The Wisdom of Fools: new molecular and morphological insights into the North American apodetiate species of Cladonia

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ABSTRACT. – The taxonomy of the North American apodetiate and superficially apodetiate species of Cladonia with persistent, primarily squamulose thalli (viz., C. apodocarpa, C. caespiticia, and C. petrophila) is revised using morphological, chemical, and ecological data in combination with molecular phylogenetic analyses of ITS1, 5.8S, and ITS2 sequence data. The results of this approach confirm some established taxonomic concepts in the group and raise doubts about others. The taxon known as C. apodocarpa is well supported by likelihood- and parsimony-based molecular phylogenetic inferences as well as morphology, chemistry, and ecology. However, molecular evidence gives an equivocal answer to the question of whether C. petrophila is a distinct monophyletic entity or if C. apodocarpa is nested within it. In addition, no phylogenetic structure is detected for the chemical races of C. petrophila with and without fumarprotocetraric acid, based on the markers used in these analyses. However, C. petrophila does remain well supported as a taxon by chemistry (i.e., the presence of sphaerophorin), morphology, and ecology. A morphologically and ecologically distinct new taxon is described as C. stipitata. We confirm the distant relationship of these taxa (C. apodocarpa, C. petrophila, and C. stipitata) to C. caespiticia, a species with stipitate or pseudopodetiate (vs. sessile) apothecia. A treatment of the North American apodetiate species of Cladonia including distribution maps, cited specimens, and illustrations is presented. A key to the taxa that includes unrelated, frequently sterile species of Cladonia found in eastern North America is also provided.

INTRODUCTION

Species of the genus Cladonia P. Browne are generally characterized by their distinctive fruticose podetia, which often bear terminal apothecia. There is, however, a small group of species currently classified in Cladonia sect. Helopodium (Ach.) S. Stenroos in which the podetia are extremely reduced or absent and whose thalli consist of conspicuous, persistent, primary squamules (Ahti 2000, Stenroos et al. 2002, Jahns & Beltman 1973). In North America, this group is represented by three described species, C. apodocarpa A. Evans, C. caespiticia (Pers.) Flörke, and C. petrophila R.C. Harris, all of which are restricted to the eastern portion of the continent. Thalli of these species are commonly encountered and frequently sterile, or at least appear so in the field. Thus, it is not surprising that these taxa are perceived as taxonomically difficult and as such often ignored by collectors because their collection and identification is seen as a futile pursuit (Harris 1992). However, as is evidenced by the description of new species by Harris (1992) and Ahti (2000), collection and study of this group is not without reward.

With the above in mind, we have made a distinct effort to collect primarily squamulose thalli of Cladonia during our field studies in eastern North America in hopes of finding a sufficient way of dealing with these problematic lichens and potentially uncovering additional, overlooked taxa. As specimens accumulated, we became aware of several entities from the Appalachian Mountains whose taxonomic status and relationships were obscured by their cryptic habit and morphology that is reduced compared to other species of Cladonia. Because we could not rely solely on so-called “traditional” characters to fully evaluate these entities, we decided to undertake the present study and utilize molecular data to provide an additional dataset against which we could test our taxonomic

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hypotheses. Toward this end, we generated ITS sequences from a geographically broad sampling of the collections that we had gathered and aligned these with sequences that we generated from an equally broad sampling of *C. apodocarpa* and *C. petrophila*.

The results of our study clearly illustrate the utility of an approach to alpha-taxonomy that involves integrating molecular data with so-called “traditional” characters of biogeography, chemistry, ecology, and morphology. A unified approach such as we have undertaken here often reveals the taxonomic value of characters that have previously been overlooked or underestimated (Amtoft et al. 2008, Argüello et al. 2007, Arup 2006, Hodkinson & Lendemer in rev., Lendemer & Hodkinson in rev.). In the present study ecological characters and vegetative morphology were found to be of unexpected value and, when combined with molecular data, led us to recognize four apodetiate species of *Cladonia* in eastern North America: *C. apodocarpa*, *C. caespiticia*, *C. petrophila*, and the newly described *C. stipitata* Lendemer & Hodkinson.

**Materials & Methods**

**Fieldwork and herbarium materials.** – Since learning of the existence of *Cladonia petrophila*, we have made a special effort to observe and collect primarily squamulose *Cladonia* thalli and their associated ecological data in conjunction with our fieldwork. The result of this effort has been the accumulation of hundreds of freshly-collected herbarium specimens from throughout eastern North America that represent an unbiased sample of the apodetiate *Cladonia* species that occur in the region. These specimens served as the primary resource for this study and have been deposited in the herbarium of The New York Botanical Garden (NY). Due to the unassuming nature of these taxa and the assumption that they are often sterile and thus unidentifiable, they are often poorly represented in herbaria. As such, we limited this study to our own collections and the holdings of undetermined sterile *Cladonia* specimens, *C. apodocarpa*, *C. caespiticia*, and *C. petrophila* at DUKE and NY.

