

Chapter 9

Plants That Aren't "Plants": Mosses and Lichens

Clayton Newberry

Department of Biological Sciences
4505 Maryland Parkway Box 454004
Las Vegas, NV 89154-4004
newberry@unlv.edu

Clayton Newberry is a graduate student at University of Nevada at Las Vegas. He received his B.S. in general botany from Brigham Young University and his M.S. in lichenology from Brigham Young University. He is currently working on his Ph.D. at University of Nevada at Las Vegas. His interests include bryophyte systematics and bryophyte floristics in western North America.

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Introduction

In many introductory biology classes and laboratories, mosses and lichens are often passed over with barely more than a nod in their direction. Larger and more charismatic gymnosperms and angiosperms are usually emphasized. Some of this neglect may be traceable to a general unfamiliarity with mosses and lichens among faculty members themselves. This laboratory exercise introduces biology teachers to some fundamental concepts and terminology in bryology and lichenology, reviews introductory literature, field guides and regional floras, and teaches them some simple techniques used to identify the species. Our objective here is for teachers to become sufficiently familiarized with mosses and lichens for further exploration either on their own or with their own students.

Materials

Compound microscope	0.8 – 1.10 x 90 mm capillary tubes, heated and drawn in half over an alcohol lamp
Dissecting microscope	Household bleach
Microscope slides & cover slips	KOH pellets
Fine forceps	Moss specimens, <i>Syntrichia papillosissima</i>
Dissecting needles	Lichen specimens, <i>Xanthoria elegans</i> (or any other lichen in the Teloschistaceae Family)
Razor blades	
Water dropper bottles	

Notes for the Instructor

Preparing good thin sections of moss leaves can be difficult, sometimes even for the expert. Although any moss species can be used to teach leaf sectioning, for the beginner I advise using a moss with large leaves (i.e., large by moss standards). Additionally, I advise using a species with distinct internal leaf tissues. We are using *Syntrichia papillosissima* for both reasons, its large leaf size and anatomical complexity. *S. papillosissima* is restricted to western North America; its sister species *Syntrichia ruralis* is just as good for demonstration purposes, and has a more cosmopolitan distribution.

Likewise, in Exercise 2, any lichen which reacts positively to any standard lichen reagent can be used to demonstrate a chemical spot test. We are using *Xanthoria elegans* for its distinct and instantaneous K^+ purple reaction. Any other lichen in the Teloschistaceae Family (generally bright orange crustose to squamulose lichens) will show the same K^+ purple reaction. If Teloschistacean lichens are not available, I advise using the C reaction on parmelioid lichens, which are very common on trunks of hardwood trees in eastern North America.

Additionally in Exercise 2, a minute droplet of reagent will be applied to the lichen surface. Use 0.8 – 1.10 x 90 mm capillary tubes to apply the reagent. Heat the capillary tube over an alcohol lamp or Bunsen burner and draw it in half. The very minute end of the attenuated tip is to be broken off before use. Reagent is drawn up into the tube by capillary action and disperses on contact with the lichen surface. You may wish to heat and draw the tubes yourself before class. Alternatively, requiring students to draw their own micropipettes teaches them another useful laboratory technique.

Student Outline

Introduction

The so-called “lower plants” include mosses, moss allies and certain green algae. These plants are also called *nonvascular plants* or *cryptogams*. A nonvascular plant is one that lacks vascular tissue—i.e., lacks xylem and phloem (vessels, tracheids, sieve cells and sieve tube cells) for internal transport. A cryptogam is any plant (or almost anything historically thought of as a plant) which reproduces by spores instead of seeds.

The two terms would be somewhat synonymous except for the ferns and their allies, which are extensively vascularized and yet reproduce by spores. Additionally, the term *cryptogam* usually refers to almost anything else remotely plant-like which reproduces by spores, as long as it is still “herbarium-able,” including lichens, nonlichenized fungi and some of the algae, even though they are not members of the Chlorobionta or Plant Kingdom.

Consider the following tables:

Table 1. “Plant” groups grouped by their vascular tissue.

NONVASCULAR PLANTS: VASCULAR TISSUE LACKING	VASCULAR PLANTS: VASCULAR TISSUE PRESENT
Algae (sens. lato)	Ferns & fern allies
Bryophytes (sens. lato)	Angiosperms & gymnosperms

Table 2. “Plant” groups grouped by their sexual disaspore.

CRYPTOGAMS: SEEDS LACKING	PHANEROGAMS: SEEDS PRESENT
Algae (sens. lato)	Gymnosperms (sens. lato)
Bryophytes (sens. lato)	Gnetales
Ferns & fern allies	Angiosperms
Fungi & lichens	

As indicated above, the fungi and lichens and many algal clades are not plants in a true phylogenetic sense. Nevertheless they are considered plants by so many in the general public, and have been covered in general botany texts and classes for so long, that they have at least squatter’s rights in a discussion on “lower plants.”

In a phylogenetic sense, the two groups which we will be looking at today sort out on a life cladogram in the following places:

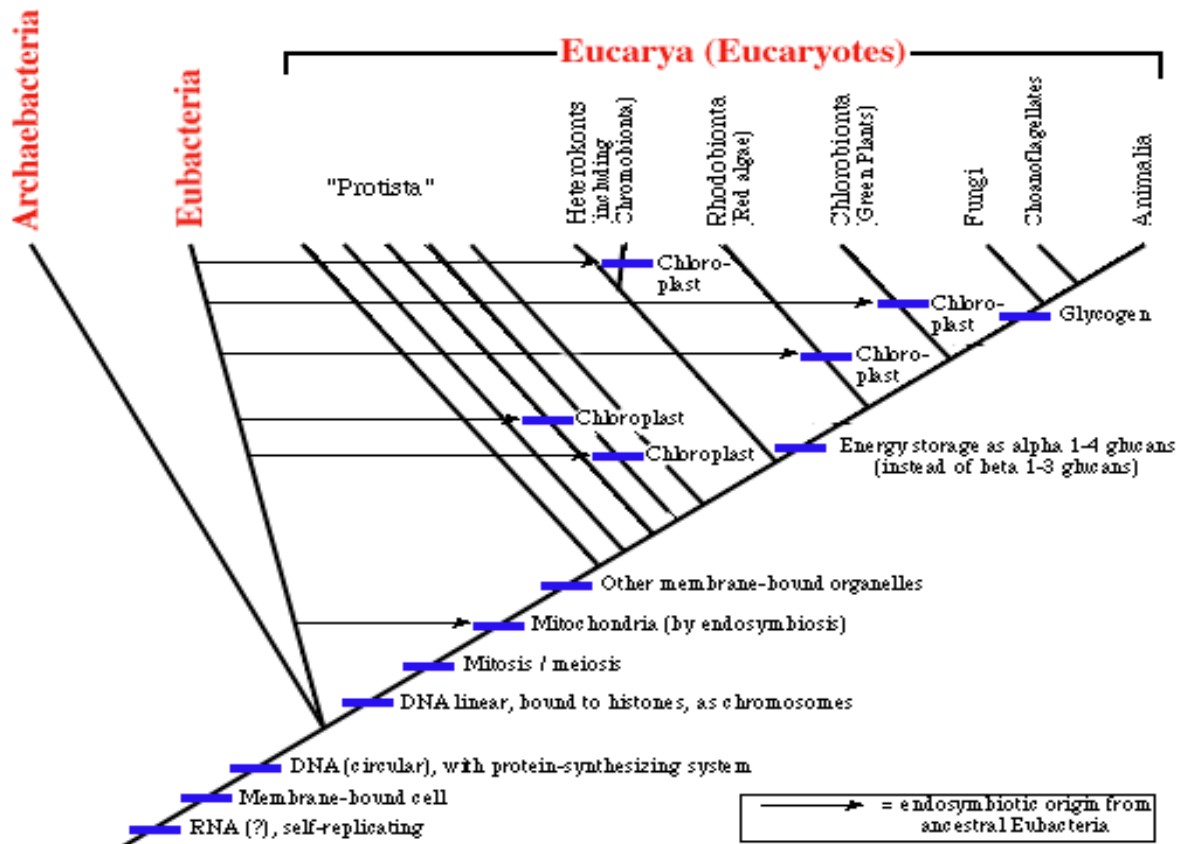


Figure 1. Phylogeny of Life Cladogram. The mosses (sens. lato) are true plants, or Green Plants, belonging to the lineage Chlorobionta. Lichens are fungi that have symbiosed with an algal partner.

(from http://www.scibridge.sdsu.edu/coursemats/introsoci/diversity/cladogram_life.html)

In this laboratory period we will be familiarizing ourselves with mosses and lichens. The objective is to provide you with enough information so that you can recognize them in the field, and even identify them to species level with a field guide or dichotomous key.

Part 1. Mosses

The mosses belong to the Green Plant Clade, or Chlorobionta. As seen in the cladogram below, they are the sister group to all vascular plants.

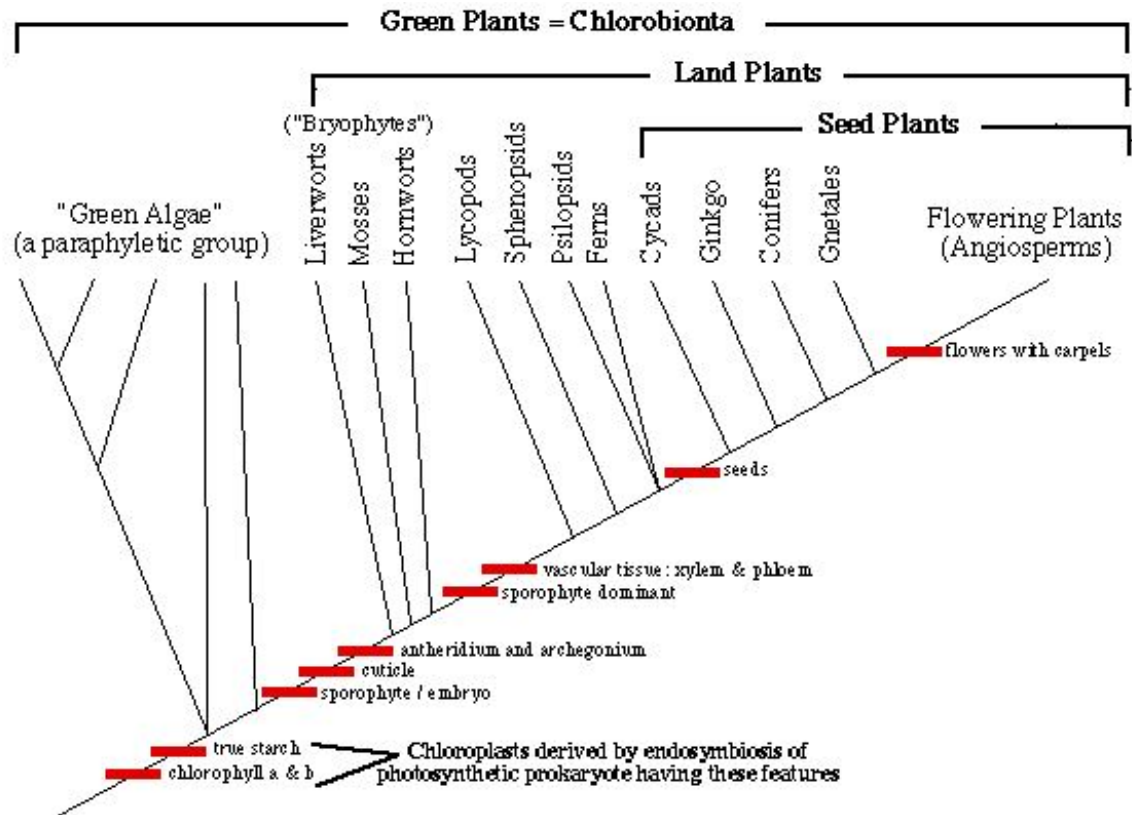


Figure 2. Phylogeny of the Chlorobionta, or Green Plants. Liverworts and hornworts are phylogenetically distinct from the mosses.

(from <http://www.scibridge.sdsu.edu/coursemats/introsci/diversity/chlorobionta.html>)

The term *moss ally* refers to the liverworts and hornworts. Moss allies are morphologically, reproductively and ecologically similar to mosses. Cladistically they sort out sister to but distinct from the mosses. We will not be covering the moss allies in this laboratory period.

