

1   **Hemiparasitic *Castilleja* presence correlated with increased plant diversity across montane plant  
2   communities.**

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30   **ABSTRACT:**

31   Root hemiparasites utilize haustoria to connect to host belowground roots and extract nutrients and water  
32   from surrounding hosts. Through parasitic symbiosis, parasitic plants disproportionately affect plant  
33   communities relative to their abundance by reducing host size, altering nutrient flow, and increasing local  
34   biodiversity. Their prevalence and potential as keystone species position hemiparasites as important

35 drivers of community structure. Here, we explored how the presence of hemiparasitic *Castilleja* is  
36 associated with differences in the structure of montane plant communities. Our objectives were to: (1)  
37 observe the relationship between three hemiparasitic *Castilleja* species and plant community diversity and  
38 composition, and (2) assess the host range and preference for each species *in situ* using direct and indirect  
39 sampling. We established a two-year study beginning in summer 2023 at the Rocky Mountain Biological  
40 Laboratory. We selected paired *Castilleja* presence and absence plots for three species across seven study  
41 sites. Percent cover of all species was recorded to assess the effect of *Castilleja* presence on plant  
42 community diversity and composition. To assess host range and preference, and to support nearest-  
43 neighbor and indicator-species analyses, we excavated *Castilleja* individuals to observe haustorial  
44 connections. Across sites, species, and years, the presence of *Castilleja* was consistently correlated with  
45 increased plant diversity, although not with consistent shifts in community composition. Excavations  
46 suggested that *Castilleja* species are generalist hemiparasites with some preference for common,  
47 dominant hosts. Our results identify a positive relationship between *Castilleja* presence and local plant  
48 community diversity and support the interpretation that these species act as facultative generalist  
49 hemiparasites.

50 **KEYWORDS**

51 *Castilleja*, parasitic plants, biodiversity, hemiparasitism, Orobanchaceae, community ecology, Rocky  
52 Mountains

53

54 **INTRODUCTION:**

55 Parasitic plants form a large and functionally diverse group of partial to full heterotrophs that  
56 have the potential to drive key community assembly processes, yet are understudied considering their  
57 prevalence across natural systems (Press and Phoenix, 2005; Nickrent, 2020). The parasitic habit has  
58 evolved independently across 13 different lineages and is unified by the formation of a unique organ  
59 called a haustorium. Parasitic plants utilize haustoria to connect to host branches and stems aboveground  
60 (aerial parasites) or to belowground roots (root parasites), extracting carbon, nutrients, and water for their  
61 growth and survival (Watson et al., 2022). Through the direct and indirect effects of parasitic symbiosis,  
62 parasitic plants disproportionately affect plant communities relative to their abundance by reducing host  
63 size, altering nutrient flow, and increasing local community diversity (Griebel et al., 2017; Craven et al.,  
64 2018). The prevalence, distinct physiology, and potential as keystone species position parasitic plants as  
65 a crucial piece in the puzzle of our understanding of ecological communities (Watson, 2009).

66 Root hemiparasites, which account for approximately 90% of parasitic plants (Nickrent, 2020),  
67 are partial parasites that maintain the ability to photosynthesize, and thus are not wholly reliant on their  
68 hosts to supply their nutrient and carbon demands (Terry, 1996; Watson et al., 2022). Recent evidence

suggests that root hemiparasites can drive plant community diversity, richness, and productivity (Press and Phoenix, 2005; DiGiovanni et al., 2017; Heer et al., 2018). While many hemiparasites are generalists with broad host ranges, they often exhibit varying degrees of specificity and show a higher preference for nitrogen-rich hosts (Gibson and Watkinson, 1989; Malcolm C. Press, 1998; Press and Phoenix, 2005). Hemiparasites may alter community structure and competitive dynamics between surrounding plants by preferentially targeting dominant host species and reducing competitive pressures (Gibson and Watkinson, 1989; Phoenix and Press, 2005; Hartley et al., 2015; McKibben and Henning, 2018; Hodžić et al., 2022). Host identity and dominance can determine whether the parasite causes a net increase or decrease in co-occurring plant diversity (Phoenix and Press, 2005; Hodžić et al., 2022). This suggests that our ability to predict the impact of hemiparasites on plant communities hinges on a more detailed understanding of parasite-host interactions (Gibson and Watkinson, 1989; Phoenix and Press, 2005).

