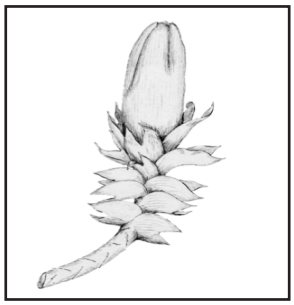

Newsletter of the FRIENDS OF THE FARLOW

Number 69 Winter 2017 Melinda A. Peterson, Editor

Stuck Like Glue: Fungi That Live on Conifer Resin

By James K. Mitchell

Conifers produce resins not only to discourage herbivory, but also to seal off damaged areas to prevent pathogens from entering the plant. When fresh, this resin is extremely sticky, flowing slowly and trapping most things that touch it. As it dries, it begins to polymerize, forming a hard, external crust; the interior becomes thicker or crystallizes and remains very sticky. It is reasonable to anticipate that this substrate is extremely inhospitable for most organisms, including fungi. Colonization of young resin could result in engulfment and death, and colonization of older resin is complicated due to its hard surface. In both cases, the resin acids and terpenes are not particularly nutritious and are likely toxic – challenges which should be ample deterrents to fungal establishment.

However, upon closer inspection, one can see that this is not the case. A few species of fairly colorful cup-fungi, such as *Lachnellula resinaria* (white and yellow), *Claussenomyces kirschsteinianus* (blue), and *Sarea resinae* (orange), are small but relatively conspicuous. One might also see a few small green lichens growing and, with the aid of a lens or microscope, some less colorful ascomycetes. *Claussenomyces olivaceus* (dark olive cups), *Sarea difformis* (black cups), and various mytilinioid fungi (black clamshells) are also frequently found, as well as fuzzy patches of various brown or black molds. For most of the surface area, though, not much activity is evident to the naked eye, nor even the eye aided by a stereomicroscope; to see further requires the use of a compound microscope.

For closer observation, a patch of resin is selected and frozen on the stage of a freezing microtome; once frozen, it is sliced very thinly and placed on a microscope slide. Even having seen the fungi growing on the surface, what we see under the microscope is still somewhat surprising: the entire surface of the resin is clearly colonized by fungal hyphae penetrating hundreds of microns into the crust, and sometimes even into the liquid resin below. Despite what we may have anticipated, fungi are flourishing on this substrate and appear to preferentially seek it out. But how is this possible and why? Unfortunately, these questions are probably not easy to answer in a broad sense. Adaptation to this resinous substrate is probably an example of convergent evolution, since most of the fungi mentioned above are not very closely related. It might be easier to explore these questions for just one or two related fungi rather than the handful of examples mentioned.

Let us focus now on the two species in the genus *Sarea*, both of which are only ever found on

FoF Annual Memberships Due!
Please see page 4 for details

resin (the other species mentioned are in genera that are not solely resinicolous).[1] *Sarea* is also in a unique position of being *incertae sedis* – of unknown placement – within the subphylum Pezizomycotina[2], which may be indicative of a completely new evolutionary lineage specialized to live on resin. These two species are also present on a wide variety of trees in both the pine and cypress families distributed across the northern hemisphere, suggesting a general adaptation to this habitat. Finally, a fair amount of research has documented these species regarding their taxonomy, distribution, and habitat. All of these facts seem to support this genus as a prime candidate for study. It may come as no great surprise that this is the genus I am currently most actively investigating.