**Molecular methods.** – DNA extractions were performed at NY using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) with the instructions modified to include a prolonged (~12 hour) incubation period in the lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed at NY using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10X PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg µl⁻¹ of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were visualized prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BDSF) for sequencing. Sequences were assembled and manually edited using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

**Taxon Sampling.** – The goal of the present study was to resolve the placement of several groups of apodetiate *Cladonia* populations from eastern North America that we considered to potentially represent distinct taxonomic entities. We conducted analyses of a dataset consisting of sequences from the nuclear ribosomal ITS region (ITS1, 5.8S, and ITS2). In addition to sequences (n=12) that we generated from populations of the putative taxa, we generated additional sequences from populations representing typical *C. apodocarpa* (n=5), *C. caespiticia* (n=1), and *C. petrophila* (n=7) to supplement those available in GenBank (one sequence for each of the aforementioned taxa). For outgroups, we used selected sequences from Stenroos et al. (2002) that (1) represented taxa for which more than one sequence of the full ITS region was available and (2) could be reasonably aligned with those from the members of section *Helopodium* that we studied without the need to exclude/recode large amounts of the analyzed nucleotide alignment (see “Sequence Alignment” and “Phylogenetic Analyses” sections below, and note that similar criteria are outlined by Hodkinson & Lendemer (in rev.) and Lendemer & Hodkinson (in rev.).)

**Sequence Alignment.** – Sequences were initially aligned using ClustalX 2.0 (Larkin et al. 2007), and were subsequently adjusted manually in Mesquite 2.6 (Maddison & Maddison 2009) taking into consideration rRNA secondary structure (Kjer 1995) based on models developed by Beiggi and Piercey-Normore (2007) for *Cladonia* sequences.
grayi G. Merr. ex Sandst. During the process of sequence alignment, it became clear (by comparing our sequences to the reference sequences from section Helopodium) that, as part of the editing process, numerous insertions representing sites that were apparently unique to a given species had inadvertently been deleted from the reference sequences. When an insertion was present in all sequences of a particular taxon in section Helopodium, except for the reference sequence, the ‘gap’ symbol (‘-’) in the reference sequence was replaced by the ‘missing’ symbol (‘?’) to offset the effect of this potentially erroneous sequence editing (since ‘gap’ was treated as a fifth character state in subsequent analyses).

Phylogenetic Analyses. – Weighted maximum parsimony (MP) inferences were made using PAUP* 4.0b10 (Swofford 2001). Introns, nucSSU/nucLSU residues, constant sites, and ambiguously-aligned regions were excluded from analyses. Ambiguously aligned regions were re-coded using INAASE 3.0 (Lutzoni et al. 2000), re-integrated as new characters, and subjected to specific step-matrices (as outlined by Hodkinson & Lutzoni (2009)); for a review of logistical details regarding re-integrating characters and implementing step-matrices, see Hodkinson & Lutzoni (in rev.)). Unambiguously-aligned portions were subjected to symmetric step matrices computed in STMatrix 3.0 (Miadlikowska et al. 2002; available at http://www.lutzonilab.net/pages/download.shtml) as outlined by Gaya et al. (2003, 2008). For each dataset, a first round of searches was performed with 1000 random-addition-sequence (RAS) replicates and TBR (tree bisection-reconnection) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. This analysis resulted in the best tree island being hit in 75.2% of the RAS replicates. Branch support was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 5 RAS per bootstrap replicate, with all other settings as above. Maximum likelihood (ML) topology and bootstrap searches were performed using RAxML 7.0.4 with GTRMIX and 1000 resamplings ( Stamatakis 2006).

Chemical and Morphological Analyses. – All specimens were studied dry using a Bausch & Lomb StereoZoom 7 dissecting microscope. Macromorphological characters of thallus size, squamule broadness, branching, aspect, and basal color were noted. All specimens were also subjected to chemical analysis using both standard spot tests (reagents abbreviated following Brodo et al. (2001)) and Thin Layer Chromatography (TLC). TLC was carried out using solvent systems A or C following the standardized methods of Culberson & Kristinsson (1970).

