

HOST-PLANT SPECIALIZATION AND NESTING BIOLOGY OF
ANTHIDIUM PLACITUM (MEGACHILIDAE) IN NORTHWEST CALIFORNIA

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ABSTRACT

HOST-PLANT SPECIALIZATION AND NESTING BIOLOGY OF *ANTHIDIUM PLACITUM* (MEGACHILIDAE) IN NORTHWEST CALIFORNIA

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- *Premise of the study:* Although the study of bees and their pollination services has grown immensely in recent years, the natural history of most solitary bee species is still largely unknown. The goal of this study was to contribute to the natural history dossier of a late-season wool carder bee, *Anthidium placitum* Cresson (Megachilidae), by establishing which plants it uses as sources of nectar and pollen as well as documenting details of its flower-handling behavior, mating behavior, and nesting biology in northwestern California.
- *Methods:* Field observations were made at five sites in Del Norte, Humboldt, Siskiyou, and Trinity counties in California, USA. Pollen use was determined via microscopic examination of samples taken from 244 foraging females and 13 larval provisions. Naturally occurring nests were difficult to find, so I deployed aerial trap nests and created clusters of artificial soil cavities in an effort to obtain nest cells and determine preferred nesting substrate. Light microscopy and SEM were used to identify nest cell trichomes and to check females for specialized clypeal and basitarsal hairs.
- *Key findings:*

- Analysis of scopal pollen loads and larval provisions revealed that *A. placitum* is oligolectic on *Cordylanthus tenuis* ssp. *viscidus* (Orobanchaceae). The pollen of this species comprised >99 % of all samples. Females collect pollen from the nototribic flowers of this species by rubbing specialized clypeal hairs against dorsally located anthers.
- Both males and females appeared to use flowers of *C. tenuis* ssp. *viscidus* as their sole source of nectar.
- Males displayed resource defense polygyny, aggressively guarding patches of *C. tenuis* ssp. *viscidus* as mating venues.
- Nine nests were discovered, all in pre-existing soil cavities.
- Natural nests were extremely cryptic and distributed in non-aggregated fashion across apparently suitable habitat; 140+ hours of searching yielded only three nests. Six of nearly 1400 artificial holes bored in the ground yielded nests.
- Nests contained one or two cells, each constructed entirely of woolly plant hairs. The source of hairs used for cell construction varied across sites, depending on the local availability and relative abundance of woolly-haired plants.
- Nest entrances were closed with an average of >300 small pebbles and plant parts, which females carried one by one in quick flights from nearby sources.
- Although the basitarsi of females were covered with dense hairs similar to the tomentum used by other species of *Anthidium* to collect extrafloral trichome

secretions, none of the nest cells showed evidence of incorporation of secretions.

- *Implications:* Effective conservation of native bees depends on understanding critical details of their life histories. Here I show that *A. placitum* depends on a single plant species for both pollen and nectar. This hemiparasitic forb, in turn, relies on its conifer host trees as a source of water and mineral nutrients. Thus a reduction in the number of these trees via fire, disease, or logging could have a negative impact on the bee. Although attempts to entice bees to use artificial soil cavities as nest sites were largely unsuccessful, modification of this approach may ultimately provide an effective approach for studying the nesting biology of other ground-nesting bees with cryptic, non-aggregated nests.

TABLE OF CONTENTS

ABSTRACT	ii
LIST OF TABLES	vi
LIST OF FIGURES.....	vii
INTRODUCTION.....	1
MATERIALS AND METHODS	8
Study Area	8
Pollen Sources	9
Nesting Biology	10
RESULTS AND DISCUSSION	13
Host-plant Specialization.....	13
Nesting Biology	16
CONCLUSIONS.....	20
LITRATURE CITED.....	23

LIST OF TABLES

Table 1. Study sites: locations, dates visited, co-flowering species, and number of scopal pollen loads samples. Note that Carmen Lake was visited in both 2017 and 2018.	29
Table 2. Artificial underground trap nests: number of holes deployed at each site and number nest cells provisioned. Holes were clustered for convenience.	30
Table 3. Aerial trap nests: number of holes deployed and number of cells provisioned. Thirty-six holes were drilled in five blocks of wood at both sites.	31
Table 4. Description of nests. N = Natural, A = Artificial. P = Pebbles, C = Conifer needles/twigs. Tunnel depth = distance from the nest entrance to the nest cell. Cell depth = the vertical distance from the tip of a nest cell to the soil surface.	32
Table 5. Description of nest cells.	33

LIST OF FIGURES

Figure 1. <i>Anthidium placitum</i> female, face view.	34
Figure 2. Map of study sites. Carmen Lake, Horse Mountain, Lassics, and French Hill were visited in 2017. Carmen Lake and Dillon Creek were visited in 2018.	35
Figure 3. Modified hairs on the clypeus of an <i>Anthidium placitum</i> female. Hairs taper into hooked, wavy ends that aid in collecting pollen grains. <i>Cordylanthus tenuis</i> ssp. <i>viscidus</i> pollen grains shown on some of the hairs.	36
Figure 4. Fore basitarsus of an <i>Anthidium placitum</i> female. Dense tomentum is used to collect extrafloral glandular secretions incorporated in nest cells. Exudates on nest cells were not observed in this study despite the presence of tomentum on basitarsi of <i>Anthidium placitum</i>	37
Figure 5. Artificial soil trap nests. Clusters of holes were made in bare soil near patches of <i>C. tenuis</i> ssp. <i>viscidus</i> . Arrows point to nest entrances.	38
Figure 6. <i>Eriophyllum lanatum</i> : light micrograph of foliar trichomes. Distinctive swellings on the trichomes (black arrows) allowed for easy identification when analyzing nest cells.	39
Figure 7. Aerial trap nest deployed at the Grouse Mountain site in 2018.	40
Figure 8. Flower-handling behavior of <i>A. placitum</i> on <i>Cordylanthus tenuis</i> ssp. <i>viscidus</i> . Females always entered COTE flowers in a position that ensured the modified hairs on the head came in contact with the dorsally located anthers. White arrows point to lower corolla lip, yellow arrows point to upper corolla lip. A. ANPL female collecting pollen from an upright COTE flower. B. ANPL female collecting pollen from an upside-down COTE flower.	41
Figure 9. Map of the range overlap of <i>Anthidium placitum</i> and <i>Cordylanthus</i> . <i>A. placitum</i> records taken from AnthWest database (Griswold and Gonzalez 2013). <i>Cordylanthus</i> records taken from several consortiums of herbaria specimens.	42

Figure 10. A single nest cell. Blue color indicates the apex of the cell. Blue powder was used to track nest tunnels during cell excavation.....43

Figure 11. Evidence of trichome scraping by *Anthidium placitum*. A. *Eriophyllum lanatum*, adaxial surfaces, French Hill. B. *Eriogonum congdonii*, abaxial surface, Carmen Lake. C. *Eriogonum strictum* ssp. *greenei*, Lassics.....44

INTRODUCTION

The life cycle biology of bees is unified by a need for flowers, which provide nutrition in the form of nectar and pollen, within flying distance of suitable nesting areas. Superimposed on this common theme are richly detailed differences in the kinds of flowers and nesting substrates required by different species. For example, most bee species can exploit a variety of unrelated plants as sources of pollen (polylectic), but others are specialists that require pollen from a particular taxonomic group (oligolectic); most species build and provision nest cells below ground, but others use or excavate cavities above ground; and some species require materials collected from the habitat for construction of nest cells while others don't. Recent concerns about the potential impact of habitat destruction and degradation on bee diversity and abundance – and on the reproductive success of the plants that depend on them – has drawn attention to the lack of detailed natural history information for the majority of native species (Rathcke and Jules 1993; Cane 2001; Brown & Paxton 2009; Lebuhn et al. 2013). Such information is vital for successful conservation and restoration efforts, especially for specialist bees that rely on a limited set of plants for larval provisioning and nest building needs.

Megachilidae is the second largest family of solitary bees, comprising more than 4,000 species in 76 genera worldwide (Michener 2000; Gonzalez 2012). Unlike most other bees, members of the family use a variety of different foreign materials for nest cell construction, including mud, leaf blades, petals, fruit pulp, small pebbles, plant trichomes, tree resins, and plastics (Grigarick & Stange 1968; Litman et al. 2011;

Gonzalez et al. 2012; MacIvor & Moore 2013; Cane 2019). A wide variety of nesting substrates are also used by different members of the family. For example, most megachilids nest above-ground in substrates ranging from cavities in wood to snail shells (Stephen et al. 1969; Martins et al. 1994; Michener 2000; Gonzalez 2012). Others nest below-ground, adopting naturally occurring cavities in soil, or less commonly, excavating their own (Cane 1991; Otto 2006; Cane et al. 2007; Gonzalez 2012). Although diverse and both ecologically and economically important, the life cycle biology of many members of this family is poorly understood (Müller 1996b; Gonzalez & Griswold 2013; Vitale, Gonzalez, & Vázquez 2017).

