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Interested in submitting an article, photo, or workshop information for an upcoming issue of the Bulletin?
The Bulletin welcomes manuscripts on technical topics in lichenology relating to western North America and on conservation of lichens, as well as news of lichenologists and their activities. The best way to submit manuscripts is by email attachments in the format of a major word processor (DOC or RTF preferred). Use italics for scientific names. Please submit figures in electronic formats with a resolution of 300 pixels per inch (600ppi minimum for line drawings); preferred minimum width for images is 2100 pixels, but widths down to 1050 pixels may be accepted. Email submissions are limited to 10MB per email, but large files may be split across several emails or other arrangements can be made. Contact Editor@californialichens.org for details of submitting illustrations or other large files. A review process is followed. Nomenclature follows Esslinger's cumulative checklist online at http://www.ndsu.edu/pubweb/~esslinge/chcklst/chcklst7.htm. The editors may substitute abbreviations of authors names, as appropriate from The International Plant Names Index - www.ipni.org/index.html. Style follows this issue. Electronic reprints in PDF format will be emailed to the lead author at no cost. The deadline for submitting material for the summer issue is April 1, 2020.

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Cover image: Collecting *Letharia vulpina* specimens on the Tahoe National Forest to be used for air quality research using the designated United States Forest Service protocol for elemental tissue analysis. See related story on p. 47. Photo by Hanna Mesraty.

Testing staining techniques for detection of basidiomycete yeasts in *Bryoria* macrolichens

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Abstract

Currently, no staining methods exist which allow for the observation of basidiomycetous yeasts within ascomycete macrolichens under simple light microscopy. Without light microscopy observation techniques, it is difficult to study the roles these yeasts play in macrolichens and to discover which lichen species have evolved this symbiosis. This study employs various histological techniques to explore the diazo dye Diazonium Blue B (DBB) as a potential stain for visualizing yeasts within the epicortical layer of *Bryoria* genus lichens. Samples of *Bryoria tortuosa, Bryoria fremontii*, and intermediate species forms were hydrated in glycerol, embedded in paraffin, and sectioned. Sections were adhered to slides, cleared with xylene, and rehydrated in a decreasing alcohol series. Four staining solutions containing DBB were tested on sections of *B. tortuosa, B. fremontii*, and the intermediate species forms, and all four solutions, were found to react with ascomycetous hyphae. These reactions show DBB to be a poor stain for differentiating basidiomycetous yeasts from ascomycetous hyphae in the epicortex of *Bryoria totuosa* and *Bryoria fremontii*. While development of microscopy stains and staining methods to target and visualize basidiomycete yeasts may be challenging, other mycological dyes and techniques such as calcofluor white and its accompanied staining protocols may still be promising.

Introduction

Lichens are composite macroorganisms formed by, at minimum, a fungus (mycobiont), and an alga or cyanobacterium (photobiont) in symbiosis with one another. The majority of known lichens are composed of an ascomycetous fungus growing a complex hyphal network that creates the thallus or body of the organism (Lücking, 2016). Basidiomycetous fungi are similarly capable of producing these same structural hyphae and forming a symbiosis with photobionts but account for less than 1% of known lichenized fungi (Lücking, 2016). Barring rare exceptions, until recently it was accepted that lichens follow a "one lichen, one fungus" rule, and that all lichens are composed of just one fungus and one photobiont.

Research in 2016 on Parmeliaceae family lichens discovered a third, common mycobiont in the epicortex of several lichen species (Spribille et al. 2016). In these lichens, basidiomycetous yeasts in the order Cyphobasidiales were found occupying an epicortical layer in *Bryoria tortuosa*, but were found in relatively low abundance in closely related *Bryoria fremontii* (Spribille et al. 2016). These yeasts were successfully imaged using fluorescent *in situ* hybridization, but under light microscopy were indiscernible against the background of other fungal structures and hyphae (Spribille et al. 2016). If methods that allowed for the viewing of such yeasts under the lens of standard light microscopy were developed, they could allow

for the study of these yeasts to encompass a greater number of lichen species.

Unicellular basidiomycete yeasts are known to stain when exposed to DBB (Hagler 1981). Filamentous ascomycetes and unicellular algae commonly have little, if any staining reaction when exposed to this dye (Hagler 1981). This compound has the potential to stain lichen basidiomycete yeasts, making them visible under light microscopy due to the dyes ability to remain nonreactive with ascomycetous hyphae, and algae. To prepare lichens for microscopy stains they must first be processed, embedded, sectioned, and fixed to slides. While these processes are well documented in plants, humans, and other animals, very little work has been performed using these techniques in lichens, and many aspects of these processes are still experimental. Published work is not readily available concerning the application of these processes to *Bryoria fremontii* and *Bryoria tortuosa*, though published work from Poland has shown how similar techniques from botany can be applied to several lichen species (Leśniewska 2008).

Materials and Methods

This study has sought to develop a protocol for identifying basidiomycetes in ascomycetous macrolichen thalli. The diazo dye Diazonium Blue B was, using multiple techniques, tested on lichens of the *Bryoria* genus to explore its potential ability to stain for basidiomycetous yeasts and make them visible for study and identification under light microscopy. Samples of *Bryoria tortuousa* and *Bryoria fremontii* were obtained through donations made by members of the California Lichen Society, and amateur and professional lichenologists from throughout the Pacific Northwest. In total, 14 specimens of *Bryoria tortuosa* and 35 specimens of *Bryoria fremontii* were obtained. Of *Bryoria fremontii* samples, 23 of the 35 obtained samples were identified as potential *Bryoria tortuosa* intermediates and were treated as a separate, third group. Sample ages varied, with collection dates of several samples being over five years old, and others only a few weeks old. As a result of long-term storage, older samples appeared completely desiccated and brittle while fresher samples still retained some of their original moisture and were malleable.

All chemicals were sourced from chemical supply companies. The Diazonium Blue B (o-Dianisidine bis[diazotized] zinc double salt) dye (DBB) used in this project was purchased from Sigma-Aldrich under the name “Fast Blue B Salt” and had a dye content of 95%. The Trizma (tris hydrochloride) solution was purchased from Sigma-Aldrich under the name “Trizma hydrochloride solution” (concentration 1.0 M, pH 7.0). The xylene was of unknown isomeric composition, but was at least 99% pure (with up to 1% of the solution being cumene) and was of suitable purity and composition for use as a deparaffinizing agent.