Results and Discussion

I. – General Remarks

As has been discussed in the introduction above, the primary goal of the present study was to evaluate the taxonomic status of some unusual apodetiate Cladonia populations from the Appalachian Mountains. These populations seemed to be distinct from the three species known to occur in the region (C. apodocarpa, C. caespiticia, and C. petrophila) and could be grouped into three putative taxonomic entities. These entities have been little discussed in print (e.g., Lendemer & Tripp 2008) and are generally not represented in herbaria because they have either been overlooked or have gone uncollected as a result of their apparent sterility and lack of podetia. We only became aware of them after several years of concentrated fieldwork led us to suspect that more than just C. apodocarpa, C. caespiticia, and C. petrophila were present in the region (although C. stipitata had been recognized in NY as a chemotype of C. petrophila). In fact, while our study did resolve the status of these putative taxa (see sections IIa-c below), the increased sampling in our molecular dataset also afforded us the opportunity to examine in detail the circumscriptions and relationships of the taxa that had already been described in the group.

The results of our phylogenetic analyses (Plate 1) confirmed the findings of Stenroos et al. (2002) that Cladonia caespiticia is distantly related to C. apodocarpa and C. petrophila, and thus that Cladonia sect. Helopodium is not monophyletic. This distant relationship is not surprising considering that C. caespiticia is morphologically dissimilar to other apodetiate species in both vegetative morphology and in the development of the apothecia (see section IIa below). The robust sampling within C. apodocarpa and C. petrophila s.l. revealed that the ITS region alone does not seem to be sufficient for resolving relationships within this species complex. In the phylogeny presented by Stenroos et al. (2002) the two species, each represented by a single terminal, were found to be sister to each other. However, increased sampling revealed that, while the sequences of C. apodocarpa form a well-supported monophyletic clade, it is not clear whether this clade is nested within C. petrophila or if the latter species is itself a monophyletic grouping that simply cannot be defined on the basis of ITS sequence data alone. Considering the equivocal nature of the molecular data, and the fact that C. apodocarpa and C. petrophila are well characterized by a suite of traditional characters (see taxonomic section below), we prefer to retain them as distinct here pending further study with molecular methods.
Plate 1. Maximum likelihood (ML) phylogenetic tree showing inferred relationships among selected members of the genus *Cladonia* based on ITS1, 5.8S, and ITS2 sequence data. Support is shown in the form of ML bootstrap proportions (BP) and maximum parsimony (MP) BP, each based on 1000 replicate resamplings. BP values \( \geq 50\% \) are shown above (ML-BP) and below (MP-BP) each internode, with branches supported by both ML-BP and MP-BP \( \geq 70\% \) thickened. Plus “+” and minus “−” symbols following the accession numbers of sequences of *C. petrophila* refer to the presence/absence of fumarprotocetraric acid. Asterisks “*” following sequences in the clade representing *C. caespiticia* indicate vouchers of the first putative taxon. Members of *Cladonia* sect. Helopodium are shaded in gray.
IIA. – *Cladonia caespiticia* s.l.

The first putative taxon that we examined was seemingly restricted to sandstone boulders in boulder fields and talus slopes in Pennsylvania. Thalli from these populations were consistently sterile but distinctive because of their large primary squamules (often > 1 cm long), otherwise resembling those of *Cladonia caespiticia* in being thin, delicate, and finely incised. While the morphological similarity of these populations to *C. caespiticia* was further correlated to chemistry (i.e., the presence of only fumarprotocetraric acid), their ecology and size were striking enough to warrant further study.

We generated sequences from two populations of this putative taxon and included them in our analysis with a sequence of *Cladonia caespiticia* from GenBank as well as one that we generated from a typical fertile population in southern New Jersey. In the results of our analysis (Plate 1), the sequences derived from the unusual saxicolous populations from Pennsylvania form a well-supported monophyletic group with those of typical *C. caespiticia*. *Cladonia caespiticia* is generally considered to be a terricolous species that can also occur on the bases of trees and on mossy shaded rocks. Our results indicate that the species has a wider ecological amplitude than previously thought. This conclusion was further supported when we examined the holdings of *C. caespiticia* at NY and found several saxicolous collections that were fertile and otherwise morphologically typical in having small squamules.

Interestingly, our results also strongly support the taxonomic value of vegetative morphology in circumscribing this taxon. The aberrant populations that we examined differed from typical *Cladonia caespiticia* only in their larger size and ecology. Our molecular analyses also confirmed the distant relationship of *C. caespiticia* to the rest of the known North American apodetiate taxa. This distant relationship is further supported by differences in the morphology of the apothecia; while the apothecia of *C. apodocarpa*, *C. petrophila*, and a new member of this group (described below) are sessile on the squamules at maturity, the apothecia of *C. caespiticia* are distinctly elevated because they form a stipe or short ecorticate pseudopodetium (Brodo et al. 2001, fig. 217).