The Orobanchaceae is the largest family of parasitic plants with a global distribution of over 2000 species (McNeal et al., 2013). Root parasitism is ubiquitous in the family, occurring in all but one genus (Schneeweiss, 2013). Most field ecological research on the family has been concentrated within the genera *Pedicularis* and *Rhinanthus* in Europe, which has shown root hemiparasites to positively or negatively impact species diversity (Gibson and Watkinson, 1989; Fibich et al., 2017; Scheidel and Borowicz, 2024), have variable effects on productivity (Davies et al., 1997; Hellström et al., 2011; Heer et al., 2018), and locally increase decomposition and nutrient availability (Malcolm C. Press, 1998; Bardgett et al., 2006; Watson, 2009; Demey et al., 2013). *Castilleja* is a widespread genus in the Orobanchaceae, comprising approximately 200 species that occur across North America, South America, and Asia. However, the highest number of species within the genus occurs in western North America (Tank et al., 2009). The diversity of *Castilleja* and its prevalence across North America position the genus as an ideal group for comparison with our understanding of European Orobanchaceae, where root hemiparasites have been more rigorously studied.

Whether *Castilleja* plays a keystone role in promoting diversity of co-occurring North American plant communities remains poorly understood. In a study on the impacts of parasitic plants in restoration, the presence of *Castilleja sessiliflora* Pursh was associated with increased species richness and soil phosphorus within an Illinois tallgrass prairie (Scheidel and Borowicz, 2024). A field-based survey assessing the impact of *Castilleja occidentalis* Torr. on diversity and productivity in the alpine tundra found *C. occidentalis* to be associated with increased biomass with minor changes in community diversity (Spasojevic and Suding, 2011). Two short-term studies conducted at the Rocky Mountain Biological Laboratory found neutral to positive associations between *Castilleja* and plant diversity. An experimental removal of *Castilleja* found no effect on plant community diversity (Reed, 2012), while an observational study found positive associations between of *Castilleja* species presence and plant diversity, across an

103 elevation gradient (McKibben and Henning, 2018). Altogether, these studies suggest that North American  
104 hemiparasites may share functional similarities with European species. While these results suggest similar  
105 ecological impacts between North American and European hemiparasites, the inconsistent results and  
106 limited number of species surveyed highlight a need for more comprehensive studies and evidence in the  
107 North American context.

108 While generalist hemiparasites have the potential to connect to a wide range of species within a  
109 community, those that exhibit a strong host preference may have impacts that are resource-, habitat-, or  
110 community-dependent (Atsatt and Strong, 1970; Gibson and Watkinson, 1989). As a result, parasites with  
111 host preference have the potential to drive and contribute to community dynamics and species interactions  
112 across different temporal and spatial scales. However, our understanding of host-parasite biology is  
113 limited by the restrictive methods available to quantify belowground parasitism *in situ*. For example, host  
114 range can be gleaned directly by extracting the host and parasite via digging (Musselman and Mann,  
115 1977; Gibson and Watkinson, 1989), which risks the loss of haustorial connections during extraction. An  
116 alternative, indirect method to understand both host range and preference is via aboveground observation  
117 to detect the frequency with which a given potential host is more closely associated with the parasite than  
118 would be expected by chance alone (Gibson and Watkinson, 1989; Lymbery, 1989). While this method is  
119 non-destructive, it is limited due to the lack of confirmation of active haustoria on closely associated  
120 plants. Ex situ methods, such as greenhouse experiments, can provide vital information about how host  
121 species influence parasite growth rates (Matthies, 2017a), carbon acquisition (Press et al., 1987;  
122 Tennakoon and Pate, 1996; Světlíková et al., 2016), and resource allocation (Matthies, 2017b), but are  
123 limited in their ability to predict host species *in situ*. Despite these difficulties, a few studies have  
124 attempted to characterize the host range of parasitic *Castilleja* in North America, both *in situ* (Musselman  
125 and Mann, 1977; Montes-Hernández et al., 2015) and ex situ (Malcolm, 1966; Montes-Hernández et al.,  
126 2019), finding support for a generalist habit. However, no study has cross-validated the direct and indirect  
127 methods for assessing hemiparasitic plant hosts *in situ*. Connecting host preference to impacts on plant  
128 community diversity *in situ* will allow for more accurate predictions of the ecological impacts of  
129 hemiparasitic plants in natural communities.