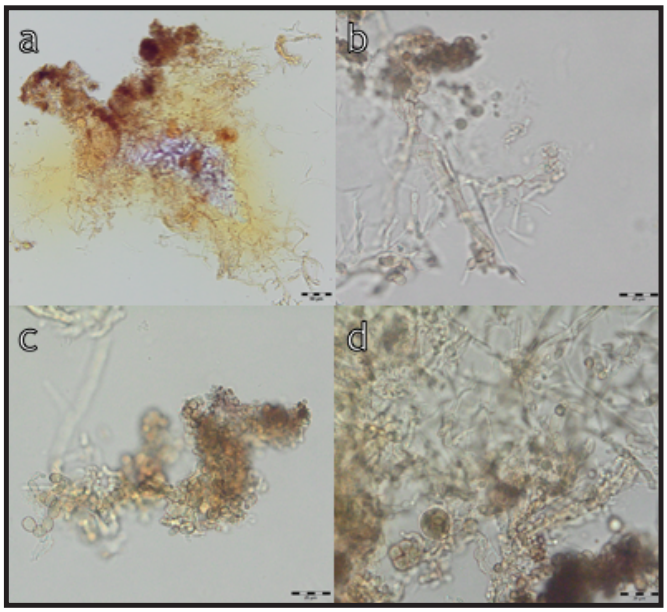

Figure 1: Asexual fungi growing on and in resin. a-d) Various fragments of a section of resin mounted in Melzer's reagent. This solution dissolves the resin and allows the large quantity of different hyphae to be seen more clearly. This resin is from a specimen of *Pinus nigra* growing on Harvard's campus within walking distance of the Farlow.

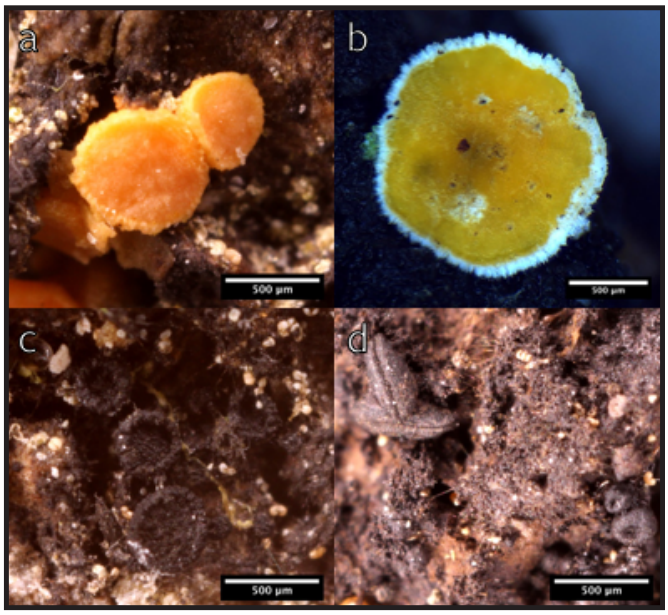

Figure 2: Some typical local resinicolous fungi. a) *Sarea resinae.* b) *Lachnellula resinaria* (photo by Luis Quijada). c) *Sarea difformis.* d) Two fungi: Small black young apothecia cups of *Claussenomyces olivaceus* (lower right) and two larger black clamshell unidentified mytilinidioid fungi (upper left).

Before studying their ecology or biochemistry in depth, I am attempting to better understand these two species by searching for them in the field on various host trees in different environments and studying them with both morphological and molecular methods. I have collected from a variety of locations primarily within New England, including the Boston Harbor Islands (Boston, MA), Appleton Farm Grass Rides (Hamilton, MA), the Eagle Hill Institute, Estabrook Woods (Steuben, ME), Arnold Arboretum (Boston, MA), Harvard Forest (Petersham, MA), Chickering Bog Natural Area (Plainfield, VT), and Unicoi (Helen, GA) and Sweetwater Creek State Parks (Lithia Springs, GA). Additionally, to date I have collected from 15 different host species belonging to 5 genera. The specimens of *Sarea resinae* and *Sarea difformis* – easily distinguishable from each other – are virtually identical morphologically within each species. There are some small variations in color (pale orange to more reddish) in specimens of *S. resinae* and variations in size in specimens of both species. However, it is unclear whether these disparities are significant, or which features might be diagnostic, since specimens look very similar under the microscope. In contrast, my molecular work thus far has yielded some encouraging and more immediately interesting results.

