Dermatocarpon (Verrucariaceae) in the Ozark Highlands, North America

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Abstract. *Dermatocarpon*, a saxicolous lichen, is common throughout the Ozarks Highlands of North America where exposed rock is abundant. *Dermatocarpon* is an understudied genus. Species delimitation is difficult because of a paucity of morphological characters and a large degree of variation within this genus. The taxonomy of *Dermatocarpon* in North America was recently thrown into flux because of a molecular study which limited the use of a once widely applied name, *D. miniatum*. The Melzer's reagent test, currently used for identifying members of the *miniatum*-complex in North America, is not useful for identifying Ozark specimens. A revision of *Dermatocarpon* for the Ozark Highlands of North America is presented based on morphological, molecular and ecological studies. The results of these studies indicate that eight taxa are present in the Ozarks. Four taxa are described new to science: *D. arenosaxi*, *D. dolomiticum*, *D. luridum* var. *xerophilum* and *D. multifolium*.

Keywords. *Dermatocarpon*, Melzer's reagent, Ozarks, Verrucariaceae.

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Dermatocarpon Eschw. is characterized by having a foliose thallus attached to the substrate by an umbilicus or cord-like holdfast and a lower cortex composed of thick-walled cells (*Dermatocarpon*-type). It is considered obligately saxicolous although at least two species are able to grow on soil over rock. *Dermatocarpon* has a worldwide distribution, occurring in both the Northern and Southern Hemispheres (http://www.biologie.uni-hamburg.de/checklists). A few species are circumpolar (Elvebakk & Hertel 1997; Øvstedal & Lewis Smith 2001). Nineteen species are reported from North America (Esslinger 1997). Three of these were either recently described or raised to species status (Breuss 2003; Heiðmarsson 2003).

The original description of *Dermatocarpon* (Eschweiler 1824) as "flexible, subcrustose, and sublobate" has been refined considerably over more than a century of study. When Eschweiler described *Dermatocarpon* he did not assign any species to the genus. The following year (Mann 1825) *Endocarpon miniatum* (L.) Ach. and several other species were

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Table 1. Voucher specimens, origin and GenBank accession numbers for ITS and LSU sequences included in ITS and combined ITS + LSU Analyses. Sequences with a GenBank ID beginning with "AF" were extracted by Heiðmarsson and downloaded from GenBank.

Taxon	Voucher	Locality	ITS GenBank	LSU GenBank
D. americanum	*Heiðmarsson 1056* (UPS)	Arizona	AF333130	–
D. americanum	*Heiðmarsson 1210B* (ASU)	Arizona	EF014153	–
D. americanum	*Nash 33616* (ASU)	Mexico	EF014156	–
D. arenosaxi	*Amtoft 1353* (NY)	Missouri	EF014187	–
D. arenosaxi	*Amtoft 1360* (NY)	Missouri	BI839503	–
D. arenosaxi	*Amtoft 1361* (NY)	Missouri	EF014161	–
D. arenosaxi	*Amtoft 1361* (NY)	Missouri	EF014188	–
D. arenosaxi	*Amtoft 1456* (NY)	Missouri	EF014164	–
D. arenosaxi	*Amtoft 1456* (NY)	Missouri	EF014189	–
D. arenosaxi	*Amtoft 559* (NY)	Missouri	EF014160	EF568796
D. arenosaxi	*Amtoft 560* (NY)	Missouri	EF014191	–
D. arenosaxi	*Buck 22179* (NY)	Georgia	EF014167	EF568800
D. arenosaxi	*Buck 35924* (NY)	Illinois	EF014166	EF568802
D. arenosaxi	*Buck 35934* (NY)	Illinois	EF014163	–
D. arenosaxi	*Buck 36675* (NY)	Georgia	EF014159	EF568788
D. arenosaxi	*Buck 42995* (NY)	Missouri	EF014190	–
D. arenosaxi	*Harris 21644* (NY)	Arkansas	EF014168	–
D. arenosaxi	*Harris 31246* (NY)	Missouri	EF014193	–
D. arenosaxi	*Harris 38701* (NY)	Georgia	EF014169	–
D. arenosaxi	*Harris 43471* (NY)	South Carolina	EF014158	–
D. arenosaxi	*Harris 44305* (NY)	Oklahoma	EF014162	–
D. arenosaxi	*Harris 44308* (NY)	Oklahoma	EF014165	–
D. arenosaxi	*Wilhelm 11220* (MOR)	Missouri	EF014186	EF568801
D. bachmannii	*Heiðmarsson 978* (UPS)	Sweden	AF333169	–
D. cf. *bachmannii*	*Heiðmarsson 1037* (AMNH)	Oregon	AF333167	–
D. deminuens	*Heiðmarsson 98* (UPS)	Sweden	AF333168	–
D. dolomiticum	*Amtoft 1002b* (NY)	Missouri	EF014212	–
D. dolomiticum	*Amtoft 1038b* (NY)	Missouri	EF014133	EF568799
D. dolomiticum	*Amtoft 3296* (NY)	Missouri	EF014135	–
D. dolomiticum	*Amtoft 3303* (NY)	Missouri	EF014136	EF568795
D. dolomiticum	*Amtoft 497a* (NY)	Missouri	EF014138	EF568791
D. dolomiticum	*Amtoft 513* (NY)	Missouri	EF014134	–
D. dolomiticum	*Amtoft 516a* (NY)	Missouri	EF014139	EF568785
D. dolomiticum	*Amtoft 518b* (NY)	Missouri	EF014210	–
D. dolomiticum	*Amtoft 572* (NY)	Missouri	EF014140	–
D. dolomiticum	*Buck 32272* (NY)	New York	EF014137	–
D. dolomiticum	*Harris 25421* (NY)	Missouri	EF014211	–
D. dolomiticum	*Parker 2496* (NY)	Missouri	EF014132	–
D. leptophyllum	*Heiðmarsson 200* (AMNH)	Sweden	AF333156	–
D. leptophyllum	*Nordin 4391* (UPS)	Sweden	AF333155	–
D. linkolae	*Westberg 16* (LD)	Sweden	AF333158	–
D. luridum	*Amtoft 1500* (NY)	Missouri	EF014196	EF568807
D. luridum	*Amtoft 1506* (NY)	Missouri	EF014195	–
D. luridum	*Amtoft 2005* (NY)	Tennessee	EF014198	EF568797
D. luridum	*Amtoft 2048* (NY)	North Carolina	EF014194	EF568789
D. luridum	*Buck 36333* (NY)	Alabama	EF014197	EF568808
D. luridum	*Heiðmarsson 100* (UPS)	Sweden	AF333132	–
D. luridum	*Heiðmarsson 1382* (AMNH)	Minnesota	AF333133	–

Table 1. Continued.

Taxon	Voucher	Locality	ITS GenBank	LSU GenBank
D. luridum var. *xerophilum*	*Amtoft 589* (NY)	Arkansas	EF014201	–
D. luridum var. xerophilum	*Amtoft 597* (NY)	Arkansas	EF014209	–
D. luridum var. *xerophilum*	*Amtoft 603a* (NY)	Arkansas	EF014204	–
D. luridum var. *xerophilum*	*Amtoft 611* (NY)	Arkansas	EF014202	–
D. luridum var. *xerophilum*	*Buck 37317* (NY)	Arkansas	EF014205	EF568804
D. luridum var. *xerophilum*	*Buck 38431* (NY)	Oklahoma	EF014200	EF568803
D. luridum var. *xerophilum*	*Harris 45427* (NY)	Arkansas	EF014203	–
D. luridum var. *xerophilum*	*Wilhelm & Ladd 22697* (MOR)	Missouri	EF014199	–
D. meiophyllizum	*Heiðmarsson 1351* (UPS)	Minnesota	AF333171	–
D. meiophyllizum	*Heiðmarsson 583* (UPS)	Finland	AF333172	–
D. meiophyllizum	*Heiðmarsson 455* (UPS)	Faeroe Islands	AF333173	–
D. miniatum var. *complicatum*	*Heiðmarsson 1340* (AMNH)	Minnesota	AF333146	–
D. miniatum var. *complicatum*	*Heiðmarsson 1350* (UPS)	Minnesota	AF333149	–
D. miniatum var. *complicatum*	*Heiðmarsson 1362* (AMNH)	Minnesota	AF333147	–
D. miniatum var. *complicatum*	*Heiðmarsson 1384* (AMNH)	Iceland	AF333141	–
D. miniatum var. *complicatum*	*Heiðmarsson 1385* (AMNH)	Iceland	AF333151	–
D. miniatum var. *complicatum*	*Heiðmarsson 302* (UPS)	Sweden	AF333150	–
D. miniatum var. *complicatum*	*Heiðmarsson 348* (UPS)	Sweden	AF333143	–
D. miniatum var. *complicatum*	*Heiðmarsson 695B* (AMNH)	Iceland	AF333142	–
D. miniatum var. *complicatum*	*Heiðmarsson 932* (UPS)	Iceland	AF333152	–
D. miniatum var. *complicatum*	*Heiðmarsson 732* (AMNH)	Austria	AF333154	–
D. miniatum var. *complicatum*	*Heiðmarsson 390* (UPS)	Norway	AF333163	–
D. miniatum var. *miniatum*	*Tibell 21893* (UPS)	India	AF333161	–
D. miniatum var. *miniatum*	*Thor 16486* (HB. THOR)	Japan	AF333160	–
D. miniatum var. *miniatum*	*Tibell 21835-36* (UPS)	India	AF333162	–
D. miniatum var. *miniatum*	*Heiðmarsson 1338* (AMNH)	Minnesota	AF333148	–
D. miniatum var. *miniatum*	*Heiðmarsson 980* (AMNH)	Sweden	AF333144	–
D. miniatum var. *miniatum*	*Heiðmarsson 587* (UPS)	Finland	AF333145	–
D. miniatum var. *miniatum*	*Heiðmarsson 466* (UPS)	Faeroe Islands	AF333159	–
D. miniatum var. *miniatum*	*Buck 47331* (NY)	Wales	EF014192	EF568786
D. miniatum var. *miniatum*	*Gueidan 387* (DUKE)	France	EF469157	EF469160
D. miniatum var. *miniatum*	*Heiðmarsson 810* (AMNH)	Austria	AF333157	–
D. miniatum var. *miniatum*	*Kristinsson 9631* (AMNH)	Iceland	AF333153	–
D. moulinsii	*Amtoft 1038a* (NY)	Missouri	EF014155	EF566984
D. muhlenbergii	*Amtoft 580* (NY)	Missouri	EF014141	–
D. muhlenbergii	*Harris 25668* (NY)	Missouri	EF014206	–
D. muhlenbergii	*Amtoft 3394* (NY)	Missouri	EF014207	–
D. muhlenbergii	*Amtoft 1122* (NY)	Missouri	EF014142	–
D. muhlenbergii	*Amtoft 1002a* (NY)	Missouri	EF014208	–
D. muhlenbergii	*Amtoft 3301* (NY)	Missouri	EF014148	–
D. muhlenbergii	*Amtoft 3262* (NY)	Missouri	EF014144	EF568792
D. muhlenbergii	*Amtoft 400* (NY)	Missouri	EF014150	–
D. muhlenbergii	*Amtoft 915* (NY)	Missouri	EF014143	–
D. muhlenbergii	*Amtoft 3356a* (NY)	Missouri	BI839443	–
D. muhlenbergii	*Amtoft 1266* (NY)	Missouri	EF014145	EF568806
D. muhlenbergii	*Amtoft 1267* (NY)	Missouri	EF014147	–
D. muhlenbergii	*Harris 48190* (NY)	Missouri	EF014146	EF568805
D. muhlenbergii	*Buck 45080* (NY)	Connecticut	EF014213	–
D. muhlenbergii	*Amtoft 3265* (NY)	Missouri	EF014149	–
D. muhlenbergii	*Amtoft 2013* (NY)	Tennessee	EF014152	–

Table 1. Continued.

