

¹ Metagenomic Barcoding of Pollen Loads Offers Insights on the
² Foraging Patterns of Queen Bumble Bees

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⁴ **Abstract**

⁵ .
⁶ 1) DNA Barcoding has been remarkably successful in nearly all kingdoms of life and has allowed for the
⁷ rapid analysis of ecological assemblies. Successful DNA barcoding in the plant kingdom has been more
⁸ difficult than other kingdoms. Due to this understanding plants in ecological contexts and understandings
⁹ of their synecology in some instances may begin to lag behind other kingdoms. 2) Here we utilize hyb-seq,
¹⁰ museum studies, and species distribution modelling, to detect the plant species present in pollen loads
¹¹ collected from Queen Bumble Bees. 3) By utilizing Species distribution modelling we allow for one
¹² to process hyb-seq data, create user specified sequence databases which may use MORE ACCURATE
¹³ alignment algorithms on personal computers over realistic time periods. 4) We show that hyb-seq using
¹⁴ the Angiosperms 353 probes, which are currently being used in the largest ever plant systematic endeavor,
¹⁵ offers significant promise to metagenomic approaches in real world scenarios. 5) We conclude that these
¹⁶ probes offer promise for the identification of plant tissue in both single sample, and metasample contexts.

¹⁷ **1 | INTRODUCTION**

¹⁸ The inability to reliably identify plants to the level of species often leaves our understanding of ecosystem
¹⁹ function and interactions wanting. Current methods to ameliorate this situation include: ignoring these
²⁰ ecologically relevant levels of detail, revisiting plots as diagnostic material becomes temporally available,
²¹ assistance from taxonomic specialists, or the use of barcoding or other molecular techniques. These approaches
²² are untenable in light of the benefits offered by: species in several morphologically difficult genera which
²³ serve as bioindicators, preferred partners in ecological interactions, as well as an increasing lack of taxonomic
²⁴ experts (Hebert *et al.* (2003)). Many genera, especially with the formalized advent of integrative taxonomy,

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25 have species which are well defined based upon ecological and behavioral rather than morphological properties,
26 the identification of these taxa in degraded areas or without their mutualistic partners is fraught with difficulty.
27 Hindering an understanding of the breadth of habitat which some species occupy, and the interactions they
28 have with other species.

29 The identification of many plant species to terminal taxon is an essential component of nearly all land
30 management programs, where many species in the same genus (e.g. Sagebrush - *Artemisia* L., Willows -
31 *Salix* L., and Sedges - *Carex* L.) serve as bioindicators (respectively for ‘rangelands’, streams, and wetlands),
32 as well as in academic research (Gage & Cooper (2013), AIM). This endeavour is often mired by lack of
33 diagnostic characters (e.g. flowers, fruits, roots or combinations thereof), and increasingly the description of
34 cryptic species (Janzen *et al.* (2017), Oliver *et al.* (2009)). Solutions to this problem are wanting, certain
35 programmes have relied increasingly upon revisiting field sites to identify material using morphological or
36 chemical approaches, whereas academic research has often used high copy number plastid genes as barcodes
37 (Rosentreter *et al.* 2021, MORE MORE). However, both approaches have significant downsides, the former
38 resource intensive at the landscape scale - and often does not work, while the latter seldom works due to a
39 lack of variability in the currently available barcodes (Liu *et al.* (2014)).

40 Recently barcoding (the identification of a sample from a single organism – e.g. a piece of leaf), and
41 metabarcoding (the identification of a sample containing a mix of organisms – e.g. soil), have shown
42 considerable promise in all Kingdoms of life (Ruppert *et al.* (2019)). With plants the identification of
43 members of certain clades using barcoding has been quite successful (REF), whereas with others results
44 have been elusive (Liu *et al.* (2014), Group *et al.* (2011), Coissac *et al.* (2012)), however metabarcoding
45 incurs additional challenges to those which exist for the currently implemented barcodes (Li *et al.* (2015),
46 Kress & Erickson (2007), Group *et al.* (2009), Coissac *et al.* (2012)). Particular challenges with barcodes
47 include the utilization of high-copy number sequences are associated with their rates of divergence, gene
48 tree conflict, and hybridization (Coissac *et al.* (2016), Fazekas *et al.* (2009)). Particular challenges with the
49 utilization of high-copy number sequences are associated with their rates of divergence, gene tree conflict,
50 and hybridization (Coissac *et al.* (2016)).

51 Currently the largest plant systematic endeavor ever undertaken, the Kew Plant and Fungal Tree of Life
52 (PAFTOL), is approaching completion (Baker *et al.* (2021)). This dataset will contain Hyb-Seq data from
53 at least one species representing each genus in the plant kingdom using the popular A353 probes (Baker *et*
54 *al.* (2021)), resulting in over 14,000 represented species. These publicly available data serve to provide a
55 taxonomically comprehensive backbone for plant metabarcoding. Data from the 10kP project, which seeks to
56 develop reference genomes from a phylogenetically diverse suite of plants will contribute many more records

57 upon it's intended completion, now slated to be by 2030, similar projects which seek to sequence high amounts
58 of genomes in regions e.g. the 'Darwin Tree of Life' are being undertaken which will contribute data applicable
59 to enormous spatial domains (Cheng *et al.* (2018), Life Project Consortium *et al.* (2022), Lewin *et al.* (2022)).

60 These data will promote the ability to apply metabarcoding to resolve a diversity of questions relevant to
61 theoretical and applied ecology (cite). However, the application of metabarcoding still face challenges relating
62 to the enormity of the genomic datasets and the computational power required to process sequence data.
63 Herein we have resolved major components of the problems of identifying plant material without diagnostic
64 morphological character states using the Angiosperms353 (A353) Hyb-Seq probes (Johnson *et al.* (2019)),
65 and custom species sequence databases derived via species distribution modelling, and temporal filtering.

66 To increase the quality of metabarcoding results in plants, we suggest reducing the number of possible plant
67 species candidates by generating user selected sequence databases relevant to the study region and its
68 ecological characteristics (CITE !?). To achieve this goal, we first create a list of candidate species using digital
69 collections gleaned from herbaria, survey work, and citizen science (e.g. iNaturalist), from a region exceeding
70 the study area. To these candidate species, modelling approaches - such as logistic regression, may be used
71 to identify taxa which warrant further exploration e.g. modelling to determine their possibility of presence
72 in metabarcoding samples. We then use species distribution models to create potential distribution maps
73 for the candidate species to limit the impact of spatial and taxonomic biases in the species list and account
74 for spatial variations in niche availability throughout the study area. Species distribution models (SDM's)
75 examine the ecological conditions associated with known occurrence of a species to identify where else in the
76 study area might suitable habitats be found. This approach has the additional benefit of greatly reducing the
77 size of a sequence database, which allows for the usage of genomic size data on personal computers. This can
78 also significantly reduce processing time, particularly as most next-generation sequence data is deposited
79 as raw-sequence reads.

80 Considerable amounts of species interactions vary along time (CaraDonna *et al.* (2021)). For the tropics,
81 contrasts in the flowering periods of many plant species, can provide an additional filter for identifying
82 material in many metagenomic samples (Janzen (1967), Newstrom *et al.* (1994)). In temperate regions,
83 pollination interactions also vary temporally (CaraDonna *et al.* (2017)), the overall shorter extent of the
84 active growing season in these systems results in the presence of few to any natural breaks in these systems
85 which subjugates the utility of these to perform as filters of post-processing results, rather than distinct
86 species assemblage for database generation. Nonetheless, we work through a process which seems applicable
87 to the tropics to utilize the temporal dimension for classifying sequencing results.

88 We apply these metagenomic and informatics approaches to determine whether the foraging record of Queen

89 Bumble Bee's is consistent across direct observations and the pollen record, an incongruity in several
90 floral visitation networks (Barker & Arceo-Gomez (2021), Zhao *et al.* (2019), Alarcón (2010)). The two
91 foraging phases of the Queen Bumble Bee life cycle is essential to 1) increase their weight before diapause, 2)
92 increase their ovary weights while establishing their recently found nests, both of these time periods represent
93 potential demographic bottlenecks in bumble bee populations (Sarro *et al.* (2022)). Bumblebees are one of
94 the only groups of insects with unequivocal quantitative evidence for numerous populations declines, while
95 simultaneously serving as the most effective pollinators in temperate montane ecosystems (Cameron & Sadd
96 (2020), Goulson *et al.* (2008), Williams (1982), Colla *et al.* (2012), Bergman *et al.* (1996), Bingham & Orthner
97 (1998)). These montane ecosystems represent some of the most ecologically resilient and resistant systems in
98 the temperate and offer unparalleled potential as refugial areas for multiple dimensions of biodiversity under
99 climate change.

100 2 | METHODS

101 Study System & Field Work

102 Observations and sample collection was conducted at The Rocky Mountain Biological Laboratory (RMBL;
103 38°57.5" N, 106°59.3" W (WGS 84), 2900 m.a.s.l.), Colorado, USA (APPENDIX 1 for site information).
104 Pollinator observations of Bombus Latreille spp. (Apidae Latreille) were conducted from June - August of 2015
105 in six study sites characterized by high-montane/subalpine Parkland vegetation communities. Observations
106 of Bombus foraging took place for one hour at each field site in three 100m transects, where all flowers were
107 also counted and placed into abundance bins. Corbiculae loads were, non-lethally, collected once from all
108 Queen individuals encountered.

109 2.1 | Spatial Analyses

110 **2.1.1 Candidate Species** We downloaded from the Botanical Information and Ecology Network 'BIEN'
111 (Maitner (2022)) all records adjacent to the field sites to develop an ecologically relevant list of vascular plant
112 species, with expected biotic pollination, which may be present at the study area . We then generated Species
113 Distribution Models (SDMs) to predict their distribution throughout the study area. These maps of potential
114 distribution served as a reference to reduce the list of species to include in the genomic sequence databases.