130 Here, we address critical knowledge gaps for North American root hemiparasitic plants in the  
131 Rocky Mountain region. First, we established the relationships between three hemiparasitic *Castilleja*  
132 species and the diversity and composition of plant communities. Second, we assessed the host range and  
133 preference of each species *in situ* using both direct and indirect sampling methods. We posed three  
134 questions: (1) Is the presence of *Castilleja* species associated with more diverse plant communities? (2) Is  
135 the presence of *Castilleja* species associated with plant community compositional differences? (3) Do the  
136 *Castilleja* species of the Colorado Rockies exhibit host preference? We hypothesized that plant diversity

137 would be higher in the presence of *Castilleja*, and community composition to differ between presence and  
138 absence plots, potentially interacting with site and species-specific effects. We expected *Castilleja* of the  
139 Colorado Rockies to have broad host ranges, but we anticipated evidence for species-specific host  
140 preferences for locally dominant species.

141

## 142 METHODS:

### 143 Study Sites

144 We conducted a two-year study in the summers of 2023-24 at the Rocky Mountain Biological  
145 Laboratory (RMBL), located in the East River valley of the West Elk mountains, approximately 10 km  
146 North of Crested Butte, Colorado (38.9592° N, 106.9898° W, elevation 2,895 m). Field sites spanned an  
147 elevation gradient of 2,637 m to 3,191 m, with ecosystems ranging from sagebrush steppe to montane and  
148 subalpine meadows. The region receives significant annual snowfall from November to May, with  
149 accumulations of 246 cm in 2023 and 164 cm in 2024 (Barr, 2025). Average summer temperature and  
150 precipitation (May – August) were 20.1 °C, 0.80 in for 2023 and 18.1 °C, 2.24 in for 2024 (Barr, 2025).  
151 We studied three species of *Castilleja* across seven study sites. All sites were surveyed in both summers  
152 of 2023 and 2024, except for Johnson Hill, Copper Creek, and Almont, which we established and  
153 surveyed in the summer of 2024 (Table I).

154 We studied communities with *Castilleja chromosa* A. Nelson at one site, “Almont,” located  
155 roughly 11 km east of CO-135 along Jacks Cabin Cutoff (Table I), south of RMBL. *C. chromosa* grows  
156 in sagebrush communities, where it receives direct sunlight and is situated on dry, rocky sediment. It  
157 occurs at lower elevations than the other species studied and flowers from late April to early July.  
158 Dominant members of this plant community included *Chrysothamnus depresus* Nutt., *Artemisia*  
159 *arbuscula* Nutt., *Chrysothamnus vaseyi* (A.Gray) Greene, and *Phlox multiflora* A. Nelson.

160 We studied communities with *Castilleja linariifolia* Benth. at three sites. Two of the sites, “Deer  
161 Creek 1 & 2”, are roughly 1.5 km from RMBL on a subalpine meadow hillslope above the Deer Creek  
162 Trail (Table I). Dominant members of the Deer Creek plant community included *Artemisia tridentata*  
163 Nutt., *Symporicarpus rotundifolius* A. Gray, *Eriogonum umbellatum* Torr. var. *majus* Hook., and  
164 *Festuca thurberi* Vasey. The third site, “Johnson Hill”, is located on a small hill near the RMBL townsite  
165 (Table I). Dominant members of the plant community included *Populus tremuloides* Michx., *Berberis*  
166 *repens* Lindl., *Heliomeris multiflora* Nutt., *Lathyrus lanszwertii* Kellogg, and *Bromus frondosus* (Shear)  
167 Wooton & Standl. *C. linariifolia* grows in both sagebrush communities and subalpine meadows  
168 dominated by perennial wildflowers and grasses. It grows at higher elevations than *C. chromosa* and  
169 flowers throughout the growing season.