Rehydration

Rehydration of samples was tested using three separate solutions: 1) distilled water, 2) an equal parts solution of distilled water and glycerol, and 3) an equal parts solution of distilled water, 95% ethanol, and glycerol. Lichen samples for rehydration were placed in solution inside of cassettes, or as free-floating thalli in the solutions. Lichens were rehydrated for a minimum of 24 hours.

Once rehydrated, samples were removed from solution. Specimens in cassettes were removed from their cassettes and transferred onto filter paper with care taken to maintain orientation.

Free-floating thalli were directly moved from solution to filter paper, cut to approximate cassette size if needed, and spread out to ensure that excess solution thoroughly drained. Excess solution appeared to adequately drain after 30 minutes.

Paraffin embedding
Tissue-Tek V.I.P. paraffin was heated until fully melted and poured as a thin layer into 0°C or colder aluminum molds. Rehydrated lichen samples were then positioned and depressed into this thin paraffin layer. Once the paraffin had solidified enough to hold these samples in place, melted paraffin was poured around the sample until the sample was completely enveloped by paraffin, and a cassette was subsequently embedded on top. These molds were then placed in a freezer until the paraffin had completely solidified and the entire paraffin block was capable of being removed.

Sectioning and dehydration
Once embedded, lichens were sectioned using both common hand sectioning techniques and a hand microtome. Hand sectioning was performed by repeatedly running a razor blade shallowly across the surface of the paraffin embedded lichen block. Level sections with a consistent, low width, and with minimal folding and other structural faults were heated in a hot water bath and submerged onto both uncharged and positively charged glass microscope slides.

Sectioning with a hand microtome was performed with a tempered steel knife and a Carolina Biological Supply hand microtome. Cassettes were cut to size and loaded into the hand microtome. Sectioning was performed with the knife cooled to 0°C, and sections were taken at varying widths between approximately 10–20 µm. Again, sections with consistent widths and with minimal folding and other structural losses were heated in hot water baths and submerged onto uncharged and positively charged glass microscope slides.

All slides were briefly passed over an open flame to aid with slide adhesion. Slides were then placed into a low heat dehydrating vessel which dehydrated the slides and removed tissue and paraffin wrinkling, as well as other artifacts from the embedding and sectioning processes.

Deparaffination and rehydration
Slides with adhered, paraffin bound tissues were deparaffinized and rehydrated. Deparaffination was achieved through three, 3-minute xylene rinses. To achieve these rinses, slides were twice placed in Coplin jars filled with 99% xylene, then once in a Coplin jar filled with equal parts 99% xylene and 95% ethanol. Slides then immediately went through a modified descending ethanol series to achieve rehydration of the sections following deparaffination. This series consisted of two 95% ethanol rinses, a 71.25% ethanol rinse, and a 47.5% ethanol rinse. These rinses were completed by placing slides inside of Coplin jars filled with corresponding solutions, and each rinse took place over four minutes. Following the final 47.5% ethanol rinse, slides were placed in two rinses of pure, distilled water lasting one minute each. Slides were then placed on slide racks and allowed to dry for approximately 12–24 hours.

Diazonium Blue B preparation
Four DBB solutions were prepared to test for the compounds ability to differentially stain basidiomycetous yeasts. These stains were prepared with the following amounts of DBB, Trizma solution, and distilled water.

	DBB (mg)	Trizma (mL)	H2O (mL)
Stain 1:	15	15	-
Stain 2:	15	3.75	11.25
Stain 3:	3.75	15	-
Stain 4:	3.75	3.75	11.25

All compounds were refrigerated prior to mixing, and solutions were kept cold in ice baths until used. When originally mixed, all solutions appeared light yellow or tan. Once solutions transitioned to a dark yellow color they were no longer used, and new solutions were mixed as needed.

Staining

Stains were applied to dried, prepared slides on a flat surface in a drop-wise fashion using transfer pipettes. Each stain was applied until the sections on each slide were fully submerged in the stain. Stains were then allowed to sit for either one second, ten seconds, thirty seconds, or one minute before being rinsed off. Removal of the stains was tested with distilled water, 95% ethanol, or Trizma solution. Rinse solutions were applied over thirty seconds using clean transfer pipettes, with slides slightly angled to allow for the draining solution and stain to run off the edge of the slides.

Once stained, slides were checked under a compound microscope for presence of stained basidiomycetous yeasts and for possible differences in sample color at the epicortical layer. Slides were checked at total magnifications of 40x, 100x, 400x, and 1,000x, with the last requiring the use of high refractive index immersion oil.

Results

Hyphae from the ascomycetous mycobiont appeared stained in all samples, and the DBB solutions were found to give similar stain results across all three tested tissue types. No differences were observed between stained *B. fremontii, B. tortuosa*, and species intermediates. Analysis for color differences at the epicortex was inconclusive as all tissue layers appeared to react identically with the DBB compound. Solutions with 15 mg of added DBB appeared to create a color change towards deep violet while those with 3.75 mg caused a color change towards light violet, however these colors were still uniformly present throughout the sectioned tissue. Outside of the severity of staining, no differences were observed between the four solutions, the different staining times, or the different rinses.

Of the rehydrating solutions experimented with, both glycerol solutions performed well as rehydrators, but pure distilled water gave poor results. Each solution was able to rehydrate thalli after 24 hours, but those rehydrated in only water did not hold their structure well during further processing and were therefore not used in additional steps. Lichens were able to be stored in both glycerol solutions for at least four months without degradation, but those in pure water appeared to degrade, and suffered from structural losses after such time. Color differences were observed between solutions storing *B. fremontii* and those storing *B. tortuosa*. Rehydrating solutions appeared to take on the color of the lichen sample they were hydrating, with *B. tortuosa* solutions turning a vibrant green/yellow color and *B. fremontii* turning tan. These differences in color may be due to the extraction of secondary metabolites into the solution, possibly vulpinic acid.

When rehydrating lichens in glycerol solutions it was beneficial to divide the thalli into cassettes prior to rehydration rather than afterwards. It is difficult to determine the anatomical orientation of free-floating thalli and cutting

lichen thalli covered in glycerol for placement into cassettes is difficult due the compound's lubricating abilities. Dividing thalli into cassettes prior to rehydration avoids these problems, though care must be taken to avoid wiping off written labels during cassette handling.