Taxon	Voucher	Locality	ITS GenBank	LSU GenBank
D. muhlenbergii	*Amtoft 3380* (NY)	Missouri	EF014151	–
D. muhlenbergii	*Mattsson 5322* (UPS)	Missouri	AF333131	–
D. muhlenbergii	*Amtoft 3384* (NY)	Missouri	–	EF568793
D. multifolium	*Amtoft 459b* (NY)	Missouri	EF014172	EF568798
D. multifolium	*Buck 42784* (NY)	Missouri	EF014173	–
D. multifolium	*Buck 32819* (NY)	Missouri	EF014170	–
D. multifolium	*Amtoft 3299B* (NY)	Missouri	EF014174	–
D. multifolium	*Amtoft 1214* (NY)	Missouri	EF014182	EF568810
D. multifolium	*Buck 36390* (NY)	Alabama	BI839581	–
D. multifolium	*Amtoft 1083* (NY)	Missouri	–	EF568794
D. multifolium	*Amtoft 440a* (NY)	Missouri	EF014175	–
D. multifolium	*Amtoft 3302* (NY)	Missouri	EF014185	–
D. multifolium	*Amtoft 3299A* (NY)	Missouri	EF014176	EF568787
D. multifolium	*Guccion s.n.* (NY)	Virginia	EF014177	–
D. multifolium	*Amtoft 3261* (NY)	Missouri	EF014178	–
D. multifolium	*Harris 33069-2* (NY)	North Carolina	EF014179	–
D. multifolium	*Amtoft 3298* (NY)	Missouri	EF014180	–
D. multifolium	*Amtoft 3269* (NY)	Missouri	EF014181	EF568790
D. multifolium	*Amtoft 397b* (NY)	Missouri	EF014183	–
D. multifolium	*Harris 33069-1* (NY)	North Carolina	EF014184	–
D. multifolium	*Amtoft 451* (NY)	Missouri	EF014171	–
D. polyphyllizum	*Heiðmarsson 976* (UPS)	Iceland	AF333170	–
D. reticulatum	*Heiðmarsson 1393*(UPS)	Arizona	AF333129	–
D. rivulorum	*Heiðmarsson 524* (AMNH)	Sweden, TL	AF333166	–
D. taminium	*Heiðmarsson 1138* (AMNH)	Arizona	AF333138	–
D. taminium	*Heiðmarsson 1292* (AMNH)	Arizona	AF333140	–
D. taminium	*Heiðmarsson 1257A* (AMNH)	Arizona	AF333135	–
D. taminium	*Heiðmarsson 1297B* (UPS)	Arizona	AF333139	–
D. taminium	*Heiðmarsson 1207* (UPS)	Arizona	AF333136	–
D. taminium	*Heiðmarsson 1256* (UPS)	Arizona	AF333134	–
D. taminium	*Nash 35378* (ASU)	Arizona	–	–
D. tomentulosum	*Amtoft 474* (NY)	Missouri	EF014154	EF568809
D. tenue	*Heiðmarsson 1137* (UPS)	Arizona	AF333128	–
D. tuzibei	*Moon 6132* (DUKE)	Japan	EF014157	EF568811
Clavascidium sp.	*Harris 46789* (NY)	Missouri	EF469156	EF469159
Placidium lacinulatum	*Gueidan & Roux CG13* (DUKE)	France	EF469155	EF469158

transferred to *Dermatocarpon* but it was not until almost over a century later (Clements & Shear 1931) that, *D. miniatum* (L.) W. Mann, was designated the type species of the genus. Many species of *Dermatocarpon* have at some point been included in *Endocarpon*, and several of the species [e.g., *D. miniatum*, *D. luridum* (With.) J. R. Laundon and *D. leptophyllum* (Ach.) K. G. W. Lång] were originally described under the genus *Lichen* L. In 1855 Körber recircumscribed *Dermatocarpon* to include only the foliose or crustose members having a dark exciple and colored muriform spores. So defined, *Dermatocarpon miniatum* and other species of the genus were returned to *Endocarpon* and just one species, *D. schaereri* (Hepp) Körber, remained. With Körber's revision *D. schaereri* implicitly became the type species. Fortunately *D. schaereri*, now referred to *Placocarpus* Trev., was not one of the original species included in *Dermatocarpon* by Mann (1825). In the mid-nineteenth century many *Endocarpon* species were moved into other genera [*Catapyrenium* (Flotow 1850), *Endopyrenium* (Körber 1855), *Placidium* (Massalongo 1855), *Placocarpus* (Trevisan 1860) and *Rhodocarpon* (Lönnroth 1858)]. Lönnroth

included *Endocarpon miniatum* in *Rhodocarpon* (described as foliose or squamulose with a hyaline exciple and simple spores) but Fries (1860) synonymized *Rhodocarpon* with *Dermatocarpon* and returned *R. miniatum* (L.) Lönnr. and some other species of *Endocarpon* and *Rhodocarpon* to *Dermatocarpon*. During the early 20th century *Dermatocarpon* included crustose members in addition to foliose and squamulose species (e.g., Servít 1952). In the last major revision of *Dermatocarpon* (Zschacke 1934) several genera previously split from *Endocarpon* were treated as synonyms or subdivisions of *Dermatocarpon* and *Dermatocarpon* included only squamulose or foliose members. Zschacke (1934) recognized four subdivisions within *Dermatocarpon* (*Catapyrenium*, *Endopyrenium*, *Entosthelia* and *Polyrhizon*) based on thallus type, pigmentation of the exciple and the presence of rhizine-like appendages; only *Entosthelia* and *Polyrhizon* are included in the current sense of *Dermatocarpon*, which is based partly on Hawksworth et al. (1980) and Harada (1993). These authors removed the squamulose members from the genus and Harada further limited the circumscription to include only foliose members with pycnidia of the *Xanthoria*-type and a lower cortex of thick-walled cells which he termed the *Dermatocarpon*-type.

Several characters were recently found to be unreliable for diagnosing species [e.g., vagrant habit (Rosentreter & McCune 1992), the presence or absence of pruina (Heiðmarsson 1996) and if the upper surface turns green when wetted (Rosentreter & McCune 1992)]. A lack of uniform, discrete characters along with a large degree of morphological variability makes species delimitation in *Dermatocarpon* difficult. Molecular data are proving to be valuable for sorting out the myriad of species for which there are few apparent morphological distinctions; yet *Dermatocarpon* remains poorly understood and largely understudied, especially outside Europe.

Ozark specimens do not fit well into current concepts of known species in terms of their morphology or ecology. Species which are potentially referable to the Ozarks are reported as growing on both acidic and alkaline substrates (Heiðmarsson & Breuss 2004; Purvis et al. 1992), yet in the Ozarks, habitat and substrate type seem to have predictive taxonomic value. Existing treatments are unsatisfactory for identifying Ozark specimens. One reason for this is that *Dermatocarpon* is understudied in North America. Additionally, species delimitation in North America has been complicated by a treatment of the *D. miniatum* complex (Heiðmarsson 2003). This treatment limited the use of a once widely applied name, *D. miniatum*. *Dermatocarpon miniatum* was once thought to be widely distributed and common throughout North America, but now several species are recognized in its place. These other species, *D. americanum* Vain., *D. tenue* (Müll. Arg.) Heiðmarsson and *D. taminium* Heiðmarsson, resemble *D. miniatum* in gross morphology and are distinguished from *D. miniatum* by one or more of the following characters: staining of the medulla with Melzer's reagent or iodine potassium iodide (IKI), color of the upper and lower surfaces, epinecral layer type and spore size. These characters, especially the Melzer's reaction, need to be reëvaluated. Morphological and ecological studies were made and DNA sequence data were obtained to better resolve the taxonomy of *Dermatocarpon* in the Ozarks.

Geographic/Ecological Description

Dermatocarpon is common in the Ozarks, sometimes lavishly covering large areas. Given its richness there, the Ozarks may in fact be a center of diversity for *Dermatocarpon* in North America. Large areas of exposed rock provide an unusual amount of suitable habitat for these lichens.

The Ozark Highlands, known for their biological richness, lie in the central part of North America across five states: Missouri, Arkansas, and to a lesser extent Oklahoma, Kansas and Illinois (**Fig. 1**). Volcanic uplift during the later part of the Pre-Cambrian laid the igneous foundation of the Ozarks and the core of the St. Francois Mountains, whose Pre-Cambrian igneous peaks are now exposed (Hawker 1992). During the Cambrian most of the Ozarks was covered by a shallow sea that extended from Mexico northward. Over time, carbonate rock was laid over the igneous foundation (Hawker 1992) due to the action of the rising and retreating sea and the metabolic processes of calcium carbonate