115 In order to minimise the number of species for which SDM's were to be generated, BIEN was queried at
116 a distance of up to 100km from our study area and all plant species records were downloaded. ***In order***
117 ***to emulate the stochasticity of botanical collecting, this dataset was bootstrap re-sampled 250***

118 *times, with 90% of samples selected, to create a testing dataset.* The median of the logistic
119 regression assessing the probability of occurrence of a species record as a function of distance from the study
120 area was used as a threshold distance, under which, to include species as candidates for distribution modelling.

121 **2.1.2 Distribution Modelling** We used all occurrence records from BIEN ($n = 23,919$) within a 50km
122 border of the Omernik level 3 ecoregion, which includes the study area (*No. 21 “Southern Rockies”*) to
123 construct the species distribution model (Omernik (1987)). These records were copied into two, initially
124 identical, sets, one for generating machine learning models (ML; Random Forest, and Boosted Regression
125 Tree’s), and the other for Generalised Linear (GLM) and Generalized Additive Models (GAM). We then
126 generated 4,029 absence points , locations where the focal taxon is anticipated missing, through a random
127 stratification of 19% of the land cover in the area and included them in (BLM CITATION - need appropriate
128 format for journal). To achieve a larger absence dataset, we generated 1,000 pseudo-absence records for each
129 taxon by randomly selecting coordinates located at least 10km away from an occurrence record. For ML
130 models, these pseudo-absences were reduced so that the ratio of presence to absence records were balanced.
131 To achieve this, we removed absence records inside of 10% of the mean sample value of the presence records;
132 the required number of absence records were then randomly sampled.

133 We used 26 environmental variables at 30m resolution to predict the potential distribution of each species,
134 six related to climate, five soil, four topographic, four related to cloud cover, with the remaining reflecting
135 assorted abiotic parameters (Wilson & Jetz (2016), Wang *et al.* (2016), Hengl *et al.* (2017), Robinson *et al.*
136 (2014)) (*APPENDIX 6*). For linear regression models these predictors underwent both *vifstep* (theta = 10,
137 max observations = 12,500) and *vifcor* (theta = 0.7, max observations = 12,500) to detect highly correlated
138 variables, and collinear features were removed leaving 16 variables (Naimi *et al.* (2014)).

139 Modelling: Random Forest and Boosted Regression Trees, were sub sampled with 30% test and two replicates
140 each before weighted ensemble based on True Skill Statistics (tss) (Naimi & Araujo (2016), @). Generalised
141 linear models (GLM) and Generalised additive models (GAM) with 30% sub sampling and three replicates
142 each were also ensembled using the tss (Naimi & Araujo (2016)). TSS was chosen as the ensemble criterion
143 as it has been shown to work across a range of species occurrences prevalences (Allouche *et al.* (2006)).
144 The results of these models were extracted on a cell-by-cell basis to a polygon feature derived from a
145 minimum-spanning tree which encompasses the study sites, and species from either ensemble with greater
146 than 50% mean habitat suitability across all cells were considered present for further purposes (Prim (1957)).
147 535 species were modelled using Generalized Linear Models and Generalized Additive Models. 534 species
148 were modelled using Random Forest and Boosted Regression Trees. To evaluate the accuracy of the species

149 distribution models, additional presence records from GBIF ($n = 61,789$), and AIM ($n = 12,730$) were used
150 as test and training sets ($n = 74,519$) for logistic regression (CITE AIM AND Ocdownload Gbif.Org (2021)).
151 Additional novel absence records were generated from the AIM dataset to create a dataset where each species
152 has balanced presence and absences. 11 or more paired presence and absence records were required for this
153 testing, resulting in 334 species being included in the logistic regression ($Mdn = 110.0$, $\bar{x} = 223.1$, max =
154 1568 record pairs used) with a 70% test split (Kuhn (2022)).

155 **2.2 | Molecular Lab Work**

156 All lab work was carried out at The Daniel F. and Ada L. Rice Plant Conservation Science Center at the
157 Chicago Botanic Garden, Glencoe, Illinois, U.S.A.

158 **2.2.1 | Reference Plant Library Generation** Using five years of observational data on *Bombus* Queen
159 Bee foraging at these studies sites, we identified the plant taxa most frequently visited by Queens across
160 all years. We sequenced the 12 most commonly visited taxa twice using samples from one site within the
161 Gunnison River Drainage and one individual from another population. In addition, for any of these 12 focal
162 species which did not have a congener pair in this filtered sample, we included a congener - or a species from
163 a closely related genus to serve as an outgroup. We also sequenced another 15 abundant taxa commonly
164 visited by *Bombus* workers, based on the aforementioned data set (*APPENDIX 4*).

165 **2.2.2 | Plant Genomic DNA Extraction** Plant genomic DNA was isolated from $\sim 1 \text{ cm}^2$ of leaf tissue
166 from silica-gel dried or herbarium material using a modified cetyltrimethylammonium (CTAB) protocol
167 (Doyle & Doyle (1987)) that included two chloroform washes. DNA was quantified using a Nanodrop 2000
168 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and Qubit fluorometer (Thermo Fisher Scientific).

169 **2.2.3 | Pollen Genomic DNA Extraction** Pollen genomic DNA was extracted from corbiculae using a
170 CTAB based protocol modified from Lahlamgiah et al. and Guertler et al. (2014, 2014). A SDS extraction
171 buffer (350 μL , 100mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS v/v., pH 7.5) was added followed
172 by vortexing to allow dissolution of corbiculae. Pollen grains were then macerated with Kontes Pellet Pestles,
173 and the tip of these washed with 130 μL of the SDS extraction buffer, samples were then incubated for 1 hour
174 at 30°C. This was followed by the addition of 10% CTAB solution (450ul, of 20 mM Tris-Cl pH. 8.0, 1.4 M
175 NaCl, 10 mM EDTA pH 7.5, 10% CTAB, 5% PVP, ~85% Deionized water) and RNase (10 uL of 10 mg/mL)
176 and samples were incubated for 40 minutes at 37°C, on heat block (Multi-Blok, Thermo Fisher Scientific,
177 Waltham Massachusetts) set to 40°C. After 20 minutes incubation, Proteinase K (15 μL of 20mg/ml) and

178 DTT (12.5 μ L of 1M in water) were added, and the samples were further incubated at 60°C for 1 hour.
179 Samples were then incubated overnight at 40°C. 500 μ L of Phenol-Chloroform-Isoamyl alcohol (25:24:1) were
180 added, vortexed, and centrifuged at 10,000 rpm for 10 minutes and the aqueous phase was pipetted to a 1.5
181 ml centrifuge tube.

182 To precipitate the DNA, chilled Isopropyl alcohol & 3 mM Sodium acetate (5:1) equivalent to 2/3 of the
183 volume of sample were added, with 1 hour of chilling at -20°C, followed by 10 minutes of centrifuging at
184 13,000 rpm. The supernatant was pipetted to a new 1.5 ml centrifuge tube, and 70% EtOH (400 μ L) were
185 added before chilling at -20°C for 20 minutes followed by centrifugation at 13,000 rpm for 10 minutes. Both
186 tubes were then washed with 75% EtOH (400 μ L), inverted, centrifuged at 13,000 rpm for 4 minutes, and the
187 solution discarded, then washed with 95% EtOH (400 μ L), inverted, centrifuged at 13,000 rpm for 4 minutes,
188 and the solution discarded. Pellets were dried at room temperature overnight before resuspension in Nuclease
189 free H₂O. Extractions were assessed using a Nanodrop 2000 (Thermo Fisher Scientific) and Qubit fluorometer
190 (Thermo Fisher Scientific). DNA extracts were then cleaned using 2:1 v./v. Sera-Mag beads (Cytiva, Little
191 Chalfont, UK) to solute following the manufacturer's protocol, eluted in 0.5x TE, and the eluent allowed to
192 reduce by half volume in ambient conditions. DNA was quantified using a Qubit fluorometer.

193 **2.2.4 | Fragmentation, Library Preparation & Target Enrichment** Library preparation was per-
194 formed using the NEBNext Ultra II FS-DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich,
195 Massachusetts, USA) using slightly modified manufacturers recommendation. Fragmentation was performed
196 at ½ volume of reagents and ¼ enzyme mix for 40 minutes at 37°C, with an input of 500 ng cleaned DNA.
197 Adapter Ligation and PCR enrichment were performed with ½ volumes, while cleanup of products was
198 performed with ½ volume of SPRI beads (Beckman Coulter, Indianapolis, Indiana, USA) and recommended
199 volumes of 80% v./v. ethanol washes. The exception was the herbarium specimens which were not fragmented
200 and only end repaired, with similar library preparation of all samples. Products were analysed on 4% agarose
201 gels, and a Qubit fluorometer. Libraries were pooled and enriched with the Angiosperms 353 probe kit V.4
202 (Arbor Biosciences myBaits Target Sequence Capture Kit) by following the manufacturer's protocol and
203 Brewer et al. 2019. Sequencing was performed using an Illumina mi-Seq with 150-bp end reads, (NUSeq Core,
204 Chicago, Illinois).

205 **2.2.5 | Computational Processes and Analyses.**

206 **2.2.5.1 | Reference Library Data Processing** Sequences were processed using Trimmomatic, which
207 removed sequence adapters, clipped the first 3 bp, discarding reads less than 36 bp, and removing reads
208 if their average PHRED score dropped beneath 20 over a window of 5 bp (Bolger & Giorgi (2014), Tange

209 (2021)). Contigs were generated using HybPiper using target files created by M353 (Johnson *et al.* (2016),
210 McLay *et al.* (2021)).

