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

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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

[1]US Fish & Wildlife Service, Kenai National Wildlife Refuge, Soldotna, Alaska, `Matt_Bowser@fws.gov`

[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**

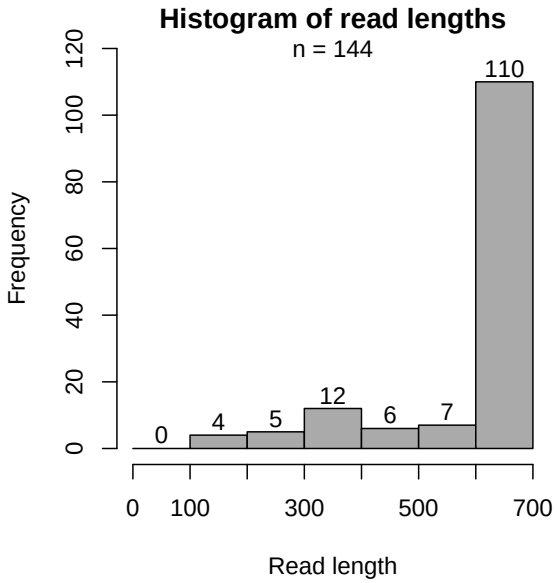

Figure 2: Histogram of read lengths of COI sequences from 144 sequences obtained via LifeScanner.

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).

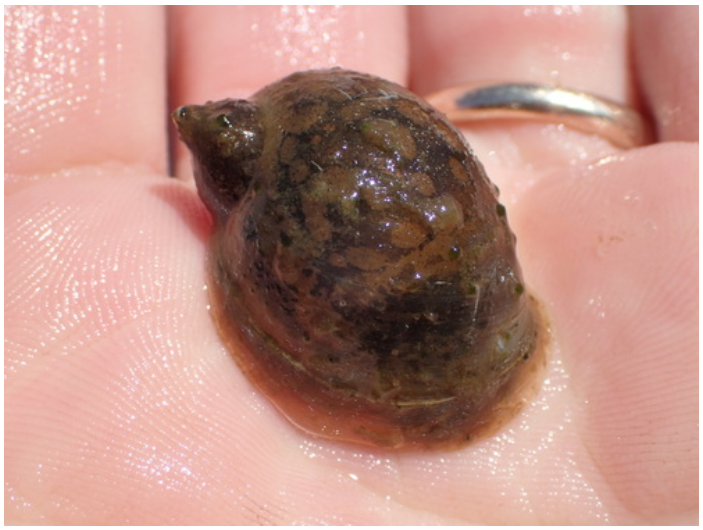

Figure 3: *Radix auricularia* at Stormy Lake, July 12, 2016 (KNWR:Ento:11102).

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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References

Baxter, R. 1987. Mollusks of Alaska: a listing of all mollusks, freshwater, terrestrial, and marine reported from the State of Alaska, with locations of the species types, maximum sizes and marine depths inhabited. Shells and Sea Life, Bayside, California.

Bland, K. P. 2001. *Rabdophaga repenticornua* sp. nov. (Diptera: Cecidomyiidae) galling *Salix repens* in Scotland. Entomologist's Gazette **52**:195–198.

Blaxter, M., J. Mann, T. Chapman, F. Thomas, C. Whitton, R. Floyd, and E. Abebe. 2005. Defining operational taxonomic units using DNA barcode data. Philosophical Transactions of the Royal Society B **360**:1935–1943. doi:10.1098/rstb.2005.1725.

Bolotov, I., Y. Bespalaya, O. Aksenova, A. Aksenov, N. Bolotov, M. Gofarov, A. Kondakov, I. Paltser, and I. Vikhrev. 2014. A taxonomic revision of two local endemic *Radix* spp. (Gastropoda: Lymnaeidae) from Khodutka geothermal area, Kamchatka, Russian Far East. Zootaxa **3869**:585–593. doi:10.11646/zootaxa.3869.5.9.

Bowser, M., 2017. The Kenai Peninsula's first exotic freshwater snail. Pp. 3–4 *in* Refuge Notebook, volume 19. USFWS Kenai National Wildlife Refuge, Soldotna, Alaska. URL https://www.fws.gov/uploadedFiles/Region_7/NWRS/Zone_2/Kenai/Sections/What_We_Do/In_The_Community/Refuge_Notebooks/2017_Articles/Refuge_Notebook_v19_n2.pdf.

Bowser, M., J. Morton, J. Hanson, D. Magness, and M. Okuly. 2017. Arthropod and oligochaete assemblages from grasslands of the southern Kenai Peninsula, Alaska. Biodiversity Data Journal **5**:e10792. doi:10.3897/BDJ.5.e10792.

Braun, A. F. 1921. Two weeks collecting in Glacier National Park. Proceedings of the Academy of Natural Sciences of Philadelphia **73**:1–23. URL http://www.jstor.org/stable/4063797.

Buddle, C. M. 2010. Photographic key to the Pseudoscorpions of Canada and the adjacent USA. Canadian Journal of Arthropod Identification doi:10.3752/cjai.2010.10, URL http://www.biology.ualberta.ca/bsc/ejournal/b_10/b_10.html.

Christophoryová, J., F. Šťáhlavský, and P. Fedor. 2011. An updated identification key to the pseudoscorpions (Arachnida: Pseudoscorpiones) of the Czech Republic and Slovakia. Zootaxa **2876**:35–48.

Clarke, A. H. 1981. The Freshwater Molluscs of Canada. National Museum of Natural Sciences, National Museums of Canada, Ottawa, Ontario, Canada.