With over 180 described species worldwide, *Anthidium* Fabricius is the fourth most diverse genus in Megachilidae. Although well-represented in the Western Hemisphere, information on the natural history of only a small number of North American species has been documented (Gonzalez & Griswold 2013). *Anthidium* are commonly referred to as “wool-carder” bees because they construct their nest cells out of cotton-like plant trichomes that females harvest from woolly leaves and stems using multi-dentate mandibles (Melander 1902; Michener 2000). The source of the trichomes used for cell construction, or whether females show selectivity, is not well known, but woolly-leaved members of Lamiaceae, Asteraceae, and Polygonaceae (*Eriogonum*) are often reported as sources (Payne et al. 2011; Gonzalez & Griswold 2013; Eltz 2015). Many *Anthidium* also have dense tomentum on the outer surface of the basitarsi that is used to absorb extrafloral trichome secretions. Females smear these secretions over the

woolly nest cell for their hydrophobic, antimicrobial, and parasitoid-repellent properties (Müller, Topfl, & Amiet 1996; Eltz 2015).

Pollen-use specialization has been well-documented for *Anthidium* in the Eastern Hemisphere (Müller 1996b), but floral relationships are still largely unknown for Western Hemisphere species (Gonzalez & Griswold 2103). Many species specialize on the nototribic flowers of Lamiaceae and Scrophulariaceae *sensu-lato*. The anthers and stigmas of nototribic flowers are positioned on the upper surface of a bilabiate corolla, so pollen is deposited on and removed from the head and dorsal surface of a pollinators body. Morphological adaptations and unique flower-handling behaviors can be associated with this floral morphology (Macior 1967; Müller 1996a). For instance, many *Anthidium* species have specialized hairs of the clypeus (area of head between antennae and labrum) that increase the efficiency of pollen collection from the dorsally located anthers. These modified hairs are generally thick at the base and taper to a wavy, hooked, or curved apex (Müller 1996a; Gonzalez & Griswold 2013). This specialized pilosity is restricted to females, who handle the flowers by inserting their heads into the tubular corolla and rubbing their clypeus over the dorsally-located anthers with rapid back and forth movements, gathering pollen grains in the specialized facial pilosity in the process (Müller 1996a). Immediately after visiting a flower, females relocate pollen from the clypeus to the abdominal scopa with their forelegs (Müller 1996b).

Aggressive male territorial behavior has been well documented for many *Anthidium* species (Jaycox et al. 1967; Alcock et al. 1977; Wirtz et al. 1988; Sugiura 1991; Villalobos & Shelly 1991; Lampert et al. 2014). Males use patches of the females'

preferred host plant as mating venues and aggressively ward off, and in some cases even kill, any other floral visitor that encroaches. The males patrol their patches and copulate with females that enter to obtain floral resources (Jaycox 1967; Eickwort 1977; Severinghaus, Kurtak, & Eickwort 1981; Wirtz 1988; Westrich 1989; Sugiura 1991; Praz 2008; Lampert et al. 2014).

Anthidium placitum Cresson, hereafter ANPL, is a late-season species (most records from late June through September) restricted to arid environments in Western North America (Grigarick & Stange 1968; Gonzalez & Griswold 2013) (Figure 1). Most collections in California are from east of the Central Valley or from southern counties, with few records from the northern part of the state (Figure 2). The life history of ANPL has not been thoroughly documented, though previous work has shown that females possess modified clypeal hairs and a dense tomentum on their basitarsi (Gonzalez and Griswold 2013) (Figures 3 & 4).

Museum collections suggest that ANPL specializes on *Cordylanthus* (Orobanchaceae), a group of late-blooming, hemi-parasitic species restricted to western North America (Grigarick & Stange 1968). These annuals have drab nototribic flowers with two fully closed corolla lips, requiring pollinators to push their way in, a morphology termed personate. All *Cordylanthus* species are hypothesized to be self-incompatible, implying a dependency on pollinators for reproduction (Chuang and Heckard 1986). With a bloom period generally lasting from mid-July through September, *Cordylanthus* are remarkable for their ability to grow and flower during hot summer months in arid habitats. The hosts of these hemiparasitic plants provide a source

of water and mineral nutrients which allows them to bloom at a time when most co-occurring plants are senescing (Chuang and Heckard 1986). *Cordylanthus tenuis* ssp. *viscidus*, hereafter COTE, was the dominant flowering plant present at all of my study sites. This subspecies occurs from Sacramento County into southern Oregon, generally occurring in dense, aggregated patches on serpentine outcrops of open slopes and flats of yellow-pine forests (Chuang and Heckard 1986) (Figure 2). Hosts include *Pinus jeffreyi*, *Calocedrus decurrens*, and *Arctostaphylos* spp. (Chuang and Heckard 1971).

The hallmark of oligolecty is consistent collection of pollen from the same taxonomically restricted subset of plants (e.g., members of the same clade, usually a genus). In the strict sense this specialization is genetically fixed and maintained even when other potential pollen sources are available at one site or across the range of the species (Sipes & Tepedino 2005). This definition is straightforward, but distinguishing true oligolecty from other forms of specialization, which are not genetically fixed, can be challenging. Most individual generalist foragers often develop short-term fidelities for conspecific flowers that may be the most abundant pollen source at a given time and place, a behavior termed “floral constancy” (Cane and Sipes 2006). Like oligolectic bees, such foragers collect “pure”, unmixed pollen loads, but unlike oligolectic species, individual foragers of generalist bees tend to specialize on different plants as the quirky outcome of their idiosyncratic foraging histories. Short-term affinities can last from a single foraging bout to up to a few days, with individuals switching to other pollen hosts when their preferred host plant is inaccessible. Since such “ecological specialization” (Fox and Morrow 1981) is expected to vary from site to site in response to differing plant

communities, a critical test for oligolecty requires documenting consistent pollen preference of a single taxon at several sites with differing sets of potential alternative pollen sources.

Observing flower visitation is not a good basis for establishing the degree of pollen specialization because oligolectic bees may visit several plant species for nectar (Michener 2000, Ritchie et al. 2016). A better method is to analyze the pollen loads collected by foraging females, but since generalist bees can show a temporary preference for a specific pollen source, a pure scopal load can reflect floral constancy and not true oligolecty. The best approach is to analyze the pollen composition of nest cell provisions, with the important stipulation that several provisions, integrating the pollen collection behavior of several different foragers, be examined from several sites across the range of the species (Cane and Sipes 2006). Analyzing nest cell pollen is more informative because pollen collected for larval provisions is a result of numerous foraging trips and can therefore better represent floral host choices throughout the provisioning of a cell. However, since finding and dissecting a large number of nest cells is neither practical nor ethical, an alternative is to combine documentation of floral visitation behavior with pollen analyses of both scopal loads and larval provisions from a large number of different females at several sites.

The goal of my thesis research was to contribute to the natural history dossier of *Anthidium placitum*, with a focus on the kind of pollen it uses and its nesting biology. My primary specific objective was to determine if ANPL is oligolectic on *Cordylanthus tenuis* ssp. *viscidus* throughout its local range in northwestern California. Other

objectives were to describe the nesting biology of ANPL as well as its flower-handling and mating behavior.

MATERIALS AND METHODS

Study Area

Populations of ANPL were studied at four sites in Northwest California from mid-July through August of 2017. The four sites were located in four counties to test for consistency in pollen preference across the species' local range (Figure 2, Table 1). ANPL had been previously observed at three of the sites (Grouse Mountain, Carmen Lake, Lassics); the French Hill site was chosen based on herbarium records of COTE (Humboldt State University Vascular Plant Herbarium). Research was conducted at three of the sites (Carmen Lake, Grouse Mountain, and Lassics) in single trips to see if ANPL consistently collected pollen from COTE at different locations at approximately the same time. For convenience, work at the French Hill site was conducted one to two days prior to each of the longer trips. COTE was clearly the dominant flowering plant at all sites, but a variety of less abundant co-flowering plants were also present (Table 1).

In 2018, research was carried out at the Carmen Lake and Dillon Creek sites where the primary focus was obtaining more data about the nesting biology of ANPL. The Dillon Creek site was visited twice; on August 9th to establish ground trap nests and on August 25th to check for occupancy by ANPL. Effort was concentrated at Carmen Lake because it had the largest population of COTE and the greatest number of ANPL in the previous year. Unfortunately, both the plants and the bees were much less abundant than expected in 2018, partly due to grazing and trampling by cattle as well as well dense smoke from the nearby Carr Fire. Dillon Creek was chosen based on reports of a large

population of COTE. Here again, however, conditions were densely smoky during the foraging season, and flowers and female bees were uncommon.