Gametophyte dominance is a key feature to distinguish the mosses from the vascular plants. The gametophyte (haploid, 1N) phase of the life cycle is perennial and the sporophyte (diploid, 2N) is seasonal and evanescent. Sporophyte dominance and gametophyte reduction is a key synapomorphic feature of the vascular plants. Refer to Figure 3 for a diagrammatic overview of the moss life cycle.

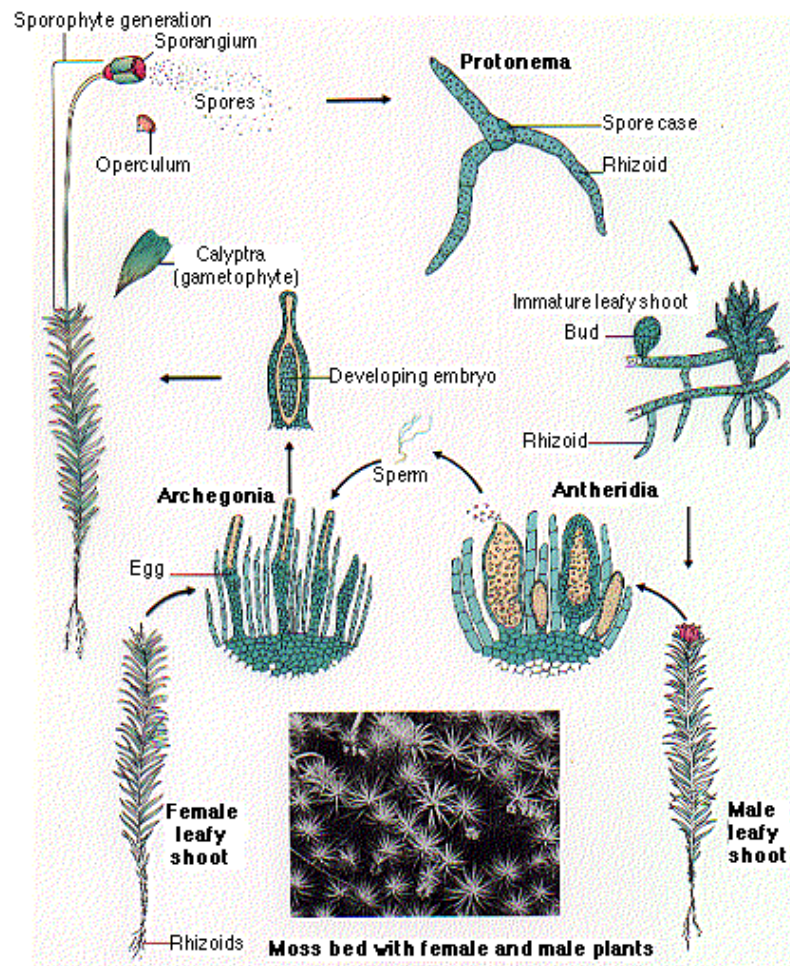


Figure 3. The moss life cycle.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/M/Mosses.html>)

The gametophyte phase of the moss life cycle begins with the germination of the haploid meiospore. Note the filamentous growth stage immediately following spore germination, the so-called *protonema*. Once this alga-like protonema achieves a certain critical size, numerous buds arise and grow together into the *gametophore*, or leafy stage. Leafy stems thus arise simultaneously and grow together, usually forming a turf, weft or thin mat over the substrate, possibly for mutual mechanical support and water relations in the boundary layer. At sexual maturity, sac-shaped male gametangia called *antheridia* develop at stem termini; a stem terminus, leaves and sterile tissue associated with antheridia, is called a *perigonium*. Flask-shaped female gametangia, usually called *archegonia*, also develop terminally; a stem terminus, leaves and sterile tissue associated with archegonia, is called a *perichaetium*.

Each antheridium produces numerous biflagellated sperm, which are discharged into ambient moisture. Each archegonium produces a single fertile egg, which remains in the archegonial base. Chemotactically attracted, sperm swim into the archegonium and fertilize the egg.

Sexuality is highly variable in mosses. Some lineages are strictly dioecious, with separate male and female plants. Various forms of monoecy also occur, as well as hermaphroditism. It should be

noted that many bryologists use the terms *dioicous* and *monoicous* instead of *dioecious* and *monoecious*, preferring the former terms for gametophyte sexuality and the latter for sporophyte sexuality.

Numerous archegonia, each with a single fertile egg, arise on a fertile stem. Many eggs may be fertilized almost simultaneously but usually only one zygote predominates and germinates, the others being hormonally suppressed or aborted.

The viable diploid zygote germinates in place and remains attached to the gametophore. Growth is initially bipolar, but growth in the direction of the gametophore ceases early as new cells at the sporophyte-gametophore interface differentiate for a haustorial function. Growth and differentiation away from the gametophore are more extensive. The mature sporophyte consists of an elongate stalk termed the *seta*, which is surmounted by a capsule.

Meiosis proceeds among sporogenous tissue inside the capsule. The capsule dehisces by means of subapical circumscission, the zone of cell wall differential thickness being termed the *annulus*. The cap-shaped tissue distal to the annulus is termed the *operculum*. Subepidermal tissue that persists after the operculum falls is called the *peristome*. The size, shape, number, ornamentation and persistence of various peristome segments are all characters of utility in cladistic analysis and dichotomous keys. Meiospore size and surface ornamentation may also be diagnostically useful.

Additionally, following fertilization, apical archegonial tissue proliferates into an outer cap over the developing sporophyte called the *calyptra*. Calyptra size, shape and surface ornamentation are also diagnostic features.

It should be noted that even though mosses lack the classical Tracheophytic xylem and phloem tissue, many moss lineages do have idiomatic conducting tissue at the center of their stems. The xylem analogs are termed *hydroids*; the phloem analogs, *leptoids*; and taken together, the hydroid and leptoid conducting tissue is termed the *central strand*. Leaf traces are mostly absent, and the hydroid xylem analog is nonlignified. The central strand is recognized in stem cross section as a cluster of small cells at the stem center.

It should be noted again that not all moss lineages even have a central strand. Water can be wicked up the stem by capillary forces along the external surface, sometimes assisted by surficial filaments and minute leaf-like appendages, called *rhizoids* and *paraphyllia* respectively—thus obviating the need for internal conduction.

The presence of the central strand is believed to represent synapomorphy in the Bryophyta, and its absence in many bryophyte lineages is believed to represent secondary loss. Central strand persistence or loss can be used as a character in cladistic analysis and dichotomous keys at the family, genus or even species level.

