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

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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

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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.

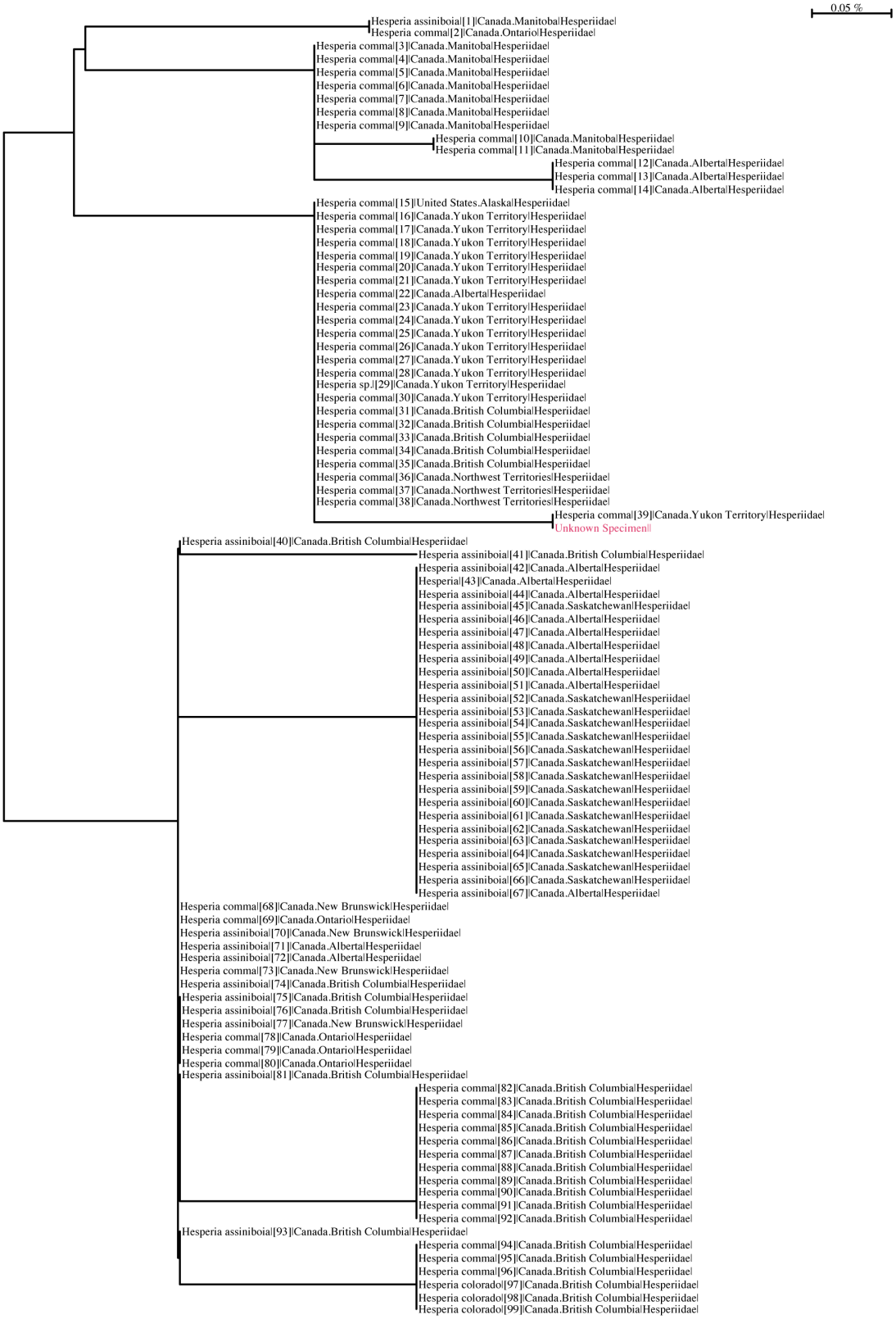

Figure 1: BOLD neighbor-joining tree for tree-based identification of KWP:Ento:36504 (indicated by purple "Unknown Specimen" in tree), *Hesperia comma*. Prepared using BOLD's full COI database, limited to sequences >200 base pairs in length, which includes records without species designation, using a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