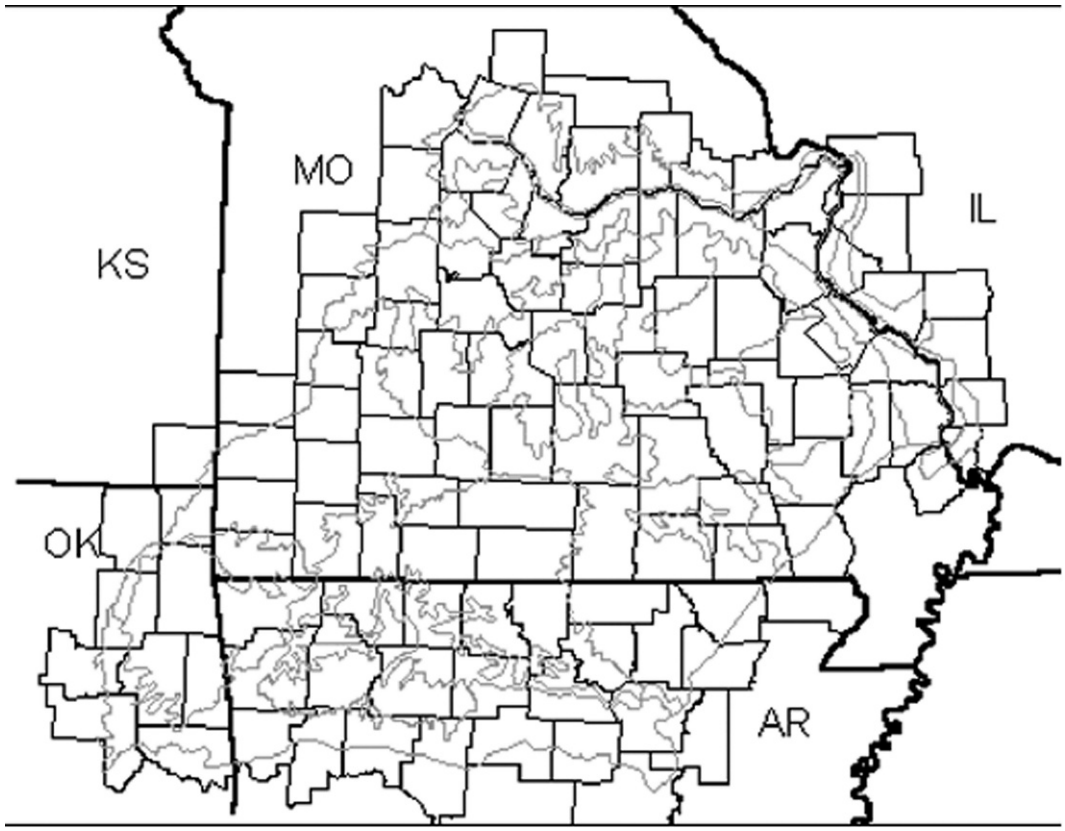


Figure 1. Map of Ozark eco-region (black lines indicate political boundaries; thin lines = counties, bold lines = states, gray lines = boundary of eco-region; KS = Kansas, MO = Missouri, IL = Illinois, OK = Oklahoma, AR = Arkansas).

sequestering organisms. This carbonate rock is abundant in the Ozarks as limestone and dolomite bluffs and glades. Ordovician silica deposits are present as sandstone bluffs and expansive sandstone glades throughout the region. Large parts of the Ozarks escaped glaciation during the Ice Age of the Pleistocene epoch. Remarkably, some of the unglaciated areas have been continuously available to terrestrial life since the late Paleozoic (Ladd 2002). Although glaciers did not enter the Ozarks proper, these Ice Age glacial periods played a large part in shaping its flora and fauna. Alternating periods of glaciation and interglacials are responsible for many of the Ozark endemics as well as Ozarks-Appalachian disjuncts separated by the Mississippi River.

Materials and Methods

Morphological study. Specimens were collected with Richard Harris, William Buck and Doug Ladd during two week-long field trips to the Ozarks each year from 2001 to 2005. A total of 494 specimens from ASU, DUKE, MOR, NY and UPS were studied (herbarium acronyms follow Holmgren & Holmgren 1998). All thalli were examined under an Olympus SZ60 dissecting microscope. Thin sections were mounted in water and examined with an Olympus BH-2 compound microscope. Images for figures and plates were taken with a Nikon DXM100F at NY and on an Olympus DP10 camera mounted on a S2X12 dissecting microscope at Appalachian State University (BOON). Thin sections were stained with lactic fuchsin to observe the epinecral layer. Lobe size was measured in millimeters with a ruler. Most of the specimens examined are deposited in NY and MOR. Specimen collection data can be found at http://sciweb.nybg.org/science2/VirtualHerbarium.asp.

Molecular study. Two loci, nuclear ribosomal large subunit (nucLSU) and the entire ITS region (ITS1, 5.8S and ITS2) were sequenced. *Placidium* A. Massal. was chosen as an outgroup based on

anatomical similarity with *Dermatocarpon* (multilocular pycnidia and a pale exciple). We obtained 130 ITS sequences (42 from GenBank) and 29 nucLSU sequences.

Genomic DNA was obtained from thallus fragments which were reduced to a powder inside a 1.5 ml Eppendorf tube. A standard DNA isolation procedure employing 2% SDS lysis buffer (Zolan & Pukkila 1986) was used. Extracted DNA was resuspended in 50–100 µl of sterile water depending on the size of the pellet.

The entire ITS region (ITS1, 5.8S and ITS2) and 1.4 kb of the nucLSU was amplified using ITS1F and ITS4 primers (Gardes & Bruns 1993) and combinations of LR0R, LR7 (Vilgalys & Hester, 1990), and LIC24R (Miadlikowska & Lutzoni, 2000), respectively.

A map and primer sequences may be viewed at http://www.lutzonilab.net/pages/primer.shtml. The amplification reactions were prepared for a final 25 µL volume containing 5.0 µL of sterile double-distilled water, 2.5 µL of 10× Taq polymerase reaction buffer (Boehringer-Mannheim), 4.0 µL 2 mM dNTP, 1.0 µL of 100× Bovine Serum Albumin (BSA; BioLabs), 0.3 µL Taq DNA polymerase (Boehringer-Mannheim), 1.25 µL for each of the 10 µM primers. PCR was performed on Peltier Thermal Cyclers PTC-200 (MJ Research) under the following conditions for ITS: step 1) one min at 95°C, 2) 45 sec at 95°C, 3) 40 sec at 52°C, 4) 1 min 30 sec at 72°C, 5) return to step 2 30 times, 6) final step of 10 min at 72°C; and for LSU: step 1) 1 min at 95°C, 2) 45 sec at 95°C, 3) 40 sec at 52°C, 4) 2 min 30 sec at 72° C, 5) return to step 2 30 times, 6) a final cycle of 10 min at 72°C. Samples were kept at 4°C until electrophoresis was performed on a 1% agarose gel prepared with TAE and visualized with Sybr®-Green (Invitrogen™). PCR reactions were cleaned using Microcon PCR cleaning kit (Millipore, Billerica MA). Cloning, when required, was performed with a TOPO TA Cloning® Kit (Invitrogen™) following the kit protocol.

Sequencing reactions were carried out on a final volume of 10 µl reaction consisting of 1.5 µl Big Dye (Big Dye Terminator Cycle sequencing Kit, ABI PRISM, Perkin-Elmer, Applied Biosystems), 2.5 µl Big Dye buffer, 1 µl of 10 µM primer and 5 µl of purified PCR product. Various sequencing primers were used for ITS (ITSF1, 5.8SR, 5.8S, ITS4) (Vilgalys & Hester 1990) and for LSU (LR0R, LIC24R, LR7, LR5)(Vilgalys & Hester 1990). Sequences were assembled using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor) and aligned manually with MacClade 4.01 (Maddison & Maddison 2001).

Phylogenetic analyses. Because we were unable to complete ITS and nucLSU sequences for the same set of specimens, analyses were carried out on ITS dataset of 132 specimens and combined ITS and LSU dataset of 31 specimens. The ITS and ITS+nucLSU datasets were subjected to weighted maximum-parsimony (MP), neighbor-joining (NJ) searches using PAUP* 4.0b.8a (Swofford 2001) and Bayesian analyses using Bayesian Metropolis coupled Markov chain Monte Carlo algorithm (B-MCMCMC) as implemented in MrBayes v3.1.1 (Huelsenbeck & Ronquist 2001). Before combining the LSU and ITS data sets, topological congruence was assessed for each data partition following the recommendation of Reeb et al. (2004). Neighbor-joining bootstrap (NJ-bs) was performed with 1000 replicates with distance measure estimated by maximum likelihood (ML). ''Best-fit'' nucleotide substitution models were estimated for all NJ analyses using hierarchical likelihood ratio tests (LRTs) as implemented in Modeltest v. 3.06 (Posada & Crandall 1998). For the ITS dataset, TrN + G model (Tamura & Nei 1993) was selected with following base frequencies: A = 0.1911, C = 0.2996, G = 0.2942, T = 0.2151; substitution rate matrix R(A-C) =1.0000, R(A-G) = 5.0611, R(A-T) = 1.0000, R(C-G) = 1.0000, R(C-T) = 11.4900, R(G-T) = 1.0000 and gamma distribution shape parameter 0.1760. For the LSU dataset a HKY + G model (Hasegawa et al. 1985) was selected with base frequencies (A = 0.4009, C = 0.2460, G = 0.1059, T = 0.2872), substitution rate Ti/tv ratio = 3.3424, gamma distribution $\gamma = 0.4337$, and proportion of invariable sites I = 0).

For the MP analyses, constant sites and ambiguously aligned sites were removed from ITS and nucLSU data matrices. However, ambiguously aligned regions were re-coded and subjected to specific step-matrices using INAASE v2.3b (Lutzoni et al. 2000) and then reïntegrated as new characters into the data set. Unambiguously aligned portions of

the ITS and LSU alignments were subjected to symmetric step matrices computed with the program STMAtrix 2.1 (written by S. Zoller and available at http://www.lutzonilab.net/pages/download.shtml) as outlined in Gaya et al. (2003). For the ITS region three separate step matrices corresponding to ITS1, 5.8S and ITS2 regions were implemented in MP analyses. Gaps were treated as a fifth character state for the unambiguous portions of the alignments.

For the ITS dataset a first round of searches was performed with 1000 random-addition-sequences (RAS) replicates, TBR (tree bisection reconnection) branch swapping, MULPARS option in effect, saving no more than two trees greater than or equal to five steps for each replicate, and collapsing zero-length branches. The 55 equally most parsimonious trees generated from this analysis were used as starting points for a second round of heuristic searches saving all most parsimonious trees without any restrictions. Swapping was performed on each of the 55 trees obtained from the first run until there were no new trees found. This procedure was necessary because of the large number of equally most parsimonious trees resulting from the inclusion of many sequences that differed only by a few mutations. For the ITS + nucLSU dataset, MP search was performed with 1000 RAS, TBR swapping, steepest descent not in effect, MULtrees option in effect, saving all trees at each step during stepwise addition and collapsing zero length branches. Branch support for MP trees derived from ITS and ITS + nucLSU datasets was estimated by bootstrap analyses (MP-bs; Felsenstein 1985) by performing 1000 bootstrap replicates with five RAS per bootstrap replicate with gaps treated as a fifth character using the same parameter settings as for the initial MP searches.

Bayesian posterior probabilities (PP) were computed on the ITS and ITS + nucLSU data sets. The ITS dataset was divided into three partitions corresponding to ITS1, 5.8S, and ITS2. For each partition (three partitions for the ITS data set and four for the ITS + nucLSU data set), a six-parameter model for the nucleotide substitution (GTR; Rodríguez et al. 1990) with a gamma distribution and invariant characters was applied. All parameters were estimated by MrBayes during the runs. Bayesian analyses were initiated using four independent chains running simultaneously for 10,000,000 generations, and sampling every 500th tree. After verifying that stationary likelihood scores and parameter estimation had been reached, the first 5,000 trees were discarded and a 50% majority-rule consensus tree was generated from the remaining 15,000 trees using PAUP.