IIB. – *Cladonia petrophila* s.l.

The second putative taxon that we examined was restricted to shaded granitic rocks at low- to middle-elevations in the southern Appalachian Mountains. The populations were sympatric with, and morphologically identical to, *Cladonia petrophila* but differed chemically in the absence of fumarprotocetraric acid. The presence of this chemotype was not mentioned in the protologue of *C. petrophila* (Harris 1992) and it does not appear to have been mentioned otherwise in print. After collecting the chemotype several times during fieldwork in North Carolina we examined the holdings of *C. petrophila* at NY and discovered several additional specimens from other portions of the southern Appalachians.

Because we could not detect any ecological or morphological differences between the two chemotypes, and both were found at the same locality, we decided to use molecular data to investigate whether a previously unrecognized geographically restricted cryptic taxon was involved. We generated five sequences from three populations of the chemotype that lacks fumarprotocetraric acid. We then generated an additional seven sequences of the typical chemotype of *Cladonia petrophila* from seven populations across its geographic range including one from a region where the chemotypes are sympatric. These additional sequences were generated both to increase the robustness of our sampling and to supplement the one sequence available in GenBank (AF4552223). Our analyses of these sequences (Plate 1) revealed that the chemotypes do not seem to represent distinct monophyletic groups and thus the hypothesis that the chemotype lacking fumarprotocetraric acid is a distinct taxon is not supported by molecular data from the ITS region. We propose that the circumscription of *C. petrophila* should be expanded to include populations that lack fumarprotocetraric acid.

While conducting background research for this study, we discovered that the chemical phenomenon observed in *Cladonia petrophila* appears to be widespread in the genus *Cladonia*. There are apparently a number of cases in which species that produce major secondary metabolites in addition to fumarprotocetraric acid are known to have chemotypes in which the latter substance is either present or absent while the primary compound is consistently present (e.g., the two chemotypes of *C. grayi*). The results of our study of *C. petrophila* would seem to support the stance that such chemotypes should not be treated as distinct taxa, even when they appear to be geographically distinct. We do not, however, believe that our results justify any taxonomic changes outside of the current taxon.

3 Unfortunately the chemical content of the voucher upon which AF455222 is neither noted in GenBank nor in print (Stenroos et al. 2002), and we have not reviewed the specimen.
Rather we believe that the status of morphologically identical chemotypes should be evaluated with molecular methods on an individual basis.

IIc. – Cladonia stipitata

The final putative taxon that we examined has an interesting history of study (see the proceeding taxonomic section) and is an excellent example of the value of a unified approach to alpha-taxonomy. Several years ago the first author (JCL) first encountered a population of this perplexing entity on an exposed low-elevation granitic bald in North Carolina. Although the thalli in this population were morphologically similar to Cladonia petrophila, they were chemically different in lacking sphaerophorin and producing atranorin as a major substance. The chemistry (atranorin and fumarprotocetraric acid) drew comparison to C. apodocarpa; however, that taxon has much larger squamules and occurs on soil or humus in disturbed habitats. Lendemer and Tripp (2008) recognized that the problem required further study and thus reported the population as C. petrophila s.l., noting the absence of sphaerophorin. During subsequent fieldwork the first author and his colleagues at NY found additional populations of this entity at other granitic balds elsewhere in the southern Appalachians. Study of the undetermined Cladonia collections at NY and DUKE also revealed the existence of several additional collections from the region (most of which had been recognized as a chemotype of C. petrophila at NY), as well as from massive granite outcrops in the Piedmont of Georgia, and one from a similar habitat in Rhode Island.

Confronted with what seemed to be an ecologically and geographically distinct entity (with a unique morphology that could have been induced by its distinctive ecology), we generated five sequences from three populations. In our analyses (Plate 1), these sequences emerged as a well-supported monophyletic group, supporting its status as a taxon distinct from Cladonia apodocarpa and C. petrophila. We thus describe these populations as a new species, C. stipitata, in the following section.

It is noteworthy that the sequences of Cladonia stipitata possess a ubiquitous (n=5) ~210 base-pair group I intron. The presence of this intron was confirmed both by gel electrophoresis and sequencing. The latter method revealed that the intron is actually found in the small portion of the nucSSU rDNA that is amplified by the ITS primers used in this study. Interestingly, the intron was entirely absent in the sequences of C. apodocarpa (n=5) and C. petrophila (n=12) examined. In C. caespiticia it was present in two of the three sequences that we generated. The situation found in C. caespiticia is apparently typical in Cladonia (Myllys et al. 2003); however, the ubiquity of this intron in one phylogenetic species (i.e., C. stipitata) and its complete absence in a closely related clade (i.e., C. apodocarpa/petrophila) suggests that there may be forces selecting for the presence and/or absence of introns in this group of species. It has been shown previously that introns can improve transcriptional and translational yield in fungi (Juneau et al. 2006), which can be especially beneficial under stressful conditions (Parenteau et al. 2008). The trend seen here may be correlated with the fact that C. stipitata lives in an environment that is more barren and receives more ultraviolet radiation than the environments inhabited by C. apodocarpa and C. petrophila; however, further study is required before any conclusions of this nature can be drawn.

