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~ Important Dates ~

April 15: Deadline: *Inoculum* 53(3)
June 22-26: MSA 2002, Corvallis OR

Editor —

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Discovery of a New Obligate Tree Canopy Myxomycete in the Great Smoky Mountains National Park

***by* Harold W. Keller and Melissa Skrabal**

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Introduction: The Great Smoky Mountains National Park (GSMNP), which comprises more than 200,000 hectares, serves as a refuge for one of the richest and most diverse biotas in the temperate regions of the world. It also has the largest remaining tracts of old growth forest in Eastern United States, estimated at 40,000 hectares. A new research initiative called the All Taxa Biodiversity Inventory (ATBI) under the rubric of a non-profit organization, Discover Life In America (DLIA), represents a research effort to inventory and identify all of the life forms in the park. Cryptogam sampling for fungi, lichens, mosses, liverworts and ferns in the park has been confined to ground sites. Life in treetops must adapt to different environmental conditions and corticolous Myxomycetes found there are often distinct from the surrounding biotic communities (Keller and Braun, 1999).

Panoramic view of forest terrain from tree canopy

METHODOLOGY: The All Taxa Biodiversity Inventory management plan established 20 one-hectare study plots scattered through the park. Site selection was based on major forest/vegetation types, elevation and relative accessibility. Our tree canopy biodiversity study emphasized champion-sized trees of certain species. Some of these trees were up to 55 meters in total height. Safety was emphasized in all of our activities. Student climbers completed a training school taught by a professional arborist. The double rope climbing technique was used because the climbers could advance to higher levels in the tree canopy. A Big Shot slingshot was used to shoot a slick line (attached to a weighted throw bag) over crotches and branches usually at heights of 18 to 24 meters. A climbing rope was attached to the slick line and pulled over the limb. This rope was tied to a climbing saddle and a friction knot used to vertically ascend the rope. Each tree climbed was entered in a database that included a tag number for each tree, numbered samples at three meter intervals up to 40 meters measured with elevation lines, diameter at breast height, total height of tree, climber's name, observations of *in situ* specimens on the tree trunk, place location description and global positioning system reading, altitude, and weather conditions. All student climbers were given tutorials that included lecture slide shows, demonstration specimens, practice keying, and field experience with experts from the multidisciplinary research team that enabled them to recognize and collect the targeted groups of organisms. A total of 240 trees representing 35 different tree species were climbed during two three-week periods in June, July and August of calendar years 2000 and 2001.

Melissa Skrabal collecting and bagging lichens.

Melissa Skrabal untying throw line from climbing rope.

LABORATORY MOIST CHAMBER CULTURES: Bark samples were scanned by student climbers in the field for plasmodial tracks and myxomycete fruiting bodies. Corticolous myxomycetes that grow and fruit on the bark surface or epiphytes on living trees are usually less than 1 mm in diameter. Sometimes the sheer number or bright colors of fruiting bodies makes it easier to see them on the bark but it takes a sharp eye and knowing what to look for to find them. Most field researchers have collected bark samples at about two meters or less so our knowledge of corticolous myxomycetes is based on samples taken near ground level. The moist chamber culture technique was used to process field-collected samples of bark and epiphytes from living trees and vines by placing the bark sample in a sterile Petri dish with sterile filter paper lining the bottom of the dish that serves as a wick. The contents were moistened with the addition of 10 ml of sterile glass distilled water. Any excess water was poured off in 24 hours and the cultures incubated at 25°C for up to four weeks. Cultures were examined daily to observe plasmodial and fruiting body development, record data on time of maturation, and harvest fruitings in a fresh fully mature state for preservation and identification (Keller and Braun, 1999).

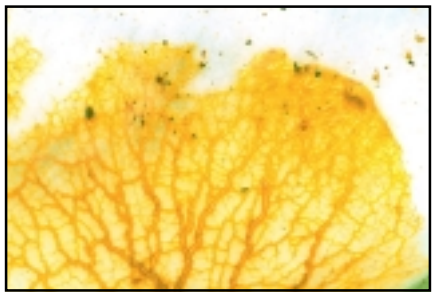
TREE CANOPY MYXOMYCETES: Are there any myxomycete species confined to the upper tree canopy? My (HWK) collecting and climbing experience dating back to the 1960s mostly with *Juniperus virginiana* (Red Cedar) trees in the midwest and southeastern United States, suggested that myxomycete taxa found on the trunks extended up into the tree canopy. These trees were climbed either with pole climbing spurs and a safety belt or free-handed.