Multiple compounds were tested for their deparaffinizing ability as potential xylene substitutes. These included acetone, kerosene, pure gasoline ("white gas"), mineral oil, ethanol, and hand soap. None of these compounds were able to completely clear paraffin from slides, and some were found to dissolve or remove sample tissue. Deparaffinizing with only heat and without chemical deparaffinizing agents was also attempted and found to be inefficient and also destructive to sample tissue. Despite its associated health hazards, xylene was found to be the only usable deparaffinizing agent and was able to clear paraffin with minimal tissue loss.

Standard hand sectioning was found to be superior to sectioning done with aid from a hand microtome. The hand microtome could have provided sections of known width and would have theoretically done so in an exact reproducible manner, but due to its bulky size it was commonly inefficient and inexact at sections under 20 µm, required continuous troubleshooting, and any movement between parts caused significant deflections in the razor blade resulting in inconsistent sample widths. While sectioning without the hand microtome meant that slides would have sections of unknown width, this technique still yielded sections thin enough to examine with a microscope and did so significantly faster than what was capable with the hand microtome. As a result, hand sectioning without the hand microtome was the favored method when preparing slides.

Standard uncharged Amscope microscope slides were initially purchased for slide preparation. During the deparaffinization and rehydration steps, significant losses in tissue occurred as there seemed to be little adhesion between the samples and glass of the slides. While passing slides over a flame prior to further processing did aid with adhesion, sample losses were still significant enough to warrant the use of more expensive, positively charged slides. Borosilicate glass microscope slides with a positively charged surface were used and found to have better tissue adherence characteristics than uncharged slides.

Discussion

Under the studied conditions, the ascomycete mycobiont in *Bryoria fremontii* and *Bryoria tortuosa* produces a positive reaction with DBB, and as a result no basidiomycetous yeasts were identified when DBB was used as a stain. However, the unexpectedly high reactivity and volatility of DBB is more likely the reason that no differential staining of basidiomycetous yeasts was observed during these experiments. Once the DBB solutions were applied as stains in a thin layer to slide sections, it appeared that rapid degradation of the compound occurred which caused the observed false positive color changes. This rapid degradation is likely the cause for false positive color changes throughout the entirety of the sectioned specimen regardless of basidiomycetous yeast presence. It is possible that before expiring, an observable color change may appear on slides only at the sites where basidiomycetous yeasts are found. However, to observe this change in *Bryoria tortuosa* one would need to be actively observing the epicortex of a sectioned specimen as DBB solution is washed over. If more stable, non-rapidly degrading solutions can be made which contain DBB, it still may be able to dif-

ferentially stain basidiomycetous yeasts in ascomycetous macrolichens, however these solutions do not currently appear to exist or are not widely available. When working with the DBB compound it quickly became apparent how volatile and sensitive to both temperature and moisture the dye is. Hagler and Ahearn (1981) who pioneered the use of DBB for identification of basidiomycetous yeasts mentioned the sensitivity of the dye, writing:

"Diazonium Blue B is unstable in warm and moist conditions, but 15-mg portions of the dye can be stored in well-sealed test tubes at 4°C for several weeks. The dissolved reagent was maintained in an ice bath and was used either before it turned dark yellow or within about 30 min".

Despite multiple attempts at storing this dye in solution, no sample was able to be preserved for more than 24 hours in cold solution regardless of how well-sealed the storage vessel was. This was found to hold true with all four previously described DBB stain preparations. Within 24 hours the dye in every sample would precipitate at the bottom of the storage vessel, after which the dye was observed to have undergone chemical reactions, as it was deeply violet in color.

Hagler and Ahearn (1981) were able to maintain usable DBB solution over a thirty minute span, and noted that solutions are no longer usable once they had undergone a color change to dark yellow. Unfortunately, during testing the dye was found to undergo intense color change to dark yellow within approximately three minutes of being mixed, and therefore was only usable for a short time. Attempts were made at maintaining solutions at temperatures below freezing but these attempts seemed to make minimal if any difference in the lifespan of the stains. Though stains containing high molar concentrations of DBB appeared to expire quicker than those with low molar concentrations, the difference was not significant enough to improve workability in a noticeable way. Though Hagler and Ahearn (1981) did use a 0.25 M Trisma solution, and their DBB was practical grade at 20% pure, "Stain 4" should have chemically been identical to their solution, having an identical end molarity of each compound once mixed, and so the observed differences in reactivity are puzzling and no satisfactory answer to the observed instability of the dye can be provided.

Conclusion

There are currently no methods available for visualizing basidiomycetous yeasts in ascomycetous macrolichens using non-fluorescent light microscopy. Due to its volatility, Diazonium Blue B in solution with Trizma hydrochloride does not appear to have many applications in microscopy as a stain. When this stain is applied to processed samples of *Bryoria fremontii* and *Bryoria tortuosa* at room temperature as a cold solution, there is no differential staining of basidiomycetous yeasts, and both species elicit an apparent false positive reaction. Non-DNA specific fluorochromes known for their ability to react with basidiomycete yeasts, like the fluorescent dye calcofluor-white, may still be capable of staining and identifying basidiomycete yeasts, and future research should explore their use for this purpose.

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Air quality biomonitoring using lichens on Tahoe National Forest

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CALS is partnering with the United States Forest Service (USFS) and the Biodiversity Research Collective (BRC) on a citizen science project using lichens to monitor air quality on the Tahoe National Forest (TNF) through the USFS CitiSci Fund grant program. The USFS CitiSci Fund encourages collaborative approaches for resource management through meaningful involvement with the public on their public lands (USFS A, n.d.).

Why Air Quality Matters on Public Lands

For public lands in America the Clean Air Act tasks Federal land managers with protecting "Air Quality Related Values" in Federal Class 1 areas (i.e. Wilderness, National Parks, National Monuments) (USFS B, n.d.). Also, "… because good air quality is prerequisite to ecosystem health, managers need to understand the status and trends in air quality on all national forest lands," (Jovan and Carlberg, 2006). Additional monitoring at areas outside Class 1 is critical to establishing context for lichen data interpretation within wilderness. In this project we aim to expand the grid of lichen surveys outside of wilderness areas to get a better feel for the air pollution, particularly nitrogen, of the area. These data will give more context to findings in wilderness areas.

In the late 1990s and early 2000s the Forest Inventory and Analysis (FIA) program conducted lichen community surveys at plots across California, including the TNF. Initially the plots were meant to be revisited every ten years, but FIA has done away with the lichen community surveys due to budget constraints. There has been much advancement with lichen bio-monitoring since the early 2000's and now collecting target lichen species for elemental analysis in a laboratory has taken hold.