Moss leaves also exhibit many diagnostically useful characters such as length and width, marginal outline, apical attenuation and basal tissue decurrency. Leaves are usually unistratose, or one cell layer thick, but bistratose leaves also occur, or bistratose regions within a single leaf. In many taxa only the leaf margin is bistratose or even multistratose, while the rest of the leaf is unistratose. Leaves may or may not exhibit a midvein, which in mosses is called the *costa*. Costal width, extent, and internal anatomy are often diagnostically useful, as is also the presence of supplementary costae. Finally the leaf cells themselves are often diagnostically useful, being differently shaped or differently ornamented in different parts of the leaf—as in the apical, mid-laminal, marginal, juxtacostal, alar and basal regions.

Unlike vascular plants, which have multicellular and relatively large meristematic tissues at stem termini, mosses have only a single meristematic cell at each stem terminus, called the

tetrahedral apical cell (acronymic TAC). Successive divisions of the TAC give rise to the stem and leaf tissues (Flowers, 1973).

Just as the angiosperm meristematic shoot tip is used up in the formation of flowers, the moss meristematic TAC is used up in the formation of gametangia. Thus gametangiogenesis signals the determinate end of that particular moss stem. If growth is to be continued, a new stem will arise from a renewal point usually located below one of the leaves. A composite stem comprised of numerous modular units successively repeating in this way (stem-gametangium-renewal stem-gametangium-renewal stem-gametangium, etc) is referred to as a *sympodial* stem. If the stem grows indeterminately and never terminates in gametangiogenesis, growth is said to be *monopodial*.

At least as far as the novice is concerned, mosses grow in one of two configurations, *acrocarpic* or *pleurocarpic*. Technically speaking, acrocarpic moss growth is orthotropic, determinate and sympodial—i.e., the stem grows perpendicular to the substrate and terminates in gametangiogenesis, then a renewal stem arises sympodially. Pleurocarpic moss growth is plagiotropic, indeterminate and monopodial—i.e., the stem grows parallel to the substrate, and, at least theoretically, does not terminate and does not renew its growth. Gametangia in pleurocarpic mosses develop atop side branches, which for that very reason do grow determinately. These fertile side branches sometimes exhibit divergent leaf and stem anatomy of diagnostic usefulness.

In less technical jargon, an acrocarp is a moss in which the main axis is terminated by archegonia (and hence, if fertilized, by the capsule); and a pleurocarp is a moss having archegonia or antheridia on a short side branch rather than the main axis. Or put another way, acrocarpic mosses form upright turfs and hemispheric mounds (think of them as a crew cut), and pleurocarpic mosses form spreading mats and deep cushiony wefts. Although these two terms may not be phylogenetically informative, nevertheless the distinction *acrocarp* vs. *pleurocarp* often constitutes the very first couplet in dichotomous keys to moss species.

A review of field guides, regional floras and introductory moss literature is provided in Appendix A. Although there is much to learn about mosses, it is hoped that the following laboratory exercise will start the student on the road to successful use of field guides and floras.

Exercise 1. Sectioning moss leaves by hand

Moss identification often requires leaf thin sections and microscopy, so it is important that you learn to make thin sections. While chemical staining, paraffin embedding and microtoming make “picture perfect” thin sections, such microtechnique is burdensome and unnecessary just to put a name on a specimen. Most bryologists identify specimens with mere hand sections. Hand sectioning can be frustrating for those not used to working with such minute samples. Advise patience.

Apparatus needed

- 2 fine forceps
- Dissecting needle
- Sharp razor blade
- Microscope slide
- Cover slip
- Dissecting microscope
- Compound microscope
- Water dropper bottle
- Moss specimen *Syntrichia papillosissima* or *S. ruralis*

Procedure

- 1 Moss leaves section best when the stem is hydrated. Usually specimens hydrate quickly if immersed in lukewarm water for only a few seconds. Some species hydrate more slowly but

immersing them in hot water for a few seconds accelerates hydration. If desirable, keep a beaker of water on a hot plate handy, just below boiling temperature. Immerse the specimen in the hot water for a few seconds, or leave it to float for a minute if more time is needed.

- 2 Place hydrated sample on a microscope slide.
- 3 Viewing through a dissecting microscope, remove leaves one by one. Grasp the stem with forceps in one hand, grasp the leaf base with forceps in the other hand, and pull the leaf in a proximal direction (i.e., downward on the stem). Leaf should come off easily.
- 4 Arrange the leaf in a drop of water on a microscope slide.
- 5 Still viewing through a dissecting microscope, right-handed individuals should depress the leaf with dissecting needle in the left hand and begin sectioning the leaf with the blade in the right hand—vice-versa for left-handed individuals. Section perpendicular to the long axis of the leaf—i.e., perpendicular to the length of the costa.
- 6 As each new section is sliced off, roll the dissecting needle back upon the leaf for the next section. Be sure to section straight and clean, as thin as possible. Try to make your hand sections no more than one cell layer thick.

(Alternatively, you may section leaves using your finger instead of dissecting needle to depress the leaf. Just depress the leaf with the thumb or a finger of the one hand, and proceed to section with the blade in the other. Your sections may not be as exact in this manner as with a dissecting needle, but by sectioning rapidly you can cut many sections, at least one of which will probably show enough features for careful diagnosis. Sometimes this method is called the “salad method” of sectioning leaves)

- 7 Once you are satisfied with the sections you’ve made, coverslip the specimens and begin viewing. You will observe the leaf cells and costal anatomy.

Either method – by dissecting needle or salad – can be used for making cross sections of leaves and also stems, which the keys call for in some genera and species. But either way, sectioning leaves by hand isn’t easy, so don’t be discouraged and give up too soon. Practice makes perfect. That which you persist in doing becomes easier—not that the nature of the thing changes, but your ability to do it improves.

Note: A glossary of all bryological terms used in this foregoing review and exercise is located in Appendix B.

Part 2. Lichens.

Lichens are a symbiosis between a fungus and an alga. The fungal symbiont is called the *mycobiont* and the algal symbiont is called the *photobiont*. Although the two symbionts remain cytologically distinct despite lichenization, they clearly function as one physiological unit and together constitute one of the tightest symbioses known to science.