Pollen Sources

Inferences about potential pollen specialization were based on analysis of pollen loads of foraging females as well as nest cell provisions. Foraging females were captured in aerial nets, placed in plastic vials, and temporarily immobilized on ice. Once they were immobile, pollen was removed from the scopa with fine forceps and mounted in fuchsian gel on a microscope slide for later analysis. Megachilids tend to relocate pollen from the head and body to the abdominal scopa periodically, placing pollen in layers that may represent flowers visited sequentially throughout a foraging trip (Stephen et al. 1969; Gonzalez 2004). In an attempt to sample pollen collected at different times throughout the individual's foraging bout, three pollen subsamples were taken from different areas and depths of the individual scopal load. To avoid re-sampling the same individual, bees were marked with a dot of acrylic paint on the thorax before releasing. Potential contamination across successively captured bees was an important concern. To minimize such contamination, I: (i) removed any obvious clumps of pollen from the nets between captures; (ii) used new plastic vials for each bee; (iii) sanitized forceps with ethanol between taking samples; and (iv) avoided sampling pollen from the immediate surface of scopal loads. Pollen from larval provisions was taken from excavated nest cells and suspended in 70% ethanol. Three sample aliquots were taken per provision and mounted on microscope slides for pollen counting. Pollen grains were counted along five

randomly chosen transects until 500 grains had been counted per subsample, totaling 1,500 pollen grains per sample. Pollen grains were identified at a magnification of 400X or 1000X with the aid of site-specific reference collections of pollen from all flowering plant species at each site. Since some contamination from wind-blown pollen or from flowers visited for nectar (but not pollen) is inevitable even for strict oligolects, a 95% threshold of congeneric pollen for oligolecty was established (Cane and Sipes 2006).

Nesting Biology

I attempted to describe the nesting biology of ANPL by locating natural nests as well as deploying trap nests. To increase chances of discovery, I watched for females entering or departing active nests and looked for telltale piles of natural materials (small pebbles, conifer needles, twigs) that many ground-nesting *Anthidium* use to plug nest entrances (Gonzalez & Griswold 2013; Mesler unpublished). In an attempt to obtain a larger sample of nest cells as well as reveal the preferred nesting substrate of ANPL, I created both above and belowground trap nests. Below-ground trap nests were made at each site by boring clusters of eight to ten holes into the soil with a 45 cm long screwdriver, an approach that has worked successfully for both *Dianthidium subparvum* and ANPL (Pow 2016 unpublished) (Figure 5). Since detailed information about cavities used by soil-nesting bees is scarce, holes were dug at varying diameters (7-9 mm), depths (10-15 mm) and angles (30, 45, 60, or 90). A small rock was placed on the west side of

each hole as a visual cue that might increase the chance of occupancy (Cane 2015). An average of 233 subterranean holes were created per site (Table 2).

Ground nest clusters were inspected during each site visit for occupancy by ANPL. Excavation of nests consisted of: (i) noting the orientation, depth, and diameter of the hole occupied; (ii) collecting all nest plug contents; (iii) digging the cell(s) from the soil; (iv) noting the number of cells per nest and (v) placing the cell(s) in a plastic vial on ice for future analysis. For each nest cell the length, width, type(s) of trichomes used for nest cell construction, and type(s) of pollen in the larval provision were recorded. Each cell was then examined and photographed with an Olympus SZX 16 to check for the presence of trichome secretions. The type and number of nest plug materials used was also recorded. A site-specific reference guide of the various trichomes of pubescent plants present at each site was created and used to help determine the source of hairs used in nest cell construction. Distinctive node-like swellings characteristic of *E. lanatum* simplified identification (Figure 6).

Aerial trap nests were made by drilling 36 holes, spaced 10 mm apart, in recycled, untreated blocks of spruce wood (Figure 7). Holes were the same diameter used for below-ground trap nests and varied from 15 to 25 cm long. Paper straws were inserted into the holes to allow for easier and less destructive removal of cells. Nest blocks were attached to trunks of trees with metal wire at a south-east facing position and heights varying from 0.8 to 1.5 meters. Ten above-ground nests were established on July 14 2107 - five at Carmen Lake and five at Grouse Mountain. Nest blocks were periodically cleared of spider webs and inspected for the presence of ANPL nests throughout the

study. Above-ground trap nests were not deployed in 2018 because observations made the previous year showed that ANPL nests in the ground.

RESULTS AND DISCUSSION

Host-plant Specialization

The flight period of ANPL coincided with the bloom period of COTE over the two years of this study. ANPL is protandrous, with males emerging before females. In 2017, the first male and female sightings were on July 14th and 17th, respectively, at Grouse Mountain. In 2018, the first males and females appeared at Carmen Lake on July 17th and 21st, respectively. The first open COTE flower was seen at the Grouse Mountain site on July 10th in 2017 and July 12th in 2018. The number of ANPL peaked in the second week of August, when most COTE were in bloom.

Analysis of pollen from 244 scopal loads and 9 larval provisions revealed that ANPL is oligolectic on COTE in Northwestern California. All scopal loads and larval provisions examined were pure, containing over 99% COTE pollen. Other grains belonged to *Polygonum douglasii* (Polygonaceae), *Eriogonum* spp. (Polygonaceae), and unidentifiable species of Asteraceae. Systematic surveys of visitors to co-flowering species were not conducted, but neither male nor female ANPL were observed visiting flowers other than COTE. Thus, we can infer exclusive dependence on COTE for both nectar and pollen at my study sites, a phenomenon not usually observed (Cane 2018).

ANPL handled COTE flowers in a stereotypic fashion. Flowers of COTE are borne in loose clusters of one to three flowers that are more or less lax on their pedicels and thus vary in their orientation on a single inflorescence (Chuang and Heckard 1976). Most flowers are upright, but some are more or less inverted on the pedicels, with

the upper lip and reproductive parts in the ventral position, and others hang laterally. Regardless of the flower orientation, ANPL females handled flowers in a manner that caused the specialized clypeal hairs to contact the dorsally located anthers (Figure 8). Females approached a flower, grabbed the calyx with all legs and positioned themselves to enter the flower in an upright fashion. Then they pushed their head into the personate corolla and rubbed the modified clypeal hairs over the anthers to collect pollen grains. Similar foraging behavior has been well-documented in Eastern Hemisphere anthidiine bees with specialized clypeal hairs (Müller 1996b).

ANPL males exhibited aggressive intra- and interspecific territoriality exclusively at patches of COTE. Throughout the season males could readily be observed loudly buzzing about COTE patches, occasionally hovering over flowers in a systematic manner. When males approached a floral visitor in their claimed patch, they increased the pitch of their buzz immediately before attacking the intruder. The buzz of the males is far more audible than the low-pitched buzz of the females and likely serves as a warning signal to conspecific males and other encroaching insects. Males aggressively warded off any insect that came in proximity of their patch, from the small *Anthidiellum notatum* ssp. *robertsonii* (6 mm) to much larger *Bombus vosnesenskii* queens (up to 22 mm). This behavior, a form of resource defense polygyny, is well-documented among many male bees that patrol the flowers of the females preferred pollen source for potential mates (Eickwort 1977; Alcock et al. 1978; Eickwort & Ginsberg 1980; Lampert et al. 2014) and is relatively common in bees with non-aggregated nests (Paxton 2005). Observations of such resource defense polygyny adds more support for oligolecty

because males generally restrict this behavior to a single host plant at a given time and place (Alcock et al. 1977; Villalobos & Shelly 1991; Lampert et al. 2014).

My observations provide direct evidence that ANPL is oligolectic on COTE at four northern California populations, but circumstantial evidence suggests that it is broadly oligolectic on the genus *Cordylanthus* throughout its range in western North America. The distributions of ANPL and *Cordylanthus* overlap almost perfectly (Figure 9), and both groups occupy xeric habitats and are active from mid-June through September (Grigarick & Stange 1968; Gonzalez & Griswold 2013). Floral records are available for only 7.5% of existing museum specimens of ANPL (Gonzalez and Griswold 2013). Approximately 25% of these were collected on *Cordylanthus* (five species in addition to COTE), but the complete list includes members of 36 other genera in 11 families. Most of these taxa are represented by a single specimen, and only three (including *Cordylanthus*) have nototribic flowers. Caution is warranted because of the paucity of floral records and because some oligoleges are known to switch pollen hosts depending on local availability (Linsley & MacSwain 1958; Wcislo & Cane 1996; Cane and Sipes 2006), but available evidence suggests that ANPL relies exclusively on *Cordylanthus* as a pollen source throughout its range. Detailed analyses of scopal loads and larval provisions from across the range of the species will be needed to make a stronger case for strict oligolecty.

Nesting Biology

ANPL nests in pre-existing soil cavities. Nine nests, containing a total of thirteen cells, were found over the course of the study, all below ground. None of the aboveground trap nests were occupied (Table 3). Three of the nests were of natural origin. Since cavity dimensions of natural nests varied and no evidence of digging was observed, such as tumulus at the nest entrance, nests were likely constructed in naturally occurring cavities and not excavated by ANPL. In fact, one nesting female was seen entering a crevice it could not have excavated in serpentine rock.

The natural nests were not aggregated, unlike the nests of many soil-nesting bees that excavate their own cavities, but instead scattered on the landscape (Batra 1978; Cane 1991; Wcislo and Cane 1996; Otto 2006). Non-aggregated nesting makes locating natural nests extremely difficult, especially because entrances of completed nests can be cryptic due to the use of natural materials to plug entrances. Approximately 144 hours of observation, over two flight seasons, yielded the three nests discovered. Natural nests were detected by noticing females making frequent trips back and forth to nests to close entrances. Nest entrances were closed with mainly small pebbles and pieces of conifer needles and twigs. Females pick up a single item at a time and return to the nest to drop it in the entrance. This continues until the nest plug just exceeds the soil surface. An average of 359 natural materials, ranging from 261 to 523, were used to close a single nest entrance. Therefore, the number of nest plug bits indicates the number of collecting trips taken to complete the task. On average, these collecting trips took 10.5 seconds each ($n = 6$), which translates to an average of 63 minutes to close a single nest.