III. – Taxonomic Section

IIIa – Key to the frequently-sterile Cladonia species of Eastern North America*

1. Squamules C+ green (strepsilin present).................................................................C. strepsilis (Ach.) Grognot
2. Squamules C- (strepsilin absent).................................................................................................2

2. Squamules extremely large (10-20 mm long) and branching; P+ red, K+ yellow (fumarprotocetraric acid and atranorin present); SE coastal plain.................................................................C. prostrata A. Evans
3. Squamules mostly smaller (<10 mm long) and not branching; chemistry various; distribution various...........3

3. On calcareous substrates; squamules thick and appressed to the substrate, appearing foliose; podetia cupped when present; northern.................................................................C. pocillum (Ach.) Grognot
4. Squamules yellowish-green, KC+ golden (usnic acid and barbatic acid present)...C. robbinsii A. Evans

4. Squamules gray or blue-gray, KC- (usnic acid and barbatic acid absent; atranorin present or absent) .........................................................................................................................5
5. Medulla UV+ blue-white (sphaerophorin present), P+ red (fumarprotocetraric acid present or absent); typical of rock outcrops in shaded forests...............................C. petrophila R.C. Harris
5. Medulla UV- (sphaerophorin absent), P+ red (fumarprotocetraric acid always present); typical of other habitats .................................................................6

6. Squamules K+ yellow (atranorin present)..........................................................................................................................7

7. Squamules long and erect, upper portions convex-reflexed to show the white underside, without a blackened narrow stipe; thallus not forming distinct cushions; typical of roadside banks and disturbed areas; widespread.......C. apodocarpa Robbins
7. Squamules shorter and not distinctly erect, upper portions concave + obscuring the underside, with a distinct blackened narrowed stipe; thallus forming low dense cushions or mats; typical of granitic outcrops in sunny openings; generally known from the central-southern Appalachians and Piedmont...C. stipitata Lendemer & Hodkinson

6. Squamules K- (atranorin absent) ................................................................................................................................8

8. Squamules large and broad, margins not finely divided; podetia rare, when present forming tall irregular cup-like structures.................C. mateocyatha Robbins
8. Squamules smaller, narrow, margins finely divided; podetia absent; apothecia when present borne on a stipe/ecorticate pseudopodetium........C. caespiticia (Pers.) Flörke

*This key does not include a number of species that normally produce podetia but can also be found sterile. The excluded species fall into two main groups: 1) species with sorediate squamules or squamules that break down into granules or micro-squamules, and 2) members of the Cladonia subcariosa group with squamules that are generally short and not easily confused with the squamules of species in this key. Additionally, the most frequently sterile members of the C. subcariosa group have distinctive ecologies (they tend to occur on soil and sand in disturbed areas) and chemistries (e.g., C. brevis (Sandst.) Sandst. contains psoromic acid, C. polycarpia G. Merr. contains stictic acid, and C. polycarpoides Nyl. contains norstictic acid). Cladonia sobolescens Nyl. ex Vain., a member of the C. subcariosa group with fumarprotocetraric acid, is rarely sterile; however, sterile specimens of this species would be almost impossible to determine in light of the numerous other species with small squamules and similar chemistries.

IIIb. – The Species

Although we recognize that the group of taxa treated here is artificial, we believe that a treatment of the four species is useful in light of their superficial similarities. It is also useful because when Harris (1992) described Cladonia petrophila he drew comparisons to C. apodocarpa and C. caespiticia. Because the aforementioned species have been well illustrated and described in recent publications (Ahti 2000, Brodo et al. 2001, Harris 1992, Hinds & Hinds 2007), we present abbreviated treatments for these taxa below and describe only the new species C. stipitata in detail.


   PUBLISHED COLOR ILLUSTRATION. – Brodo et al. (2001: 238 [fig. 210]).

   CHEMISTRY. – Atranorin (major), fumarprotocetraric acid (major). Spot tests: K+ yellow, C-, KC-, P+ red, UV-.