211 **2.2.5.2 | Sequence Identification** A custom Kraken2 database was created by downloading representative
212 species of each genus indicated as being present in the study area by the spatial analyses from the Sequence
213 Read Archive (SRA) NCBI (Wood *et al.* (2019)). These sequences were processed in the same manner as
214 our novel sequences before being placed into the database. The Kraken2 database was built using default
215 parameters. Kraken2 was run on sequences using default parameters (*APPENDIX 5*). Following Kraken2,
216 Bracken was used to classify sequences to terminal taxa (Lu *et al.* (2017)). Results from both Kraken2 and
217 Bracken, results were reclassified manually to identify terminal taxa. For example, when only a single species
218 of a genus was known in the study area, but our database used a representative of another taxon in the genus,
219 this species was coded as the result. The re-coding of sequences from another representative species for the
220 genus to the sole RMBL representative allowed the identification of XX & % more species.

221 **2.2.5.3 | Identification of Sequence Matching Loci** A local NCBI database was built using the same
222 processed novel and downloaded sequences (Camacho *et al.* (2009)).

223 **2.2.5.4 | Morphological Pollen identification**

224 To develop a reference library of pollen grains which may be present in corbiculae loads, an image reference
225 collection of fuchsin-jelly stained (Beattie (1971)) slides was assembled from slides previously prepared by the
226 authors (n = 21), and other researchers (n = 38) (Brosi & Briggs (2013)). Using five years of observational
227 data on *Bombus* Queen Bee foraging at these studies sites (Ogilvie unpublished), as well as the Vascular
228 Plant Checklist (Frase & Buck (2007)), an additional 62 voucher slides for species were prepared and imaged
229 at 400x (Leica DMLB, Leica MC170 HD Camera, Leica Application Suite V. 4.13.0) from non accessioned
230 herbarium collections to supplement the number of species and clades covered (Appendix 3).

231 We used Divisive Hierarchical Clustering techniques to determine which plant taxa were distinguishable via
232 light microscopy, and to develop a dichotomous key to pollen morphotypes. Ten readily discernible categorical
233 traits were collected from each specimen in the image collection. These traits were transformed using Gower
234 distances, and clustered using Divisive Hierarchical clustering techniques (Maechler *et al.* (2022)). Using
235 the cluster dendrogram, elbow plot, and heatmaps (Hennig (2020)), of these results morphological groups of
236 pollen which could not be resolved via microscopy were delineated, and a dichotomous key was prepared
237 (*APPENDIX NO.*). This key was then used to identify the pollen grains sampled from corbiculae loads to
238 morphotypes in a consistent manner. To prepare the pollen slides from corbiculae, all corbiculae loads were

239 broken apart and rolled using dissection needlepoints to increase heterogeneity of samples. *Cerca* 0.5mm² of
240 pollen was placed onto a ~4mm² fuchsin jelly cube (Beattie (1971)) atop a graticulated microscope slide,
241 with 20 transects and 20 rows (400 quadrants) (EMS, Hartfield, PA). The jelly was melted, with stirring, until
242 pollen grains were homogeneously spread across the microscope slide. Slides were sealed with Canada Balsam
243 (Rublev Colours, Willits, CA) followed by sealing with nail polish; all samples are noted in *APPENDIX 3*.
244 To identify the pollen present in corbiculae loads, light microscopy at 400x (Zeiss Axioscope A1) was used. In
245 initial sampling in three transects, each pollen grain was identified to morphotype and counted; an additional
246 two transects were scanned for morphotypes unique to that slide, if either transect contained an unique
247 morphotype than all grains in that transect were also identified and counted. Subsequent to the first round
248 of sampling, non-parametric species richness rarefaction curves (Oksanen *et al.* (2022)), and non-parametric
249 species diversity rarefaction curves were used to assess the completeness of sampling (Chao *et al.* (2014),
250 Hsieh *et al.* (2020)). Slides not approaching the asymptote of the rarefaction curve were then re-sampled,
251 and analysed iteratively for up to a total of seven transects *APPENDIX 2*.

252 2.3 | Temporal Analyses

253 To estimate the duration of dates in which plant species were flowering weibull estimates of several phenological
254 parameters all spatially modelled taxa were developed (Belitz *et al.* (2020), Pearse *et al.* (2017)). Only
255 BIEN records which occurred in the Omernik Level 4 Ecoregions within 15km of the study area ($n = 5$
256 Level 4 Ecoregions, or conditionally 6 if enough records not be found in the nearest 5), and which were from
257 herbarium records were included. To remove temporally irrelevant herbarium records, i.e. material collected
258 during times which flowering is impossible at the study area due to snow cover, we used the SnowUS dataset
259 (Iler *et al.* (2021), Tran *et al.* (2019)) from 2000-2017 was analyzed for the first three days of contiguous snow
260 absence, and the first three days of contiguous snow cover in Fall. Herbarium records after the 3rd quantile
261 for melt, and the 1st quantile for snow cover of these metrics were removed. Species with > 10 records had
262 their weibull distributions generated for the date when 10% of individuals had begun flowering, when 50%
263 were flowering, and when 90% of individuals had flowered.

264 2.4 | Floral Observations

265 3 | RESULTS

266 3.1 | Spatial Analyses

267 [Table 1 about here.]

269 The median (25.009 km) of the logistic regression assessing the probability of occurrence of a species record as
 270 a function of distance from the study area was used as a threshold distance to include species for distribution
 271 modelling. A 2-sample test for equality of proportions with continuity correction (χ^2 -squared = 13.254, df
 272 = 1, p-value = 0.000136, 95% CI 0.04-1.00) was used to test whether more of the records located in the
 273 broad ecological sites present at the field station, between the distance of the median (25.009 km) to the
 274 third quantile (ca 43.830 km) of the regression distance, where true presences at the field station. Including
 275 these records would have resulted in modelling an additional 222 species distributions of which 30 are true
 276 presences these taxa were not modelled.

277 Across the entire spatial domain of modelling all ensembled models ($n = 968$) had an accuracy of 0.84 (95%
 278 CI 0.8356 - 0.8443), kappa 0.68, p-value < 0.001, sensitivity = 0.80, specificity = 0.87, AUC = 0.92.

279 At the field site, of the 554 vascular plants with biotic pollination syndromes, the 493 ML ensembles accurately
 280 predicted the presence of 362 (65.3%), incorrectly predicted the presence of 64 (11.6%), incorrectly predicted
 281 34 true presences (6.1%) as being absent, and correctly predicted the true absence of 33 (6.0%). The balanced
 282 accuracy of the ensembled models is 0.627 (Sensitivity = 0.340, Specificity 0.914). Of the 554 vascular plants
 283 with biotic pollination syndromes, the 475 LM ensembles accurately predicted the presence of 286 (51.6%),
 284 incorrectly predicted the presence of 41 (14.3%), incorrectly predicted 93 true presences (16.8%) as being
 285 absent, and correctly predicted the true absence of 55 (9.9%). The balanced accuracy of the ensembled
 286 models is 0.664 (Sensitivity = 0.573, Specificity 0.754). Of the 554 vascular plants with biotic pollination
 287 syndromes in the flora 13 (2.3%) were in the Orchid family and 41 (7.4%) are non-natives, both of which are
 288 restricted from the database, and can only reduce the number of true predicted presences by roughly 10%.

289 At the six study plots, of the 117 plant species identified to the species level across the spatial extents of all
 290 plots and duration of queen bee activity, the ML ensembles predicted the presence of 105 (89.7%) of them,
 291 and LM ensembles 102 (87.2%). Of the missing species two (1.7%) are Orchids, six (5.1%) are non-native,
 292 and one (0.85%) is of contested taxonomic standing, all of which (7.65%) are restricted from the initial query
 293 database.

294 3.2 | Microscopic Pollen identification

295 Using the fuchsin jelly preparation and light microscopic analyses of grains and scoring of 10 character
 296 states resulted in the establishment of 28 morphotypes which grains could be classified into. APPENDIX
 297 7. 60 samples were counted and based on rarefaction **had over % of expected morphotypes found**

298 (morphotype richness, $\bar{x} = 4.5$, Mdn = 4, min = 1, max = 9), all samples had expected morphotype diversity
299 reach the asymptote APPENDIX 8. The number of counted pollen grains in each sample range from (MIN -
300 16,293, $\bar{x} = 2788.685$, Mdn = 1453).

301 3.3 | Metabarcoding Pollen identification

302 54 corbiculae loads had DNA extracted and underwent various steps towards hyb-seq, in the end a total of 44
303 corbiculae samples were sequenced, 7,752,353 reads were recovered from sequencing. The number of reads
304 per sequence varied widely (range = 76 - 508,795, $\bar{x} = 176,189.8$, Mdn = 138,395). Of the possible 353 loci,
305 the number which were recovered from each sample, and informative to BLAST were range = 24 - 353, $\bar{x} =$
306 305.5, Mdn = 331. The number of reads per loci from across all samples had a range of 178 - 506,653, $\bar{x} =$
307 20,688, Mdn = 12,616.

308 APPENDIX X.

309 After trimming 7,865,680 sequences remained. 10,682,538 reads were matched using Kraken, of the reads
310 classified by Kraken 10,160,768 reads were matched using Bracken, of the reads classified by Kraken 7,302,876
311 reads were matched using BLAST.

312 Based upon subjective review of the three classifiers, BLAST was chosen as the classification method which
313 yielded the most probable results, and it's values were used for all subsequent analyses.

