a tapeworm (Diphyllobothriidae: *Schistocephalus solidus* Müller, 1776) and received a cecidomyiid sequence, perhaps from one of many cecidomyiid specimens that I had submitted.

I also learned from talking with Elizabeth Graham (USFS, Juneau) and Garret Dubois (USFS, Anchorage, see Moan et al., 2017) that they had similar experiences with LifeScanner sequences and data getting swapped.

The other inconvenience with the LifeScanner workflow was with the user interface and management of the data. The LifeScanner app and user experience appeared to be designed for a narrow purpose of providing DNA-based identifications to the user. The app returned identifications, but there was no way to extract the actual COI sequences from the app. There was also no way for the user to update or correct erroneous specimen data other than contacting the LifeScanner team and sending them corrected data. However, the LifeScanner website now offers a web app for use with newer kits. I was not able to test this because the older LifeScanner kits that I have lack the 6-digit kit codes required by the web app.

Upon request the LifeScanner team does enable access through BOLD, where the user can view, download, and work with these data via BOLD's workbench interface. The LifeScanner team retains ownership of the records, however, so the user still cannot correct or update the records. Any updated data must be sent in by e-mail. Thankfully, the people at LifeScanner have been responsive to these requests.

Pros

We at KNWR lack any molecular lab capabilities, so we needed a complete service from DNA extraction through sequencing. The LifeScanner kits offered a far greater degree of convenience and flexibility than the 95-well plates we had submitted to the CCDB in the past, enabling us to send in small numbers of specimens at any time. Also, the CCDB had required all 95 samples in a plate be of the same order (e.g., a plate of only Diptera). There are no such restrictions with LifeScanner.

The cost for this service was competitive at $40 CAD per kit, which comes to $10 CAD per vial, a cost of $7.62 USD at the current exchange rate (February 21, 2017).

Turn-around time was generally quick. We usually had COI sequence results within about three weeks of mailing off the specimens.

I was surprised to learn that the Centre for Biodiversity Genomics (`http://biodiversitygenomics.net/`) retains the specimens submitted, at least when possible. I found this out when a high-quality image appeared on BOLD for a specimen I had sent in without a good photo (MOBIL1042-15). When I inquired about this, I received the response below from Sujeevan Ratnasingham, principle investigator of the LifeScanner project.

> LifeScanner was developed to provide citizen scientists access to DNA barcoding technology and the massive barcode library constructed by the research community. In exchange, the research community would gain novel occurrence points for previously barcoded species. However, as the project progressed, we discovered that some citizen scientists and professional scientists were submitting completely new species to the library. In recognition of the value of such contributions we moved to preserve the samples representing novel species, where possible, and add information like a high quality images.

I had assumed that specimens submitted to LifeScanner were ground up; I was pleased to learn that I had been wrong.

The LifeScanner workflow performed well even for fragmentary and degraded material such as the two specimens that had been stomach contents listed in our methods section.

Conclusions

Overall, these LifeScanner kits offered a far greater degree of convenience and flexibility than in my previous experience sending in 95-well plates to CCDB, enabling us to send in small numbers of assorted specimens at any time at a reasonable cost. These easy-to-use kits are just so handy to have around that I have become accustomed to this ability to learn from DNA barcodes and I am more than willing to deal with some of the problems described above in order to get those sequences.

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Highlights of the 10th annual meeting of the Alaska Entomological Society

by Kathryn Daly[1]

Figure 1: Members present at the end of the meeting. Back row, from left: Michael Baldwin, Kathryn Daly, Adam Haberski, Renee Nowicki, Steve Swenson, Jason Moan, Garret Dubois, Steven Burr and Alexandria Wenninger. Front row, from left: Logan Mullen, Isaac Davis, Derek Sikes, Matt Bowser and Roger Burnside.

The tenth annual meeting of the Alaska Entomological Society took place at the Alaska Department of Natural Resources building in Fairbanks on Saturday, February 4th, 2017. We are grateful to Steven Burr of the Forest Health Protection Service for offering us the use of this space. Every year, our annual meetings allow us to exchange information about statewide monitoring efforts, discoveries, and updates on long-running projects and events of potential interest to Alaskan entomologists. In the three years that I've attended, I have always left the meetings awed by the vast range of topics which our membership is involved in. I found this year to be no exception, and thoroughly enjoyed learning about the diverse research that has occurred since our last meeting. It was my favorite meeting yet because of the musical performances of the first and last presentations; for those who were unable to attend, audio recordings and PDFs of all presentations can be found on our website (`http://www.akentsoc.org/`).

Presentations

Matt Bowser's opening presentation, "Willow Rose Cecids via LifeScanner," left us amazed both with his continued progress in documenting the immense diversity of Cecidomyiidae (gall Midges) using COI sequences obtained from LifeScanner kits, and with his talented musicianship of sharing the research as a song while accompanying himself on guitar! Thank you very much for such an enjoyable start to our meeting, Matt!