   ECOLOGY. – This species typically occurs on soil and humus in recently disturbed habitats or other areas such as forest openings where succession is arrested because of natural factors such as periodic fire. It typically occurs in edge habitats where it is not fully exposed to the sun but rather protected by shade, at least periodically. Although we have not observed saxicolous populations of Cladonia apodocarpa, several specimens we examined listed the ecology as “vertical rock faces” or “boulders in light shade”. Thalli in these specimens appeared to represent typical C. apodocarpa and almost certainly occurred on a layer of soil or humus rather than directly on rock.

PLATE 2 (PAGE 87).
**Distribution.** – *Cladonia apodocarpa* is known primarily from eastern North America, where it is common and widespread at low to middle elevations. Although the species is most common south of the boreal region, records from as far north as Quebec have been reported (Thomson 1967). Ahti (2000) also reported the species from a single locality in Uruguay and noted a questionable literature report by Thomson (1967) from Haiti.

**Discussion.** – Due to the large size of its squamules, *Cladonia apodocarpa* is not easily confused with any of its close relatives, namely *C. petrophila* and *C. stipitata*. Rather, it is likely to be confused with sterile thalli of members of the *C. subcariosa* group, with which it frequently occurs. Members of the *C. subcariosa* group that occur in eastern North America differ chemically from *C. apodocarpa* in lacking the combination of atranorin and fumarprotocetraric acid. The primary squamules of taxa in the *C. subcariosa* group also tend to be coarser, less fragile, and shorter than those of *C. apodocarpa* although these characters are variable and distinguishing among species in the field (i.e., without access to chemical data) can be difficult. In most cases, however, careful examination of a given, apparently sterile population will reveal the presence of some podetia. Several species of *Cladonia* with cushion-forming primary squamules that could be confused with *C. apodocarpa*, particularly *C. robbinsii* and *C. strepsilis*, are distinguished in the above key.

Although the apothecia of *Cladonia apodocarpa* have generally been described as being sessile on the primary squamules of the thallus (Brodo et al. 2001, Evans 1930, Hinds & Hinds 2007, Robbins 1925, Thomson 1967), they were recently noted to be borne on short podetia by Ahti (2000). Unfortunately, we cannot confirm either interpretation because mature apothecia were not present on any of the specimens that we examined.

Plate 2. Cladonia apodocarpa. Figure 1, typical habitat (Lackawanna Co., Pennsylvania, USA). Figure 2, thallus (Harris 24794, scale = 5 mm). Figure 3, detail of primary squamules (Harris 24794, scale = 10 mm).
Plate 3. Figure 1, mature apothecia of *Cladonia caespiticia* (Harris 52734A; scale = 1 mm). Figure 2, mature apothecia and primary squamules of *C. caespiticia* (Buck 53753; scale = 1 mm). Figure 3, pycnidia of *C. caespiticia* (Harris 52734A; scale = 0.5 mm). Figure 4, pycnidium of *C. petrophila* (Harris 10730; scale = 0.1 mm). Figure 5, mature apothecia and primary squamules of *C. petrophila* (Harris 10730; scale = 1 mm). Figure 6, detail of mature apothecia of *C. petrophila* (Harris 10730; scale = 0.25 mm).
As part of our study, we generated ITS sequences from several sterile generally collected only when it is fertile because, as noted by Harris (1992), “only fools collect sterile Cladonia". In fact, the apothecia of Cladonia caespiticia are not sessile but rather borne on very short ecticate podetia that give the appearance of a stipe (the reader will note that we have referred to the apothecia as “stipitate” or “pseudopodetiate” throughout this paper). Cladonia caespiticia is clearly unrelated to the others discussed here because it is only superficially apodetiate. In fact, the apothecia of Cladonia caespiticia is widespread and common throughout eastern North America with scattered occurrences in the Great Plains. It has also been reported from every other continent except Australia and Antarctica (see distribution map in Litterski & Ahti (2004)).

Discussion. – Morphologically, Cladonia caespiticia can be recognized by its thallus composed of delicate, finely divided primary squamules. The two other species with small squamules discussed in the proceeding sections differ in having robust simple squamules that never become finely divided. Cladonia caespiticia is clearly unrelated to the others discussed here because it is only superficially apodetiate. In fact, the apothecia of Cladonia caespiticia are not sessile but rather borne on very short ecticate podetia that give the appearance of a stipe (the reader will note that we have referred to the apothecia as “stipitate” or “pseudopodetiate” throughout this paper). Cladonia caespiticia is generally collected only when it is fertile because, as noted by Harris (1992), “only fools collect sterile Cladonia”. As part of our study, we generated ITS sequences from several sterile Cladonia specimens with incised squamules
that were filed only to genus at NY because, although distinctive, they could not be identified with certainty. These sequences formed a monophyletic group with those generated from fertile specimens of C. caespiticia. Thus, we conclude that sterile Cladonia thalli on soil and humus from shaded habitats in eastern North America can be referred to this taxon on the basis of the presence of only furanprotopercaric acid and delicate, esorediate primary squamules.