Results and Discussion

The final alignment for the combined ITS + nucLSU dataset consisted of 1,529 characters. A total of 1,292 ambiguously aligned and constant characters were excluded. Of the 237 remaining characters included in the analysis, 132 were from LSU (including 7 INAASE-coded characters) and 105 from ITS (40 from ITS1, 16 from 5.8S, 37 from ITS2 and 12 INAASE- coded characters). In this dataset 157 characters were parsimony-informative (63 from ITS and 94 from nucLSU).

The final alignment for the ITS dataset consisted of 591 characters. A total of 441 ambiguously aligned and constant characters were excluded. Of the 150 remaining characters included in the analysis, 49 were from ITS1, 33 from 5.8S, 60 from ITS2 and 8 INAASE-coded. In this dataset, 108 characters were parsimony-uninformative.

For specimens in which cloning was necessary, the contaminant sequences most often blasted (GenBank) as Dothideales and Chaetothyriales.

The ITS region is variable and useful for species delimitation. Some basal clades are resolved in the combined analysis with LSU but without strong statistical support (**Figs. 2, 3**). The combined ITS + LSU analysis however proved useful for resolving some relationships that ITS alone did not. Based on both the ITS and combined ITS + LSU analyses there are eight taxa in the Ozarks which include three new species and one new variety. LSU sequences were obtained for *D. miniatum* but not for *D. americanum* and therefore the status of *D. americanum* is based solely on ITS sequences.

Phylogenetic relationships. Based on molecular data, *Dermatocarpon miniatum* and *D. americanum* are not present in the Ozarks, and it appears that *D. americanum sensu* Heiðmarsson is polyphyletic. Each of the three specimens of *D. americanum* in the ITS analysis were resolved in different positions on the

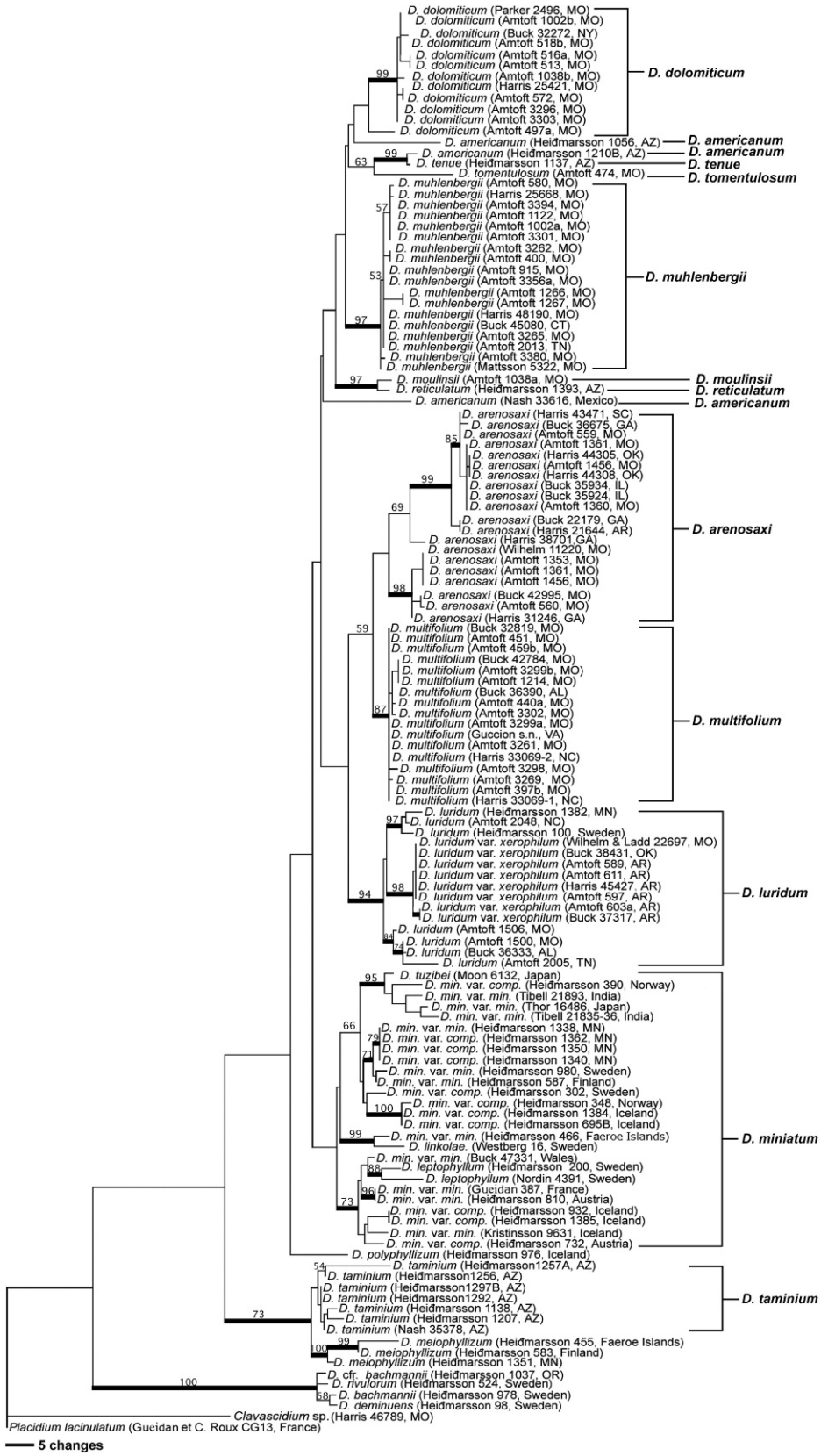
tree (**Fig. 2**). One *D. americanum* specimen from Arizona is sister to *D. tenue* (*Heiðmarsson 1137*, ASU) with a bootstrap value of 99%, and of the remaining two specimens of *D. americanum*, *Heiðmarsson 1056* (ASU) from Arizona and *Nash 33616* (ASU) from Mexico, one is sister to *D. dolomiticum* and the other is sister to the rest of the *D. americanum* group (= *D. americanum*, *D. muhlenbergii*, *D. tenue*, *D. moulinsii*, *D. reticulatum*, *D. tomentulosum* and *D. dolomiticum*), respectively, but without significant support. We have not examined the type of *D. americanum* but since the type is described from Mexico, *D. americanum* might not be as widespread in North America as has recently been suggested (Heiðmarsson 2003), but instead may have a southwestern distribution. It is likely that specimens from eastern North America identified and filed under *D. americanum* belong to *D. muhlenbergii*. Because *D. muhlenbergii* reacts variably with Melzer's reagent (see below), *D. americanum* cannot be distinguished from *D. muhlenbergii* by the Melzer's reagent test alone as was suggested by Heiðmarsson (2003). Specimens of *D. americanum* from Arizona (ASU) and the specimens sequenced here are distinct from *D. muhlenbergii* in the appearance of the perithecia and upper surface. These distinctions are discussed further under the description of *D. muhlenbergii*.

Despite extensive sampling, no Ozark specimens were affiliated with the *D. miniatum* clade (**Figs. 2, 3**). This includes specimens which were tentatively identified as *D. miniatum*, either based on morphology or because the medulla did not stain with Melzer's reagent. Molecular data suggest that *D. miniatum* may have a northern or boreal distribution in North America. Although collections from thirteen states were sampled, only four Minnesota specimens clustered with GenBank sequences of *D. miniatum* (**Fig. 2**).

Some Ozark specimens provisionally identified as *D. tenue* based on the upper surface color and reaction of the medulla to Melzer's reagent are actually referable to either *D. muhlenbergii* or *D. luridum*. The pale blue-green color of the specimens referred to *D. tenue* seems to be an ecological response to low light conditions. *Dermatocarpon tenue sensu* Heiðmarsson therefore is not present in the Ozarks. An isosyntype of *D. tenue* (*Wright 187b*, Cuba; NY) appears to be conspecific with *D. muhlenbergii*. Heiðmarsson raised *D. muhlenbergii* var. *tenue* to species level and assigned the name *D. tenue* to specimens from Arizona with an inflated epinecral layer type and a medulla that stains red with Melzer's reagent. Molecular data (Heiðmarsson 2003) supported the recognition of *D. tenue* but specimens representing *D. tenue* are strictly based on recent material of Heiðmarsson's concept of the species since the type of *D. tenue* is more than a century old. Specimens from Arizona identified and sequenced as *D. tenue* were examined and found to be different from the type specimen of *D. muhlenbergii* var. *tenue*. As with the Melzer's reagent test, the epinecral layer type does not appear to be a diagnostic character. Specimens from the Southwest identified as *D. tenue* and *D. americanum* represent a single species which is either new to science or referable to *D. americanum* but not to *D. tenue* (**Fig. 2**). The type of *D. americanum* was not examined.

Molecular data support the recognition of four new taxa: *D. arenosaxi*, *D. dolomiticum*, *D. luridum* var. *xerophilum* and *D. multifolium*. The distinctiveness of *D. dolomiticum* is well supported in the combined analysis (**Fig. 3**). One specimen of *D. dolomiticum* (*Amtoft 497a*, NY) has bootstrap support less than 50% in the ITS analysis for inclusion in the *D. dolomiticum* clade (**Fig. 2**). One sequence (*Heiðmarsson 1056*, UPS; SH33) belonging to *D. americanum* appears to be a close relative of *D. dolomiticum* but there is no support in the ITS analysis for its inclusion in the *D. dolomiticum* clade. No LSU sequence was obtained for SH33 so this relationship could not be resolved in the combined analysis. However, the ITS sequence for SH33 is quite different from *D. dolomiticum* (**Fig. 2**). Morphologically, specimen SH33 resembles *D. dolomiticum* in having small perithecia with a black ostiole, but differs in having a thicker thallus, a pale lower surface, an upper cortex with very little melanin and an evenly well-developed epinecral layer.