Collecting lichen tissue to analyze its elemental profile helps make determinations about the air quality of these sensitive places and is part of the mandated monitoring efforts. There are many wilderness areas that are too remote to use mechanical monitoring equipment. Lichen bio-monitoring helps fill in these gaps. In addi-

One of the sites selected where Citizen Scientists will be brought for specimen collection which has been logged to reduce fire fuels on the Tahoe National Forest.

tion, the USFS Region 5 wilderness program has a need to monitor air quality in all wilderness areas in the state of California.

The collection process is a relatively simple procedure that is a great way for volunteers to assist trained experts conducting the lichen community survey while also providing key lichen data and providing information about air quality. A subset of the FIA plots where lichen community surveys were conducted had lichen tissue collected in the mid 2000s, so it will be very informative to compare results approximately 15 years later.

Why Citizen Science

Citizen science is a form of community science where nonprofessional participants engage in scientific research, guided and/or trained by professional experts. These opportunities not only help advance scientific research, but also bridge connections between participants and science through real world experiences. In today's changing world personal connections and understanding of science and the natural world are vital to ensure effective stewardship of natural resources and ecosystems on a global scale. The emergence of global citizens rooted in a shared scientific practice could be an opportunity to evolve policy around resource management and ecosystem health standards.

How Volunteers Participate in the Scientific Process

Citizen science opportunities make science and nature accessible while encouraging participants to contribute first-hand. This project connects more people with lichens on their public lands while highlighting the ecological role these indicator species play. Participation allows for a larger conversation on lichens, biodiversity, forest ecology, and land management to emerge among volunteers and experts, advancing CALS, BRC, and USFS missions.

Left: *Letharia vulpina* grows right above the snow line on conifers, approximately five feet from the ground. Center: Tools used for collecting lichen tissue for air quality monitoring include gloves to prevent contamination, special plastic bags to hold lichen tissue, a scale to weigh the bags once filled, and a sharpie to label the bags. Right: Developing and maintaining good organizational habits is important when in the field to ensure the integrity of specimens and accuracy of information.

Kovasi [pictured] and Mesraty scouted locations on the TNF this summer while also collecting lichen tissue to further contribute to the baseline of lichenological data for the area.

The project aims to empower community scientists to critically engage with lichens and the ecology of the TNF by learning how and why air quality monitoring is important, and how it could impact the health of the forest and surrounding areas. Volunteers will be trained to collect *Letharia vulpina* lichen tissue, record data, and support the lichen expert[s] performing the lichen community surveys. Additionally they may learn how to clean lichen tissue as preparation for analysis and prepare herbarium quality specimens.

The volunteer engagement process puts participants in the driver's seat of their experience. The project offers various entry points into science through air quality monitoring processes, ecological observation, herbarium collection methods, trainings, dialogic discourse, and real world experience working with experts on their public lands. Participants will engage with the scientific process in the field, trainings, and with scientists directly to offer a variety of accessibility points as not everyone is field-able. This human connection with scientists and scientific practices builds accessible relationships and trust between society and science.

We know lichens play a vital role in a larger ecological story, this opportunity could help us communicate and engage publics with that story directly by providing the USFS with key data. This work is interesting in California, especially with increased wildfires, urbanization, habitat loss, and of course the lichens.

What Happens Next?

At a time when climate issues are impacting all systems on a global scale, there is a unique opportunity to share knowledge and resourceful practices. Innovative uses of data results could alter human impact by cultivating a sense of accountability between people.

This project is in the ideation phase and the team will be developing and designing the specifics to launch a pilot in spring/summer of 2020, conditions depending, while working with community members and partners through the process.

This project's collected data will be available for the general public to access, and will be entered into the National Lichens and Air Quality Database and Clearinghouse that is managed by the USFS (USFS C, n.d.). Historic and current datasets will be reviewed to assess changes in air quality and species presence.

It is difficult to make a tangible argument with society that biodiversity matters; it is more tangible to make an argument that air quality matters because everyone breathes air and is impacted by air quality. One of the neatest things about air quality and lichens are how relatable they are. Air quality monitoring is a practical application of lichenology that is not mainstream news but has big impacts. This project helps the lichenological field at-large, benefits from increased awareness to critical issues highlighting relevant applications of lichens, and current USFS programs on air quality gain exposure and participation at a societal level.

The project team includes Donald Schweizer, Air Resource Specialist with the USFS, Adrienne Kovasi, Biological Science Technician with the USFS, and CALS member, and Hanna Mesraty, Project Director with the BRC and CALS Vice President. Schweizer, Kovasi, and Mesraty all bring unique skills to the project and are trying to utilize strengths within their partnerships to engage volunteers in a community science project using lichens to discuss critical issues in air quality and ecosystem health on public lands.

Curious to learn more about the project? Stay connected!

Website — lichenscitisci.org

Instagram — @lichenscitisci

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Calicium brachysporum, a rare California endemic

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Calicium brachysporum (Nádv.) K. Knudsen, Kocourk. & Lendemer, comb. nov. MB 833014 (Fig. 1).

=*Cyphelium brachysporum* Nádv., Ann. Myc. 40: 133 (1942). TYPE: U.S.A. California, Riverside County, near Murrieta, *H.E. Hasse*, 1903 (Holotype, PRM. Isotype, BRA).

In the 1890s and early 20th century, pioneer Californian lichenologist H.E. Hasse, while collecting lichens in southern California, often liked to end his day relaxing in hot springs. In Riverside County he stayed at resorts at Murrieta Hot Springs in Temecula Valley, Eden Hot Springs in the Badlands bordering Moreno Valley, and Palm Springs. In 1903 in the Temecula Valley near Murrieta Hot Springs Hasse collected on the dead branches of chamise, *Adenostomas fasciculatum*, a specimen he identified as *Cyphelium tigillare* (Ach.) Ach. (Hasse 1913).

In 1903, the Temecula Valley was covered with chaparral dominated by chamise. Hasse came back and collected more *Cyphelium* in 1905. Since the 1970s the area has been suburbanized. Tract homes and malls have spread out around a freeway that links Los Angeles with San Diego. Most of the chaparral and coastal sage shrubs are gone except on hills around the edges of the Temecula Valley. Despite much searching since 2000 we never found any *Cyphelium* in the remaining chaparral areas or nearby on the Santa Rosa Plateau or the Santa Ana Mountains.