PUBLISHED COLOR ILLUSTRATION. – Hinds and Hinds (2007: 195 [fig. 75]).


ECOLOGY. – As discussed by Harris (1992), Cladonia petrophila is restricted to shaded non-calcareous boulders and rock outcrops in intact hardwood forests, often near streams, streambeds, or habitats with high humidity. In particularly humid localities, the species can also occur on the roots and bases of hardwood trees; however, typical saxicolous thalli are always present in the immediate vicinity.

DISTRIBUTION. – Cladonia petrophila is known only from eastern North America where it is widely distributed in the Appalachian Mountains, Piedmont, and Ozark Ecoregion. A chemotype lacking fumarprotocetraric is restricted to the southern Appalachian Mountains (see specimens cited below) and outlying granite outcrops in the Piedmont of the southeastern United States.

DISCUSSION. – Cladonia petrophila is a common species throughout eastern North America and the Ozarks. It cannot be confused in the lab with any other Cladonia in the region because of its diminutive usually imbricate primarily squamulose thallus and the presence of sphaerophorin in the medulla. In the field, it could be confused with sterile thalli of species with esorediate primary squamules, particularly Cladonia ochrochlora Flörke; however, it can be readily distinguished from these by its distinctive medullary chemistry. The distinction between C. petrophila and C. stipitata is addressed in the following section. It should be noted that examination of the holotype of C. petrophila revealed that the figure published as part of the protologue (Harris 1992: 327, fig. 1) illustrates pycnidia rather than apothecia (see plate 3, herein).


Plate 4. *Cladonia petrophila*. Figure 1, typical habitat (Pike Co., Pennsylvania, USA). Figure 2, detail of the primary squamules (*Buck 50325*, scale = 1 mm). Figure 3, thallus (*Buck 53025*, scale = 5 mm).
Plate 5. *Cladonia stipitata*. Figure 1, thallus (*Harris 30810, scale = 5 mm*). Figure 2, detail of the primary squamules (*Harris 38010, scale = 1mm*). Figure 3, typical habitat (Jackson Co., North Carolina, USA, photo by E.A. Tripp). Figure 4, detail of apothecium (*Lendemer 7615, scale = 2 mm*).
4. *Cladonia stipitata* Lendemer & Hodkinson sp. nov.

Mycobank. #515515

Similis *C. petrophila* apothecis sessilibus sed differt squamulis stipitatibus, acidum sphaerophoricum nullum et acidum atranoricum contineat.


**Description.** – Primary thallus squamulose, forming distinct cushion-like colonies up to several centimeters in diameter. Squamules digitate, dichotomously branching, crowded, erect, forming tight dense cushions, not distinctly recurved near the tips, 0.7-1.25 mm wide, (2.5)-5-8.75 mm long, with multiple central cartilaginous strands; upper surface blue-gray though often discolored to brownish near the tips; lower surface white; margins and tips crenate. Podetia absent. Apothecia frequently present, although, mature apothecia not observed, sessile, plane, reddish-brown darkening to blackish-brown, ca. 0.5 mm in diameter. Pycnidia sessile on the squamules, reddish-brown, darkening to blackish, subglobose to pyriform, ca. 0.5 mm diam, occasionally elongating apically to becoming ca. 1.0 mm tall. Conidia hyaline, short and rod-like, often slightly curved, (7.7)-8.9-(10.2) x 0.75-1 µm.

**Etymology.** – The epithet “stipitata” refers to the distinctive stipitate morphology of the primary squamules of the species.

**Chemistry.** – Atranorin (major), fumarprotocetraric acid (major). Spot tests: K+ yellow, C-, KC+ weak purple, P+ orange-red (especially near the growing edge of the squamules), UV-.

**Ecology.** – *Cladonia stipitata* is essentially restricted to fully exposed rock slabs and boulders on granitic balds at middle to low elevations (e.g., 2000-3000 ft.) of the southern Appalachian Mountains. To date, the species has not been found in the humid shaded hardwood forests that typically surround the granitic balds where it occurs. It has also not been found in similar habitats at the highest elevations of the southern Appalachians (e.g., >3000 ft.).

**Distribution.** – *Cladonia stipitata* is presently known only from a handful of localities in the southern Appalachian Mountains (in Georgia, Kentucky, North Carolina, and South Carolina) and from a single disjunct population on an exposed granite outcrop near sea level in Rhode Island. The occurrence of this disjunct population of *C. stipitata* suggests that the species may actually have a typical Appalachian-Great Lakes distribution and that exhaustive searching of regional herbaria or additional fieldwork would turn up additional records. It should be noted, however, that our attempts to locate the species in seemingly appropriate habitats outside of its currently documented range (e.g., in Maryland, Pennsylvania, and Virginia) were unsuccessful.