Dermatocarpon moulinsii and *D. reticulatum* formed a highly supported clade in the ITS analysis but the position of *D. tomentulosum* Amtoft was not


D. dolomiticum (Parker 2496, MO)
D. dolomiticum (Amtoft 1002b, MO)
D. dolomiticum (Buck 32272, NY)
D. dolomiticum (Amtoft 518b, MO)
D. dolomiticum (Amtoft 516a, MO)
D. dolomiticum (Amtoft 513, MO)
D. dolomiticum (Amtoft 1038b, MO)
D. dolomiticum (Harris 25421, MO)
D. dolomiticum (Amtoft 572, MO)
D. dolomiticum (Amtoft 3296, MO)
D. dolomiticum (Amtoft 3303, MO)
D. dolomiticum (Amtoft 497a, MO)
D. dolomiticum
D. americanum (Heiðmarsson 1056, AZ)
D. americanum (Heiðmarsson 1210B, AZ)
D. tenue (Heiðmarsson 1137, AZ)
D. tomentulosum (Amtoft 474, MO)
D. americanum
D. tenue
D. tomentulosum
D. muhlenbergii (Amtoft 580, MO)
D. muhlenbergii (Harris 25668, MO)
D. muhlenbergii (Amtoft 3394, MO)
D. muhlenbergii (Amtoft 1122, MO)
D. muhlenbergii (Amtoft 1002a, MO)
D. muhlenbergii (Amtoft 3301, MO)
D. muhlenbergii (Amtoft 3262, MO)
D. muhlenbergii (Amtoft 400, MO)
D. muhlenbergii (Amtoft 915, MO)
D. muhlenbergii (Amtoft 3356a, MO)
D. muhlenbergii (Amtoft 1266, MO)
D. muhlenbergii (Amtoft 1267, MO)
D. muhlenbergii (Harris 48190, MO)
D. muhlenbergii (Buck 45080, CT)
D. muhlenbergii (Amtoft 3265, MO)
D. muhlenbergii (Amtoft 2013, TN)
D. muhlenbergii (Amtoft 3380, MO)
D. muhlenbergii (Mattsson 5322, MO)
D. muhlenbergii
D. moulinsii (Amtoft 1038a, MO)
D. reticulatum (Heiðmarsson 1393, AZ)
D. americanum (Nash 33616, Mexico)
D. moulinsii
D. reticulatum
D. americanum
D. arenosaxi (Harris 43471, SC)
D. arenosaxi (Buck 36675, GA)
D. arenosaxi (Amtoft 559, MO)
D. arenosaxi (Amtoft 1361, MO)
D. arenosaxi (Harris 44305, OK)
D. arenosaxi (Amtoft 1456, MO)
D. arenosaxi (Harris 44308, OK)
D. arenosaxi (Buck 35934, IL)
D. arenosaxi (Buck 35924, IL)
D. arenosaxi (Amtoft 1360, MO)
D. arenosaxi (Buck 22179, GA)
D. arenosaxi (Harris 21644, AR)
D. arenosaxi (Harris 38701 GA)
D. arenosaxi (Wilhelm 11220, MO)
D. arenosaxi (Amtoft 1353, MO)
D. arenosaxi (Amtoft 1361, MO)
D. arenosaxi (Amtoft 1456, MO)
D. arenosaxi (Buck 42995, MO)
D. arenosaxi (Amtoft 560, MO)
D. arenosaxi (Harris 31246, GA)
D. arenosaxi
D. multifolium (Buck 32819, MO)
D. multifolium (Amtoft 451, MO)
D. multifolium (Amtoft 459b, MO)
D. multifolium (Buck 42784, MO)
D. multifolium (Amtoft 3299b, MO)
D. multifolium (Amtoft 1214, MO)
D. multifolium (Buck 36390, AL)
D. multifolium (Amtoft 440a, MO)
D. multifolium (Amtoft 3302, MO)
D. multifolium (Amtoft 3299a, MO)
D. multifolium (Guccion s.n., VA)
D. multifolium (Amtoft 3261, MO)
D. multifolium (Harris 33069-2, NC)
D. multifolium (Amtoft 3298, MO)
D. multifolium (Amtoft 3269, MO)
D. multifolium (Amtoft 397b, MO)
D. multifolium (Harris 33069-1, NC)
D. multifolium
D. luridum (Heiðmarsson 1382, MN)
D. luridum (Amtoft 2048, NC)
D. luridum (Heiðmarsson 100, Sweden)
D. luridum var. xerophilum (Wilhelm & Ladd 22697, MO)
D. luridum var. xerophilum (Buck 38431, OK)
D. luridum var. xerophilum (Amtoft 589, AR)
D. luridum var. xerophilum (Amtoft 611, AR)
D. luridum var. xerophilum (Harris 45427, AR)
D. luridum var. xerophilum (Amtoft 597, AR)
D. luridum var. xerophilum (Amtoft 603a, AR)
D. luridum var. xerophilum (Buck 37317, AR)
D. luridum (Amtoft 1506, MO)
D. luridum (Amtoft 1500, MO)
D. luridum (Buck 36333, AL)
D. luridum (Amtoft 2005, TN)
D. luridum
D. tuzibei (Moon 6132, Japan)
D. min. var. comp. (Heiðmarsson 390, Norway)
D. min. var. min. (Tibell 21893, India)
D. min. var. min. (Thor 16486, Japan)
D. min. var. min. (Tibell 21835-36, India)
D. min. var. min. (Heiðmarsson 1338, MN)
D. min. var. comp. (Heiðmarsson 1362, MN)
D. min. var. comp. (Heiðmarsson 1350, MN)
D. min. var. comp. (Heiðmarsson 1340, MN)
D. min. var. min. (Heiðmarsson 980, Sweden)
D. min. var. min. (Heiðmarsson 587, Finland)
D. min. var. comp. (Heiðmarsson 302, Sweden)
D. min. var. comp. (Heiðmarsson 348, Norway)
D. min. var. comp. (Heiðmarsson 1384, Iceland)
D. min. var. comp. (Heiðmarsson 695B, Iceland)
D. min. var. min. (Heiðmarsson 466, Faeroe Islands)
D. linkolae. (Westberg 16, Sweden)
D. min. var. min. (Buck 47331, Wales)
D. leptophyllum (Heiðmarsson 200, Sweden)
D. leptophyllum (Nordin 4391, Sweden)
D. min. var. min. (Gueidan 387, France)
D. min. var. min. (Heiðmarsson 810, Austria)
D. min. var. comp. (Heiðmarsson 932, Iceland)
D. min. var. comp. (Heiðmarsson 1385, Iceland)
D. min. var. min. (Kristinsson 9631, Iceland)
D. min. var. comp. (Heiðmarsson 732, Austria)
D. miniatum
D. polyphyllizum (Heiðmarsson 976, Iceland)
D. taminium (Heiðmarsson1257A, AZ)
D. taminium (Heiðmarsson1256, AZ)
D. taminium (Heiðmarsson1297B, AZ)
D. taminium (Heiðmarsson1292, AZ)
D. taminium (Heiðmarsson 1138, AZ)
D. taminium (Heiðmarsson 1207, AZ)
D. taminium (Nash 35378, AZ)
D. taminium
D. meiophyllizum (Heiðmarsson 455, Faeroe Islands)
D. meiophyllizum (Heiðmarsson 583, Finland)
D. meiophyllizum (Heiðmarsson 1351, MN)
D. cfr. bachmannii (Heiðmarsson 1037, OR)
D. rivulorum (Heiðmarsson 524, Sweden)
D. bachmannii (Heiðmarsson 978, Sweden)
D. deminuens (Heiðmarsson 98, Sweden)
Clavascidium sp. (Harris 46789, MO)
Placidium lacinulatum (Gueidan et C. Roux CG13, France)
5 changes

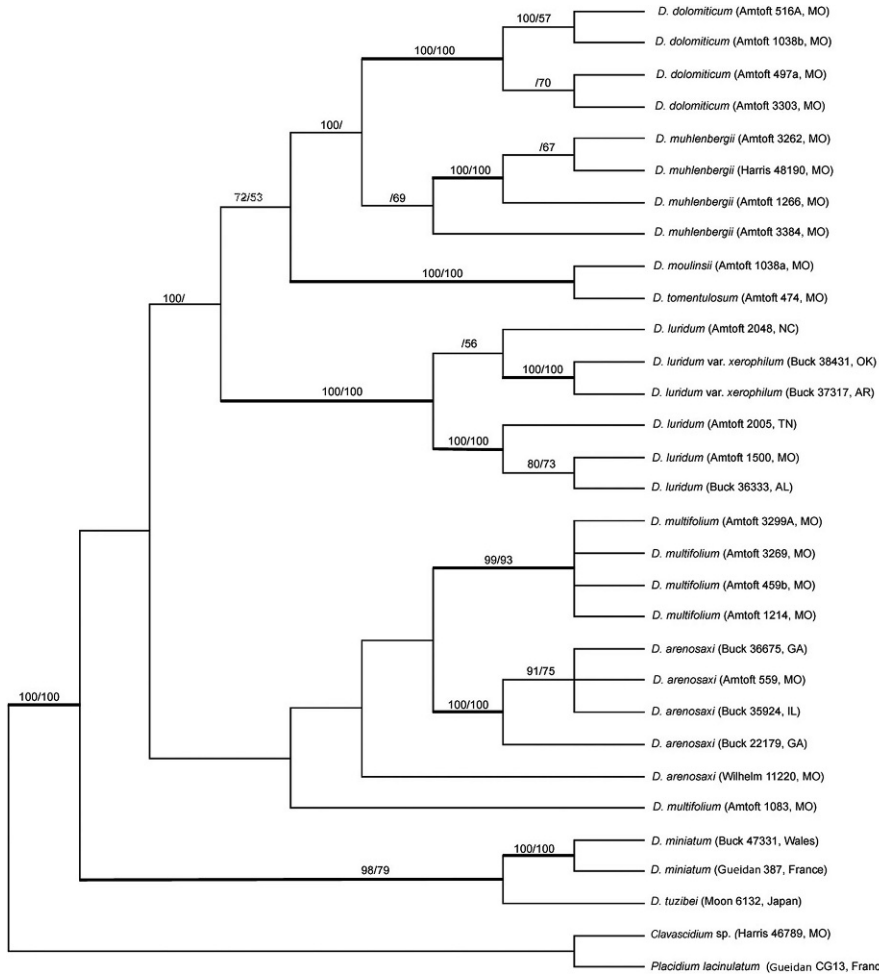


Figure 3. Strict consensus of 15,000 equally parsimonious trees resulting from a combined analysis of LSU and ITS data sets for 29 specimens of *Dermatocarpon*. Tree length = 804 steps, CI = 0.765, RI =0.839. Numbers above branches indicate posterior probability/bootstrap values. Significantly supported (PP ≥ 95% and bootstrap ≥ 70%) internodes are indicated with thicker lines.

resolved. These species are distinct from one another in their unique lower surface topographies or cortical outgrowths, but as in *D. dolomiticum* they all have small perithecia with immersed ostioles.

Four taxa with multiple lobes and holdfasts are present in the Ozarks: *D. luridum*, *D. luridum* var. *xerophilum* var. nov., *D. arenosaxi* sp. nov. and *D. multifolium* sp. nov. *Dermatocarpon taminium* is not present in the Ozarks and appears to be restricted to the Southwest. Specimens that were tentatively identified as *D. taminium* prior to sequencing are now assigned to the new variety, *Dermatocarpon luridum* var. *xerophilum*. This variety is distinguished from typical *D. luridum* largely by habitat preference and to some degree by morphology. *Dermatocarpon luridum* var. *xerophilum* was nested within var. *luridum*, suggesting that two distinct lineages of *D. luridum* var. *luridum* exist, but these relationships are

←

Figure 2. Strict consensus phylogram of 1872 equally most parsimonious trees derived from unequally weighted maximum parsimony analysis of ITS for 130 specimens of *Dermatocarpon*. Tree length = 717.24, CI = 0.637, RI = 0.901. Maximum parsimony bootstrap values are shown above branches. Significantly supported (i.e., MP-bs ≥ 70%) internodes are indicated with thicker lines.

not statistically supported. There are no morphological, ecological or geographical differences that reflect the genetic differences found in the two *D. luridum* var. *luridum* lineages. One group is composed of specimens from Tennessee, Missouri and Alabama and the other from North Carolina, Sweden and Minnesota. More specimens and another locus should be sequenced in order to resolve the relationships within *D. luridum*.