The initial Hasse collection in 1903 ended up in Europe in the herbarium of the National Museum of the Czech Republic (PRM), where it was discovered by the curator Jana Kocourková in 2007. We do not know how the specimen got to Prague but Hasse studied medicine there for part of his doctorate and possibly corresponded with friends made during his stay (Knudsen 2010). During World War II and the Nazi occupation of Czechoslovakia, the Czech lichenologist Josef Nádvorník described the species *Cyphelium brachysporum* from the Hasse collection (Nádvorník 1942). In 2003, based upon an isotype in Europe in the Slovak National

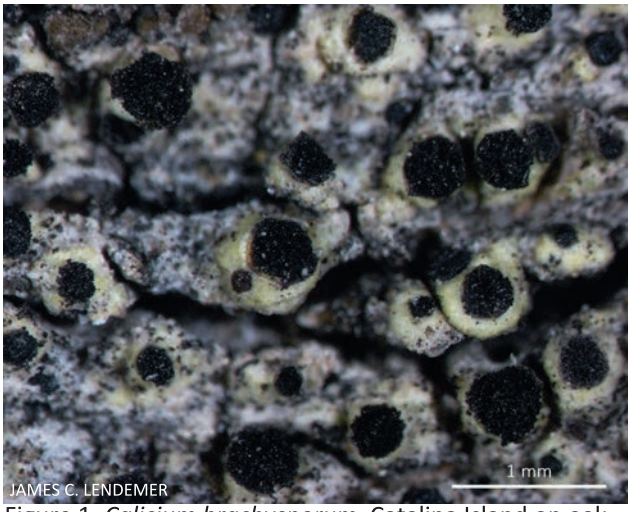

Figure 1. *Calicium brachysporum*. Catalina Island on oak snag. *Knudsen 15272* (NY).

Museum (BRA), L. Tibell synonymized *C. brachysporum* with *C. notarisii* (Tul.) Blomb. & Forssell (Tiebel et al. 2003). Tibell assumed *C. brachysporum* was immature *C. notarisii* based on rare slanted septa that rarely occur in the top cell (Lendemer et al. 2008). Examining Hasse's collections from 1903 and 1905 and a modern collection from Los Osos Nature Reserve in central California, the authors resurrected *Cyphelium brachysporum* (Lendemer et al. 2008; Knudsen & Kocourková 2008).

Cyphelium brachysporum has apothecia yellow with rhizocarpic acid and can be confused with *C. tigillare* and *C. notarisii*. It differs from *C. tigillare* in having smaller dark 1-septate ascospores 10–15 × 10– 12 µm with a punctate surface (best seen at 1000X when mature) vs. ascospores 17–21 × 9–11 µm with a smooth surface. *Cyphelium brachysporum* differs from *C. notarisii* in having smaller 1-septate ascospores 10–15 × 10–12 µm with a punctate surface, that are not constricted at septum vs. ascospores 17–24 × 13–18 µm with a smooth surface and constricted at center septum. *Cyphelium notarisii* when immature has 1-septate ascospores, becoming submuriform to muriform and eventually forming up to 14 cells. *Cyphelium brachysporum* had in one collection by Hasse in 1905 (NY) a single oblique septum formed in top cell dividing it in two but this character was lacking in the holotype and in recent collections. The report of *C. notarisii* in southern California is based on the Hasse specimens and possibly collections identified by Bruce Ryan from Santa Rosa Island as *C. brachysporum* that were probably revised by Tibell as immature *C. notarisii* (Tibell & Ryan 2004; CNALH 2019). These specimens are apparently lost. It is possible *C. notarisii* does not even occur in California. The final version of the *Cyphelium* treatment in the Sonoran Flora was published after Bruce Ryan's death and is based on an initial manuscript drafted by Ryan from his early unpublished flora in flora's format and revised by L. Tibell (Tibell & Ryan 2004). Since 2008, *Cyphelium brachysporum* has been collected on Catalina Island in southern California and in central California from Santa Barbara to San Luis Obispo Counties as well as Kern County in the southern Sierra Nevada Mountains. All recent specimens examined had 1-septate ascospores of similar size. The species is considered endemic to California.

In a molecular study of *Cyphelium*, a number of species were transferred to the genus *Calicium* including *C. tigillare* and *C. notarisii* which are morphologically similar to *C. brachysporum* (Lendemer et al. 2008; Prieto & Wedin 2017; Esslinger 2019). *Cyphelium brachysporum* was not included in this study. No species occur in the genus *Cyphelium* anymore in North America and we transfer *Cyphelium brachysporum* to the genus *Calicium*.

Selected specimens. U.S.A. Los Angeles Co., Catalina Island, Bulrush Canyon, 232 m, on dry oak snag, Jan. 19, 2013, *K. Knudsen 15214 & Ben Carter* (FH, UCR), *15272* (NY, UCR). Kern Co., southern Sierra Nevada Mountains, Caliente Ranch, 1227 m, on dry stump of blue oak, Oct. 11, 2013, *K. Knudsen 16229* (NY, UCR). Riverside Co., Murrieta, on dead chamise wood, 1905, *H.E. Hasse* (FH, NY). San Luis Obispo Co. Hind Ranch, 230 m, on oak bark, Nov. 12, 2011, *S. Sharnoff 4012* (UCR); Los Osos Oak Reserve, on Morro Manzanita, 57 m, Dec. 16, 2005, *K. Knudsen 4615* (UCR); San Simeon State Park, San Carpoforo Creek, on wood fence, June 19, 2006, *K. Knudsen 6510* (UCR). Santa Barbara Co., Sedgwick UC Reserve: 294m, on wood fence (destroyed), *K. Knudsen 15717* (NY, SBBG, UCR).