Natural nests differed in size and the number and arrangement of cells. The diameter of natural nest entrances ranged from 10 to 18 mm, with cells ranging from 47 to 68 mm below soil level at angles varying from 15 to 60 degrees from vertical (Table 4). Of the three natural nests, one contained a single cell and the others had two cells. The nests with two cells differed in their arrangement of the cells. In one case, the two cells were oriented vertically, with a transverse partition of woolly hairs in-between the tip of the first cell and the base of the second cell. The other nest contained two cells oriented in a “T” like configuration with one cell connected at a right angle from the other. The difference in the arrangement of the two-celled nests could be a reflection of the idiosyncrasies of natural cavities.

Of the 1,395 artificially-made holes, only six (0.43%) were utilized by ANPL as nest sites (Table 2). Five of the artificial nests contained a single nest cell. The sixth hole contained three cells, each in its own cavity. In this case, the artificial hole likely intersected pre-existing cavities, creating separate chambers for cell construction.

There was more variation in the size of natural nest cells compared to artificial nest cells (Table 4). Natural nest cells varied from 12 to 27 mm in length and 9 to 18 mm in width. One female was seen closing a natural nest entrance that was eighteen mm in diameter (two and a half times larger than the diameter of an ANPL female) at Carmen Lake in 2017. This nest contained a single cell that was almost twice as large as most artificial cells. Cells from artificial holes were more consistent in size and shape, varying from 14 to 19 mm in length and 7-12 mm in width.

All nest cells were made exclusively of woolly plant trichomes as has been reported for all other *Anthidium* species that have been studied (Grigarick & Stange 1968; Michener 2000; Gonzalez & Griswold 2013; Vitale, Gonzalez, & Vázquez 2017) (Figure 10). Observations of scrape-marks on stems and leaves of woolly plants as well as microscopic examination of nest cells suggests that ANPL collects trichomes from only one to two plant species in a given area. The choice of plant species used varied amongst sites, depending on the relative abundance of plants offering woolly hairs. For example, *Eriophyllum lanatum* (Asteraceae) trichomes were found in all five nest cells from French Hill, where it was the only abundant plant offering woolly hairs. The Carmen Lake site did not yield any nests, yet scrape-mark evidence was restricted to leaves of *E. lanatum* despite the co-occurrence of other woolly plants (Figure 11A) (Table 5). At the Carmen Lake site, *Eriogonum congdonii* was the most abundant woolly plant. Scrape marks were seen on leaves of this species as well as *E. lanatum* at this site (Figure 11B), yet only *Eriogonum* trichomes were found in the five nest cells collected. At the Lassics site, the most abundant plant offering woolly trichomes was *Eriogonum strictum* var. *greenei*. All three cells from this site were constructed of *Eriogonum* hairs. Although other *Eriogonum* species were present at this site (*E. nudum* and *E. umbellatum*), scrape marks were seen only on *E. strictum* ssp. *greenei*, the most abundant of the three (Figure 11C). Although ANPL females possess specialized tomentum on the basitarsi for collecting trichome secretions, none of the nest cells obtained showed evidence of exudates on their exterior surfaces (Figure 10). This finding was surprising since COTE was abundant at my study sites and its glandular hairs could presumably supply exudates.

It is possible that bees did not apply protective exudate because of the apparent absence of nest parasites at my study sites (Pow, unpublished).

CONCLUSIONS

Natural history information exists for only a small portion of North American species of *Anthidium*, most focusing on nesting biology and mating behavior (Gonzalez and Griswold 2013). My study provides the first documented case of oligolecty by a North American *Anthidium* species. Little evidence is available on the pollen sources of other North American species, but four additional species have been collected on *Cordylanthus* (Gonzalez and Griswold 2013), three of which have modified clypeal hairs. Moldenke and Neff (1974) proposed that *Anthidium palliventris* is oligolectic on Hydrophyllaceae and Fabaceae (Moldenke and Neff 1974), but observations by other authors are not consistent with this claim (Villalobos and Shelly, 1991; Reid, 2010).

A prerequisite for oligolecty is the availability of a host plant that provides predictably abundant floral resources throughout its flight season (Minckley & Roulston 2006), a condition that seems unlikely to be satisfied by a late-season annual plant subject to vagaries of soil moisture availability in xeric habitats. But, in fact, COTE forms large populations of thousands of individuals that reliably begin to bloom in mid-summer each year, thus providing an abundant and predictable source of pollen and nectar for ANPL at sites where few other plants are in still in bloom (Mesler, unpublished observations). The ability of COTE to flourish when soil water potentials are extremely low is almost certainly linked to its hemiparasitic habit; woody host plants supply the water required for its development and flowering (Chuang and Heckard 1986). As such, the success of ANPL is indirectly linked to the success of these woody host plants. In recent decades, the western United States has witnessed increased rates of tree mortality related to

climate change, forest fires, and bark beetle outbreaks (van Mantgem et al. 2009; Bentz et al. 2010; Edburg et al. 2012; Hicke et al. 2015). These threats may cascade down to ANPL. Increased rates of tree mortality can translate to a reduction in the parasitic plants that rely on these trees for their nutritional needs, which could ultimately impact pollinators that depend on their flowers for pollen and nectar.

As in many other cases, the relationship between ANPL and COTE is asymmetrical (Vázquez & Aizen 2004; Minckley & Roulston 2006). ANPL depended completely on the flowers of COTE, yet the latter was observed being visited and likely pollinated by three other bees throughout the study, including two other megachilids (*Megachile angelarum* Cockerell and *Anthidiellum notatum ssp. robertsonii* Cockerell) and queens of *Bombus vosnesenskii* Radoszkowski (Apidae). *Bombus vosnesenskii*, widespread generalist bumblebees, were present at all sites, visiting several flowering plants including COTE, *Phacelia corymbosa*, *Eriogonum* spp., *Helenium bigelovii*, and *Pyrrocoma racemosa*. *Megachile angelarum* is also polylectic (Bosch, Maeta, & Rust 2014), yet seemed to restrict its pollen-collecting to COTE at all of my study sites. Females consistently carried cream-yellow scopal loads, indicative of COTE pollen in my study areas. In fact, *M. angelarum* were seen visiting COTE flowers more frequently than ANPL in this study, and in a 2013 survey of the bee fauna of Horse and Grouse Mountain (Lopez 2017). Floral records from the 2013 Grouse Mountain survey indicate that *A. notatum ssp. robertsonii* visit the flowers of *Polygonum douglasii*, *Eriogonum nudum*, and *Cirsium occidentale* (Asteraceae) (Lopez 2017). *Anthidiellum notatum ssp. robertsonii* was present at all study sites in 2017 and 2018, mainly visiting

COTE and were seldom seen on *P. douglasii* and *E. nudum*. Like ANPL, males of *M. angelarum* and *A. notatum* ssp. *robertsonii* displayed territorial mating behavior at patches of COTE.

All but one *Anthidium* species studied to date nest in pre-existing cavities (*A. palliventre* excavates its own nests in sand) (Hicks 1928, Gonzalez & Griswold 2013). A majority of these species nest in above-ground cavities, and many will accept artificial trap nests, providing an opportunity to study their nesting biology (Vitale, Gonzalez, & Vázquez 2017). To my knowledge, my study is the first attempt to create artificial soil trap nests. Although fewer than 1% of the holes created were utilized (Table 2), more nest cells were obtained using this method than by searching for natural nests. This approach to obtaining nest cells would be especially useful for studying bees that nest in non-aggregated, pre-existing soil cavities, as this style of nesting makes locating nests difficult. Future research utilizing artificial soil trap nests should experiment with alternative ways to make cavities. For example, larger cavities might increase occupancy. The diameter of the holes I created ranged from 7-9 mm, but the three natural nests were found in crevices exceeding this diameter, reaching up to 18 mm.

LITERATURE CITED

- Alcock, J., Eickwort, G. C., & Eickwort, K. R. 1977. The reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. *Behavioral Ecology and Sociobiology* 2: 385-396.
- Alcock, J., Barrows, E. M., Gordh, G., Hubbard, L. J., Kirkendall, L., Pyle, D. W., & Zalom, F. G. 1978. The ecology and evolution of male reproductive behaviour in the bees and wasps. *Zoological Journal of the Linnaean Society* 64: 293-326.
- Batra, S. W. 1978. Aggression, territoriality, mating and nest aggregation of some solitary bees (Hymenoptera: Halictidae, Megachilidae, Colletidae, Anthophoridae). *Journal of the Kansas Entomological Society* 51: 547-559.
- Bentz, B. J., Régnière, J., Fettig, C. J., Hansen, E. M., Hayes, J. L., Hicke, J. A., Kelsey R.G., Negron J.F., & Seybold, S. J. 2010. Climate change and bark beetles of the western United States and Canada: direct and indirect effects. *BioScience* 60: 602-613.
- Brown, M. J., & Paxton, R. J. 2009. The conservation of bees: a global perspective. *Apidologie* 40: 410-416.
- Cane, J. H. 1991. Soils of ground-nesting bees (Hymenoptera: Apoidea): texture, moisture, cell depth and climate. *Journal of the Kansas Entomological Society* 64: 406-413.
- Cane, J. H. 2001. Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology* 5: 3.
- Cane, J. H., & Sipes, S. 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. pp. 99-122 in: *Plant- pollinator Interactions: from specialization to generalization*. (eds Waser NM, Ollerton J). The University of Chicago Press, Chicago.
- Cane, J. H., Griswold, T., & Parker, F. D. 2007. Substrates and materials used for nesting by North American *Osmia* bees (Hymenoptera: Apiformes: Megachilidae). *Annals of the Entomological Society of America* 100: 350-358.
- Cane, J. H. 2015. Landscaping pebbles attract nesting by the native ground-nesting bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Apidologie* 46: 728-734.