ITS (internal transcribed spacer) sequences from Europe and Japan procured from publicly accessible sequence databases[3, 4] reveal fairly significant differences between *Sarea* species in these two lo-

cations. This is perhaps not surprising due to the geographic separation of Europe and Japan; there is likely to have been speciation since the two populations could not mix unless they were transferred by humans. However, what is more surprising is that for each of these locations there are significant differences (~4%) among the sequences of different specimens from fairly close locations. My own data show the same pattern. My sequences are different by about 4% or more from specimens from Japan and most of the specimens from Europe, and there are a few clusters of my own sequences that are about 4% different from each other. This is significant because the ITS gene is considered the barcode gene for fungi. This means that this sequence is generally used to identify species, with a 3% difference considered the standard cutoff-point for species differentiation. Therefore, there are strong indications that multiple Asian, European, and American species of these fungi exist, rather than just two as previously thought. Furthermore, when the clusters of similar sequences are compared, another pattern starts to emerge. Genetically similar specimens appear to grow on the same type of tree. For example, two "species" from Japan are found on trees in the genus *Chamaecyparis,* but specimens from *Tsuga*, *Larix*, and *Pinus* seem to belong to other "species"; one of the "species" from New England has so far only been found on *Picea*, whereas others have only been found on *Pinus*. At this stage sampling is too sparse to tell if this is truly a pattern. However, it does point to interesting possibilities.

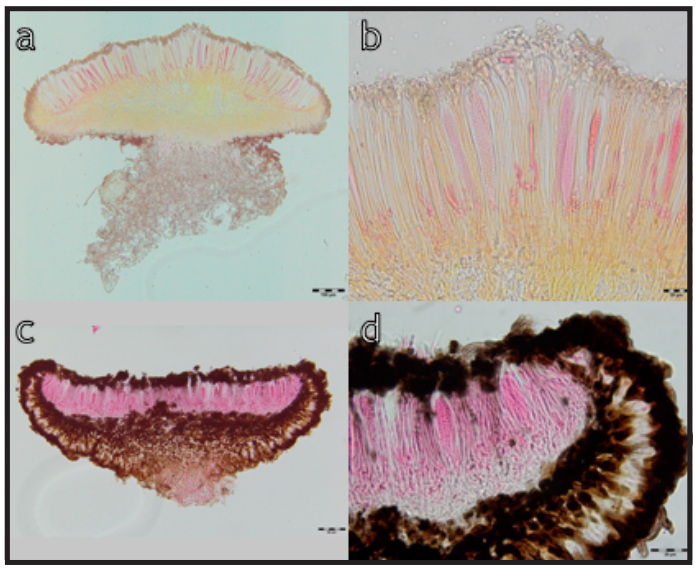

Figure 3: *Sarea* specimens. a) A 15µm thick section of *Sarea resinae* mounted in a solution of eosin dye in glycerol. The eosin gives the pink color to the insides of the asci. b) A close up of some asci in various stages of maturity. It is evident that these asci – when mature – contain hundreds of small, spherical spores. c) A 15 µm thick section of *Sarea difformis* mounted in a solution of eosin and glycerol. Instead of just the asci, it appears that the eosin stains the entire hymenium. d) A close up of asci from the same specimen, where the asci again are clearly polysporous (containing many spores).

The possibility of the existence of many previously unrecognized species is exciting. If these species have all truly adapted to species of different genera of conifers, they may also have different ecophysiologies. Even multiple species adapted to the same genus or species of tree may have significantly different biochemistry and ecology. One could try to grow them artificially on various types of resin and record growth rates to see if they differ. Chemical tests might also be devised to distinguish the species. It might even be possible to match the evolution of various conifers with that of these different species of fungus.

Of course, a significant amount of work still remains. At this time, I am focusing on acquiring additional specimens to sequence – both locally and internationally – including those from a variety of host trees. This is aimed at verifying my preliminary hypotheses. If you or any of your friends happen upon either of these species, and you are in a place where you do not need a permit to collect, I would be more than happy to accept any contributions of specimens for my research.