**History of study.** – We were first alerted to the existence of *Cladonia stipitata* when the first author (JCL) and Erin Tripp collected it during a survey of the lichens of Gorges State Park in North Carolina. Considerable study by JCL and Richard Harris failed to resolve its status, and despite its aberrant ecology, chemistry, and distinctly stipitate squamules it was reported by Lendemer & Tripp (2008) as “*Cladonia petrophila* s.l.”. In an effort to better understand the species, a specimen that had been deposited at DUKE was studied with molecular methods as part of the AFTOL project and sequences generated from that specimen were included in the dataset used to produce the inferred phylogeny of the Lecanoromycetes by Miadlikowska et al. (2006). As a result of that study, this species is now represented in GenBank by nucSSU, nucLSU, RPB1, RPB2, and mitSSU sequences. Unfortunately, Miadlikowska et al. (2006) included this sample under the name “*Cetradonia* sp.” based on the specimen label and the initial communication from JCL suggesting that molecular data might resolve whether the taxon should be included in *Cetradonia* J.C. Wei & Ahti or *Cladonia*. It was only after additional collections of this taxon were discovered and we generated additional sequences for it and other apodetiate *Cladonia* species that we decided that formal recognition was required.
Plate 6. Geographic distributions of *C. apodocarpa* (shaded region is distribution mapped by Brodo et al. (2001)), *C. caespiticia* (shaded region is distribution mapped by Brodo et al. (2001) excluding disjunction in Nebraska), *C. petrophila* (stars = chemotype fumarprotocetraric acid lacking), and *C. stipitata* (arrow points to Rhode Island population).

Discussion. – Among the apodetiate species of *Cladonia* with sessile apothecia borne on the squamules found in eastern North America, *C. stipitata* can be recognized by its unique ecology, restricted distribution, and stipitate squamules with a blackened base. Morphologically, the species resembles *C. petrophila*, which also occurs on non-calcareous rocks; however, *C. petrophila* is restricted to shaded habitats in intact forests, consistently produces sphaerophorin in the medulla, and produces atranorin only as a minor substance that is usually detectable as only a trace with TLC. Chemically, the species is identical to *C. apodocarpa* in producing high concentrations of atranorin and fumarprotocetraric acid; however, that species occupies an entirely different habitat (soil and humus in disturbed open areas) and has much longer squamules that are not distinctly stipitate or blackened at the base.

The discovery of a new species of *Cladonia* that is essentially restricted to exposed granitic balds in the southern Appalachian Mountains is not surprising when one considers how poorly collected the middle and low elevations of the mountain range are in that region (Lendemer & Tripp 2008). An endemic monotypic genus, *Cetradonia* J.C. Wei & Ahti, is already known from shaded granitic seepage faces in the same region and ongoing studies by the authors and their colleagues continue to result in the discovery of previously unrecognized disjunct


**Acknowledgements**

We thank Richard Harris for his helpful discussions of *C. petrophila* and other sterile *Cladonia*. Thanks to Teuvo Ahti and Erin Tripp for reviewing the manuscript. The second author thanks François Lutzoni and Jolanta Miadlikowska for training in phylogenetic methods. Special thanks to Sean Beech, Bill Buck, Richard Harris, Malcolm Hodges, Andy Moroz, and Erin Tripp for their companionship and help with logistics associated with fieldwork. Fieldwork carried out by the first author was funded in part by the Department of Conservation and Natural Resources of the Commonwealth of Pennsylvania (DCNR), New York Botanical Garden (NYBG), Western Pennsylvania Conservancy (WPC), and the Zalk Travel Fund of the City University of New York (CUNY). Thanks also to the following for providing the first author with permission to collect in their respective regions: United States Forest Service (Nantahala National Forest), United States National Park Service (Delaware Water Gap National Recreation Area, Great Smoky Mountains National Park), DCR of Virginia, DCNR of Pennsylvania, Pennsylvania State Game Commission, The Nature Conservancy, and Western Pennsylvania Conservancy. Molecular data was gathered using the facilities of the Lewis B. and Dorothy Cullman Laboratory at NYBG.

**Literature Cited**


**APPENDIX I**

DATA FOR MOLECULAR VOUCHERS AND GENBANK ACCESSIONS FOR ITS SEQUENCES GENERATED AS PART OF THIS STUDY.

<table>
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<th>GenBank No.</th>
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