- Cane, J. H. 2018. Co-dependency between a specialist *Andrena* bee and its death camas host, *Toxicoscordion paniculatum*. *Arthropod-Plant Interactions* 12: 657-662.
- Cane, J. H. 2019. Scientific note: cavity-nesting *Osmia bruneri* bees (Megachilidae) can use fruit pulp for nest construction. *Apidologie* 50: 1-4.
- Chuang, T. I., & Heckard, L. R. 1971. Observations on root-parasitism in *Cordylanthus* (Scrophulariaceae). *American Journal of Botany* 58: 218-228.
- Chuang, T. I., & Heckard, L. R. 1976. Morphology, evolution, and taxonomic significance of the inflorescence in *Cordylanthus* (Scrophulariaceae). *American Journal of Botany* 63: 272-282.
- Chuang, T. I., & Heckard, L. R. 1986. Systematics and evolution of *Cordylanthus* (Scrophulariaceae-Pediculariaceae) (including the taxonomy of Subgenus *Cordylanthus*). *Systematic Botany Monographs* 12: 1-105.
- Edburg, S. L., Hicke, J. A., Brooks, P. D., Pendall, E. G., Ewers, B. E., Norton, U., Gochis D., Gutmann E.D., & Meddens, A. J. 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. *Frontiers in Ecology and the Environment* 10: 416-424.
- Eickwort, G. C. 1977. Male territorial behaviour in the mason bee *Hoplitis anthocopoides* (Hymenoptera: Megachilidae). *Animal Behaviour* 25: 542-554.
- Eickwort, G. C., & Ginsberg, H. S. 1980. Foraging and mating behavior in Apoidea. *Annual Review of Entomology* 25: 421-446.
- Eickwort, G. C., Matthews, R. W., & Carpenter, J. 1981. Observations on the nesting behavior of *Megachile rubi* and *M. texana* with a discussion of the significance of soil nesting in the evolution of megachilid bees (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society* 54: 557-570.
- Eltz, T., Küttner, J., Lunau, K., & Tollrian, R. 2015. Plant secretions prevent wasp parasitism in nests of wool-carder bees, with implications for the diversification of nesting materials in Megachilidae. *Frontiers in Ecology and Evolution* 2: 86.
- Gonzalez, V. H., & Chavez, F. 2004. Nesting biology of a new high Andean bee, *Anthophora walteri* Gonzalez (Hymenoptera: Apidae: Anthophorini). *Journal of the Kansas Entomological Society* 77: 584-592.

- Gonzalez, V. H., Griswold, T., Praz, C. J., & Danforth, B. N. 2012. Phylogeny of the bee family Megachilidae (Hymenoptera: Apoidea) based on adult morphology. *Systematic Entomology* 37: 261-286.
- Gonzalez, V. H., & Griswold, T. L. 2013. Wool carder bees of the genus *Anthidium* in the Western Hemisphere (Hymenoptera: Megachilidae): Diversity, host plant associations, phylogeny, and biogeography. *Zoological Journal of the Linnaean Society* 168: 221-425.
- Grewell, B., DaPratro, M., Hyde, P., & Rejmánková, E. 2003. Reintroduction of endangered soft bird's beak (*Cordylanthus mollis* ssp. *mollis*) to restored habitat in Suisun Marsh. Final Report for CALFED Ecosystem Restoration Project 99-N05.
- Grigarick, A.A., & Stange, L.A. 1968. The pollen-collecting bees of the Anthidiini of California (Hymenoptera, Megachilidae). *Bulletin of the California Insect Survey* 9: 1-113.
- Griswold, T., Gonzalez, V.H., & Ikerd, H. 2014. AnthWest, occurrence records for wool carder bees of the genus *Anthidium* (Hymenoptera, Megachilidae, Anthidiini) in the Western Hemisphere. *ZooKeys* 408: 31-49.
- Hicke, J. A., Meddens, A. J., & Kolden, C. A. 2015. Recent tree mortality in the western United States from bark beetles and forest fires. *Forest Science* 62: 141-153.
- Hicks, C. H. 1928. Notes on *Anthidium palliventris* Cresson. *The Pan-Pacific Entomologist* 5: 51-52.
- Jaycox, E. R. 1967. Territorial behavior among males of *Anthidium banningense* (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society* 40: 565-570.
- Lampert, K. P., Pasternak, V., Brand, P., Tollrian, R., Leese, F., & Eltz, T. 2014. 'Late' male sperm precedence in polyandrous wool-carder bees and the evolution of male resource defence in Hymenoptera. *Animal Behaviour* 90: 211-217.
- Linsley, E. G., & MacSwain, J. W. 1958. The significance of floral constancy among bees of the genus *Diadasia* (Hymenoptera, Anthophoridae). *Evolution* 12: 219-223.
- Litman, J. R., Danforth, B. N., Eardley, C. D., & Praz, C. J. 2011. Why do leafcutter bees cut leaves? New insights into the early evolution of bees. *Proceedings of the Royal Society B* 278: 3593-3600.

- Lopez, C. 2017. The bee fauna of the Horse Mountain and Grouse Mountain Region, Humboldt County, California. M.A. Thesis, Humboldt State University, Arcata, California, USA
- Macior, L. W. 1967. Pollen-foraging behavior of *Bombus* in relation to pollination of nototribic flowers. *American Journal of Botany* 54: 359-364.
- MacIvor, J. S., & Moore, A. E. 2013. Bees collect polyurethane and polyethylene plastics as novel nest materials. *Ecosphere* 4: 1-6.
- Martins, R. P., & de Almeida, D. A. 1994. Is the bee, *Megachile assumptionis* (Hymenoptera: Megachilidae), a cavity-nesting specialist? *Journal of Insect Behavior* 7: 759-765.
- Melander, A. L. 1902. The nesting habits of *Anthidium*. *The Biological Bulletin* 3: 27-32.
- Michener C.D. 2000. The bees of the world, The Johns Hopkins University Press, Baltimore.
- Minckley RL, & Roulston TH. 2006. Incidental mutualisms and pollen specialization among bees. pp. 69-98 in: *Plant-pollinator Interactions: from specialization to generalization*. (eds Waser NM, Ollerton J). The University of Chicago Press, Chicago.
- Moldenke, A.R., and J.L. Neff. 1974. The bees of California. Board of studies in Biology Technical report 74-1. University of California, Santa Cruz, CA, USA.
- Müller, A. 1996a. Convergent evolution of morphological specializations in Central European bee and honey wasp species as an adaptation to the uptake of pollen from nototribic flowers (Hymenoptera, Apoidea and Masaridae). *Biological Journal of the Linnean Society* 57: 235-252.
- Müller, A. 1996b. Host-plant specialization in Western Palearctic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs* 66: 235-257.
- Müller, A., Topfl, W., & Amiet, F. 1996. Collection of extrafloral trichome secretions for nest wool impregnation in the solitary bee *Anthidium manicatum*. *Naturwissenschaften* 83: 230-232.
- Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy, C., & Dorn, S. 2006. Quantitative pollen requirements of solitary bees: implications for bee conservation and the evolution of bee–flower relationships. *Biological Conservation* 130: 604-615.