Melissa Skrabal was the Central Missouri State University undergraduate student who discovered the first obligate tree canopy myxomycete, a new species in the genus *Diachea.* Melissa kept a field diary of her discovery and excerpts are reproduced here.

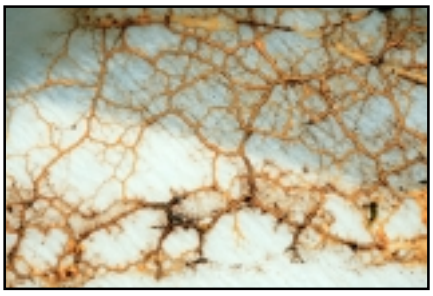
The drive was only about 10 minutes from the Cades Cove House to the Turkey Pen Ridge Trailhead. We slung our 70-pound gear bags containing a climbing harness, 120-foot rope, slick line, gloves, and more, onto our backs and began the hike up the trail. After hiking slightly more than a mile we came to a fork in the trail. We were impatiently itching to climb the White Oak trees in the area but the trees were about 30 meters tall. I figured we would be up and down the trees in no time and with little accomplished due to their small size. We gave our "Big Shot" slingshot a test run on a White Oak tree. The throw line was shot up so accurately over the first high branch it took only a few minutes to rig the climbing rope. Coordinating all my body parts in a rhythmic upward motion with synchronized pull-ups and pelvic thrusts I was able to reach a height of 6 meters where I secured number 88 to the tree. After filling a small white bag halfway I folded the top edge over and let it cruise to the ground. The ground crew member labeled the sample bag with the appropriate data. An elevation line attached to each climbers harness monitored collection height. These lines were handmade by our ground crew team out of paper dog tags attached every foot to a lightweight line and labeled with a permanent marker. I continued to climb and paused at about 9 meters to take

another sample. During my usual scanning of the bark before removal I noticed an unusual snake-like pattern on the bark. Suddenly flashbacks of Dr. Keller's slide show on the myxomycete life cycle vividly appeared in my mind. Just the night before, Dr. Keller had presented a lecture to all of the tree canopy research team members on the different stages in the myxomycete life cycle. The tracks on the bark in front of me looked identical to the remains of plasmodial tracks left by developing myxomycetes. I kept climbing higher as the twisted patterns on the bark seemed to follow my ascent to almost 24 meters. I heard a lot of hooting, hollering, and celebrating as Dr. Keller confirmed the discovery of a very unusual myxomycete. Dr. Keller enthusiastically asked that I come down in smaller steps and sample more bark every 3 meters. Finally, after two hours up in the treetop I reached solid ground. When we arrived back at the Cades Cove House, provided by Discover Life in America and the GSMNP service, I anxiously sorted out the days dazzling findings and placed samples on the porch railing with the plasmodial tracks and the myxomycete sporangia. Dr. Keller confirmed that this was probably a new species of *Diachea* but additional collections and moist chamber culture work would have to be completed. Without any real jaw-dropping fireworks display, we truly had ourselves a very explosive 4th of July.

The great adventure phase of our research project was over and now the laboratory work began. I grew to enjoy the moist chamber cultures seeing the diverse beauty of life forms. In about two-weeks the white oak bark produced the growth of a magnificent yellow plamodial fan. It spread all over the bark and bottom of the Petri dish leaving distinct veinlike plasmodial tracks just like on the surface of the tree bark. Finally I was able to observe all of the developing stages of the sporangia culminating in a sphere of iridescent gold with a blending of the colors of the rainbow. I never realized such beauty existed in microscopic proportions. This project taught me that trees are made of more than branches and leaves. They consist of a mosaic of mosses, lichens, liverworts, Myxomycetes, bugs, and more. And Myxomycetes are glorious!