314 3.4 | Temporal Analyses

315 The first date of modeled snow melt in the Gothic area ($n = 17$, $\bar{x} = 137.9$, Mdn = 135, 3rd quantile = 151),
316 and the first date of a consistent winter snow base ($n = 17$, $\bar{x} = 299.9$, Mdn = 300, 1st quantile = 291) from
317 2000-2017, were used as delimiters for the inclusions of herbarium records in modelling. Of the 500 species
318 predicted likely present in the area via logistic regression, 332 species (64.4%) with more than 10 records
319 in the focal level 4 ecoregions ($\bar{x} = 35.01657$, Mdn = 35, max = 96) had weibull estimates calculated, an
320 additional 56 species (11.2%) with enough contributing records from the 'Sedimentary Mid-Elevation Forests',
321 a large ecoregion in general just beneath the elevation bands occupied by the five ecoregions around the study
322 area had weibull estimates also calculated ($\bar{x} = 13.86885$, Mdn = 13, max = 24).

323 Only 58 of these 388 species ($n = 34.56897$, Mdn = 31) were able to be compared to plot based observational
324 data from the long term (1974–2012) dataset. Of these species relatively high accord was observed between
325 the long-term ground truthed dataset, and the modelled species. There was very strong evidence that the
326 weibull estimates were positively associated with the observed onset ($r^2 = 0.72$, $p < 0.0001$, tau = 0.61) and

327 peak ($r^2 = 0.70$, $p < 0.0001$, $\tau = 0.65$) of flowering, and that the number of herbarium samples had a
328 moderate effect on the estimates ($p = 0.004$ and $p = 0.034$ respectively). There was very strong evidence
329 that the weibull estimates had a positive association with the observed cessation of flowering ($r^2 = 0.4339$, p
330 < 0.0001 , $\tau = 0.489$), however there was no evidence that sample size had an effect ($p = 0.349$). There was
331 moderate evidence that the weibull estimates, with an effect of sample size, had a weak positive association
332 with the observed duration of flowering ($p = 0.0401$, $r^2 = 0.07$, $\tau = 0.17$).

333 [Figure 1 about here.]

334 3.5 | Floral Observations

335 The six sites were surveyed for a total of 52 hours from May 27-July 27. A total of 723 queen-pollen foraging
336 interactions were observed (range per bee species by week range = 1 - 18, $\bar{x} = 3.46$, Mdn = 2), with a range
337 of total observed interactions per bee species across this time period (min = 1, $\bar{x} = 59.08$, Mdn = 19, max =
338 184). Plants varied widely in the number of interactions which they partook in with each species of bee (range
339 per plant species by week min = 1 - 20, $\bar{x} = 3.51$, Mdn = 2), with a range of total observed interactions per
340 plant species over this time period (min = 1, $\bar{x} = 20.26$, Mdn = 4, max = 141). The number of plant species
341 which bees were observed interacting with varied more narrowly (range = 1 - 18, $\bar{x} = 8$, Mdn = 6).

342 A total of 66 corbiculae loads were collected from Bees, 64 of them from Queens.

343 4 | DISCUSSION

344 Although we were able to use an actually fine scale flora to determine the species present at the field site,
345 we suspect a similar approach may be accomplished via quick species richness inventories at sites, and then
346 utilizing a bootstrap approach akin to ours, to the taxa returned from databases to derive these estimates.

347 ** Mention in here that the general effect of sample size on predictions means that larger samples sizes are
348 required for this to work!!!! ** Although our temporal results were lackluster, we note that our study area
349 has an incredibly brief growing period. and we suspect these temporal results would be useful in sub-tropical
350 and tropical ecosystems. FURTHER, the sites used for ground truthing the temporal flowering periods
351 were not randomly selected across the study area, and cannot be used to make inference to the population
352 across the entirety of the study area as we did here. Regardless both show good agreement on flower onset,
353 peak flowering, and moderate agreement with flowering cessation. The disagreement in flowering cessation
354 is perhaps due to more microclimates which retain water, rather than microclimates which allow the early
355 accumulation of heat.

356 Fewer modelling runs for SDM's likely to be effective for determining inclusion, elastic inclusion criteria. The
357 actual dataset which was used for training and testing all of the models incorporated into SDM's represented
358 only roughly one quarter of the records available for such purposes. We consciously chose to do this in order
359 to showcase the possibility of this approach working in less data rich areas.

360 Bayesian framework

361 Future Directions:

362 While at the time of writing this there are limited A353 sequence data, the Plant and Fungal Trees of Life
363 (PAFTOL) project, which is sequencing at least a species of each genera in the plant Kingdom will produce
364 sequence data from over 14,000 species. Given the extant publicly available genomic data, we conservatively
365 estimate that upon completion of PAFTOL there will be no fewer than 15,500 species (4.4% of all ca. 350,000
366 plant species) for which sequence data of a majority of these loci exist (Govaerts *et al.* (2021)). Accordingly,
367 projects in the near future may increase the number of metagenomics samples while decreasing the need to
368 create their own plant sequence reference libraries. As a result of PAFTOL the first ever comprehensive
369 phylogenetic hypotheses of all plant genera will be presented. In tandem with an increased number of
370 digitised and geo-referenced herbarium specimens, and monitoring programs in natural areas, we believe that
371 geo-informatics, and phylogenetic inference will increase the ability of researchers applying this technique
372 to identifying sequence reads. While our approach emphasises the use of this metagenomic technique for
373 the purpose of identifying pollen, I argue the template and resources we provide here make this approach a
374 suitable candidate for many plant metagenomic tasks. While we did not have the resources to explore the
375 possibility of characterising infraspecific characteristics, preliminary results from others (Wenzell *et al.* (2021),
376 Loke *et al.* in prep) indicate a possibility for these probes to also collect data at the level of populations and
377 individuals. **

378 In regards to better understanding the foraging preferences of *Bombus* feeding in subalpine ecosystems.

379 JANE AND PAUL SET UP FOR NEAR FUTURE RESULTS?

380 **AUTHOR CONTRIBUTIONS:** R.C.B conducted botanical collections, conducted all molecular lab work,
381 lead all analyses, and writing. J.E.O conceived, designed, and conducted all ecological fieldwork, assisted
382 with analyses, and writing. E.J.W. prepared, imaged, and collected trait data on pollen reference slides, and
383 assisted with analysis of trait data and writing a dichotomous key. S.T. assisted with spatial analyses and
384 writing. P.J.C assisted with ecological analyses and writing. J.B.F. conceived, and designed all lab work,
385 analyses, assisted with writing, and secured funding for molecular work.

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397 **CONFLICT OF INTERESTS** The authors declare no conflicts of interest.

398 **PEER REVIEW** The peer review history for this document is available at ...

399 **DATA AVAILABILITY STATEMENT** The queries required to download all data used in this project
400 are located in... All novel sequencing data are located at NCBI...

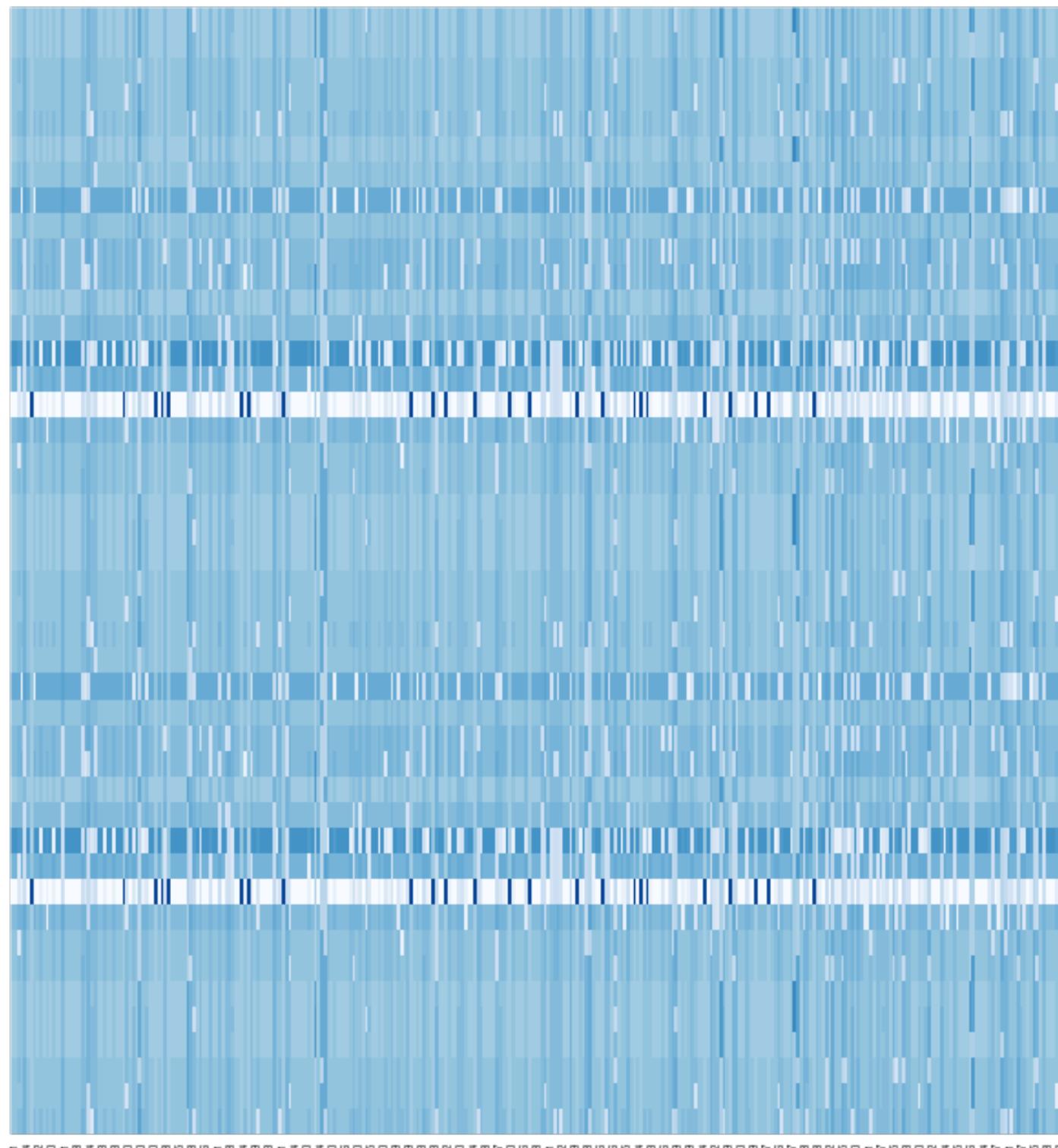
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406 **References**