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News & Events from the Farlow

2017 Annual Meeting

On Saturday, November 4th, we held the 2017 FoF Annual Meeting at the Harvard Herbaria. **Camille Truong**, a postdoctoral fellow from the University of Florida presented her research on ectomycorrhizal fungi associated with the Southern beech in Tierra del Fuego. In "Gondwanan fungi: mycological explorations in Patagonia," Camille shared her experiences and findings from her work from the end of the earth. A reception was held Farlow Library following the lecture. Camille was jointly sponsored by the Boston Mycological Club where she also gave a lecture. During her stay, Camille worked with the Patouillard herbarium containing 19th century collections from Mexico. This is of particular interest to Camille because she will be beginning a new position in Mexico. With her assistance, we were able to more accurately catalog a set of drawings of fungi illustrated by Paul Maury in Mexico.

FoF Book Sale Update

In early October of this year, we held the 2017 Friends of the Farlow book sale. Of the 97 available titles, 53 books were sold, yielding $668.59. Book sales have annually been a prominent source of funds for the FoF, and its success is largely in part to the generosity of our membership in both the purchase and donation of books. Thank you again to all those who participated in the book sale this year.

Membership & Dues

The FoF will begin collecting membership dues at the end of the calendar year. A notice will be issued in December reminding the membership.

Special Thanks

One of the noteworthy achievements of the Friends of the Farlow has been in the development of several research initiatives to bring scholars to study the collections of the Farlow Library and Herbarium. Initially, the program was designed to support graduate students with small fellowships of $500-$1000; the program later expanded to permit the inclusion of foreign scholars to visit our facilities. Established in 1981, the **Geneva Sayre Fund** was endowed to allow senior researchers to study at the Farlow, in honor of an integral former member of the Farlow team and research associate in bryology. The **Harvey Pofcher Fund** was later founded to support young professionals in their study at the Farlow. These programs have not only facilitated important work on fungi, lichens, algae, and bryophytes, but have greatly improved our collections through the contributions and expert annotations of these researchers. We are proud to express that the generosity of our members has made it possible for us to support more than 35 researchers to date. Thank you, friends, for your continued support!

Greetings, Farewell & Best Wishes

As some of you may know, **Genevieve Lewis-Gentry Tocci** – our principle curatorial assistant at the Farlow Herbarium and former FoF newsletter editor – has been on maternity leave. In August, Gen and her husband welcomed their child, a beautiful and healthy baby named Isadora. In Gen's absence, curatorial assistant **Hannah Merchant** has been filling in with the assistance of **Giovanna Bishop**, a recent graduate of Wheaton College. We look forward to Gen's return in the new year.

In September, we said goodbye to **Summaira Shaheen**, who returned to Pakistan to resume her studies. During the six months she was here studying lichens, she enhanced her identification skills and learned invaluable sequencing techniques. We wish Summaira the best in her future endeavors.

In the wake of Barbara Hanrahan's retirement this spring, we are thrilled to welcome new HUH receptionist and staff assistant **Claire Gallagher**. Claire moved to Cambridge from the Bay Area after working in business development for many years. She began working for the HUH over the summer in a temporary capacity, before officially joining the staff in October. Claire has had a deep and abiding love for plants ever since childhood and we couldn't be happier to have her be a part of the HUH. Welcome Claire!

Visitors & Researchers

Afshan Wahab

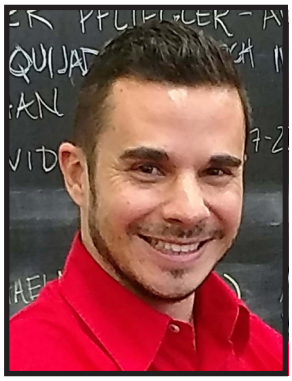
In September, we welcomed **Afshan Wahab** to the Pfister Lab. Afshan is a visiting graduate student from Pakistan who is working on polypores.

In October, she was joined by two additional visitors from Pakistan: **Tayyaba Qasim** and **Munazza Kiran.** Tayyaba and Munazza are working on various aspects of the agarics of Pakistan, and will be visiting the Farlow Herbarium for six months.

Munazza Kiran

Tayyaba Qasim

Luis Quijada, who we introduced in the previous newsletter, has returned to the Farlow in October on a fellowship from the Fundación Ramón Areces in Spain. Luis will continue his work on the *Tympanaceae*, but is also collecting and examining a range of small inoperculate discomycetes that he has been studying locally. Many of the fungi that Luis is studying have not been well investigated in North America; therefore, his contributions will significantly benefit those interested in the fungi.