Jacque Shade's presentation on the 2016 field season of the Alaska Cooperative Agricultural Pest Survey shared the positive news that no Asian Gypsy moths, exotic wood boring bark beetles, or exotic mollusks were found during their intensive survey work across the central and southeastern regions. In the coming 2017 season, the CAPS project will also survey nurseries between Fairbanks and Anchorage for invasive pests.

Next, I shared an update on the Kenelm W. Philip Lepidoptera Collection. In 2016, I had the opportunity to teach 17 people (15 students & 2 technicians) to assist in curating both papered and pinned specimens. As of March 6th, 2017, a total of 24,296 specimens have now been associated with a barcode linking the specimen to its digitized record in ARCTOS (`http://arctos.database.museum`), representing 21% of the Beringian material within Dr. Philip's collection.

Logan Mullen presented a summary of his phylogenetic studies and subsequent revision of the Rove Beetle genus *Phlaeopterus*. He described the distributions of these snowfield-loving beetles, which are under threat of extinction due to climatic warming. Logan's meticulous analyses of both COI data and morphology has resolved multiple species problems which existed within the genus.

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Steven Burr shared an overview of the 2016 Forest Health Protection's pest mapping efforts, beginning with the Northern Spruce Engraver beetle, *Ips perturbatus*. He described efforts to mitigate damage caused by leaf mining sawflies (Tenthredinidae) using an ichneumonid wasp *Lathrolestes thomsoni*. Birch Leaf Roller moths have caused substantial damage to Interior Alaska, as has the Eastern Spruce Budworm, *Choristoneura fumiferana* and the Willow Leafblotch Miner, *Micurapteryx salicifoliella*. Steven also shared his research on leaf gas exchange and water relationships of trees damaged by the Aspen Leafminer. He shared positive news that as of yet, there is no evidence that the Brown Marmorated Stink Bug (*Halyomorpha halys*), a potential agricultural pest, has established within Alaska.

Jason Moan's presentation on the 2016 Forest Insect Impacts in Southcentral Alaska was an excellent overview of the Alaska Division of Forestry's monitoring efforts. Species of interest included the spruce beetle, speckled green fruitworm moth, and the spruce aphid. Insect prevalence was monitored through aerial surveys and ground traps, along with collaborative reports of pest appearances from communities off the road system.

Derek Sikes was the last to present, sharing an overview of the 2016 NPS Centennial Bioblitzes from Alaska's National Parks, where arthropods were collected in Anaktuvuk Pass (within Gates of the Arctic Nat'l Park), Serpentine Hot Springs (in Bering Land Bridge Nat'l Preserve) and Denali National Park. Bioblitzes offer the chance for community members, including children, to participate in the documentation of Alaska's diversity. The Serpentine Hot Springs bioblitz was a collaborative effort between the National Park Service and children from the Seward Peninsula communities of Wales, Shishmaref, and Nome. A highlight of this trip was the discovery of a new species of *Boreus* sp. found by a 13 year old, a momentous occasion which was captured in a song written and performed by Heidi Kristenson, of the Fairbanks NPS. Derek played a recording of this catchy tune to end his presentation, the conclusion of our general meeting.

Slides and audio for these presentations are available on our website at `http://www.akentsoc.org/archives/1128`.

Business Meeting Highlights

Our business meeting began with a summary from our Treasurer, Roger Burnside, who successfully motioned for a resolution to make the membership dues payment cutoff at the end of the calendar year. If the cutoff is missed, we will give a grace period until March 1, after which the member is dropped from the roster if dues are not received. Our Secretary and Treasurer will set a dues reminder prior to the calendar year deadline, and will send a follow up reminder before the March 1 cutoff.

To continue our Alaska Science Fair awards for children who create an entomology-focused project, this year Alaska Entomological Society members will judge entomological entries in the Juneau, Anchorage, and Fairbanks fairs. Our 2017 editorial board now consists of Roger Burnside, Garrett Dubois, Matt Bowser, Jason Moan and Derek Sikes.

Matt Bowser successfully motioned that we apply digital object identifiers (DOI's) to *AKES Newsletter* articles starting in 2017 through Arctos if specimens are cited within the article. This will allow for a persistent link to our publications and allow them to be directly linked to the specimens as a media item in specimen records.

To conclude our meeting, 2017 officers were elected: Kathryn Daly (President), Garrett Dubois (Vice President), Adam Haberski (Secretary), Roger Burnside (Treasurer).

It is my honor to serve the Society as President for the next year, and I look forward to seeing you at the next annual meeting!

Sincerely,
Kathryn Daly