407 **Supporting**

Percent matched reads per locus by sample



Locus

409

- 1a: Pollen shed in clumps (tetrads/polyads); grains generally triangular, with an annulus subtending the porate apertures (go 34)
- 1b: Pollen generally dispersed as single units (monads); grains seldom if ever with annulus.
- 2a: Apertures porate, always lacking colpi
- 3a: grain outline from equatorial view circular
- 4a: Pores distributed along the equator.
- 5a: Pores > 5 (stephanoporate)
- 6a: Ornamentation homobrochate (~ *MENTZELIA*)
- 6b: Ornamentation otherwise (~ *POLYGALA*)
- 5b: Pores < 5 (*CURRENTLY OPEN*)
- 4b: Pores +/- distributed across grain (pantoporate)
- 7a: Ornamentation with striate ornamentation (~ *POLEMONIUM*)
- 7b: Ornamentation otherwise
- 8a: Ornamentation, slightly irregular - without regularly repeating features (scabrate) (~ *STELLARIA*)
- 8b: Ornamentation forming regularly repeating (reticulate) cells of varying shapes.
- 9a: spacing between the grid cells large (lophate), the walls of the cells with another set of projecting ornamentation (~ *OPUNTIA*)
- 9b: spacing between cells small, the wall of the cells without projecting features.
- 10a: Pores extending beyond the reticulate grids (~ *ARENARIA*)
- 10b: Pores extending beyond the reticulate grids (~ *PHLOX*)
- 3b: Outline from equatorial view otherwise (usually slightly triangular)
- 11a: Outline elliptic (*CURRENTLY EMPTY*)
- 11b: Outline not elliptic, grains often with acute, if rounded, angles along sides (e.g., triangular, polygonal) (*EMPTY*)
- 2b: Apertures with colpi, occasionally also with pores in addition (coporate)
- 12a: Grains with bristles tapering to points (echinate), and tri-colporate.
- 13a: Grains uniformly echinate, less the apertures. (Asteraceae 1)
- 13b: Grains with echinate bristles on ridges of lophae (Asteraceae 2)
- 12b: Grains without echinate ornamentation - this lead includes projections with ornamentation with round tips.
- 14a: Grains with either less than 3 apertures, or with two distinct ornamentation types (generally $\frac{1}{2}$ psilate, $\frac{1}{2}$ reticulate).
- 15a. Grains apparently lacking any apertures. (~ *IRIS*)
- 15b. Grains aperturate
- 16a. Ornamentation on one face of grain psilate, the other homobrochate (~ *ZIGADENUS + ANTICLEA*)
- 16b. Ornamentation psilate across both faces of grain (~*ERYTHONIUM*)
- 14b. Grains with either 3 or more apertures, or with an elongated spiral like aperture
- 17a. Grain with spiral like colpi
- 18a. Spiral with deep well-defined furrows (~ *ERYTHRANTHE GUTTATA*, syn. obsolete. *MIMULUS*)
- 18b. Spirals without well-defined grooves, ornamentation evidently perforate (~ *RANUNCULUS ALISMIFOLIUS*)
- 17b. Grains with colpi these not forming irregular spiral motifs.
- 19a. Grains elliptic, essentially perfectly cylindrical along longest axis, except for minor inundations along equatorial region. Apertures, of two types (heteroaperturate). (~ *BORAGINACEAE*)
- 19b Grains shaped similar or not, but never heteroaperturate.
- 20a. From a polar view, grains notably polygonal (hexagonal), also evident when seldom seen from a equatorial view. (~ *PHACELIA*/ maybe *Hydrophyllaceae*, *Hydrophyllum* not sampled)
- 20b. From a polar view, grains not with 6 convex apices

- 21a. Grains elliptic, with a short colporate aperture on each psilate face, the edges of each face and the apices with a distinct (homobrochate) textured ornamentation. (~ POLYGONUM)
- 21b. Grains otherwise, not featuring a mix of ornamentations independent of the apertures.
- 22a. Ornamentation perforate, the three colpi very short, their longest axis parallel to the equator rather than perpendicular. These colpi often times almost appearing to be slightly raised on an annulus like feature (~ LONICERA)
- 22b. Grains not as described in all aspects of the above.
- 23a. Apertures colporate
- 24a. Outline of grain in equatorial view circular, ornamentation smooth. (~MORPHOTYPE A).
- 24b. Grains otherwise
 - 25a Grains distinctly triangular from polar view (go 26)
 - 25b Grains elliptic (go 27)
 - 26a Grains very large, clearly strongly triangular in cross section. (~ GERANIUM)
 - 26b Grains smaller (SIZE), weakly triangular in cross section (~ POTENTILLA/DASIPHORA in part)
 - 27a Grains elliptic to weakly circular (~MORPHOTYPE B)
 - 27b Grains elliptic, much longer pole to pole than across equator.
 - 28a Grains with evident protrusions of the pore, colpi short, scarcely noticeable (~ APIACEAE)
- 23b. Apertures colpate
 - 30a Ornamentation psilate (~MORPHOTYPE C).
 - 30b Ornamentation otherwise
 - 31a Ornamentation homobrochate (~ MORPHOTYPE D)
 - 31b Ornamentation otherwise
 - 32a. Ornamentation bacculate, grains large, (~LINUM)
 - 32b. Ornamentation otherwise
 - 33a. Ornamentation of minor cross-corrugated grooves (fossulate) (~CORYDALIS)
 - 33b. Ornamentation of scarcely perceptible irregular features (scabrate) (~RANUNCULUS IN PART)

34a: Annula subtending the apertures – making grains appear more or less triangular; Pollen often with viscin threads (ONAGRACEAE)

34b: Apertures not annulate – grains appear more or less circular (~ERICACEAE)

Morphotype A: Trifolium, Lupinus, Glycrrhiza, Mitella, Geum

Morphotype B: Lupinus, Lathyrus, Potentilla, Androsace, Bistorta, Vicia

Morphotype C: Jeffersonia, Micranthes, Prunus, Delphinium, Androsace, Penstemon, Orthocarpus, Scutellaria, Aquilegia, Castilleja, Draba)