Approximately 15000 lichen species have been described worldwide, almost all of which (98%) belong to the Fungal Division Ascomycota. As the fungus seems to be the physiologically dominant symbiont, and most of the lichen biomass is fungal tissue, classification and nomenclature always follow that of the fungus; therefore these 15000 lichen species names are actually fungal species

names. Additionally, in almost all cases investigated, the fungal species does not occur unlichenized; therefore these 15000 fungal species are obligate lichen mycobionts.

The photobiont usually belongs to either the Chlorophyceae or Cyanobacteria. Only about 500 algal species in 40 genera are known to lichenize, and almost 60% of all known photobionts belong to the single genus *Trebouxia* (St. Clair, 1999). In other words, the same algal species will lichenize with numerous different fungal species. Unlike the mycobionts, the photobiont species have been recovered in a free-living state; therefore, as far as is known, these 500 species of lichen algae are merely facultative lichen photobionts.

Lichens are widely thought of as the poster boy of mutualism symbiosis, the classic textbook case of a mutually beneficial relationship between two disparate species. This widespread characterization represents that kind of conventional wisdom which, when challenged empirically, is sometimes found wanting. Some now argue that a lichen is a controlled parasitism, in which the alga has been lichenized (or in the vernacular, “shanghaied”) for fixing carbon to serve the needs of the fungus, and receives little in return which it couldn’t obtain on its own.

With 15000 different species, the actual nature of the symbionts’ relationship to each other might vary from mutualism to controlled parasitism. Lichenization has evolved independently in several Ascomycete and Basidiomycete lineages, and to date none of the mycobiont species has been found free-living. Thus, whatever the nature of the initial symbioses in the several clades, by now it has evolved into a somewhat obligate relationship at least for the fungus.

In terms of its ecological amplitude and adaptability, the lichen symbiosis is one of the most successful relationships on earth. Lichens are found throughout all climatic zones and in almost all every habitat.

The term *thallus* is used to refer to any unvascularized plant tissue, whether fungus, alga, bryophyte or lichen. Lichenologists commonly use it to refer to the lichen body. Morphologically, a lichen thallus consists of three tissue layers: cortex, algal layer and medulla. The cortex constitutes the external surface of the lichen, and consists of tightly woven, often highly pigmented fungal hyphae. Algal symbionts usually do not occur in the cortex, but in a layer immediately beneath it. The photobiont occurs as single cells or small clumps or abbreviated filaments, tightly enveloped or enmeshed by fungal haustoria. The medullary layer lies beneath the algal layer. The medulla consists of loosely woven, cottony fungal hyphae, usually unassociated with photobiont tissue.

In some lichens these three tissue regions are poorly defined, so that mycobiont and photobiont tissues are spread uniformly throughout the thallus, a condition termed *homoiomerous*. Many of the cyanolichens are characterized by homoiomerous development. The more common stratified condition is termed *heteromerous*.

Lichen architecture follows one of four generalized *Bauplans*: fruticose, foliose, crustose and leprose. Fruticose lichens grow pendent or cespitose to the substrate and are symmetric in cross section, exhibiting an encircling cortex, subcortical algal layer and internal medulla. Foliose lichens grow loosely attached to or cespitose from the substrate, and are dorsiventral in cross section, exhibiting an upper cortex, algal layer, medulla and lower cortex, the latter occasionally invested with rhizines or haptera for substrate attachment. As fruticose and foliose lichens can achieve relatively large size, they are often referred to as *macrolichens*.

Crustose lichens grow tightly appressed to the substrate and are dorsiventral in cross section, exhibiting an upper cortex, algal layer and medulla. Crustose lichens lack a lower cortex; their medullary hyphae penetrate and matriculate into the substrate so tightly that removal is usually impossible. Leprose lichens grow as mealy, powdery or farinaceous granules over the substrate and

lack any tissue differentiation altogether. Although in some species a single crustose or leprose individual can cover a fairly large area, nevertheless crustose and leprose forms often referred to as *microlichens*.

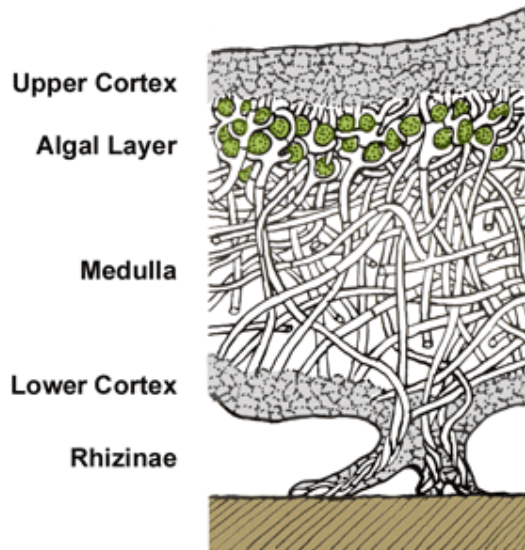


Figure 4. Diagrammatic representation of foliose lichen anatomy. (<http://www.arctic.uoguelph.ca/cpl/organisms/plants/Terrestrial/lichens/basicmorph.htm>)

These four categories are somewhat artificial tags; variations and overlaps between them are common, and the category edges blur. Some foliose species grow as numerous tiny folia, approaching the crustose habit—a condition termed *squamulose* or *areolate*. Other foliose species attach to the substrate at a single point, a central undersurface holdfast or hapter—a condition termed *umbilicate*. Some crustose species are marginally effigurate-lobate and centrally areolate, a condition called *placodioid*. And while some crustose species develop as erect lobules off the substrate, approaching the fruticose habit, some minutely fruticose species grow like a tight turf over the substrate, almost resembling the crustose habit. Descriptive phytography can always be improved but never perfected: Nature mocks human categories.

Sexual reproduction in the photobiont has never been reported and is thought to be suppressed by the fungus. It is thought that the fungus regulates even the photobiont cell and mitotic cycle. To the extent that it does, the lichen symbiosis may be analogized to a dairy farm, the mycobiont as owner and the photobiont as cow. Some have coined a clever term for such an arrangement: *helotism*, recalling the Messenian helots or serfs of ancient Sparta, who toiled their lives away for the support and pleasure of the Spartan warrior class.