170 We studied communities with *Castilleja septentrionalis* Lindl. at three sites. The first site, “Avery  
171 Picnic”, is roughly 2.3 km north of RMBL in a meadow by the Avery Picnic parking area (Table I). At  
172 Avery, dominant species included *Taraxacum officinale* F.H. Wigg, *Potentilla pulcherrima* Lehm., *Poa*  
173 *pratensis* L., *Erigeron subtrinervis* Rydb. Ex Porter & Britton, and *Ipomopsis aggregata* (Pursh) V.E.  
174 Grant. The second site, “Copper Creek”, was located roughly 1.5 km east of RMBL along the Copper  
175 Creek trail (Table I). Dominant species at Copper Creek included *Eremogone congesta* (Nutt.) Ikonn.,  
176 *Taraxacum officinale*, *Poa pratensis*, *Thalictrum fendleri* Engelm. ex A Gray, and *Maianthemum*  
177 *stellatum* (L.) Link. The third site, “Emerald Lake”, roughly 7.2 km north of RMBL, was located in a  
178 meadow 50 m southeast of Emerald Lake (Table I). Dominant members of the plant community included  
179 *Potentilla pulcherrima*, *Fragaria virginiana* Mill., *Senecio crassulus* A. Gray, *Deschampsia cespitosa*  
180 (L.) P.Beauv., and *Anemone multifida* Poir. In general, *C. septentrionalis* grows in moist subalpine  
181 meadows dominated by perennial wildflowers at moderate to higher elevations and flowers all summer.

182 Although we did not conduct community diversity or composition analysis for *Castilleja miniata*  
183 Douglas ex Hook. var. *miniata* is a common and abundant species that occurs across a wide range of  
184 habitats throughout the study area, and we were able to conduct host assessments for the species.

185

#### 186 Community Diversity Sampling

187 We estimated community composition and species abundance at each study site from 20 50 x 50  
188 cm (0.25 m<sup>2</sup>) paired plots with *Castilleja* (treatment) or without *Castilleja* (control) (40 plots  
189 total/site/year). At each study site, we selected 20 random sampling points along a 50 m transect to place  
190 a 0.25 m<sup>2</sup> quadrat centered around the closest *Castilleja*. We then selected a paired plot within 2 m, which  
191 was absent of *Castilleja* or any other hemiparasite. In each plot, we recorded the number of plant species,  
192 the number of individuals of each species, and the percent cover of each species, including bare ground.  
193 In *Castilleja* present plots, species growing within a 10 cm radius of *Castilleja* were identified and  
194 documented as nearest neighbors (NN) (Gibson and Watkinson, 1989). In each year, 20 plot pairs were  
195 sampled within each study site. All 2023 sites were resampled in 2024, in addition to those established in  
196 the second year (total  $n = 220$  paired-plots) (Table I). Samples of all plant community members were  
197 vouchered at the RMBL Herbarium to ensure the accuracy of species identifications. As population  
198 density and habitat varied between sites and species, we occasionally modified the specific data collection  
199 methods to ensure accurate surveys of all sites (see below).

200 We sampled *C. chromosa* without modifications to the methods above (total  $n = 20$  paired  
201 plots). *C. linariifolia* populations required several modifications. Given their lower density relative to  
202 other *Castilleja* species, we found it more effective to sample every *Castilleja* that we encountered along  
203 the transect at both Deer Creek Sites. In denser clumps of *Castilleja* (<1 m apart), the focal *Castilleja*

204 plant was selected via a random number generator. At Johnson Hill, the population was too sparse for  
205 transect sampling. Instead, every *Castilleja* individual in the population was sampled.