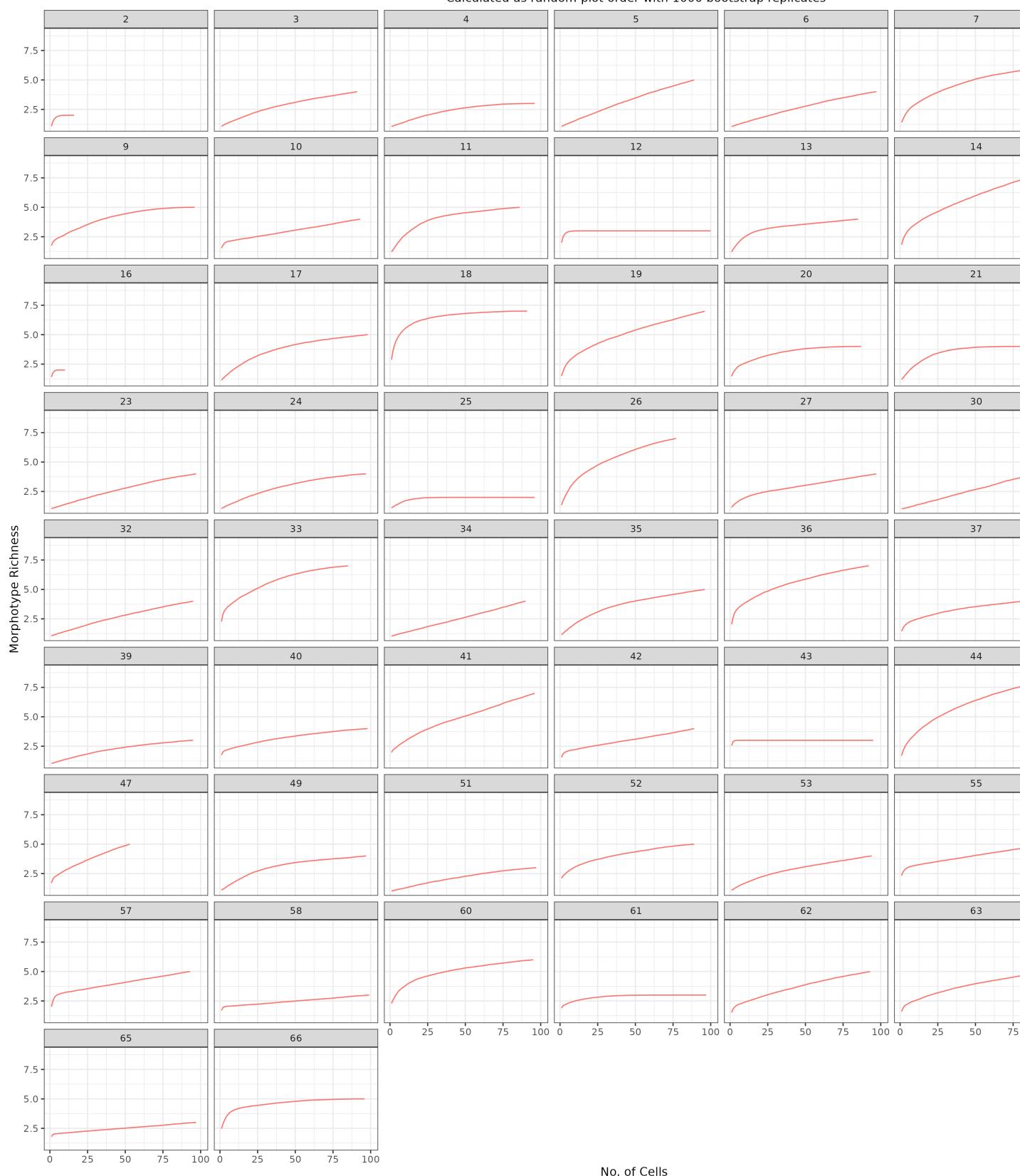
Morphotype D: Salix, Boechera

⁴¹³ Appendix XX - Species Distribution Models Predictors

Layer	Description	Source
1.	Mean annual cloudiness - MODIS	Wilson et al. 2016
2.	Cloudiness seasonality 1 - MODIS	Wilson et al. 2016
3.	Cloudiness seasonality 2 - MODIS	Wilson et al. 2016
4.	Cloudiness seasonality 3 - MODIS	Wilson et al. 2016
5.	Beginning of the frost-free period	Wang et al.
6.	Climatic moisture deficit	Wang et al.
7.	Degree-days above 5C from	Wang et al.
8.	Mean annual precipitation	Wang et al.
9.	Mean annual precipitation as snow	Wang et al.
10.	Temperature seasonality	Wang et al.
11.	2015 Percent Grass/Herbaceous cover - MODIS	(MOD44B)
12.	2015 Percent Tree cover from Landsat 7/8	(GLCF)
13.	Soil probability of bedrock (R Horizon)	SoilGrids
14.	Soil organic carbon (Tonnes / ha)	SoilGrids
15.	Surface soil pH in H ₂ O	SoilGrids
16.	Surface soil percent sand	SoilGrids
17.	Soil USDA class	SoilGrids
18.	Topographic elevation	EarthEnv DEM
19.	Topographic elevation, moving window.	EarthEnv DEM
20.	Topographic percent slope	EarthEnv DEM
21.	Topographic wetness index	EarthEnv DEM
22.	Topographic aspect from	EarthEnv DEM
23.	Annual potential solar radiation computed	r.sun
24.	Estimated actual (w/-cloud) solar radiation r	.sun / Wilson et al. 2016
25.	Log-transformed distance to surface water Gl	obal Surface Water Explorer
26.	Percent surface water Gl	obal Surface Water Explorer

⁴¹⁴ APPENDIX XX - Pollen Morphotype Richness Rarefaction Curves

Rarefaction Curves of Species Richness
Calculated as random plot order with 1000 bootstrap replicates



Used to assess completeness of subsampling. VEGAN package 'specaccum' function used

⁴¹⁶ Appendix XX - Pollen Morphotype Abundance Rarefaction Curves



Table 1: samples used in creating the Reference Library

Taxon	Family	Accession	Pres.	Locality	Date Col.	GenBank	Dist. (km)
<i>Cirsium parryi</i> (A. Gray) Petr.	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Cirsium parryi</i> (A. Gray) Petr.	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Ericameria parryi</i> (A. Gray) G.L. Nesom & Baird	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Erigeron speciosus</i> (Lindley) De Candolle	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Erigeron subtrinervis</i> Rydb. Ex Porter & Britton	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.VII.2020	tba	3.6
<i>Helianthella quinquenervis</i> (Hook.) A. Gray	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Helianthus multiflora</i> Nutt.	Asteraceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	CHIC tba	S	Colorado, Gunnison	20.IX.2020	tba	3.6
<i>Senecio sera</i> Hook.	Asteraceae	CHIC tba	P	Idaho, Idaho	26.VII.2020	tba	105.0
<i>Symplytrichum foliacum</i> (Lindl. Ex D.C.) G.L. Nesom	Asteraceae	CHIC tba	S	Illinois, McHenry	28.VII.2020	tba	1624.6
<i>Taraxacum officinale</i> F.H. Wigg.	Asteraceae	CHIC tba	S	Illinois, McHenry	28.VII.2020	tba	1624.6
<i>Mertenia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	ID 1754185	S	Idaho, Valley	18.VI.2018	tba	979.3
<i>Mertenia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	ID 169837	P	Idaho, Adams	10.VII.2014	tba	991.5
<i>Mertensia fusiformis</i> Greene	Boraginaceae	RMH 720522	P	Colorado, Gunnison	7.VI.1997	tba	44.8
<i>Campanula rotundifolia</i> L.	Campanulaceae	RMH 720600	P	Colorado, Gunnison	9.VII.1997	tba	38.9
<i>Lathyrus lanszwertii</i> Kellogg var. leucanthus (Ryd.) Dorn	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Lathyrus lanszwertii</i> Kellogg var. leucanthus (Ryd.) Dorn	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Lupinus argenteus</i> Pursh	Fabaceae	CHIC tba	P	Nevada, Pershing	29.V.2018	tba	3.6
<i>Lupinus argenteus</i> Pursh	Fabaceae	ISU 10387	P	Colorado, Gunnison	29.VI.2010	tba	971.2
<i>Lupinus bakeri</i> Greene	Fabaceae	ISU 10142	P	Colorado, Gunnison	15.VIII.2010	tba	0.2
<i>Vicia americana</i> Muhl. ex Willd.	Fabaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	2.6
<i>Vicia americana</i> Muhl. ex Willd. var. minor Hook.	Fabaceae	CHIC tba	S	Montana, Carbon	4.VII.2019	tba	10020.8
<i>Frasera speciosa</i> Douglas ex Griseb	Gentianaceae	RMH 721930	P	Colorado, Gunnison	20.VI.1997	tba	66.2
<i>Frasera speciosa</i> Douglas ex Griseb	Gentianaceae	RMH 719305	P	Colorado, Gunnison	7.VII.1997	tba	19.8
<i>Hydrophyllum capitatum</i> Douglas ex. Benth	Hydrophyllaceae	RMH tba	P	Colorado, Mesa	30.VI.2011	tba	64.6
<i>Hydrophyllum capitatum</i> Douglas ex. Benth	Hydrophyllaceae	RMH tba	P	Colorado, Delta	8.VI.2011	tba	65.3
<i>Hydrophyllum fendleri</i> (Gray) Heller	Hydrophyllaceae	ID 161100	P	Washington, Yakima	9.VI.2008	tba	1429.7
<i>Hydrophyllum fendleri</i> (Gray) Heller	Hydrophyllaceae	ID 164040	P	Idaho, Idaho	27.V.2009	tba	1014.4
<i>Agastache pallidiflora</i> (Heller) Rydberg	Lamiaceae	CHIC tba	S	Arizona, Coconino	17.VII.2020	tba	617.7
<i>Chamerion angustifolium</i> (L.) Holub	Lamiaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Delphinium barbeyi</i> (Huth) Huth	Ranunculaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Delphinium nuttallianum</i> Pritz.	Ranunculaceae	ID 166162	P	Idaho, Gem	15.VI.2011	tba	9825.5
<i>Delphinium nuttallianum</i> Pritz.	Ranunculaceae	ID 179376	P	Idaho, Gooding	29.IV.2017	tba	733.7
<i>Potentilla fruticosa</i> Pursh	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Potentilla fruticosa</i> Pursh	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6
<i>Potentilla hippiana</i> Lehman.	Rosaceae	CHIC tba	S	New Mexico, Catron	15.VIII.2020	tba	573.8

(Continued on Next Page)

Table 1: samples used in creating the Reference Library (*continued*)

Taxon	Family	Accession	Pres.	Locality	Date Col.	GenBank	Dist. (km)
Potentilla pulcherrima Lehman.	Rosaceae	CHIC tba	S	Colorado, Gunnison	18.VII.2020	tba	3.6

^a Accession includes both Herbarium and Accession number

^b Pres. refers to Preservation method. 'S' denotes silica gel dried, 'P' denotes pressed

^c All Localities are in the United States of America

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST)

Order	Family	Taxon
Alismatales	Potamogetonaceae	<i>Potamogeton wrightii</i>
Apiales	Apiaceae	<i>Osmorhiza aristata</i>
Asparagales	Amaryllidaceae	<i>Allium stamineum</i>
	Asparagaceae	<i>Streptopus amplexifolius</i>
Asterales	Asteraceae	<i>Anaphalis margaritacea</i> <i>Antennaria carpatica</i> <i>Antennaria dioica</i> <i>Artemisia sibirica</i> <i>Brickellia dentata</i> <i>Chrysanthemus greenei</i> <i>Cirsium pannonicum</i> <i>Cirsium parryi</i> <i>Cirsium vulgare</i> <i>Crepis pygmaea</i> <i>Ericameria parryi</i> <i>Erigeron ecuadoriensis</i> <i>Erigeron grandiflorus</i> <i>Erigeron rosulatus</i> <i>Erigeron uniflorus</i> <i>Helianthella quinquenervis</i> <i>Heterotheca villosa</i> <i>Hieracium avilae</i> <i>Hieracium jubatum</i> <i>Hymenoxys hoopesii</i> <i>Leucanthemum graminifolium</i> <i>Microseris lindleyi</i> <i>Omalotheca supina</i> <i>Packera quercetorum</i> <i>Pseudognaphalium attenuatum</i> <i>Pseudognaphalium frigidum</i> <i>Pseudognaphalium lacteum</i> <i>Pseudognaphalium oxyphyllum</i> <i>Rudbeckia hirta</i> <i>Scabrethia scabra</i> <i>Senecio adenophyllus</i> <i>Senecio algens</i> <i>Senecio apolobambensis</i> <i>Senecio candollei</i> <i>Senecio chionogeton</i> <i>Senecio formosus</i> <i>Senecio funcii</i> <i>Senecio gilliesii</i> <i>Senecio humillimus</i> <i>Senecio nutans</i> <i>Senecio puchei</i> <i>Senecio rufescens</i> <i>Senecio spinosus</i> <i>Senecio tephrosioides</i>

