Acarospora brattiae: Current assessment of a rare western North American taxon

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ABSTRACT
New records of Acarospora brattiae with updated species description, substrate preference, distribution, GenBank numbers and chemistry.

INTRODUCTION
For the Acarospora treatment in Volume 3 of the Sonoran Flora (Knudsen 2007a), the 2nd author (K.K.) was finishing the final version of his manuscript when he received a specimen collected by Cherie Bratt in 1990 from the lichen herbarium of the Santa Barbara Botanic Garden (SBBG). The specimen, from Los Alamos in northern Santa Barbara County, was labelled as growing on soil (Bratt 6521). The areoles were mostly small, about 0.5 mm or less, with a few larger areoles, all with one apothecium each. In section, the cortex and parathecium around the apothecia had a red reaction to potassium hydroxide (K+R) and produced abundant crystals of norstictic acid. Though it had a tall hymenium, there was no interrupted algal layer visible in the small fertile areoles. With these characters K.K. determined it was not Myriospora smaragdula (Wahlenb. Ex Ach.) Nägeli ex Uloth, a taxon also containing norstictic acid, which he had reported from a Coos County, Oregon collection by Bruce McCune (Knudsen 2007b), and instead represented an undescribed taxon.

Acarospora brattiae was formally described in Volume 3 of the Sonoran Flora (Knudsen 2007a). The taxon was named in honor of the collector Cherie Bratt for the important work she did in California lichenology, including starting the lichen herbarium at SBBG and being an important leader of the California Lichen Society. She taught many lichen classes around California and generated awareness of lichens among government managers of reserves, national forests, and national parks. The holotype specimen was designated as Bratt 6521 and housed at SBBG, while a duplicate at the University of California, Riverside (UCR) was designated as the isotype. Both specimens were rather scant, consisting of a few pieces of soft crumbly rock, each glued to card stock. During our specimen review for this study, the isotype was confirmed present at UCR (Chris Wagner, personal communication, June 2022) but the holotype was not found at SBBG (Danielle Ward, personal communication, June 2022). The UCR Lichen Herbarium is being transferred to SBBG, so soon the isotype will be back in Santa Barbara County and there is hope that the holotype will be found.

In Volume 3 of the Sonoran Flora A. brattiae was included in the keys as a terricolous species based on the holotype packet notation and appearance of the specimens where the substrate looked soft and glued together on card
stock. Subsequent to publication, Bratt informed K.K. that the specimen was actually collected on rock. To date, the exact location of this collection in Los Alamos has not been identified. The 1st author (J.D.) visited the described location along Palmer Road in Los Alamos in June 2022 and found the area to be characterized by low shelves of white, soft, non-calcareous sandstone eroding out of hillsides dominated by coastal scrub habitat.

**COLLECTION HISTORY**
A species described from a single type collection is a hypothesis. Giving a lichen a name and a description preserves valuable biological information. It may take decades, or even over a hundred years like *Trimmatothelopsis versipellis* (Nyl.) Zschacke in France, but usually new collections are made. Eventually the taxon is either verified as a species or it is found to be a synonym of a species which was already described. After the 2007 publication of *A. brattiae* in the Sonoran Flora, K.K. never examined the type specimens again, and these specimens remained the only confirmed records for the species.

Ten years after being described, in 2017, J.D. collected brown *Acarospora* specimens (Dart 847.1, 856) on crumbling shale bedrock in the Cholame Hills of southeastern Monterey County. The *Acarospora* specimens had mostly large areolate to subsquamulose thallus units which were 1–2 mm wide. He misdiagnosed the spot test results and passed over *A. brattiae* as it was reported only from soil. Examining a duplicate of Dart 847.1 in 2021, K.K. saw in the large areoles, especially when sterile and the apothecia did not fill the areoles, an interrupted algal layer (Figure 1). He thought it might be *Myriospora smaragdula* or maybe a new *Myriospora* taxon. Thinking it was new to science, K.K. recognized it as the same taxon he identified as *M. smaragdula* from Coos County, Oregon, collected by Bruce McCune (22267) in 1995 (Knudsen 2007b). He then wrote an extensive description of the 2017 collection before he ultimately determined it was *A. brattiae*.

In May 2022, J.D. confirmed another record of *A. brattiae* (Dart 2319) from the central coast of California, in Arroyo Grande, San Luis Obispo County. The specimen is areolate, orange-brown, rarely with subsquamulose lobes, numerous apothecia per areole, and with reddish staining around the apothecia inner margin. The cortex contains norstictic acid (K+R) and the algal layer has regular interruptions from hyphal bundles (Figure 1).

One additional specimen listed in the Consortium of North American Lichen Herbaria (CNALH 2022) as *A. brattiae* was reviewed for this study. The specimen, housed at SBBG, was collected on soil at Mount Laguna in San Diego County in 2001 (Pigniolo 13), and was later determined as *A. brattiae*. J.D. confirmed the specimen lacked norstictic acid (K-) and had a C+R reaction in the lower cortex indicating likely presence of gyrophoric acid, and thus made the determination that this was in fact *Acarospora obpallens* (Nyl. ex Hasse) Zahlbr., a common *Acarospora* on soil and rock in Southern California.
Figure 1. *Acarospora brattiae* recent collections and diagnostic characters. (A) Habit, *A. brattiae* (Dart 847.1), Monterey County, California, 2017. (B) Habit, *A. brattiae* (Dart 2319), with strong red pigmentation around apothecia, San Luis Obispo County, California, 2022. (C) Section of thallus areole with apothecium at 100x (Dart 856), in potassium hydroxide (KOH), showing red reaction and norstictic acid crystals. (D) Detail of interrupted algal layer in thallus section (Dart 856), 400x in water. All photos by J.D.