Sexual reproduction in the mycobiont is common—or at least meiosis, spore production and ascoma development are common. Most lichen species produce meiospores, often called ascospores, in typical Ascomycete apothecia or perithecia. Ascospores are discharged into ambient air and presumably dispersed by air currents. Actual recruitment by ascospores, however, is problematic—that is to say, exactly how the propagule of an obligate symbiont manages to find the right photobiont and germinate together, has not been satisfactorily explained.

Asexual reproduction by lichens is far less problematic. Mechanical fragmentation of the thallus is easy to envision. The term *isidium* refers to minute lobules apparently specialized for such

mechanical fragmentation and dispersal. Isidia are corticated propagules, arising from the cortex and variously shaped—granular, spherical, ellipsoid, fusiform, cylindrical, branching. Isidium presence and shape are often diagnostic characters in dichotomous keys.

While isidia are lichenized fragments of the cortex, soredia are lichenized fragments of the medulla. Soredia are therefore noncorticated, nonpigmented and whitish, like the medullary tissue from which they arise. Each soredium consists of a few medullary hyphae enmeshed about a few algal cells. Soredia may arise from beneath cortical fissures or may be produced in a specialized tissue of erumpent medulla termed a *soralium*. The absence or presence of soralia and their thalline disposition and shape are diagnostically useful in field guides and dichotomous keys.

Lichens with cyanobacterial photobionts are often called *cyanolichens*. Cyanolichens tend to be anatomically homoiomerous in cross section, usually dark brown in color, and, (when wet) gelatinous in texture. Some predominantly Chlorophycean lichens incorporate nitrogen-fixing cyanobacteria as supplementary symbionts for nitrogen fixation. In these cases the cyanobacter may be sequestered in specialized mycobiont chambers called *cephalodia*—thus representing a remarkable symbiosis among species from three disparate kingdoms.

A review of field guides, regional floras and introductory lichenological literature is provided in Appendix C. As with the mosses, there is much to learn about lichens, but it is hoped that the following laboratory exercise will start the student on the road to successful use of field guides and floras.

Exercise 2. Lichen Chemical Spot Tests.

Lichen genera and species are often distinguished on the basis of their secondary chemistry. Lichenologists use thin-layer chromatography or high-performance liquid chromatography (TLC, HPLC) for very careful identification of secondary acids, but chemical spot tests are usually used for a “quick and dirty” approximation of the secondary compounds. Most lichen keys employ such expressions as “K+ red” or “C+ yellow” to refer to spot tests.

Four reagents are commonly used in chemical spot tests: potassium hydroxide (KOH or abbreviated K), sodium hypochlorite (household bleach—NaClO or abbreviated C), alkaline iodine (KOH + KI or abbreviated AI) and paraphenylenediamine ((CNH₂)₂(CH)₄ or abbreviated PD). Protocols for the preparation of the less toxic three reagents are given in Appendix E. In this laboratory exercise we will be using only the K reagent for a spot test on a lichen in the Teloschistaceae Family.

Apparatus needed

- Razor blade
- Porcelain watch plate
- 0.8 – 1.10 x 90 mm capillary tube heated and drawn in half over an alcohol lamp
- Half-dram (12 x 35 mm) glass vial
- KOH pellets
- Distilled water
- Dissecting microscope
- Specimen of *Xanthoria elegans* (or any other member of lichen family Teloschistaceae)

Procedure

- 1 To prepare the K reagent, simply dissolve 2-3 KOH pellets in 1-2 mL distilled water in a half-dram (12 x 35 mm) glass vial.

2. Excise a portion of the thallus of the lichen *Xanthoria elegans*. Place it pigmented side up on the porcelain watch plate.
- 2 Apply reagent to the excised portion of the lichen cortex. Advise carrying out the actual spot test under the dissecting microscope for careful observation. If the lichen tissue is to show a positive color reaction to the reagent spot, the color change should be immediate. Color changes should be to very dark purple or dark brown, almost black, on the applicated spot.
- 3 Record your observation.

Note: A glossary of all lichenological terms used in this foregoing review and exercise is located in Appendix D.

Hazard warning

The reagent KOH is mildly toxic, and if used inappropriately can be extremely dangerous. You are responsible to ensure that reagents are stored properly, used safely, and discarded according to lab or herbarium protocols. The specimen fragment used for the spot test should also be discarded, and any filter paper, porcelainware, glassware or capillary tubes used in the spot test should also be either discarded or cleaned according to lab or herbarium protocol.

Acknowledgments

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Appendix A: Annotated Bryological Literature

- Conard, H. 1979. How to know the mosses and liverworts. Second edition revised by P. Redfearn, Jr. William C. Brown, Dubuque, Iowa, 302 pp. – *Pictorial key to the mosses of North America. Keys and short descriptions. Often a good place to start.*
- Crum H. and L. E. Anderson. 1981. Mosses of eastern North America. Volumes 1 and 2. Columbia University Press, New York, 1328 pp. – *The most encyclopedic treatment of mosses for this continent, oriented to the east but usable in the west. Probably not to be recommended for the novice, but an essential for any bryological library in North America.*
- Flowers, S. 1973. Mosses: Utah and the west. Edited by A. Holmgren. Brigham Young University Press, Provo, Utah, 567 pp. – *Regional moss flora for the Intermountain, Great Basin and Colorado Plateau regions of the west. Contains keys, descriptions, comments, detailed illustrations. Out of print but available from the publisher upon request.*
- Lawton, E. 1971. Moss flora of the Pacific Northwest. Hattori Botanical Laboratory, Nichinan, Japan, 362 pp. + 195 plates. – *Regional moss flora with good descriptions and line drawings. A must for working in northwest North America.*
- Malcolm, B. and N. Malcolm. 2000. Mosses and other bryophytes: an illustrated glossary. Micro-Optics Press, Nelson, New Zealand, 220 pp. – *Probably the best illustrated glossary of bryophyte terminology ever published.*
- McQueen, Cyrus. 1990. Field guide to the peat mosses of boreal North America. University of New England Press, Hanover and London, 138 pp. – *Manual to the genus Sphagnum. Unavoidably technical given the nature of the genus, but, along with Crum & Anderson 1981, essential for understanding and identifying Sphagnum in North America.*
- Reese, W. 1984. Mosses of the Gulf South from the Rio Grande to the Appalachicola. Louisiana State University Press, Baton Rouge, Louisiana, 252 pp. – *Regional moss flora for southeastern Gulf Coast with descriptions, drawings, comments.*
- Schofield, W. 1969. Some common mosses of British Columbia. British Columbia Provincial Museum Handbook No. 28. Victoria, British Columbia, 262 pp. – *Brief descriptions and line drawings of the most common or noteworthy moss species of the Canadian west coast.*
- Schofield, W. 1985. Introduction to bryology. Macmillan & Co., New York, 431 pp. – *Outstanding introduction to the study of mosses and liverworts.*
- Sharp, A., H. Crum and P. Eckel. (eds.). 1994. The moss flora of Mexico. Volumes 1 and 2. Memoirs of the New York Botanical Garden vol. 69. – *Encyclopedic treatment of Mexican moss flora. Highly recommended for working in the American Southwest. Good but possibly too technical for the novice.*
- Shaw, J. and B. Goffinet. (eds.) 2000. Bryophyte biology. Cambridge University Press, 476 pp. – *Introduction to the study of mosses, but rather more technical than Schofield 1985.*
- Vitt, D., J. Marsh, and R. Bovey. 1988. Mosses, lichens and ferns of northwest North America. Lone Pine Publishing, Edmonton, 296 pp. – *Field guide with outstanding pictures, keys, descriptions, comments and distribution maps. Out of print.*