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New and interesting lichen finds in California

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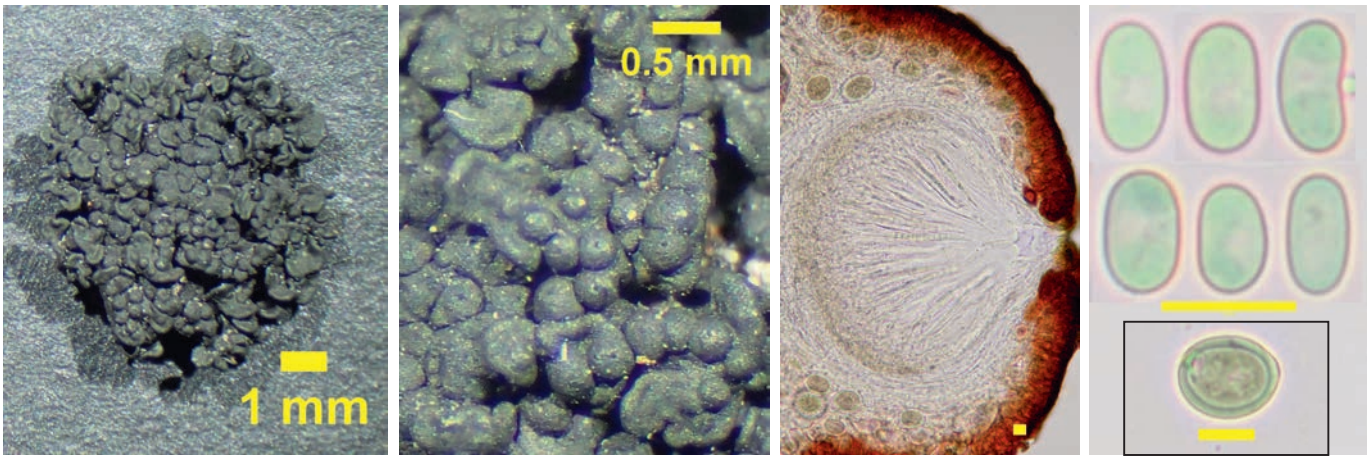
Phylliscum demangeonii (Moug. & Mont.) Nyl.
On siliceous roadside boulder in Klamath mixed conifer forest, Del Norte County, Six Rivers National Forest, SSW of Haystack Peak. *Carlberg 05930*.

A small squamulose gelatinous cyanolichen in the Lichinaceae, *Phylliscum demangeonii* grows in small 2–10 mm rosettes directly on the rock surface (Figure 1), attached by an umbilicus. As with other taxa in that family, it has a thick-walled single-celled *Chroococcus*-type cyanobacterium, unlike many gelatinous macrolichens, which contain *Nostoc*. The globular apothecia have thalline margins and are immersed or sessile on the rounded lobes (Figure 2). They resemble perithecia (Figure 3), with a tiny pinhole for an opening, visible in the field with a hand lens. With a light microscope, the spore size (Figure 4), thick sheath surrounding the photobiont cells (inset, below), and slender pointed tips of the asci are a good confirmation.

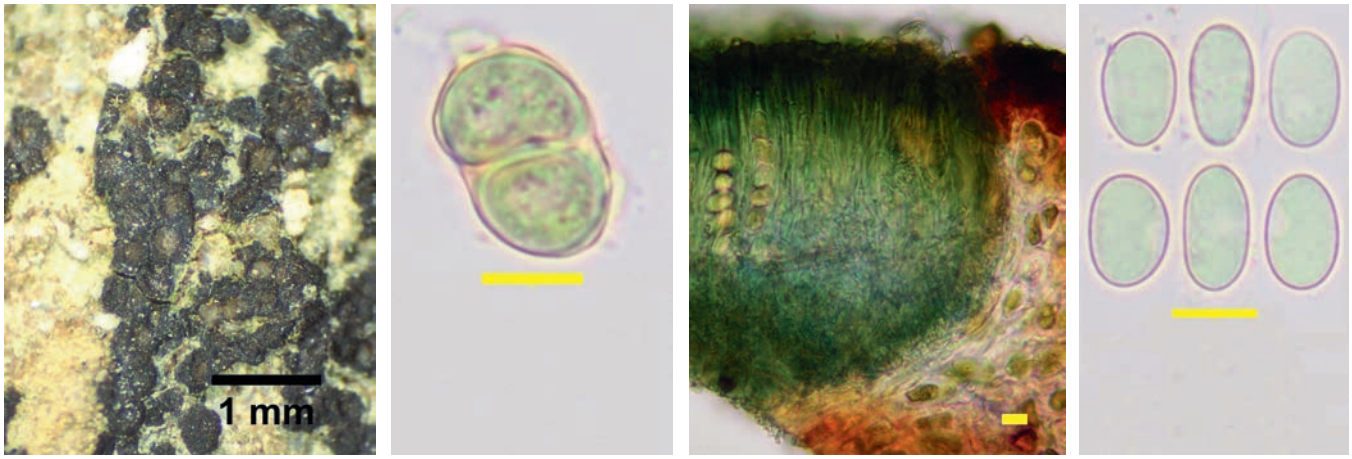
Pyrenopsis furfurea (Nyl.) Nyl.
On small weathered HCl- sandstone immersed in soil on a gravelly bald with scattered invasive annual grasses, in a clearing in a Douglas-fir forest on the UC Berkeley Angelo Coast Range Reserve, Mendocino County. *Carlberg 05825*.

Some authors consider *Pyrenopsis* to be a genus in need of thorough revision, with characters similar to those found in *Euopsis, Cryptothele, Psorotichia* and other genera, and inconsistencies within itself (McCune 2017, Smith et al. 2009, Schultz 2007, Jorgenson 2007). This specimen of *Pyrenopsis furfurea* appears very much to be a crustose lichen, although it sometimes develops a squamulose habit. It has expanded apothecia with a thalline margin and reddish-brown disk (Figure 5). Its photopartner



Figures 1-4. *Phylliscum demangeonii*. From left: habit; perithecium-like apothecium; section of apothecium showing pointed ascus tips; spores. Inset: *Chroococcus*-type photobiont. Scale bars = 10μm unless noted. Photos by Tom Carlberg.

is similar to that found in *Phylliscum demangeonii*, but lacks a thick gelatinous sheath (Figure 6). The spores are similar in size and shape (Figure 7), but the asci are clavate and not pointed. The reaction in IKI is markedly different, the hymenium reacting strongly IKI+ green or deep reddish-brown (Figure 8). In the field, *Pyrenopsis furfurea* might be mistaken for *Peltula patellata*, which sometimes grows on rock, but that species is polysporous, the hymenium reaction is never green, and it prefers calcareous substrates.



Figures 5-8. *Pyrenopsis furfurea*. From left: habit; cyanobacterial photobiont; section of apothecium showing thalline margin and vivid green reaction in IKI; spores. Scale bars = 10µm unless noted. Photos by Tom Carlberg.

Pertusaria islandica Bratt, Lumbsch & Schmitt
On HCl- sandstone roadbank in riparian forest along Gazos Creek Road, San Mateo County. *Kellman #8585*.