Specimens which were tentatively identified as *D. miniatum* var. *complicatum* are here referred to the new species, *D. multifolium*. As discussed above, *D. miniatum* is not present in the Ozarks. The *D. multifolium* clade is well supported by both the ITS and combined analyses (**Figs. 2, 3**).

Specimens from the Ozarks which closely resemble *Dermatocarpon intestiniforme* (Körb.) Hasse or *D. tuzibei* M. Satô morphologically are referred to the new species *D. arenosaxi*. Neither *D. intestiniforme* nor *D. tuzibei* are present in the Ozarks. *Dermatocarpon tuzibei* is nested in *D. miniatum*, close to specimens from East and South Asia and Norway (**Fig. 2**). The ITS dataset alone gave low support for *D. arenosaxi* as circumscribed here. This species has the highest level of genetic variation of all species included in this study (**Fig. 2**). At least two well-supported lineages are present (**Fig. 2**). There is one motif in the highly conserved 5.8S region that is unique to both lineages and distinguishes them from other species of *Dermatocarpon*, but there is also another motif in the same region, which distinguishes the two lineages from each other. They are similar morphologically, ecologically and geographically (e.g., *Amtoft 559* and *560*, NY, were growing side by side in a chert glade). It is possible that these two lineages emerged in the Ozarks during the interglacials but did not have sufficient time between alternating periods of geographical isolation to become reproductively isolated or phenotypically distinct. Molecular data support the recognition of two (potentially three) distinct lineages; because no morphological or ecological criteria exist to separate them, they are both treated here as *D. arenosaxi*.

Morphological characterization of Ozark species is largely based on thallus growth form, spore size, perithecial characters, ecological preference and to a lesser degree upper surface color and lower surface topography. Details of these characters are discussed below.

Melzer's reagent and iodine potassium iodide. The use of iodine potassium iodide (IKI) or Melzer's reagent as a taxonomic tool was discussed in depth by Orange (1998) and Common (1991). Orange discovered that out of seven *Dermatocarpon* species (*D. miniatum*, *D. meiophyllizum*, *D. leptophyllodes*, *D. deminuens* Vain., *D. intestiniforme*, *D. luridum* and *D. polyphyllizum*) the medullae of only two, *D. luridum* and *D. polyphyllizum*, stained with IKI or Melzer's reagent. Heiðmarsson (2003) later reported that the medullae of *D. americanum* and *D. tenue* also stained red with Melzer's reagent. Preliminary study testing the medullae of specimens with Melzer's reagent proved problematic because the variation in staining (red, brownish-red, yellow-brown turning red, yellow-brown, yellowish) left one guessing if the result should be interpreted as a positive reaction or a negative reaction. The Melzer's problem was exacerbated with the discovery that depending on where the test is applied a single thallus can sometimes react both positively (red) or negatively (yellow) to the reagent. There appears to be a correlation with high light exposure and a negative reaction to Melzer's reagent. For example, there were sometimes two different reactions to Melzer's reagent in a single thallus (especially in *D. muhlenbergii* and *D. arenosaxi*) with a folded-under margin which is consequently exposed to lower light levels: the pale, folded-under margin often stained red with Melzer's, and the exposed parts of the thallus did not stain. Specimens growing in very shaded habitats with reduced melanin in the upper surface also usually stained red with Melzer's reagent. This suggests that the presence of the carbohydrate which stains red with Melzer's reagent and melanin production are perhaps interconnected.

False positive reactions are problematic and tend to occur in thalli with a loose medulla or medullary hyphae with large lumina. If the medulla is loose the IKI or Melzer's reagent fills the interhyphal spaces and appears as a positive stain. Preparing a thin section and observing the staining of hyphae under the compound microscope is often not helpful. This is largely because the reagent itself can obscure the

results, and because light passing through the section dilutes the intensity of the stain.

In addition to non-sequenced specimens, 93 of the 130 specimens included in the ITS analysis were tested with Melzer's reagent. The remaining 27 specimens were not tested because the sequences were downloaded from GenBank and the specimens were not seen. The reaction of the medulla to Melzer's reagent for each of these 93 specimens was mapped on the ITS tree (**Fig. 4**). Instead of arbitrarily assigning a positive or negative designation to ambiguous reactions (e.g., brown, reddish-brown, yellowish-brown) the color of the stain to Melzer's reagent is mapped instead. All Ozark taxa are polymorphic for the Melzer's reagent medullary stain.

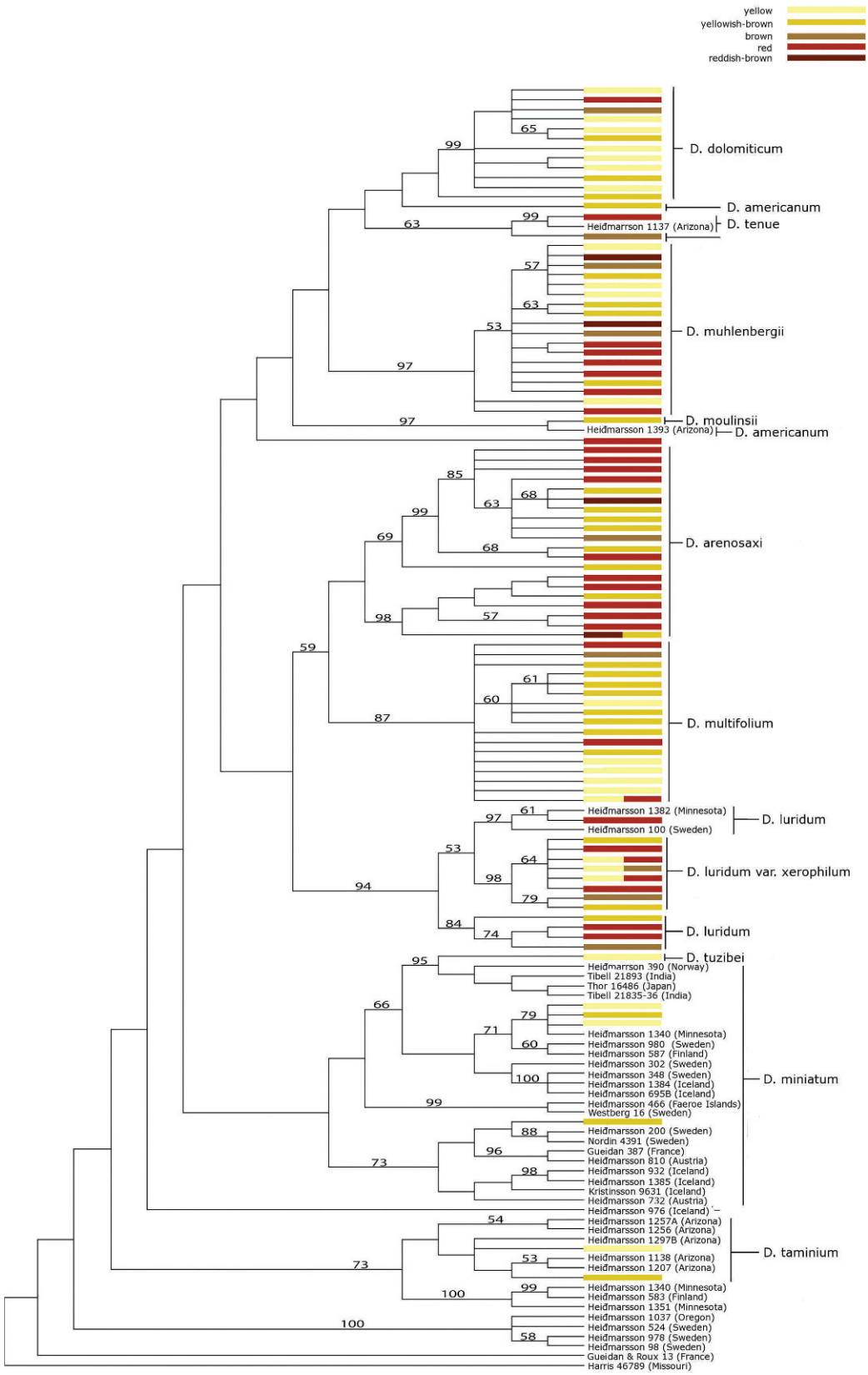
The response of *D. muhlenbergii* to Melzer's reagent was highly variable, with many specimens reacting either truly negative (yellow) or truly positive (red). This is significant because Heiðmarsson (2003) used the Melzer's reagent test to distinguish *D. muhlenbergii* from *D. americanum* and to raise *D. muhlenbergii* var. *tenue* to species status. According to Heiðmarsson, *D. americanum* stains red with Melzer's reagent while (the type of) *D. muhlenbergii* does not stain with Melzer's reagent. An isotype (PH) of *D. muhlenbergii* was tested and found that the medulla stained red with Melzer's reagent. In his study, Heiðmarsson (2003) named *Mattson 5322*, UPS (SH32) *D. americanum* and not *D. muhlenbergii* because the medulla stained red with Melzer's reagent and because the lower surface is black and not reddish. We included the SH32 sequence in the ITS analysis and examined the specimen. The SH32 specimen does have a medulla that stains red with Melzer's reagent but it also agrees with *D. muhlenbergii* morphologically. Additionally, the molecular data (**Fig. 2**) supported the inclusion of specimen SH32 in *D. muhlenbergii*. *Dermatocarpon muhlenbergii* therefore cannot be distinguished from *D. americanum* by the Melzer's reagent test. For this same reason the current status of *D. tenue* is thrown into question.

The Melzer's reagent test has more traditionally been used to positively identify *D. luridum* (Purvis et al. 1992). Among all the taxa studied, *D. luridum* appeared to most consistently stain with Melzer's reagent with three specimens staining yellow-brown, two staining both yellow and red on the same thallus, and five staining red for the twelve specimens sequenced.

Only six specimens of *D. miniatum* included in the molecular study were tested with Melzer's reagent. None of the six *D. miniatum* specimens stained red or reddish-brown. It is possible that the Melzer's reagent test could in fact be used for *D. miniatum* but more specimens should be tested. The Melzer's reagent test cannot be used to diagnose *D. dolomiticum*, *D. americanum*, *D. muhlenbergii*, *D. arenosaxi* or *D. multifolium*, and possibly *D. luridum*.