**Sequence Data**
Eva Hodková in the Jana Kocourková lab at the Czech University of Life Sciences in Prague sequenced the Dart 847.1 specimen. It was not a *Myriospora* but an *Acarospora*. Sequences of ITS and mtSSU were blasted to the *Acarospora* group and in an unpublished phylogeny it was recovered in a lineage with *Acarospora minuta* K. Knudsen, Hodková & J. McCarthy, nom. prov., a new species being described from Canada. GenBank numbers: ITS (ON303959), SSU (ON303850), LSU (ON303964).

**Updated Description**
Hypothallus in substrate. Thallus of areoles (0.5-)1–2 mm wide, ca. 0.5 mm thick, lobulate with mycelial base over one half width of areole (subsquamulose), up to 1 mm high. Upper surface orange-brown to pale brown, shiny, with some creases and 1 to 8 apothecia, epruinose. Lower surface white, ecorticate. Epicortex 10 μm thick. Cortex 40 μm thick of round to irregular cells 2–5 μm wide, upper layer orange to orange-brown, lower layer hyaline varying in thickness. Algal layer 120–140 μm thick, algal cells 10–12 μm wide, scattered under apothecia.
Hyphal bundles 10–20 μm wide, interrupting algal layer, best observed in sections not including the apothecia. The algal layer is uninterrupted in smaller areoles. Medulla 250–300 μm thick, obscure, of variable hyphae, intricate, narrow to 4–5 μm wide with globose cells constricted at septa, easily breaking apart (at least in sections) and paraplectenchymatous, continuous with attaching hyphae.

Apothecia up to eight per areole, punctiform to 0.5 mm wide, immersed, brown, epruinose, reddish when wet, with red stain uneven on cortex around the apothecia. Parathecium 40 μm at mid-level of hymenium expanding to up to 100 μm around the disc, merging with cortex. Hymenium 180–200 μm tall, epihymenium light reddish brown, 15 μm tall, paraphyses 1 μm wide, some branching, apices unexpanded or in pigment cap, hymenial gel IKI+ red or blue turning red, hemiamyloid. Asci mostly 120–200 × 30–40 μm, ascospores thin, 2–5 × 1 μm, with one oil drop at each end. Subhymenium ca. 20 μm tall, IKI+ dark blue, easily bleeding upward into lower part of hymenium in sections. Hypothecium 40 μm tall. Pycnidia not observed.

**Chemistry:** TLC performed by J. Maliček (A, B, C): norstictic acid, connorstictic acid. Cortex and parathecium with ample norstictic acid for spot tests. In section a strong red reaction is evident after application of potassium hydroxide (K+R), with production of norstictic acid crystals (Figure 1).

**EcoLOGY AND DISTRIBUTION**
The six known specimens of *Acarospora brattiae* represent four localities stretching from Santa Barbara County, California (type locality) to Coos County, Oregon (Figure 2). The three localities in central coastal California span approximately 74 miles north to south, and the Oregon locality is disjunct, at 559 miles further north. We expect the center of distribution of *A. brattiae* is the central coast of California, but the Oregon specimen and another potential specimen from Humboldt County, California (Pike 1400, OSC) suggest there is more to learn. The species occupies the coastal zone, with one specimen occurring 60 miles from the Pacific Ocean (Cholame Hills, Monterey County), while the remaining specimens occur between 0 and 16 miles from the ocean. Substrate preference is non-calcareous sandstone and shale bedrock outcrops near ground level, in open areas with full sun. The rock is generally soft, fractured, or crumbly.

![Figure 2. Range map for *Acarospora brattiae* verified specimens.](image-url)
SPECIMENS EXAMINED
Oregon: Coos County, 14 km SW of Coos Bay, Simpson Beach near Shore Acres State Park, 43º 19’ N, 124º 22’ W, 3 m. elevation, on soft sandstone outcrops by the ocean, June 1995, McCune 22267 (hb. McCune). California: Monterey County, Cholame Hills, 35.861038, -120.326721, 459 m., on low crumbling siliceous (HCL-) shale, August 17, 2017, Dart 847.1 (OBI, duplicate at SBBG); Monterey County, Cholame Hills, 35.861038, -120.326721, 459 m., on low crumbling siliceous (HCL-) shale, September 6, 2017, Dart 856 (PRM); San Luis Obispo County, Badger Canyon Lane, Arroyo Grande, 35.155625, -120.563059, 121 m., on soft white siliceous (HCL-) sandstone bedrock, May 5, 2022, Dart 2319 (OBI); Santa Barbara County, Palmer Road in Los Alamos, on crumbling white sandstone, February 20, 1990, Bratt 6521 (Holotype at SBBG, Isotype at UCR); Santa Barbara County, Los Alamos, 4 Deer Lease, on sandstone, June 27 1991, Bratt 7461 (ASU).

DISCUSSION
In the 32 years since Cherie Bratt first collected Acarospora brattiae in Santa Barbara County, California, only five additional verified specimens have been found. The dearth of specimens resulted in early confusion about the species substrate preference and morphological description. An updated key to 90 Acarosporaceae of southwestern North America to be published in 2023 by Knudsen and co-authors accurately places A. brattiae as occurring on rock and having an interrupted algal layer within the areoles. It is very possible that additional specimens could be in herbaria under misidentified names such as Myriospora smaragdula, a taxon also containing norstictic acid. A renewed effort should be made to find additional localities and misidentified specimens. The authors believe A. brattiae is a rare taxon worthy of legal protections, but further work is needed before a conservation proposal can be made.

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CITED LITERATURE