This is one of the brown spored species of *Pertusaria*, although the original description by Schmitt et al. allows for hyaline spores which are K+ violet or purple (Figure 9). Per the authors, there are 8 spores per ascus, but I was only able to see six. In this specimen, they measured 51–73 x 27–30µm, with walls 5.5–7.5 µm thick. The black apothecia are buried in thick areolate warts and are exposed by more or less radial cracks (Figure 10). The sterile areoles are beige, the fertile ones off-white. The epithecium is K+ purple (figure 11). All other spot tests are negative. It is separated from the

Figures 9-11. *Pertusaria islandica*. From left: thick-walled spores in K; habit; hymenium and asci. Photos by Ken Kellman.

other brown (K+ purple) spored *Pertusaria*, *Pertusaria occidentalis* Bratt, Lumbsch and Schmitt by its elliptical spores. *P. occidentalis* has globose to subglobose spores. Some authors place these two species in the genus *Melanaria*. This species is most common on the islands of Ventura County, and this collection represents a modest range extension.

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California Lichen Society Grants Program

The California Lichen Society offers small grants to support research pertaining to the lichens of California. No geographical constraints are placed on grantees or their associated institutions, but grantees must be members in good standing of the California Lichen Society. The Grants Committee administers the grants program, with grants awarded to an individual only once during the duration of a project. Grant proposals should be brief and concise.

Grant Applicants should submit a proposal containing the following information:

- Title of the project, applicant's name, address, phone number, email address, and the date submitted.
- Estimated time frame for project.
- Description of the project. Outline the purposes, objectives, hypotheses where appropriate, and methods of data collection and analysis. Highlight aspects of the work that you believe are particularly important and creative. Discuss how the project will advance knowledge of California lichens.
- Description of the final product. We ask you to submit an article to the Bulletin of the California Lichen Society, based on the results of your work.
- Budget. Summarize intended use of funds. If you received or expect to receive other grants or material support, show how these fit into the overall budget. The following list gives examples of the kinds of things for which grant funds may be used if appropriate to the objectives of the project: expendable supplies, transportation, equipment rental or purchase of inexpensive equipment, laboratory services, salaries, and living expenses. CALS does not approve grants for outright purchase of capital equipment or high-end items such as computers, software, machinery, or for clothing.
- Academic status (if any). State whether you are a graduate student or an undergraduate student. CALS grants are also available to non-students conducting research on California lichens. CALS grants are available to individuals only and will not be issued to institutions.
- Two letters of support from sponsors, academic supervisors, major professors, professional associates or colleagues should be part of your application. These should be submitted directly from the author to the committee Chair.
- Your signature, as the person performing the project and the one responsible for dispersing the funds. All of the information related to your application may be submitted electronically.

Review: Members of the Grants Committee conduct anonymous evaluation of grant proposals once a year based on completeness, technical quality, consistency with CALS goals, intended use of funds, and likelihood of completion. Grant proposals received by November 1 each year will be considered for that year's grant cycle. The Grants Committee brings its recommendations for funding to the Board of Directors of the California Lichen Society, which has final say regarding approval or denial.

Grant Amounts: CALS typically offers two grants of $750.00 and $1000.00 each year. Typically grants are awarded to two separate individuals, however depending on the quality of the applications and the amount of funding available, the committee maintains the option to disburse funds as appropriate. All grants are partially dependent on member contributions, therefore the amounts of these awards may vary from year to year.

Obligations of recipients: 1) Acknowledge the California Lichen Society in any reports, publications, or other products resulting from the work supported by CALS. 2) Submit an article to the Bulletin of the California Lichen Society. 3) Submit any relevant rare lichen data to California Natural Diversity Data Base using NDDB's field survey forms. See http://californialichens.org/conservation for additional information.

How to submit an application: Please email submissions or questions to the committee Chair at grants@californialichens.org **by November 1 of the current calendar year**. The current Chair is Rikke Reese Næsborg.

Upcoming Events

26th Annual Meeting of the California Lichen Society
Dates: January 24–26, 2020
Location: Hastings Natural History Reservation, Monterey County, California
Fees: Attendance is free, but lodging is $20/night/person

CALS' 26th Annual Meeting will be held at Hastings Reserve in Carmel Valley January 24th - 26th, 2020. The weekend celebration will include field trips on Friday and Saturday. Additionally, on Saturday there will be an open meeting of the Board of Directors and a social hour followed by a pot-luck dinner. In your free time between all these activities, participate in lichen identification sessions and enjoyable lichen chatter. This event connects CALS members with California lichens.

After Saturday's pot-luck, there will be a presentation by Dr. Jessica R. Coyle, an ecologist and Assistant Professor at St. Mary's College in Moraga. Her current research projects examine both communities of lichens and microorganismal communities within lichens, but her presentation will describe research projects executed by her undergraduate students.

Hastings Reserve is part of the Big Sur Wilderness in central, coastal California. The site is

unique, with some rare flora given that the site has not been used for grazing in over 70 years. Additionally, the site has been observed, researched, and protected for just as long, offering insights into the natural ecological systems. The reserve is primarily a mixture of live oaks and redwoods with perennial stream riparian zones mixed in. With a rich non-vascular flora, join CALS in identifying the lichens at Hastings Reserve as we celebrate 26 years of lichenology in California.

If interested in participating, email memberat-large@californialichens.org for more information or to register. Lodging options are on a first come first serve basis.

Introduction to Lichen Identification and Ecology

Dates: February 8–9, 2020
Instructors: Jesse Miller and Allie Weill
Location: UC Berkeley and Marin Municipal Water District Field Site
Course Fee: $275/$305

Lichens are all around us and they have fascinating stories to tell. This two-day workshop will focus on developing skills for identifying common Bay Area macrolichens (foliose and fruticose lichens) to genus. We will begin with an introductory classroom session, where we will cover basic lichen anatomy and terminology, and discuss the roles lichens play in ecosystems such as supporting wildlife. We'll then divide the rest of the class time between field trips to nearby natural areas and lab time, so that students can observe lichens in their natural habitats and then bring collections back to the lab for study. Students will learn to recognize and distinguish between pollution-tolerant lichen communities that we often see in cities and the more pristine communities that occur in places with high air quality. After taking this course you will be sure to observe lichens, big or small, almost everywhere you go!