(Continued on Next Page)

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST) (*continued*)

Order	Family	Taxon
		<i>Solidago chilensis</i>
		<i>Stilpnolepis intricata</i>
		<i>Symphyotrichum foliaceum</i>
		<i>Taraxacum cucullatum</i>
		<i>Taraxacum officinale</i>
		<i>Tonestus lyallii</i>
		<i>Townsendia formosa</i>
	Campanulaceae	<i>Campanula argaea</i>
		<i>Campanula rotundifolia</i>
Boraginales	Boraginaceae	<i>Cynoglossum amplifolium</i>
		<i>Cynoglossum anchusoides</i>
		<i>Cynoglossum pringlei</i>
		<i>Mertensia ciliata</i>
		<i>Mertensia fusiformis</i>
	Hydrophyllaceae	<i>Hydrophyllum canadense</i>
		<i>Hydrophyllum capitatum</i>
		<i>Hydrophyllum fendleri</i>
		<i>Nemophila menziesii</i>
Caryophyllales	Caryophyllaceae	<i>Arenaria globiflora</i>
		<i>Arenaria serpyllifolia</i>
		<i>Cerastium arvense</i>
		<i>Cerastium lanceolatum</i>
		<i>Minuartia recurva</i>
		<i>Odontostemma leucasterium</i>
		<i>Pseudostellaria heterophylla</i>
		<i>Sagina procumbens</i>
		<i>Schizotechium monospermum</i>
		<i>Shivparvatia glanduligera</i>
		<i>Stellaria graminea</i>
		<i>Stellaria holostea</i>
		<i>Stellaria obtusa</i>
	Polygonaceae	<i>Rumex induratus</i>
		<i>Rumex spinosus</i>
Celastrales	Celastraceae	<i>Parnassia faberi</i>
		<i>Parnassia palustris</i>
		<i>Paxistima canbyi</i>
Ericales	Ericaceae	<i>Gaultheria prostrata</i>
		<i>Moneses uniflora</i>
		<i>Orthilia secunda</i>
		<i>Vaccinium vitis-idaea</i>
	Polemoniaceae	<i>Collomia grandiflora</i>
		<i>Ipomopsis aggregata</i>
		<i>Phlox douglasii</i>
	Primulaceae	<i>Androsace studiosorum</i>
		<i>Androsace vitaliana</i>
Fabales	Fabaceae	<i>Astragalus pelecinus</i>
		<i>Lupinus argenteus</i>
		<i>Lupinus sericeus</i>

(Continued on Next Page)

Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST) (*continued*)

Order	Family	Taxon
Gentianales	Gentianaceae	<i>Vicia americana</i> <i>Frasera speciosa</i> <i>Gentiana cruciata</i>
Hyphomicrobiales	Xanthobacteraceae	<i>Azorhizobium caulinodans</i>
Lamiales	Lamiaceae	<i>Agastache pallidiflora</i>
Liliales	Colchicaceae	<i>Prosartes smithii</i>
	Liliaceae	<i>Erythronium dens-canis</i>
	Melanthiaceae	<i>Anticlea elegans</i> <i>Veratrum viride</i>
Malpighiales	Hypericaceae	<i>Hypericum perforatum</i>
	Salicaceae	<i>Populus alba</i>
	Violaceae	<i>Viola odorata</i>
Myrtales	Onagraceae	<i>Chamaenerion angustifolium</i> <i>Epilobium canum</i> <i>Epilobium parviflorum</i>
Ranunculales	Berberidaceae	<i>Berberis sibirica</i>
	Papaveraceae	<i>Corydalis aitchisonii</i>
	Ranunculaceae	<i>Actaea heracleifolia</i> <i>Anemone anemonoides</i> <i>Anemone obtusiloba</i> <i>Aquilegia ecalcarata</i> <i>Caltha palustris</i> <i>Delphinium barbeyi</i> <i>Delphinium gracile</i> <i>Delphinium nuttallianum</i> <i>Pulsatilla chinensis</i> <i>Thalictrum thalictroides</i> <i>Thalictrum tuberosum</i> <i>Trollius europaeus</i>
Rosales	Elaeagnaceae	<i>Shepherdia argentea</i>
	Rosaceae	<i>Crataegus bipinnatifida</i> <i>Dasiphora fruticosa</i> <i>Geum ternatum</i> <i>Hedlundia austriaca</i> <i>Holodiscus argenteus</i> <i>Karpatiosorbus devoniensis</i> <i>Micromeles japonica</i> <i>Potentilla anserina</i> <i>Potentilla pulcherrima</i> <i>Potentilla tetrandra</i> <i>Rubus chingii</i>
Sapindales	Sapindaceae	<i>Acer campestre</i>
Saxifragales	Crassulaceae	<i>Rhodiola rosea</i> <i>Sedum nudum</i>
	Grossulariaceae	<i>Ribes rubrum</i>
	Saxifragaceae	<i>Lithophragma parviflorum</i> <i>Saxifraga biflora</i> <i>Saxifraga fortunei</i>

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Table 1: All species present in the Reference Sequence Databases
(Kraken and BLAST) (*continued*)

Order	Family	Taxon
		Saxifraga maderensis
		Saxifraga oppositifolia
		Saxifraga portosanctana
		Saxifraga x geum

x geum* \end{longtable}

426 Appendix XX - All Pollen Reference Slides Used to Establish Morphotypes

Table 1: All Pollen Voucher Slides Consulted

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Cymopterus lemnoides</i> (J.M. Coulter & Rose) Dorn	Apiaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heracleum sphondylium</i> L.	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ligusticum porteri</i> J.M. Coulter & Rose	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Osmorhiza depauperata</i> Phil.	Apiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Maianthemum stellatum</i> (L.) Link	Asparagaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Achillea millefolium</i> L.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Achillea millefolium</i> L.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Acourtia wrightii</i> (A. Gray) Reveal & King	Asteraceae	NV, Clark, Gold Butte	tba	Novo	E.J.W.	2021
<i>Antennaria racemosissima</i> Hook.	Asteraceae	WY, Park, Ishawooa Mesa	tba	Novo	E.J.W.	2021
<i>Arnica latifolia</i> Bong.	Asteraceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Artemisia scopulorum</i> A. Gray	Asteraceae	CO, Hinsdale, Uncompahgre Peak	tba	Novo	E.J.W.	2021
<i>Canadanthus modestus</i> (Lindl.) G.L. Nesom	Asteraceae	ID, Idaho, Whiskey Creek	tba	Novo	E.J.W.	2021
<i>Chaenactis douglasii</i> (Hook.) Hook. & Arn.	Asteraceae	MT, Carbon, Pryor Mtn. Rd.	tba	Novo	E.J.W.	2021
<i>Erigeron corymbosus</i> Nutt.	Asteraceae	MT, Carbon, Pryor Mtn. Rd.	tba	Novo	E.J.W.	2021
<i>Erigeron flagellaris</i> A. Gray	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Erigeron speciosus</i> (Lind.) DC.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Erigeron speciosus</i> (Lindl.) DC.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Helianthella quinquenervis</i> (Hook.) A. Gray	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heliotropium multiflorum</i> Nutt.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heliotropium multiflorum</i> Nutt.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Heterotheca villosa</i> (Pursh) Shinners	Asteraceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Hymenoxys hoopesii</i> (A. Gray) Bierner	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Hymenoxys rusbyi</i> (A. Gray) Cockerell	Asteraceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Ionactis stenomeria</i> (A. Gray) Greene	Asteraceae	ID, Idaho, Marshall Mountains	tba	Novo	E.J.W.	2021
<i>Senecio hydrophilus</i> Nutt.	Asteraceae	ID, Custer, E. fl. Salmon River	tba	Novo	E.J.W.	2021
<i>Senecio integrerrimus</i> Nutt.	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Senecio serrula</i> Hook.	Asteraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Senecio wootonii</i> Greene	Asteraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Solidago lepida</i> DC.	Asteraceae	ID, Idaho, American River	tba	Novo	E.J.W.	2021
<i>Sympotrichum foliacum</i> (Lindl. ex DC.) G.L. Nesom	Asteraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Sympotrichum subspicatum</i> (Nees) G.L. Nesom	Asteraceae	ID, Custer, E. fl. Salmon River	tba	Novo	E.J.W.	2021
<i>Taraxacum officinale</i> F.H. Wigg	Asteraceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Taraxacum officinale</i> F.H. Wigg	Asteraceae	IL, McHenry, Barrington	tba	Novo	E.J.W.	2021
<i>Lappula squarrosa</i> (Retz.) Dumort.	Boraginaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Mertensia ciliata</i> (James ex Torr.) G. Don	Boraginaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Mertensia fusiformis</i> Greene	Boraginaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Boechera</i>	Brassicaceae	NV, Washoe, Mt. Rose	tba	Novo	E.J.W.	2021
<i>Boechera stricta</i> (Graham) Al-Shehbaz	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Cardamine cordifolia</i> A. Gray	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Draba aurea</i> Vahl. Ex Hornem	Brassicaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014