- Otto, C. M. 2006. Natural history and habitat selection by *Osmia lanei*, a solitary bee. M.A. Thesis, Humboldt State University, Arcata, California, USA
- Parsons, L. S., & Zedler, J. B. 1997. Factors affecting reestablishment of an endangered annual plant at a California salt marsh. *Ecological Applications* 7: 253-267.
- Paxton, R. J. 2005. Male mating behaviour and mating systems of bees: an overview. *Apidologie* 36: 145-156.
- Payne, A., Schildroth, D. A., & Starks, P. T. 2011. Nest site selection in the European wool-carder bee, *Anthidium manicatum*, with methods for an emerging model species. *Apidologie* 42: 181-191.
- Praz, C. J., Müller, A., & Dorn, S. 2008. Host recognition in a pollen-specialist bee: evidence for a genetic basis. *Apidologie* 39: 547-557.
- Rathcke, B. J., & Jules, E. S. 1993. Habitat fragmentation and plant–pollinator interactions. *Current Science* 10: 273-277.
- Reid, A. 2010. Morphometric influence on pollinator sharing between native and invasive plant species along the North Spit of Humboldt Bay, California. M.A. Thesis, Humboldt State University, Arcata, California, USA
- Ritchie, A. D., Ruppel, R., & Jha, S. 2016. Generalist behavior describes pollen foraging for perceived oligolectic and polylectic bees. *Environmental Entomology* 45: 909-919.
- Schwarz, H. F. 1928. Bees of the subfamily Anthidiinae, including some new species and varieties, and some new locality records. *Journal of the New York Entomological Society* 36: 369-419.
- Severinghaus, L. L., Kurtak, B. H., & Eickwort, G. C. 1981. The reproductive behavior of *Anthidium manicatum* (Hymenoptera: Megachilidae) and the significance of size for territorial males. *Behavioral Ecology and Sociobiology* 9: 51-58.
- Sipes, S. D., & Tepedino, V. J. 2005. Pollen-host specificity and evolutionary patterns of host switching in a clade of specialist bees (Apoidea: *Diadasia*). *Biological Journal of the Linnean Society* 86: 487-505.
- Stephen, W. P., Bohart, G. E. & Torchio, P. F. 1969. *The Biology and External Morphology of Bees, with a Synopsis of the Genera of Northwestern America*. Agricultural Experimental Station, Oregon State University, Corvallis, OR.

- Stockhammer, K. A. 1966. Nesting habits and life cycle of a sweat bee, *Augochlora pura* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 39: 157-192.
- Sugiura, N. 1991. Male territoriality and mating tactics in the wool-carder bee, *Anthidium septemspinosum* Lepeletier (Hymenoptera: Megachilidae). *Journal of Ethology* 9: 95-103.
- Turell, M. J. 1976. Observations on the mating behavior of *Anthidiellum notatum* and *Anthidiellum perplexum*. *Florida Entomologist* pp. 55-61.
- van Mantgem, P. J., Stephenson, N. L., Byrne, J. C., Daniels, L. D., Franklin, J. F., Fulé, P. Z., Harmon M. E., Larson A. J., Smith J. M, Taylor A. H, & Veblen, T. T. 2009. Widespread increase of tree mortality rates in the western United States. *Science* 323: 521-524.
- Vázquez, D. P., & Aizen, M. A. 2004. Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology* 85 1251-1257.
- Villalobos, E. M., & Shelly, T. E. 1991. Correlates of male mating success in two species of *Anthidium* bees (Hymenoptera: Megachilidae). *Behavioral Ecology and Sociobiology* 29: 47-53.
- Vitale, N., Gonzalez, V. H., & Vázquez, D. P. 2017. Nesting ecology of sympatric species of wool carder bees (Hymenoptera: Megachilidae: *Anthidium*) in South America. *Journal of Apicultural Research* 56: 497-509.
- Wilson, B. L., Brainerd, R. E., Otting, N., Knaus, B. J., & Nelson, J. K. 2014. Identification and Taxonomic Status of *Cordylanthus tenuis subsp. pallescens* (Orobanchaceae). *Madroño* 64-76.
- Wcislo, W. T., & Cane, J. H. 1996. Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology* 41: 257-286.
- Wirtz, P., Szabados, M., Pethig, H., & Plant, J. 1988. An extreme case of interspecific territoriality: male *Anthidium manicatum* (Hymenoptera, Megachilidae) wound and kill intruders. *Ethology* 78: 159-167.
- Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., & Dorn, S. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation* 143: 669-676.

Table 1. Study sites: locations, dates visited, co-flowering species, and number of scopal pollen loads samples. Note that Carmen Lake was visited in both 2017 and 2018.

Study site	County	Elevation (m)	Coordinates	Dates visited	Co-occurring flowering plants (in order of abundance)	# scopal samples
Carmen Lake	Siskiyou	1,690	41° 18' 2.91" N, -122° 40' 56.79" W	<u>2017</u> Jul 21, 22, 28, 29 Aug 5, 11, 19, 26 <u>2018</u> Jul 17, 21, 22, 24, 25, 28 Aug 4, 5, 9, 10, 16, 24	<i>Eriogonum congdonii</i> <i>Eriogonum nudum</i> ssp. <i>nudum</i> <i>Polygonum douglasii</i> <i>Eriophyllum lanatum</i> ssp. <i>lanceolatum</i> <i>Pyrrocoma racemosa</i> ssp. <i>pinetorum</i> <i>Helenium bigelovii</i>	76
Grouse Mtn.	Humboldt	1,505	40° 48' 31.32" N, -123° 41' 13.16" W	<u>2017</u> Jul 14, 17, 23, 29, 30 Aug 4, 10, 20	<i>Eriogonum nudum</i> ssp. <i>nudum</i> <i>Eriogonum umbellatum</i> ssp. <i>nelsoniorum</i> <i>Eriophyllum lanatum</i> ssp. <i>lanceolatum</i> <i>Crepis pleurocarpa</i> <i>Polygonum douglasii</i> <i>Achillea millefolium</i>	71
Lassics	Trinity	1,646	40° 17' 23.064" N -123° 33' 44.136" W	<u>2017</u> Jul 20, 21, 27, 28 Aug 3, 9	<i>Eriogonum strictum</i> ssp. <i>greenei</i> <i>Eriogonum nudum</i> ssp. <i>nudum</i> <i>Minuarita nuttallii</i> ssp. <i>fragilis</i> <i>Phacelia corymbosa</i> <i>Lupinus lepidus</i> ssp. <i>lobbii</i>	69
French Hill	Del Norte	568	41° 49' 33.24" N, -123° 59' 28.95" W	<u>2017</u> Jul 19, 25 Aug 1, 7	<i>Perideridia oregana</i> <i>Eriophyllum lanatum</i> ssp. <i>lanceolatum</i> <i>Horkelia sericata</i> <i>Pyrrocoma racemosa</i> ssp. <i>congesta</i>	28
Dillon Creek	Humboldt	570	41° 33' 18.1" N, -123° 33' 34.62" W	<u>2018</u> Aug 9, 25	<i>Eriogonum umbellatum</i> ssp. <i>polyanthum</i> <i>Eriogonum nudum</i> ssp. <i>nudum</i>	0
Total						274

Table 2. Artificial underground trap nests: number of holes deployed at each site and number nest cells provisioned. Holes were clustered for convenience.

	Study site	Aggregations	Holes	Holes/aggregation	Nests	Success rate (%)
2017	Carmen Lake	36	305	8	0	0.00
	Grouse Mountain	17	135	8	0	0.00
	Lassics	11	96	9	1	1.04
	French Hill	23	216	9	3	1.39
2018	Carmen Lake	49	516	11	2	0.39
	Dillon Creek	14	127	9	0	0.00
Total		150	1395	9 (avg.)	6	0.43 (avg.)

Table 3. Aerial trap nests: number of holes deployed and number of cells provisioned. Thirty-six holes were drilled in five blocks of wood at both sites.

Study site	Trap nests established	Holes	Nest cells obtained	Success rate (%)
Carmen Lake	5	200	0	0
Grouse Mountain	5	200	0	0

Table 4. Description of nests. N = Natural, A = Artificial. P = Pebbles, C = Conifer needles/twigs. Tunnel depth = distance from the nest entrance to the nest cell. Cell depth = the vertical distance from the tip of a nest cell to the soil surface.

Site	Year	Nest	Origin	Orientation (°)	Entrance diameter (mm)	Tunnel Length (mm)	# cells	Cell Depth (mm)	Plug materials	# plug bits
Lassics	2017	1	N	~ 60	11	68	2	54	P C	523
		2	A	~ 45	8	69	1	55	P C	480
French Hill	2017	1	A	~ 30	8	59	3	41	P C	325
		2	A	~ 45	8	48	1	34	P C	287
		3	A	~ 30	9	56	1	40	P	312
Carmen Lake	2017	1	N	~ 45	18	65	1	40	P	446
	2018	2	N	~ 15	10	47	2	24	P C	261
		3	A	~ 45	9	46	1	32	P C	293
		4	A	~ 15	8	49	1	37	P	304

Table 5. Description of nest cells.

Site	Nest #	Dimensions (mm)	Trichomes
Lassics	1	16x10	<i>Eriogonum strictum</i> ssp. <i>greenei</i>
	1	12x10	<i>Eriogonum strictum</i> ssp. <i>greenei</i>
	2	16x10	<i>Eriogonum strictum</i> ssp. <i>greenei</i>
French Hill	1	21x12	<i>Eriophyllum lanatum</i>
	1	15x11	<i>Eriophyllum lanatum</i>
	1	19x12	<i>Eriophyllum lanatum</i>
	2	16x11	<i>Eriophyllum lanatum</i>
	3	18x7	<i>Eriophyllum lanatum</i>
Carmen Lake	1	27x18	<i>Eriogonum congdonii</i>
	2	21x9	<i>Eriogonum congdonii</i>
	2	18x9	<i>Eriogonum congdonii</i>
	3	15x8	<i>Eriogonum congdonii</i>
	4	14x8	<i>Eriogonum congdonii</i>

Figure 1. *Anthidium placitum* female, face view.