Thallus. The thalli of Ozarkian *Dermatocarpon* are generally either composed of one lobe (e.g., *D. moulinsii*, *D. muhlenbergii* and *D. tomentulosum*) or of many lobes (e.g., *D. arenosaxi*, *D. luridum*, *D. xerophilum* and *D. multifolium*). Sometimes a thallus is composed of a single lobe but the lobe is divided (e.g., *D. dolomiticum*) such that the thallus appears to be composed of multiple lobes; the distinction between this and a thallus composed of many lobes is occasionally difficult to make, especially if thalli are growing crowded together. Species with multiple lobes typically have multiple holdfasts on the lower surface and species composed of a single lobe are typically centrally or eccentrically umbilicate. A thallus composed of a single lobe with many holdfasts is uncommon and is generally only seen in aberrant specimens of multiple-lobed, multiple-holdfasted species; this happens most often in *D. multifolium* but it also occurs in *D. luridum* and *D. arenosaxi*. The holdfasts in multiple-holdfasted species are often elongate, and are smaller and narrower than an umbilicus (**Fig. 12C, D**). Rarely a species that is typically umbilicate produces a few additional holdfasts or a species that typically has many holdfasts produces only one holdfast. In the latter condition the single holdfast is then usually more like a secondary holdfast than an umbilicus.

Two Ozark species are erratically vagrant, *D. arenosaxi* and *D. dolomiticum*. Vagrant species in the Ozarks are usually prolific and locally abundant where present. The vagrant condition is most common in exposed areas with poor drainage but the vagrant response to these conditions appears to be an inherited trait. Prior to 1992 only one species with


yellow
yellowish-brown
brown
red
reddish-brown
D. dolomiticum
D. americanum
D. tenue
D. muhlenbergii
D. moulinsii
D. americanum
D. arenosaxi
D. multifolium
D. luridum
D. luridum var. xerophilum
D. luridum
D. tuzibei
D. miniatum
D. taminium
Heiðmarrson 1137 (Arizona)
Heiðmarsson 1393 (Arizona)
Heiðmarsson 1382 (Minnesota)
Heiðmarsson 100 (Sweden)
Heiðmarsson 390 (Norway)
Tibell 21893 (India)
Thor 16486 (Japan)
Tibell 21835-36 (India)
Heiðmarsson 1340 (Minnesota)
Heiðmarsson 980 (Sweden)
Heiðmarsson 587 (Finland)
Heiðmarsson 302 (Sweden)
Heiðmarsson 348 (Sweden)
Heiðmarsson 1384 (Iceland)
Heiðmarsson 695B (Iceland)
Heiðmarsson 466 (Faeroe Islands)
Westberg 16 (Sweden)
Heiðmarsson 200 (Sweden)
Nordin 4391 (Sweden)
Gueidan 387 (France)
Heiðmarsson 810 (Austria)
Heiðmarsson 932 (Iceland)
Heiðmarsson 1385 (Iceland)
Kristinsson 9631 (Iceland)
Heiðmarsson 732 (Austria)
Heiðmarsson 976 (Iceland)
Heiðmarsson 1257A (Arizona)
Heiðmarsson 1256 (Arizona)
Heiðmarsson 1297B (Arizona)
Heiðmarsson 1138 (Arizona)
Heiðmarsson 1207 (Arizona)
Heiðmarsson 1340 (Minnesota)
Heiðmarsson 583 (Finland)
Heiðmarsson 1351 (Minnesota)
Heiðmarsson 1037 (Oregon)
Heiðmarsson 524 (Sweden)
Heiðmarsson 978 (Sweden)
Heiðmarsson 98 (Sweden)
Gueidan & Roux 13 (France)
Harris 46789 (Missouri)

a vagrant habit, *D. vagans* Imsh., was known in *Dermatocarpon*. *Dermatocarpon vagans* was synonymized (Rosentreter & McCune 1992) with *D. reticulatum* because the type of *D. vagans* has the papillose lower surface diagnostic of *D. reticulatum*. At the same time Rosentreter and McCune reported a second vagrant species which they loosely called "*D. miniatum*." The specimens cited in Rosentreter and McCune's study were not examined but it seems likely that what they call "vagrant *D. miniatum*" is in fact not *D. miniatum*. We concur with Rosentreter and McCune that it is not necessary to recognize these vagrant forms of *Dermatocarpon* at any taxonomic level because species which possess the ability to become vagrant are not always vagrant. It does however seem significant that a species possesses the ability to become vagrant. This seemingly intrinsic ability is not present in all *Dermatocarpon* species but instead appears to have evolved independently in distantly related species which typically grow in habitats with poor drainage, such as glades. Vagrant forms of *D. muhlenbergii* have not been observed despite the fact that it occasionally grows in areas of poor drainage. Similarly, the semi-aquatic species *D. luridum* is not found vagrant.

Each species tends to exhibit a characteristic range of thallus thickness. The thalli of *Dermatocarpon dolomiticum* and *D. arenosaxi* are consistently noticeably thin. *Dermatocarpon multifolium* is intermediate in thickness; while *D. muhlenbergii*, *D. luridum* and especially *D. luridum* var. *xerophilum* are often thick and less fragile than the species with thinner thalli. The degree of development of the medulla often determines the thallus thickness but other variables participate. These variables include the thickness of the algal layer, thickness of the lower and upper cortices, and the presence of an extra layer of thick-walled fungal cells between the algal layer and the medulla. The latter is occasionally present in all species of Ozarkian *Dermatocarpon* (**Figs. 6F, 13D**). In some respects the cells of this layer appear to be former compartments for algal cells. This layer is most common in *D. muhlenbergii* and both varieties of *D. luridum*. It is largely absent in thin specimens (**Fig. 14C, D**). In thick specimens of *D. dolomiticum* this extra layer is often present as narrowly rectangular cells in a columnar arrangement (bottom of **Fig. 5D**). A similar columnar layer of rectangular cells (although interspersed with algae) can sometimes predominate to the exclusion of the medulla in *D. luridum* var. *xerophilum* (e.g., in *Buck 38431*, NY).

Thick specimens are often rigid. The medulla and the lower cortex seem to contribute the most to rigidity. The medulla imparts rigidity to the thallus in two possible ways: by being composed of compact or conglutinate hyphae and/or by thick-walled hyphae. Rigidity can additionally be attributed to a lower cortex composed of cells in a regular columnar arrangement.

Upper surface color. The upper surface color is dependent on the amount of melanin in the cortical cell walls, the presence or absence of a brown extracellular granular deposit, the development of an epinecral layer and the evenness of the upper cortex. Cyanobacteria growing on the upper surface can sometimes obscure the true color of the lichen.

Melanin production in the cells of the upper cortex appears to be proportionally influenced by light intensity. Specimens with a blue-green, melanin-free upper surface were usually found growing in underhangs or low light conditions. Folded-under lobe margins (e.g., *Amtoft 946*, *Amtoft 3307*, *J. K. Small s.n.*, *Amtoft 568a*, NY) have less melanin than the exposed central parts of the thallus. Melanin decreases light transmittance to the algal layer (Dietz et al. 2000) and probably protects the algal layer from photoöxidative stress. Even though there is a correlation between degree of pigmentation and light intensity, most Ozark species exhibit a characteristic range of melanization. This suggests that sensitivity to light and the subsequent amount of melanin produced could be in part genetically

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Figure 4. Results of the Melzer's reagent test mapped on strict consensus tree of 1872 equally parsimonious trees derived from maximum parsimony analysis of ITS for 130 specimens. Tree length = 717.24, CI = 0.637, RI = 0.901. Maximum parsimony bootstrap values are shown above branches.

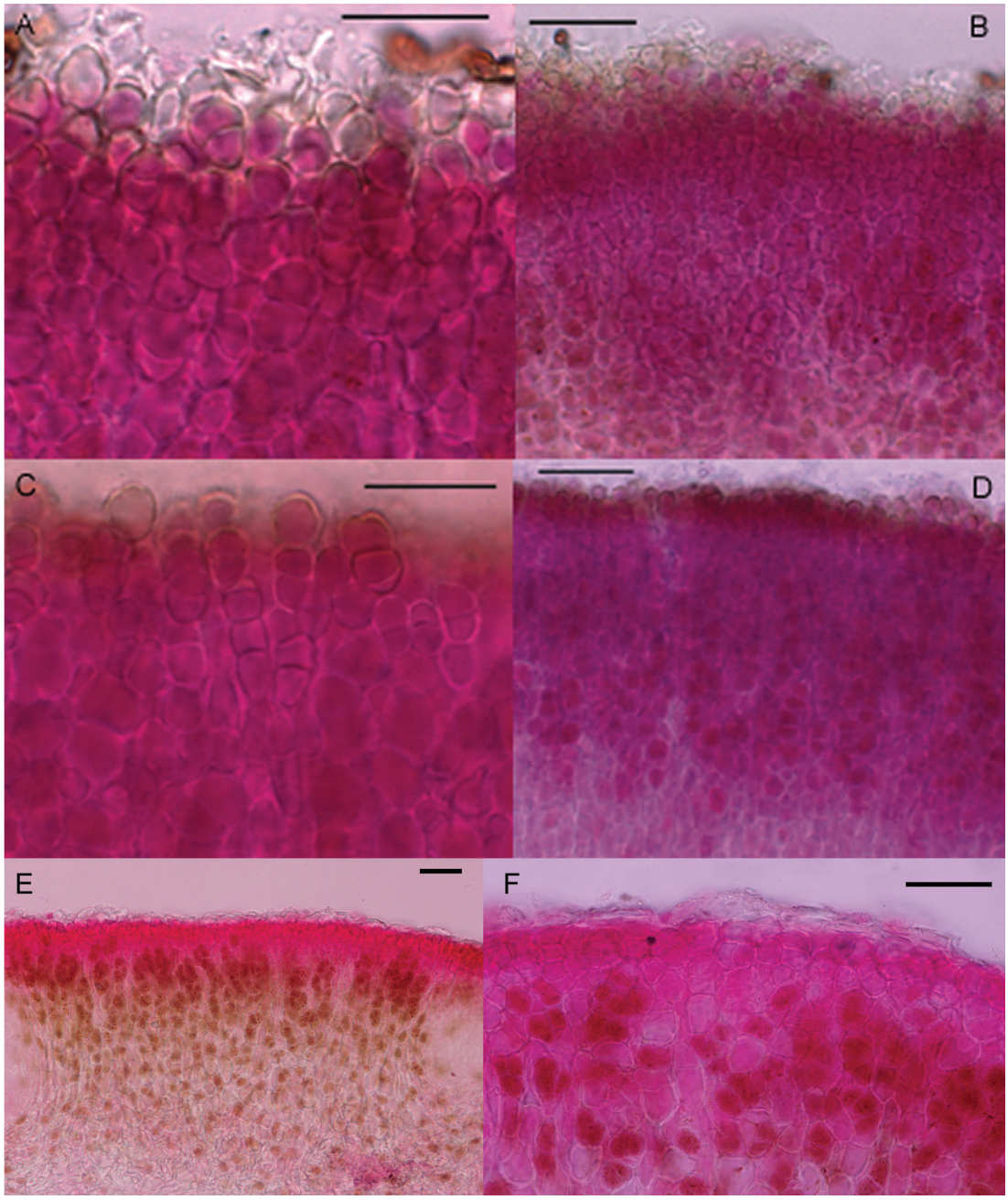


Figure 5. Thallus cross section of *Dermatocarpon* (stained with lactic fuschin) showing presence or absence of an epinecral layer, (E, F) showing compressed epinecral layer. **A, B.** *D. dolomiticum* (*Amtoft 1011*, NY). **C.** *D. dolomiticum* (*Ladd 8050*, MOR). **D.** *D. dolomiticum*, lower left corner shows columnar layer of rectangular fungal cells separating the algal layer from the medulla (*Ladd 8050*, MOR). **E, F.** *D. luridum* with compressed epinecral layer (*Amtoft 1499*, NY). Scale bars: A, B, E = 20 µm; C, D, F = 10 µm.

determined. *Dermatocarpon muhlenbergii*, for example, is infrequently very dark brown even in exposed areas but is often bluish-green (melanin-free) in shaded areas. *Dermatocarpon dolomiticum* is frequently very dark brown and only rarely has an entirely blue-green upper surface (*Amtoft 509*, *568*, NY), even in shaded habitats.