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Table 1: All Pollen Voucher Slides Consulted (*continued*)

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Draba spectabilis</i> Greene	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Erysimum capitatum</i> (Douglas ex Hook.) Greene	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Lepidium montanum</i> Nutt.	Brassicaceae	NM, Catron, Zuni Dry Lake	tba	Novo	E.J.W.	2021
<i>Smelowskia americana</i> Rydb.	Brassicaceae	ID, Blaine, Pioneer Mtns Crest	tba	Novo	E.J.W.	2021
<i>Thlaspi arvense</i> L.	Brassicaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Campanula rotundifolia</i> L.	Campanulaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Campanula rotundifolia</i> L.	Campanulaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Downingia</i>	Campanulaceae	CA, Nevada, Truckee Meadows	tba	Novo	E.J.W.	2021
<i>Lonicera involucrata</i> (Richardson) Banks ex Spreng.	Caprifoliaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Minuartia nuttallii</i> (Pax.) Briq.	Caryophyllaceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Stellaria longifolia</i> Muh. Ex. Willd.	Caryophyllaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Parnassia kotzebuei</i> Cham. ex Spreng	Celastraceae	ID, Lemhi, Terrace Lakes	tba	Novo	E.J.W.	2021
<i>Sedum lanceolatum</i> Torr.	Crassulaceae	ID, Lemhi, Terrace Lakes	tba	Novo	E.J.W.	2021
<i>Astragalus robbinsii</i> (Oakes) A. Gray	Fabaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Glycyrrhiza lepidota</i> Nutt.	Fabaceae	ID, Butte, Warm Springs Creek	tba	Novo	E.J.W.	2021
<i>Lathyrus eucomus</i> Butters & H. St. John	Fabaceae	NM, Catron, Zuni Salt Lake	tba	Novo	E.J.W.	2021
<i>Lathyrus lanszwertii</i> var. <i>leucanthus</i> (Rydb.) Dorn	Fabaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Lathyrus lanszwertii</i> var. <i>leucanthus</i> (Rydb.) Dorn	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Lupinus argenteus</i> Pursh	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Lupinus argenteus</i> Pursh	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Lupinus argenteus</i> Pursh	Fabaceae	NV, Pershing, Star Peak Canyon	tba	Novo	E.J.W.	2021
<i>Lupinus crassus</i> Payson	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Lupinus sericeus</i> Pursh	Fabaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Melilotus albus</i> Medik.	Fabaceae	NM, Catron, Hwy 159	tba	Novo	E.J.W.	2021
<i>Trifolium hybridum</i> L.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Trifolium pratense</i> L.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	UT, Cache, Spawn Creek	tba	Novo	E.J.W.	2021
<i>Vicia americana</i> Muhl. Ex Willd.	Fabaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Geranium</i>	Geraniaceae	NM, Catron, Jim Smith TH. Rd.	tba	Novo	E.J.W.	2021
<i>Geranium richardsonii</i> Fisch. Trautv.	Geraniaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Phacelia</i> sp.	Hydrophyllaceae	NV, Nye, Toiyabe Crest	tba	Novo	E.J.W.	2021
<i>Iris missouriensis</i> Nutt.	Iridaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Agastache palliflora</i> (A. Heller) Rydb.	Lamiaceae	AZ, Coconino, Lake Mary Rd. & 209	tba	Novo	E.J.W.	2021
<i>Erythronium grandiflorum</i> Pursh	Liliaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Linum lewisii</i> Pursh	Linaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Zigadenus elegans</i> Pursh	Melanthiaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Anticlea elegans</i> (A. Gray) Zomlefer & Judd	Melanthiaceae	ID, Blaine, Wildhorse Canyon	tba	Novo	E.J.W.	2021
<i>Cistanthe/Calyptidium</i>	Montiaceae	NV, Nye, Toiyabe Crest	tba	Novo	E.J.W.	2021
<i>Chamerion angustifolium</i> (L.) Holub	Onagraceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Epilobium obcordatum</i> A. Gray	Onagraceae	ID, Lemhi, Bighorn Crags	tba	Novo	E.J.W.	2021
<i>Castilleja miniata</i> Douglas ex Hook.	Orobanchaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010

(Continued on Next Page)

Table 1: All Pollen Voucher Slides Consulted (*continued*)

Taxon	Family	Locality	Accession	Type	Prepared by	Date
<i>Castilleja sulphurea</i> Rydb.	Orobanchaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Pedicularis groenlandica</i> Retz.	Orobanchaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Pedicularis racemosa</i> Douglas ex Benth.	Orobanchaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Corydalis aurea</i> Willd.	Papaveraceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Erythranthe guttata</i> (DC.) G.L. Nesom	Phrymaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Penstemon wilcoxii</i> Rydb.	Plantaginaceae	MT, Missoula, Mission Mtns vic.	tba	Novo	E.J.W.	2021
<i>Collomia linearis</i> Nutt.	Polemoniaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Phlox condensata</i> (A. Gray) E.E. Nelson	Polemoniaceae	CO, Hinsdale, Uncompahgre Peak	tba	Novo	E.J.W.	2021
<i>Polemonium foliosissimum</i> A. Gray	Polemoniaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Polemonium occidentale</i> Greene	Polemoniaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Polemonium viscosum</i> Nutt.	Polemoniaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2010
<i>Bistorta bistortoides</i> (Pursh) Small	Polygonaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Eriogonum</i> spp.	Polygonaceae	NV, Washoe, Hwy 445	tba	Novo	E.J.W.	2021
<i>Polygala barbeyana</i> Chodat	Polygonaceae	NM, Eddy, Yeso Hills	tba	Novo	E.J.W.	2021
<i>Polygonum polygaloides</i> L.	Polygonaceae	MT, Missoula, Mission Mtns	tba	Novo	E.J.W.	2021
<i>Androsace filiformis</i> Retz.	Primulaceae	ID, Custer, Bradshaw Creek	tba	Novo	E.J.W.	2021
<i>Aquilegia coerulea</i> E. James	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Aquilegia coerulea</i> E. James	Ranunculaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Aquilegia elegantula</i> Greene	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Delphinium barbeyi</i> (Huth) Huth	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Delphinium nuttallianum</i> Pritz. Ex Walp.	Ranunculaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Ranunculus alismifolius</i> Geyer ex Benth.	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ranunculus glaberrimus</i> Hook.	Ranunculaceae	ID, Lemhi, Agency Creek	tba	Novo	E.J.W.	2021
<i>Ranunculus inamoenus</i> Greene	Ranunculaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Ranunculus</i> spp.	Ranunculaceae	NV, Washoe, Mt. Rose	tba	Novo	E.J.W.	2021
<i>Thalictrum sparsiflorum</i> Tuzc. Ex Fisch. & C.A. Mey.	Ranunculaceae	ID, Custer, E. fk. Salmon River	tba	Novo	E.J.W.	2021
<i>Dasiphora fruticosa</i> (L.) Rydb.	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Dasiphora fruticosa</i> (L.) Rydb.	Rosaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Fragaria virginiana</i> Duchesne	Rosaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Geum triflorum</i> Pursh	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Potentilla biennis</i> Greene	Rosaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Potentilla hippiana</i> Lehm.	Rosaceae	NM, Catron, Jim Smith TH.Rd.	tba	Novo	E.J.W.	2021
<i>Potentilla pulcherrima</i> Lehm.	Rosaceae	CO, Gunnison, Gothic	None	Physical	P.J.C.	2014
<i>Potentilla pulcherrima</i> Lehm.	Rosaceae	CO, Gunnison, Washington Gulch	tba	Novo	E.J.W.	2021
<i>Salix bebbiana</i> Sarg.	Salicaceae	ID, Custer, Lake Creek	tba	Novo	E.J.W.	2021
<i>Salix geyeriana</i> Andersson	Salicaceae	ID, Butte, Clyde	tba	Novo	E.J.W.	2021
<i>Mitella stauropetala</i> Piper	Saxifragaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Valeriana occidentalis</i> A. Heller	Valerianaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010
<i>Viola canadensis</i> L.	Violaceae	CO, Gunnison, Gothic	None	Image	H.M.B.	2010

* All Localities are in the United States of America

† Accession refers to whole-plant vouchers, all specimens are deposited at CHIC.

‡ Type Refers to whether both a physical and digital copy exist; 'Image' denotes only digital

§ Date refers to the Date of preparation.

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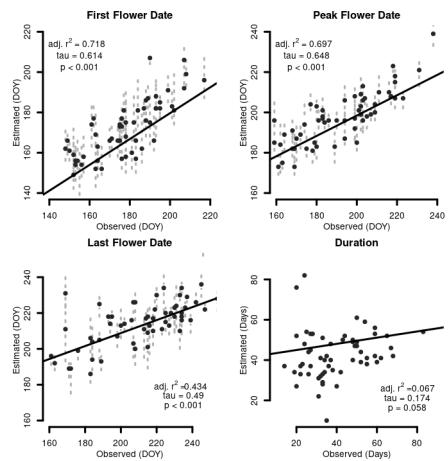


Figure 1: A caption

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Table 2: Logistic regression assessing accuracy of SDMs

Metric	Value	Metric	Value
Accuracy (Training)	83.75	F-Score	0.84
Accuracy (Test)	84.00	AUC	0.92
Recall	81.03	Concordance	0.92
True Neg. Rate	86.97	Discordance	0.08
Precision	88.04	Tied	0.00

Table 3: SDM evaluation contingency table

		Training		Testing	
		Absence	Presence	Absence	Presence
Absence	Absence	25620	3838	11130	1653
	Presence	6614	28248	2758	12024