206

207 **Host identity and haustoria analysis**

208 We collected *Castilleja* nearby but not within our study plots (Table SI). To identify hosts, we  
209 excavated soil and roots to a depth of ~15 cm within a 10 cm radius of a focal *Castilleja* plant. Excavated  
210 plants were then transported to the RMBL Herbarium and soaked in water. Typically, soils with a higher  
211 clay content were soaked for longer, usually over 12 hours. We then agitated the root mix to release  
212 adhered soil particles and prepared collections for observation. Root mats were examined under a  
213 dissecting microscope to identify haustoria. If haustoria were found, we recorded the host species as a  
214 confirmed host. When attached haustoria could not be traced back to a host plant, either due to severed  
215 root connections or high root density, haustoria segments with unidentified host roots were collected and  
216 preserved with the specimen. The *Castilleja*, host, and haustoria were then vouchered as an extended  
217 specimen for the RMBL Herbarium (Teixeira-Costa et al., 2023). In total, we made 29 collections of *C.  
218 chromosa*, *C. linariifolia*, *C. miniata*, and *C. septentrionalis* from 2023 to 2025 (Table SI).

219

220 **Statistical Analysis**

221 Data curation, analysis, and visualizations were conducted using R Statistical Software version  
222 4.5.1 (R Core Team, 2024) in RStudio (Posit team, 2025). *Castilleja* species were removed from the  
223 dataset for all diversity and compositional analyses to determine differences between the co-occurring  
224 plant community (Wickham, 2016). To assess differences in Shannon diversity, plant richness, and  
225 Pielou's evenness between *Castilleja* presence and absence plots, we used a linear mixed-effect model in  
226 the lme4 package (Bates et al., 2003) with *Castilleja* presence, species, and year as fixed effects, and pair-  
227 plot ID and site as random intercepts [lme4 package (Bates et al., 2003)]. Model assumptions were  
228 assessed using the performance package (Lüdecke et al., 2019). Post-hoc analyses were then conducted  
229 using the emmeans package (Lenth, 2017) to investigate pairwise differences between sites, presence-  
230 absence plots, and their interactions. We used the Anova function from the car package (Fox et al., 2001)  
231 to conduct null hypothesis tests for all fixed effects in the mixed models. To determine differences in  
232 species composition, we first conducted an indicator species analysis (De Cáceres et al., 2010) for each  
233 site to assess which species were associated with *Castilleja* presence or absence communities. We then  
234 performed a Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray-Curtis  
235 dissimilarities and the adonis() function in the vegan package (Oksanen et al., 2001) with *Castilleja*  
236 presence, species, year, and site, and their interactions as predictor variables. Data were visualized using  
237 ggplot2 (Wickham et al., 2007), and ggpubr (Kassambara, 2016) packages.

238 To analyze nearest neighbor data, we conducted a linear regression of relative NN frequency (the  
239 number of observations of a species as a NN divided by the total number of NN observations) on the  
240 relative total frequency of all species. This way, the total expected frequency is 100% for both NN and  
241 total. We then calculated 95% prediction intervals alongside 95% Confidence intervals. Species that fell  
242 outside the 95% prediction interval were significantly more (or less) abundant as a NN than expected by  
243 chance alone.

244

## 245 **RESULTS:**

### 246 **Community diversity is higher in association with *Castilleja***

247 Across sites, species, and years, we found that the presence of *Castilleja* was positively  
248 associated with plant community Shannon diversity ( $\chi^2 = 13.57, p < 0.001$ ) (Fig. 1a), which was driven by  
249 increased species richness ( $\chi^2 = 18.32, p < 0.001$ ) (Fig. 1b), but not evenness ( $\chi^2 = 2.06, p = 0.151$ ) (Fig.  
250 1c). For species richness averaged across hemiparasite species, our post-hoc analysis showed higher  
251 species richness in *Castilleja* presence plots by about half a species (estimate = 0.590) ( $p = 0.08$ ) in 2023  
252 and by 0.844 species ( $p < 0.001$ ) in 2024. These results support our hypothesis that plant diversity is  
253 higher in the presence of *Castilleja*. Contrary to our expectations, we found no significant differences  
254 among *Castilleja* species in their impact on co-occurring plant Shannon diversity, species richness, or  
255 species evenness. These results suggest that the presence or absence of *Castilleja* is a stronger predictor of  
256 plant community diversity than the potential variation among species within the genus. Sampling year,  
257 most likely driven by interannual changes in snowpack, temperature, and precipitation, was significantly  
258 correlated with changes in Shannon diversity ( $\chi^2 = 35.36, p < 0.001$ ), species richness ( $\chi^2 = 141.69, p <$   
259  $0.001$ ), and species evenness ( $\chi^2 = 6.07, p < 0.05$ ) (Fig. 1).