Figure 2. Map of study sites. Carmen Lake, Horse Mountain, Lassics, and French Hill were visited in 2017. Carmen Lake and Dillon Creek were visited in 2018.

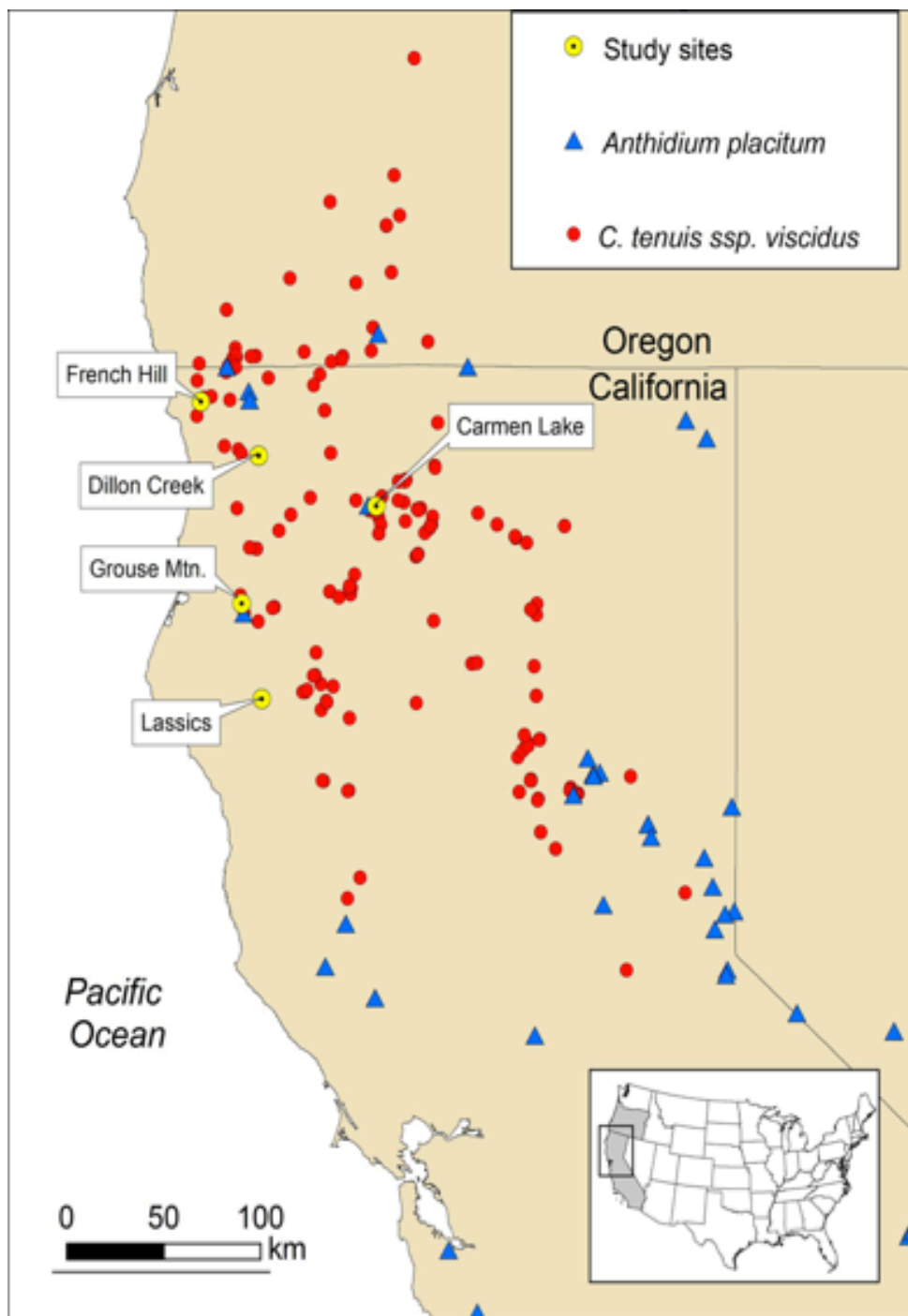


Figure 3. Modified hairs on the clypeus of an *Anthidium placitum* female. Hairs taper into hooked, wavy ends that aid in collecting pollen grains. *Cordylanthus tenuis* ssp. *viscidus* pollen grains shown on some of the hairs.

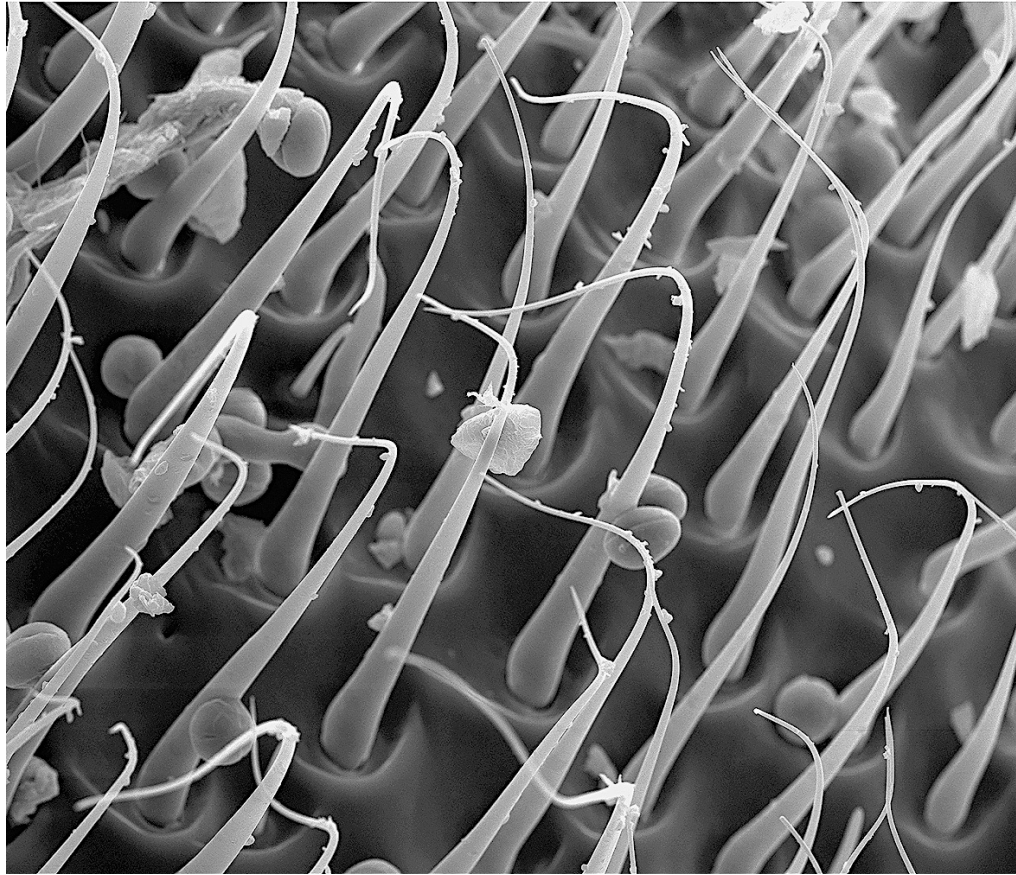


Figure 4. Fore basitarsus of an *Anthidium placitum* female. Dense tomentum is used to collect extrafloral glandular secretions incorporated in nest cells. Exudates on nest cells were not observed in this study despite the presence of tomentum on basitarsi of *Anthidium placitum*.



Figure 5. Artificial soil trap nests. Clusters of holes were made in bare soil near patches of *C. tenuis* ssp. *viscidus*. Arrows point to nest entrances.



Figure 6. *Eriophyllum lanatum*: light micrograph of foliar trichomes. Distinctive swellings on the trichomes (black arrows) allowed for easy identification when analyzing nest cells.



Figure 7. Aerial trap nest deployed at the Grouse Mountain site in 2018.