Register for this workshop here:
http://ucjeps.berkeley.edu/workshops/regform.html

Charismatic Microflora: The Ecology and Management of Biological Soil Crusts

Dates: February 20–23, 2020
Instructors: Matt Bowker, Kirsten Fisher, Brent Mishler, Tom Carlberg, and Mandy Slate.
Location: Desert Studies Center, Zzyzx, CA
Course Fee: $75

Biological soil crusts (biocrusts) are communities of cryptic organisms, including cyanobacteria, mosses, and lichens that typically stand less than 0.5 cm in height. Biological soil crusts have a significant impact on the world because of their extensive global distribution and their regulation of ecosystem functions. They also provide the opportunity to study amazing biological traits such as desiccation tolerance. These communities are easily damaged or destroyed by human activities such as cattle grazing and off-road vehicle use and are of considerable concern in managing dryland environments.

This workshop will cover the basics, including: What is a biocrust? What are biocrusts composed of? How are biocrust organisms identified? Where are biocrusts found? How do the organisms in biocrusts manage to survive and reproduce in such a seemingly harsh environment? What role do biocrusts play in ecosystems? How can biocrusts be managed? How and where to find compelling, charismatic, and crucial biocrusts? We will combine classroom lecture with hands-on activities at the microscope, and visits to the field.

The Desert Studies Center at Zzyzx is located at Soda Springs on the northwestern edge of the Mojave National Preserve. The surrounding landscape supports a range of plant communities, including halophytic vegetation, marsh communities, ponds and springs with pondweed, cattail and sedges, extensive creosote bush scrub and saltbush scrub stands, crescent sand dunes, and plants stabilized by mesquite thickets and the rocky slopes and ravines of the Soda Mountains.

Register for this workshop here:
http://ucjeps.berkeley.edu/workshops/regform.html

Northwest Lichenologists Annual Spring Meeting with Northwest Science Association

Dates: March 24–27, 2020
Location: Univ. of Oregon, Eugene, Oregon
Fees: See Northwest Science link below

Annual general meeting (March 24-27, 2020, Eugene, Oregon at the University of Oregon. We are meeting with the Northwest Scientific Association (NWSA). Registration is through NWSA. The meeting has talks, a poster session, workshops, and one or more field trips.

Support for students. For 2020 we are offering 3, $100 gifts for students (undergraduate or graduate) travelling to present in the lichen-bryophyte session at the meeting. First priority will be given to those giving oral presentations, second priority to poster presentations. Within those groups, awards will be given in the order that abstracts were received. Also, check for opportunities with your college or university -- most of those have grants for student travel to meetings.

Field trips: TBA. We plan to have at least two field trips near Eugene. We will update this page as details become available. http://northwest-lichenologists.wildapricot.org/page-1816534

Workshop: TBA. Full meeting Details at: https://www.northwestscience.org.

President's Message

Dear CALS members – It seems that in recent years some of our best affiliations have been increasingly in connection with students. Our grants program continues to grow, and with it the contributions to the Bulletin. Due largely to the efforts of **Jesse Miller**, our distinguished guest speakers at last year's annual meeting were three students. This year at Hastings, Dr. **Jes Coyle** will speak about some of the original research her undergraduate students are working on. Given Jes's committment to lichens and academic professionalism, I am certain we will leave Hastings much wiser than when we arrived. We can thank **Julene Johnson** and **Ken Kellman** for organizing most of this year's meeting, by the way!

As some of you had undoubtedly noticed, we had some difficulties with our website in the past year; something to do with an "infinite loop"... I don't know what that is, but it cost our webmaster **Eric Peterson** a lot of patient work to put it back together. Thank you, Eric!

On Santa Cruz Island in 2016, trying to figure it all out.

In August of this year, CALS members and the CALS Board of Directors decided to make our Bulletin an Open Access journal, and now even the most recent issues are available for everyone at no charge. In January, when it comes time for most of our members to renew, we will learn whether this was a fiscally sound decision or not. It certainly is in keeping with CALS' mission statement, to "*promote the appreciation, conservation, and study of California lichens*", and is in accord with the many free and paid workshops, meetings and field trips we have initiated over the past 26 years.

But it comes down to this: we still need the support and generosity of our members to continue as a society. I am deeply hopeful that many of you have availed yourselves of our meetings, website, field trips, etc., and that you think well of what the Society does, and that you will continue your memberships without second thoughts. To those of you who have made donations above and beyond your membership level, I thank you personally, and hope to be able to express those thanks face-to-face sometime soon.

Do you have any plans for late January...?

Hoping to see you soon,
Tom

Tom Carlberg
President@californialichens.org

California Lichen Society

PO Box 472, Fairfax, California 94978

The California Lichen Society (CALS) seeks to promote the appreciation, conservation, and study of lichens. The interests of the Society include the entire western part of the continent, although the focus is on California.

Members receive the Bulletin of the California Lichen Society (print and/or online access), voter rights in society elections, access to the CALS community, and notices of meetings, field trips, lectures, and workshops.

Membership Dues (in $US per year)
Student and fixed income (online eBulletin only) - $10
Regular - $20 ($25 for foreign members)
Family - $25
Sponsor and Libraries - $35
Donor - $50
Benefactor - $100
Life Members - $500 (one time)

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To join or renew online, please visit www.californialichens.org/membership

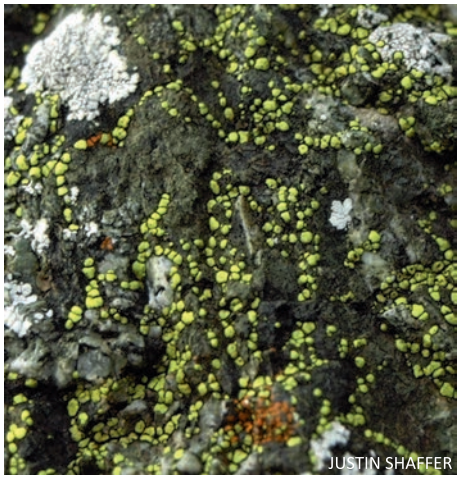
Names for some of the lichen species pictured on the back cover. All determinations are by the authors of the photographs. **Foliose lichens:** *Sticta limbata; Hypotrachyna revoluta; Parmotrema austrosinense.* **Fruticose species:** *Ramalina farinacea; Xanthoria pollinarioides; Cladonia macilenta.* **Crustose lichens:** a species of *Ochrolechia* and an unknown crust with black apothecia; another unknown crust; *Calicium viride.*

Lichens from around California

A variety of lichen forms and species from far northern California all the way down to the Santa Barbara coast. Can you identify any of them? Some of the species' names appear on the inside back cover...

Foliose lichens.

Fruticose lichens.



Crustose lichens.