Research & Other Activities

This summer, **Don Pfister** and **James Mitchell** traveled to attend the 2017 MSA Annual Meeting in Athens, Georgia, from July 16-19.

A great deal of Don's activities this year have focused on **Harvard's Glass Flowers** collection. Last year, Don was featured in a video regarding the Harvard Museum of Natural History Glass Flower Gallery renovation that took place last year. At this time, there is a temporary mycological exhibit featuring rotten apples and apple diversity. We hope that you will visit the Ware Collection exhibit and see not only the diseased apple models, but a super-sized model of an *Aspergillus* species. For a preview of the exhibit narrated by Don, please visit: *https://news.harvard.edu/gazette/story/2017/10/rotten-apples-opens-inside-harvards-glass-flowers-gallery*

Luis Quijada collecting on Great Brewster Island, Boston, MA (2017). Photo courtesy of D. Haelewaters.

In September, **Melinda Peterson**, our newsletter editor, celebrated her first-year anniversary with the Farlow. In addition to her work as a research lab coordinator with the Pfister and Davis Labs, she has been meticulously undertaking much of the work for the Friends of the Farlow in order to keep us on track.

A still of Don from the Harvard Gazette story, "Rotten Apples Return to Harvard's Glass Flowers Gallery." Video courtesy of KJ Wang (October 5, 2017)

James Mitchell, our recent addition from the Physics Department, has been actively collecting and studying the genus *Sarea*, which he chronicles in the lead article of this newsletter. James's research on these species has been largely supported through a Friends of the Farlow grant.

Graduate student **Danny Haelewaters** travelled to Panama and spent his summer doing fieldwork at the Smithsonian Tropical Research Institute in Gamboa and the Chucantí Nature Reserve cloud forest in the Darién Gap. He also spent two weeks in David, conducting research at the Universidad Autónoma de

Clockwise from lower left: The bat processing table at the Chucantí Nature Reserve in Panama; an *Artibeus* leaf-nosed bat; Danny Haelewaters holding a leaf-nosed *Artibeus watsoni* bat; An undescribed species of *Gloeandromyces* on a *Trichobius joblingi* bat fly, collected from a *Carollia perspicillata* leaf-nosed bat; A rare specimen of *Antennopsis* sp. on a *Nasutitermes* sp. termite.

Chiriquí. The purpose of the excursion was to screen parasitic flies on bats for the presence of Laboulbeniales fungi, contruct a phylogeny with the Panamanian data, and identify traits associated with parasitism by Laboulbeniales. Also accompanying Danny on the Harvard-Smithsonian funded research endeavor were **Kirk Silas** (Pennsylvania), **Lauren Meckler** (University of Delaware), **Walter Pfliegler** (University of Debrecen, Hungary), **Annabel Dorrestein** (Utrecht University, the Netherlands), **Melissa Walker** (La Trobe Institute for Molecular Science, Australia) and fellow graduate student **Jasmin Camacho** (Tabin Lab, Harvard Medical School).

In October, Danny also attended the 47th North American Symposium on Bat Research (NASBR) in Knoxville, Tennessee, where he presented preliminary results on his work on bats, parasitic bat flies, and Laboulbeniales fungi. In addition to actively writing and publishing, Danny has also been working on compiling an inventory of the fungi of the Boston Harbor Islands in collaboration with **James Mitchell** and **Luis Quijada**. These collections are being actively sequenced to help document the mycota of the islands.

Philip May Fund

To date, friends and colleagues of the late **Philip May** have contributed more than $3500 to a fund established in Phil's honor to promote the study of lichens. We reported the sad news of Phil's passing in a previous newsletter. That fact that his associates and friends have chosen to remember Philip in this way is a testament to how dedicated Phil himself was to the partnership with the Friends of the Farlow and the study of lichens.

Join us!

Receive the FoF Newsletter, notification of the annual book sale, discounts on Farlow publications and services, invitations to the annual meeting and other events, and a special welcome when visiting the Farlow. *Formal dues notices will be issued in December.*

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