Collet, D. M. 2002. Willows of Southcentral Alaska. Kenai Watershed Forum, Soldotna, Alaska. URL http://web.acsalaska.net/~kenaiwatershed.forum/willowguide.html.

Dashdamirov, S., and S. I. Golovatch. 2005. Miscellanea chernetologica (Arachnida: Pseudoscorpiones), based on the collection of the Natural History Museum in Vienna, Part 1. Arthropoda Selecta **14**:299–301. URL http://kmkjournals.com/upload/PDF/ArthropodaSelecta/14/14_4%20299_301%20Dashdamirov,%20golovatch.pdf.

Ellis, W. N., 2017. Leafminers and plant galls of Europe. URL http://www.bladmineerders.nl.

Eskelin, L., 2016. Kenaitze youth dig into their past at Susten Archaeology Camp. Pp. 51–52 *in* Refuge Notebook, volume 18. USFWS Kenai National Wildlife Refuge, Soldotna, Alaska. URL https://www.fws.gov/uploadedFiles/Region_7/NWRS/Zone_2/Kenai/Sections/What_We_Do/In_The_Community/Refuge_Notebooks/2016_Articles/Refuge_Notebook_v18_n26.pdf.

Ferris, C. D., J. J. Kruse, J. D. Lafontaine, K. W. Philip, B. C. Schmidt, and D. S. Sikes. 2012. A checklist of the moths of Alaska. Zootaxa **3571**:1–25. URL http://www.mapress.com/zootaxa/2012/f/zt03571p025.pdf.

Gagné, R. J. 1989. The Plant-Feeding Gall Midges of North America. Cornell University Press, Ithaca, New York.

Gagné, R. J. 2010. Update for a catalog of the Cecidomyiidae (Diptera) of the world. Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Washington, DC. URL https://www.ars.usda.gov/ARSUserFiles/12754100/Gagne_2010_World_Catalog_Cecidomyiidae.pdf.

Glime, J. M., 2013. Arthropods: Harvestmen and pseudoscorpions. Chapter 8 *in* J. M. Glime, editor. Bryophyte Ecology. Volume 2. Bryological Interaction. Michigan Technological University, Houghton, Michigan. URL http://www.bryoecol.mtu.edu/chapters_2011/8Arthropods_Harvestmen.pdf.

Griffiths, G. C. D., 2004. Anthomyiidae, Part 2, No. 15. Pp. 2485–2635 *in* G. C. D. Griffiths, editor. Flies of the Nearctic Region, volume 8. Schweizerbart, Stuttgart.

Kenai National Wildlife Refuge biology staff. 2017. Kenai National Wildlife Refuge Species List, version 2017-01-23. Kenai National Wildlife Refuge, Soldotna, Alaska. URL https://www.fws.gov/uploadedFiles/Kenai_NWR_Bio_2017-01-23.pdf.

Koster, J. C., and S. Y. Sinev. 1996. A revision of the *divisella* group of the genus *Mompha*, with the description of *Mompha confusella* spec. nov. (Lepidoptera: Momphidae). Entomologische Berichten **56**:137–148.

Lawrence, A. P., and M. A. Bowers. 2002. A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biology and Biochemistry **34**:549–552. doi:10.1016/s0038-0717(01)00211-5.

Moan, J., G. Dubois, and S. Swenson. 2017. *Orthosia hibisci* (Guenée), the speckled green fruitworm: confirmed causing extensive hardwood defoliation in Southcentral and Western Alaska. Newsletter of the Alaska Entomological Society **10**:4–8. doi:10.7299/X7P55NPX, URL http://www.akentsoc.org/doc/AKES_newsletter_2017_n1_a03.pdf.

Nelson, S. 1984. The genus *Microbisium* in North and Central America (Pseudoscorpionida, Neobisiidae). The Journal of Arachnology **12**:341–350. URL http://www.jstor.org/stable/3705364.

Osten Sacken, C. R., 1862. V. On the North American Cecidomyidae. Pp. 173–205 *in* H. Loew, editor. Monographs of the Diptera of North America. Part I. Number 141 in Smithsonian Miscellaneous Collections 6, Smithsonian Institution, Washington, D.C.

Pitkin, B., W. Ellis, C. Plant, and R. Edmunds, 2017. The leaf and stem mines of British flies and other insects. URL http://www.ukflymines.co.uk/.

Pohl, G. R., R. A. Cannings, J. F. Landry, D. G. Holden, and G. G. E. Scudder. 2015. Checklist of the Lepidoptera of British Columbia, Canada. Number 3 in Entomological Society of British Columbia Occasional Paper, Lulu Publishing Services. URL http://journal.entsocbc.ca/index.php/journal/article/view/917.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes **7**:355–364. doi:10.1111/j.1471-8286.2007.01678.x.

Ratnasingham, S., and P. D. N. Hebert. 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE **8**:e66213. doi:10.1371/journal.pone.0066213, URL http://dx.doi.org/10.1371%2Fjournal.pone.0066213.

Robinson, G. S., P. R. Ackery, I. J. Kitching, G. W. Beccaloni, and L. M. Hernández, 2017. HOSTS - A Database of the World's Lepidopteran Hostplants. URL http://www.nhm.ac.uk/hosts.

Schawaller, W. 1989. Pseudoskorpione aus der Sowjetunion, Teil 3 (Arachnida: Pseudoscorpiones). Stuttgarter Beiträge zur Naturkunde, Serie A (Biologie) **440**:1–30. URL http://biostor.org/reference/95811.

Sikes, D. S., M. Bowser, J. M. Morton, C. Bickford, S. Meierotto, and K. Hildebrandt. 2017. Building a DNA barcode library of Alaska's nonmarine arthropods. Genome **60**:248–259. doi:10.1139/gen-2015-0203.