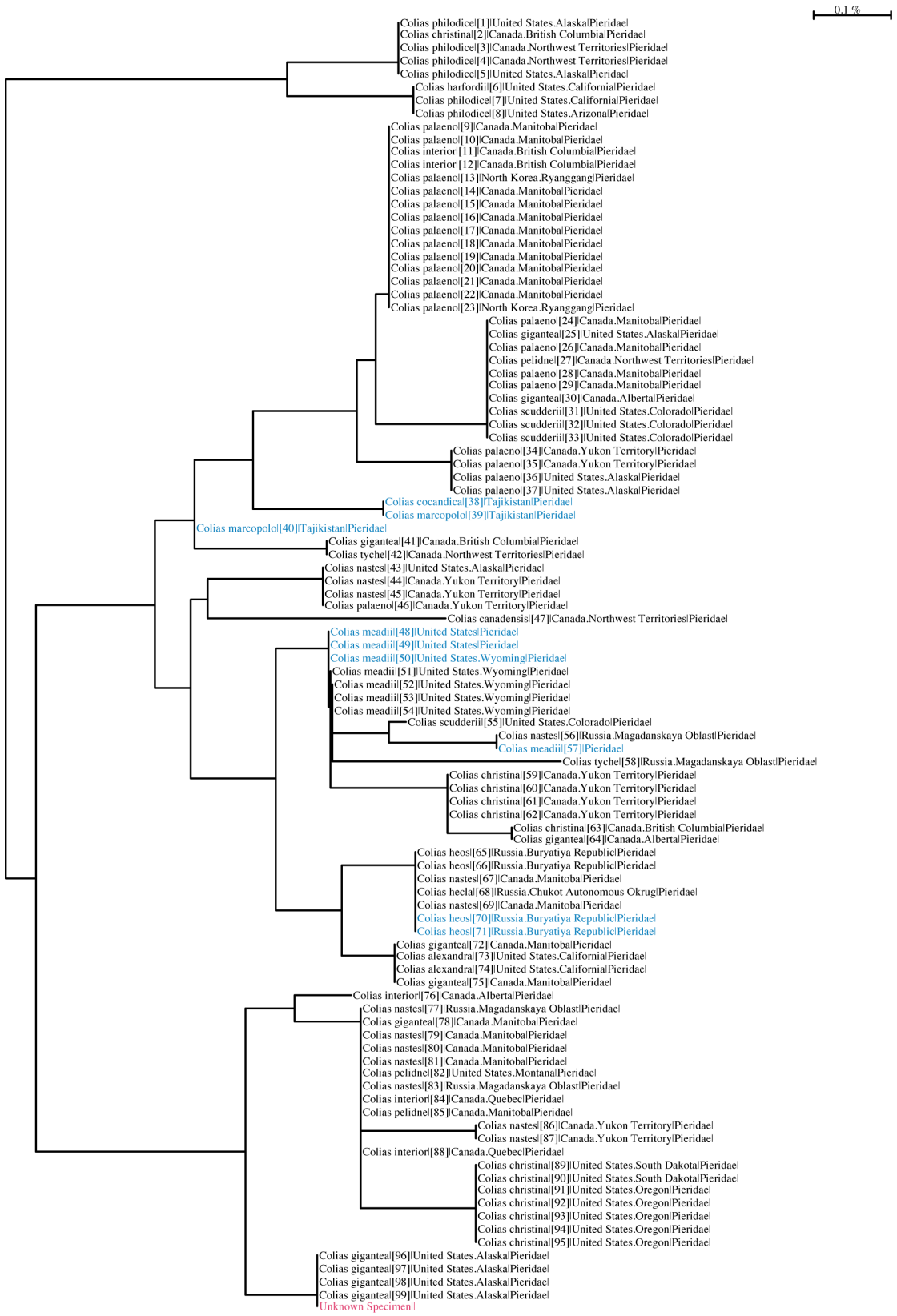

Figure 2: BOLD neighbor-joining tree for tree-based identification of UAM:Ento:19009 (indicated by purple "Unknown Specimen" in tree), *Colias gigantea*. Prepared using BOLD's full COI database, limited to sequences >200 base pairs in length, which includes records without species designation, using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

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