Figure 8. Flower-handling behavior of *A. placitum* on *Cordylanthus tenuis* ssp. *viscidus*. Females always entered COTE flowers in a position that ensured the modified hairs on the head came in contact with the dorsally located anthers. White arrows point to lower corolla lip, yellow arrows point to upper corolla lip. A. ANPL female collecting pollen from an upright COTE flower. B. ANPL female collecting pollen from an upside-down COTE

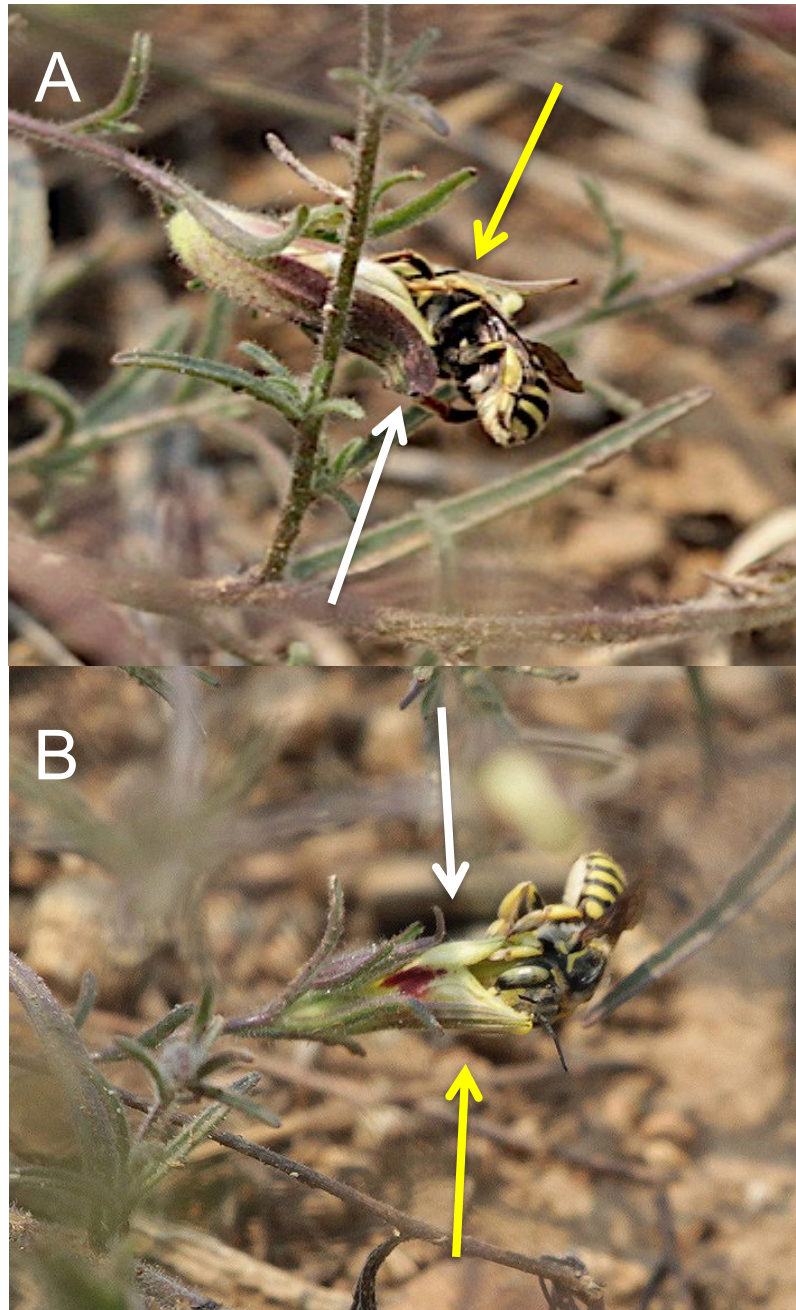


Figure 9. Map of the range overlap of *Anthidium placitum* and *Cordylanthus*. *A. placitum* records taken from AnthWest database (Griswold and Gonzalez 2013). *Cordylanthus* records taken from several consortiums of herbaria specimens.

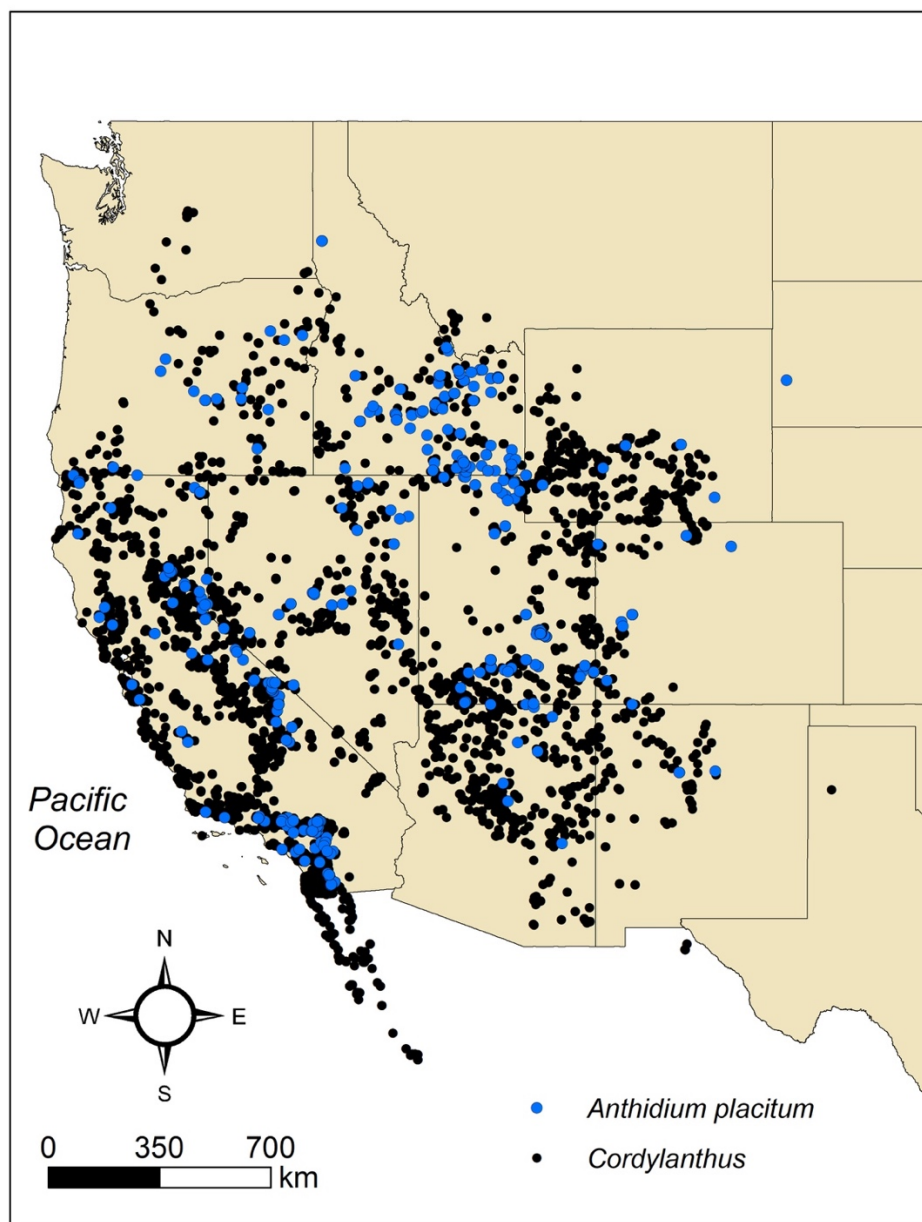


Figure 10. A single nest cell. Blue color indicates the apex of the cell. Blue powder was used to track nest tunnels during cell excavation.

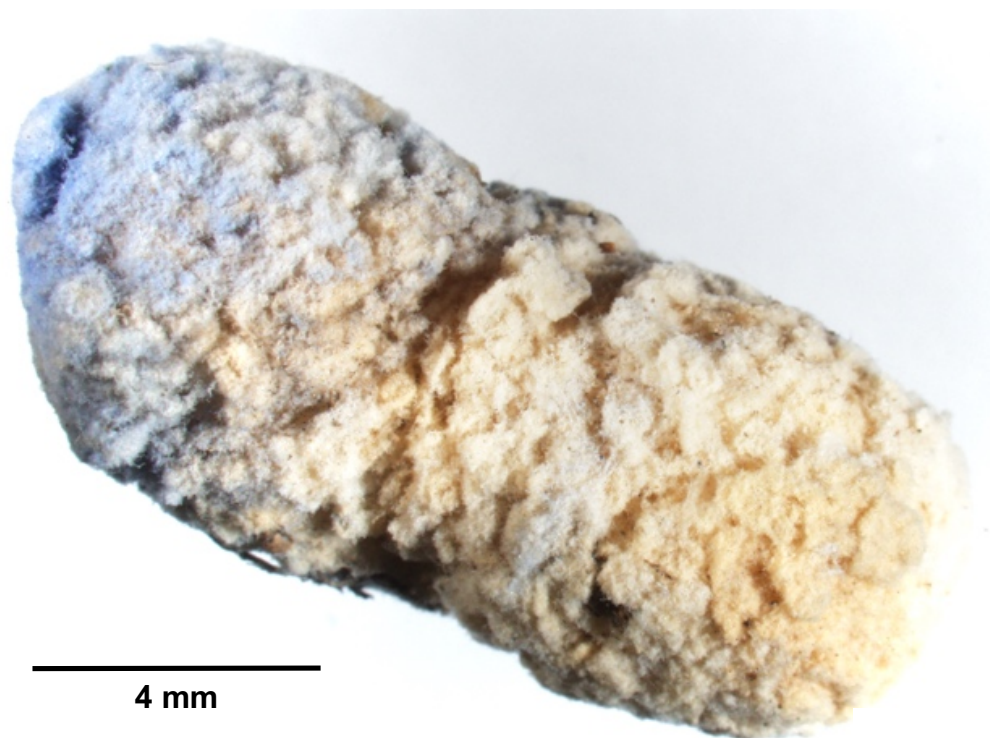


Figure 11. Evidence of trichome scraping by *Anthidium placitum*. A. *Eriophyllum lanatum*, adaxial surfaces, French Hill. B. *Eriogonum congdonii*, abaxial surface, Carmen Lake. C. *Eriogonum strictum* ssp. *greenii*, Lassics.