Appendix B: Bryological Glossary

NB: phytography means botanical terminology. For a more encyclopedic and richly illustrated bryophytography, see Malcolm & Malcolm 2000.

alar: marginal basal region of a moss leaf.

annulus: subapical circumscissile zone of cell wall differential thickness in the epidermal tissue of the moss capsule; site of operculum dehiscence.

antheridium: banana-shaped male gametangium, site of sperm formation.

archegonium: flask-shaped female gametangium, site of egg formation.

bistratose: two cell layers thick, as in *bistratose* leaf.

calyptra: apical archegonial tissue which, on hormonal cue from fertilization of the egg, proliferates into a hood-shaped covering over the developing sporophyte, and dries and dehisces as the capsule matures.

capsule: cylindrical to spherical swelling at sporophyte apex, site of meiosis and sporogenesis.

central strand: vascular analog in bryophyte stems.

costa: midvein analog in bryophyte leaves; usually a multistratose spine with thick-walled fiber cells and vascular analogs; also called the nerve in some texts.

decurrent: with the leaf basal margins extending below the point of leaf insertion, often as vertical ridges or flanges down the stem.

dioecious: with separate male and female plants; more specifically, separate male and female sporophytic plants.

dioicous: separate male and female gametophytic plants.

gametangiogenesis: formation of gametangia.

gametangium: site of gamete formation; in mosses, antheridia and archegonia.

gametophore: the leafy stage of the moss gametophyte, as opposed to the filamentous protonemal stage.

gametophyte: the haploid or 1N phase of a plant life cycle; in mosses, the stem- and leaf-producing phase, which is the dominant and perennial phase. See also *sporophyte*.

haustorium: a tissue differentiated for drawing water and/or nutrients from one organ to another.

hermaphroditism: having both male and female gametangia in the same bud.

hydroid: nonlignified xylem analog in moss stems.

juxtacostal: immediately adjacent to the costa, as in *juxtacostal* cells of the leaf.

leptoids: nonlignified phloem analog in moss stems.

marginal: at or along the leaf edge.

mid-laminal: at or within the middle region of the leaf.

monoecious: with male and female gametangia on the same plant but on different stems, specifically as applied to sporophytic plants.

monoicous: with male and female gametangia on the same plant but on different stems, specifically as applied to gametophytic plants.

monopodium: a growth pattern with numerous secondary lateral branches arising from a main stem. Monopodial growth is indeterminate or theoretically infinite, because the gametangia are borne laterally and so can't halt the growth of the main stem.

moss allies: liverworts and hornworts

operculum: the lid covering the peristome and sporogenous tissue of the capsule.

paraphyllia: tiny photosynthetic leaf-like appendages, scales or filaments along the stem surface of some pleurocarpous mosses.

perichaetium: the female inflorescence, including archegonia and associated sterile hairs and specialized leaves.

perigonium: the male inflorescence, including antheridia and associated sterile hairs and specialized leaves.

peristome: subdermal tissue at the capsule apex which usually persists and splits into 8-16 teeth after the operculum deciduates.

pleurocarpic: producing gametangia and sporophytes on secondary lateral stems; pleurocarps are usually matted, prostrate and branched.

protonema: initial stage of moss spore germination, usually resembling a branching filamentous alga.

rhizoid: root-like and usually densely pigmented appendage to the moss surface.

seta: the sporophytic stalk that arises from the basal point of attachment to the gametophyte and is surmounted by the capsule.

sporophyte: the diploid or 2N phase of a plant life cycle; in mosses, the seta- and capsule-producing phase, which is evanescent and seasonal. See also *gametophyte*.

sympodial: branching repeatedly with stems of limited growth which are continually replaced with lateral branches.

unistratose: one cell layer thick, as in *unistratose* leaf.

Appendix C: Annotated Lichenological Literature

Brodo, I., S. Sharnoff and S. Sharnoff. 2001. *Lichens of North America*. Yale University Press, New Haven & London, 795 pp. – *Outstanding treatment of the more common and noteworthy one-third of North American lichen species. Includes well written introductory material; then keys to the species with descriptions, comments, ecological remarks, distribution maps, and extraordinarily beautiful photographs. Introduction includes good ethnobotanical write-up and lichen-animal interaction write-ups. Useful for both novice and expert. This book is a must. Fairly inexpensive via Internet.*

Hale, M. 1979. *How to know the lichens*. Second edition. Willam C. Brown, Dubuque, Iowa. 246 pp. – *Pictorial key to the lichens of North America. Keys and short descriptions. Often a good place to start. Includes simple effective guides for chemical spot tests in introduction.*

McCune B. and L. Geiser. 1997. *Macrolichens of the Pacific Northwest*. Oregon State University Press, Corvallis, 386 pp. – *Outstanding keys, illustrations, comments and pictures. A must for macrolichen studies in the Pacific Northwest. Largely supersedes Vitt et al. 1988.*

Nash, T. (ed.) 1996. *Lichen biology*. Cambridge University Press, 303 pp. – *Good introduction to the study of lichens.*