260

### 261 **Plant community composition is robust against *Castilleja*'s effect on species diversity**

262 Despite differences in diversity, we found that *Castilleja* presence was not associated with  
263 differences in plant community composition (PERMANOVA  $F_{1,424} = 0.77, p = 0.72$ ), contrary to our  
264 expectations (Fig. 2). However, community composition was significantly predicted by species  
265 (PERMANOVA  $F_{2,424} = 91.90, p = 0.0001$ ), site (PERMANOVA  $F_{4,424} = 40.97, p = 0.0001$ ), and year  
266 (PERMANOVA  $F_{4,424} = 21.85, p = 0.0001$ ). Our indicator species analysis revealed several significant  
267 associations between co-occurring plant species and *Castilleja* species at the site level (Table II). This  
268 suggests that while differences in plant community diversity and site-level compositional changes exist,  
269 they were either insufficient in magnitude or consistency to alter community composition between  
270 hemiparasite presence and absence plots across Rocky Mountain plant communities.

271

272 **Rocky Mountain *Castilleja* are generalist hemiparasites**

273 Plants within the *Castilleja* genus appeared to have a broad host range with some host preference  
274 for common and dominant members within montane plant communities (Fig. 3). Direct excavation of  
275 host-parasite complexes revealed 10 confirmed host species with which *Castilleja* species formed  
276 haustoria (Fig. 3). Our nearest neighbor analysis identified 13 plant taxa more likely to occur within a 10  
277 cm radius of *Castilleja* than by chance alone (Fig. 4). By functional group, forbs (11 taxa) and grasses (7  
278 taxa) represent the most common potentially preferred host species (Fig. 3). In total, our analysis shows  
279 that only 21 of the 249 plant taxa found in our dataset appear to be potential preferred hosts. Direct  
280 observations of haustoria were confirmed on only three of the 13 species that appeared in our Nearest  
281 Neighbor analysis, and two of the three species that emerged as indicator species in *Castilleja* presence  
282 plots. These results suggest *Castilleja* species are generalist hemiparasites capable of connecting to a  
283 broad range of host species.

284

285 **DISCUSSION:**

286 We found a consistent and positive relationship between *Castilleja* presence and co-occurring  
287 plant community diversity. Importantly, this was a general pattern across three *Castilleja* species, seven  
288 sites, and two years that differed strongly in abiotic conditions and overall plant diversity. These results  
289 expand on and confirm the findings of McKibben and Henning (2018), which similarly found higher  
290 plant community diversity across an alpine gradient in the presence of *Castilleja* species at RMBL.  
291 However, our results contrast with two other studies on North American *Castilleja*, which found no  
292 correlation between parasite presence and community diversity (Spasojevic and Suding, 2011; Reed,  
293 2012). One possible explanation for these differences is the observational nature of our study, which  
294 contrasts with the experimental removal (Reed, 2012), as that approach may require a longer duration to  
295 develop an effect.

296 Our results support the theory that the presence of hemiparasitic plants leads to increases in co-  
297 occurring plant diversity, and suggest potential similarities between the functioning of European and  
298 North American root-hemiparasites (Phoenix and Press, 2005; Fibich et al., 2017; Heer et al., 2018). In  
299 addition, our results lay the groundwork for future experimental studies to confirm the mechanisms by  
300 which root-hemiparasites facilitate increases in plant community diversity.

301

302 **Plant community composition is robust against *Castilleja*'s effect on species diversity**

303 Our results suggest that the consistent, albeit small, changes in diversity did not translate into  
304 consistent differences in community composition between *Castilleja* presence and absence plots, contrary  
305 to our hypothesis. These results contrast with the significant and small effect of *Castilleja* presence on

underlying plant community composition found by McKibben and Henning (2018). Interestingly, our indicator species analysis revealed several species that increased in relative abundance in association with the presence of *Castilleja* (Table II). These differences suggest small-scale compositional changes are likely specific to host-parasite relationships within a given site (Cameron and Phoenix, 2013). Across our study, we observed over 230 species, characterized by high turnover in species among sites. As plant communities were heterogeneous at the plot level, within the landscape, one would expect inconsistent changes in species composition, which could explain the lack of an overall effect of *Castilleja* presence on plant community composition.