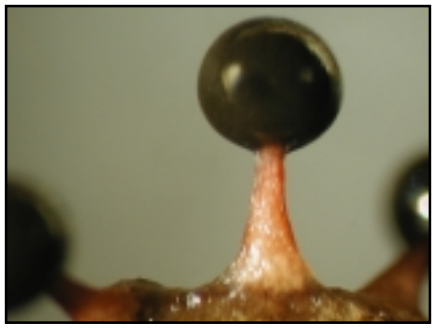


Yellow phaneroplasmodium growing on white filter paper in moist chamber culture. Note the feeding, advancing fan and trailing veins that leave plasmodial tracks on the surface of tree bark. This is the plasmodial stage of a new species of Diachea.



Yellow veins spreading and covering white filter paper in moist chamber culture. Melissa Skrabal observed plasmodial tracks covering extensive areas of a living white oak tree over a distance of 15 meters.

Results: There are three trees, *Quercus alba* (White Oak), *Juniperus virginiana* (Red Cedar) and *Fraxinus americana* (White Ash), in the GMSNP where this taxon was found. All of the sporangia collected in the field were above 3 meters. Approximately 20 moist chamber cultures of bark collected under 3 meters have not yielded the new *Diachea*. These trees have been climbed at least twice and each time the sporangia were found above 9 meters on the White Oak and 4.6 meters on the Red Cedar. The plasmodial tracks on the White Oak tree extended a distance from 9 to 24 meters. This bright yellow phaneroplasmodium migrates all over the upper and lower surfaces of bark, mosses, and the filter paper in the bottom of Petri dishes. Sporangia fruit on the upper and lower bark surface, on the moss phyllidia, on the filter paper and on the side of the plastic Petri dish. All of the *Diachea* species have gorgeous iridescent peridia but this one is striking because of its glittering golden colors and the contrasting pink to reddish orange stalk. The capillitium is attached to the apex of the columella and the spore ornamentation as seen with scanning electron microscopy is unique in the Myxomycetes. What a great experience for an undergraduate student!



Immature sporangium of Diachea *on bark in moist chamber culture.*

Literature Cited: Keller, HW and KL Braun. 1999. Myxomycetes of Ohio: Their Systematics, Biology and Use in Teaching. Ohio Biological Survey Bulletin New Series Volume 13, Number 2 xvi + Pp. 182.

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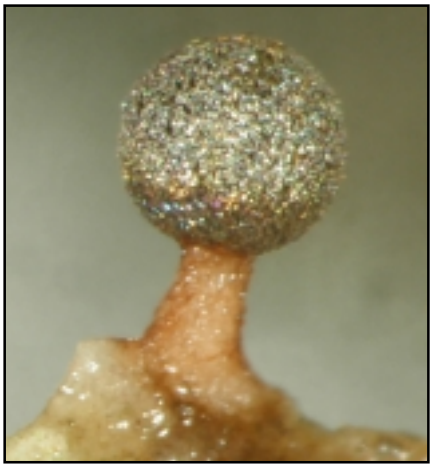

Mature sporangium with iridescent peridium exhibiting the colors of a rainbow. This new species of Diachea *is an obligate tree canopy species never observed or cultured below 5 meters. This is the first documented obligate tree canopy species in the Myxomycetes (plasmodial slime molds). Melissa Skrabal observed sporangia of this* Diachea *at heights of 25 meters on a living white oak tree.*

instruction and certification for climbers. More images are displayed on our webpage at **http://www.cmsu.edu/biology/Faculty/keller.html** and Discover Life in America "Tree Canopy Biodiversity in the Great Smoky Mountains National Park" **http://www.discoverlife.org/nh/tx/Fungi/**. Mike Ferro spent many hours preparing our web page. James Murray served as our research project photographer. Keith Langdon from the GSMNP and Jeanie Hilten from Discover Life in America provided assistance with equipment, housing and logistics. The multidisciplinary research team included: Drs. Alex Ciegler, lichenologist, Paul Davison, bryologist (mosses and liverworts), Professor Uno Eliasson, Göteborg University, Sweden, Myxomycetes and vascular plants, Professor Thomas Gaither, Myxomycetes and macrofungi, Ken Nelson, volunteer ecologist, Drs. Jay Raveill, expert on the flora of the GSMNP, David Smith, bryologist, Ted Stampfer, volunteer moist chamber culture specialist. This research project is funded by the National Science Foundation, Division of Environmental Biology, Biotic Surveys and Inventories Program, Award # DEB-0079058 and Discover Life in America Award #2001-26.