- Nash, T. *et al.* (eds.) 2002. Lichen flora of the Greater Sonoran Desert Region. Vol. 1. Lichens Unlimited, Arizona State University, Tempe, Arizona, 532 pp. – *Outstanding regional flora, including keys, illustrations, comments and pictures. Covers Arizona, southern California, Sonora, Baja California and adjacent regions in southwest USA and northwest Mexico.*
- St. Clair, L. 1999. A color guidebook to common Rocky Mountain lichens. M. L. Bean Life Science Museum, Provo, Utah, 242 pp. – *Outstanding keys, illustrations, comments and pictures. Essential for both macrolichen and microlichen studies in the Rocky Mountains.*
- Vitt, D., J. Marsh, and R. Bovey. 1988. Mosses, lichens and ferns of northwest North America. Lone Pine Publishing, Edmonton, 296 pp. – *Field guide with excellent pictures, keys, descriptions, comments and distribution maps. Out of print.*

Appendix D: Lichenological Glossary

NB: phytography means botanical terminology.

- apothecium (pl. apothecia):* cup-shaped ascoma.
- areolate:* thallus type divided by fissures into many small tightly appressed scales.
- ascoma (pl. ascomata):* spore-producing organ in Division Ascomycota.
- ascospore:* meiotically produced spore in Ascomycota, usually numbering 8 per ascus.
- ascus (pl. asci):* meiospore sac in Ascomycota, usually containing 8 spores.
- cephalodium (pl. cephalodia):* hyphal chamber containing lichenized cyanobacteria.
- cortex:* mycobiont layer of tightly woven hyphal cells, usually lacking algae.
- rhizines:* multihyphal root-like tissue, usually arising from the lower cortex.
- crustose:* thallus type consisting of cortex, algal layer and medulla, and lacking a lower cortex and rhizines, and usually highly appressed to the substrate surface.
- cyanolichen:* lichen with a cyanobacterium as its principal photobiont.
- foliose:* thallus type with leaf-like growth and complete dorsiventral orientation.
- fruticose:* thallus type with 3-dimensional growth form, often branched and pendulous, cespitose, erect or shrubby, symmetric in cross section and lacking dorsiventrality.
- hapter:* attachment structure produced by some lichens
- heteromerous:* referring to a lichen thallus with photobiont and mycobiont tissues in distinct layers.
- homoiomorous:* referring to a lichen thallus with photobiont and mycobiont tissues evenly dispersed together instead of stratified into distinct layers.
- hypha (pl. hyphae):* fungal cells, usually filamentous in shape.
- isidium (pl. isidia):* corticated lichen propagules, detachable from the upper cortex.
- leprose lichens* grow as mealy, powdery or farinaceous granules
- lichenization:* the symbiosis of algae and fungi.
- macrolichens:* fruticose and foliose lichens.

medulla: interior, subcortical fungal tissue within the lichen thallus, consisting of loosely interwoven, cottony hyphae.

microlichens: crustose, leprose and areolate lichens.

perithecium (*pl. perithecia*): flask-shaped ascoma.

photobiont: the algal symbiont in the lichen thallus.

placodioid: type of crustose lichen with lobed or effigurate margins and areolate or mosaic center.

soralium (*pl. soralia*): medullary tissue erumpent through the cortex, the site of soredium production.

soredium (*pl. soredia*): asexual propagules, noncorticated and usually nonpigmented, containing both photobiont and mycobiont as small, medullary granules.

squamulose: thallus type characterized by minute lobes or scales.

symbiont: one of the partners in symbiosis.

symbiosis: association of two or more species in a tight relationship.

thallus: unvascularized plant or fungal tissue.

umbilicate: foliose thallus type, characterized by a single organ of attachment to the substrate.

Appendix E. Lichen Chemical Spot Tests

Four reagents are commonly used in chemical spot tests: potassium hydroxide (KOH or abbreviated K), sodium hypochlorite (household bleach—NaClO or abbreviated C), alkaline iodine (KOH + KI or abbreviated AI) and paraphenylenediamine ((CNH₂)₂(CH)₄ or abbreviated PD).

- To prepare the K reagent, simply dissolve 2-3 KOH pellets in 1-2 mL distilled water in a half-dram (12 x 35 mm) glass vial.
- The C reagent is just household bleach. It is photosensitive, so it needs to be recharged daily if left out.
- The AI reagent is best prepared in a stock solution of 7g KOH and 15 KI diluted to 100mL, and stored in the dark.
- The PD reagent is highly carcinogenic and should be used with utmost care. Instructions for its use are found in Brodo, Sharnoff & Sharnoff (2001). Better still, learn from a trained and expert lichenologist.

- Reagents are applied by a 0.8 – 1.10 x 90 mm capillary tube heated and drawn in half over an alcohol lamp or Bunsen burner. The very end of the attenuated tip is broken off before use. Reagent is drawn up into the tube by capillary action.
- Reagent is then applied to an excised portion of the lichen cortex or medulla, as directed by the key. If the lichen tissue is to show a positive color reaction to the reagent spot, the color change should be immediate. Color changes will be to red, orange, yellow or purple on the spot, according to the key. Some spot color changes also fade quickly.
- Advise carrying out the actual spot test under the dissecting microscope for careful observation. Record your observation.
- For herbarium specimens, affix an annotation label onto the specimen envelope or inside the packet, indicating the spot test result, your consequent identification, your name and the date, as seen in the sample annotation label below:

HERBARIUM OF THE UNIVERSITY OF NEVADA

Teloschistes contortuplicatus

Cortex K+ purple, C-. Medulla K-, C-. ann. Joe Smith, 13 June 2003.

Hazard warning

These reagents are toxic and/or carcinogenic, and if used inappropriately can be extremely dangerous. You are responsible to ensure that reagents are stored properly, used safely, and discarded according to lab or herbarium protocols. The specimen fragment used for the spot test should also be discarded, and any filter paper, porcelainware, glassware or capillary tubes used in the spot test should also be either discarded or cleaned according to lab or herbarium protocol. Especial care must be taken whenever using the PD reagent, paraphenylenediamine.

Appendix F: Mycological Literature

Arora, D. 1986. – *Excellent field and lab manual for identifying mushrooms and other fleshy basidiomycetes and ascomycetes of prairie and forest. Oriented towards western North America, but also useful in the east. Probably the best field guide for fleshy fungi in North America today. Used by both novices and experts.*