314

### 315 **Rocky Mountain *Castilleja* are generalist hemiparasites**

Our findings provide the first list of putative and confirmed host species for Rocky Mountain *Castilleja* (Fig. 3). We found that Rocky Mountain *Castilleja* species parasitize a broad range of hosts, with no consistent preference for functional groups or species. Our findings suggest that *C. septentrionalis*, *C. linariifolia*, and *C. chromosa* behave as facultative generalist hemiparasites, consistent with hemiparasitic plants of the Orobanchaceae and in support of our hypothesis (Matthies, 2017c). Our work provides support for using both direct and indirect methods to assess host-parasite relations, as putative host species suggested by each method minimally overlapped (Fig. 3). Of particular interest are species, such as *C. septentrionalis*, which we found to parasitize *Fragaria virginiana*, and *C. linariifolia*, which we found to parasitize *Viola praemorsa* and *Eremogone congesta*. These were the only hosts identified through both direct haustoria observation and indirect analysis, which suggests these species may serve as preferential or higher-quality hosts and could be helpful in predicting the ecological impacts of *Castilleja*.

Our study highlights four considerations for reducing bias and maximizing inference when identifying hosts of hemiparasites. First, excavation of root systems can be particularly challenging for larger or woody host species. However, the avoidance of large plants can bias host species lists towards herbaceous and grassy species. In future studies, sampling efforts should prioritize sampling from the observed range of functional groups and growth forms to limit potential bias. Second, as haustoria connections can be delicate, excavation can often separate host and parasite root segments, thereby limiting the ability to trace roots back to aboveground tissue and identify hosts without the use of molecular methods. For our project, whenever possible, we collected and preserved host-parasite root segments, cataloging them alongside herbarium specimens for future molecular identification. Third, direct excavation can be limited by the persistence and potential inactivity of haustoria. To date, we are unaware of any study that has demonstrated how long haustoria persist and remain active on an individual host in the field, which would significantly increase our understanding of host dependency and

340 persistence. Fourth and finally, in our study, we often observed *Castilleja* parasitizing multiple species  
341 within a given excavation effort (Table SI), which makes host identity via attached or severed root  
342 segments more challenging to confirm. The most rigorous approach, therefore, would be to use a  
343 combination of molecular and tracing techniques (Světlíková et al., 2016).

344 Nearest neighbor analysis, especially when paired with additional analyses such as indicator  
345 species, provides an ecologically informed estimate of potential hosts. This is because parasitic  
346 Orobanchaceae are known to have limited root growth (Van Hoveln et al., 2011), so species detected as  
347 nearest neighbors are likely to be hosts, particularly for herbaceous hosts. However, this statistical method  
348 is less likely to detect host species with deep or sprawling root systems, such as woody species that occur  
349 outside the 10 cm radius. Nevertheless, our host species list provides useful information for future studies  
350 interested in assessing host-parasite relationships in the field. This enables us to better understand how  
351 host identity influences parasite fitness, the ecological impacts of parasitism, and ultimately, to increase  
352 our ability to predict the fate of natural communities.

353

## 354 CONCLUSIONS:

355 Our study reveals a positive correlation between the presence of *Castilleja* and the diversity of  
356 Rocky Mountain plant communities. The results of this study provide evidence for the ubiquitous nature  
357 of hemiparasitic plants as potential keystone species. Future work should incorporate assessments of  
358 productivity alongside diversity, composition, and host identity within plots to better connect host and  
359 diversity studies. Additionally, there is a great need for experimental manipulations to identify the  
360 underlying mechanisms between the presence of hemiparasitic plants and increased plant community  
361 diversity. This would provide a more complete investigation into the effect of hemiparasites on  
362 community composition.

363

364

## 365 CRediT Statement:

366 **Jordan Argrett:** Conceptualization, Methodology, Validation, Formal analysis, Investigation,  
367 Supervision, Project administration, Funding acquisition, Resources, Data Curation, Writing - Original  
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