The pruina found in some species of *Dermatocarpon* is not pruina *sensu* Hale and Cole (1988), i.e., composed of calcium oxalate crystals. A

''pruinose'' upper surface in *Dermatocarpon* indicates the presence of an epinecral layer (Heiđmarsson 1996). The epinecral layer, somewhat of a misnomer, refers (*sensu* Büdel 1990) to the layer of dead, often ruptured, fungal cells on the upper surface which is identifiable with lacto-phenol blue or lactic fuchsin, both of which selectively stain the cytoplasm of cells. Presumably dead fungal cells lack a cytoplasm and so the epinecral cells remain unstained. According to Heiđmarsson (1996), there are two types of epinecral layers in *Dermatocarpon*, one of air-filled hyphae (Type 1) and another of compressed hyphae (Type 2). An epinecral layer of air-filled cells renders the upper surface grayish and a compressed epinecral layer renders the upper surface brown. Heiđmarsson used epinecral layer type to delimit species of *Dermatocarpon*. As previously mentioned, part of the reason for raising *D. muhlenbergii* var. *tenue* to species level was that the epinecral layer of the type specimen is composed of inflated cells while the type specimen of *D. muhlenbergii* has an epinecral layer of compressed cells. We examined type specimens of both species and found the upper cortices to be similar (**Fig. 6A–D**) with very few to no epinecral cells. The difference in upper surface color between the two specimens, pale tan in *D. muhlenbergii* var. *tenue* and brown in *D. muhlenbergii*, is attributable to the degree of melanization and not to the epinecral layer type. Specimens identified as *D. tenue sensu* Heiđmarsson (e.g., *Heiđmarsson 1210b*, ASU) have an evenly gray upper surface with a better developed epinecral layer (**Fig. 6G, H**).

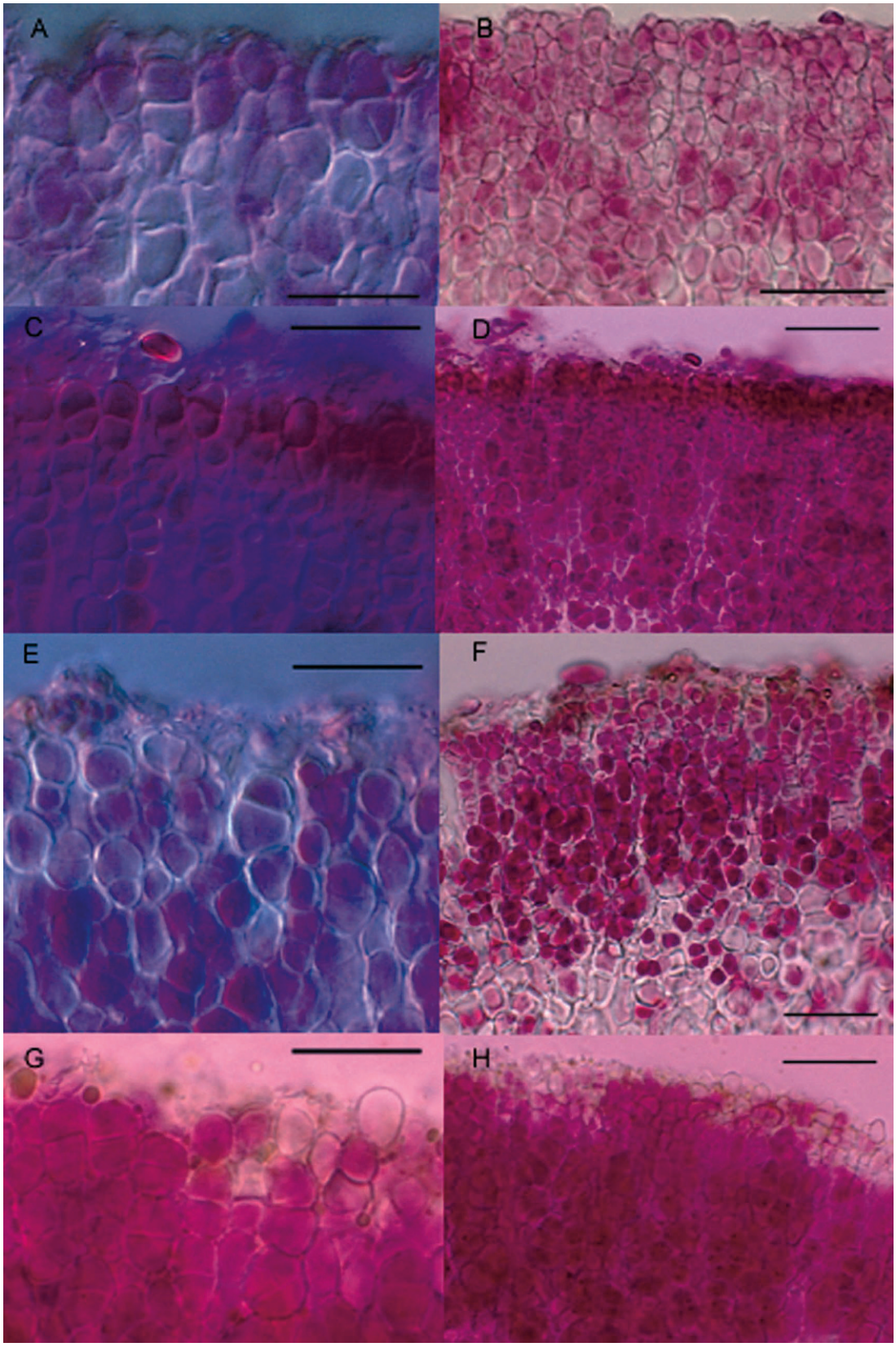
The epinecral layer gives the upper surface a farinose quality, and can be scraped off easily with a razorblade. However, the upper surface also appears grayish and with a farinose quality if the cells in the topmost layer of the upper cortex are irregularly arranged (i.e., scatter cells projecting beyond all others) as in **Fig. 5C**.

In Ozark species the formation of an epinecral layer appears to be a response to insolation because thalli growing in exposed areas often have an epinecral layer and thalli growing in shaded areas do not. For this reason, in addition to the discussion of the *D. tenue* problem above, the presence or absence of an epinecral layer does not appear to be strictly diagnostic. With this said, there are a few notable observations: *D. luridum* rarely has a farinose upper surface and often has a compressed epinecral layer (**Fig. 5E, F**); *D. muhlenbergii* only has a farinose upper surface if growing in exposed areas; *D. dolomiticum* almost always has an upper surface that is partly farinose with a pruinose appearance (**Figs. 5A, B,11A–D**); and *D. arenosaxi* often has a somewhat shiny upper surface which can be partly farinose.

The presence or absence of a farinose upper surface or an inflated epinecral layer may have more significance for species occurring outside the Ozarks. For example, in the few specimens of *D. miniatum* var. *complicatum* examined, all invariably had an evenly farinose upper surface either composed of an irregular upper cortex (e.g., *Buck 20860*, Minnesota; NY) or of an epinecral layer (e.g., *Merrill 265*, Maine; NY). In addition all specimens (ASU) identified as *D. americanum sensu* Heiđmarsson from Arizona have an evenly farinose upper surface.

Color of lower surface. Lower surface color is determined by the amount of melanin deposited in the cells of the lower cortex or by the presence of a brown extracellular deposit. The thickness or depth of the melanized layer does not affect the darkness of the lower surface and is variable within a species. Melanin tends to be mostly restricted to the basal cells of the lower cortex. Melanin in or along the cell wall or an unidentified brown extracellular deposit often forms a ''coating'' over the lower surface. Melanin in the lower cortex might function in increasing water capacity but this has not been studied. Light intensity does not affect the degree of pigmentation in the lower surface and thus melanin in the lower cortex does not appear to be photoprotective (from refracted light?) in function. For example, some specimens of *D. muhlenbergii* growing in very low light conditions (e.g., *Amtoft 353*, *580*, *581*, *972*, NY) such as in underhangs of bluffs can have a dark brown to black lower surface while others (e.g., *Amtoft 886*, *Harris 48190*, NY) have a pale tan to light brown lower surface. In some cases two thalli of the same species, growing side by side, and seemingly under the same ecological conditions, will have differently colored lower surfaces, one pale and the other dark brown.

Each species of *Dermatocarpon* in the Ozarks has a lower surface that is more often one color or shade


A
B
C
D
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F
G
H

than another, but there are often many exceptions. The lower surface of *D. dolomiticum* is frequently brown-black to black, but specimens growing along the periphery of glades sometimes have a brown to light brown or reddish-brown lower surface. The lower surface of *D. luridum* is most often pale tan with a pinkish tinge. In *D. arenosaxi* the lower surface is often golden brown, a color very infrequently observed in other Ozark taxa, but it can also be brown or dark brown.

The color of the lower surface is of limited use taxonomically. This contradicts Heiðmarsson (2003) who suggested that specimen SH32 (*Mattsson 5322*, UPS) from Missouri might be a new species because of its black lower surface. As previously mentioned, the placement of specimen SH32 in the *D. muhlenbergii* clade is well supported. The lower surface color of *D. muhlenbergii* ranges from pale tan to black.

Lower surface topography. The lower surface can be variable degrees of veined, wrinkled, smooth or verrucose, and may or may not possess rhizinomorphs. The lower surface topography in Ozark species without rhizinomorphs varies within taxa, but as with other characters in *Dermatocarpon* a single character-state usually predominates. The topography of the lower surface is sometimes affected by the presence of perithecia which can produce bulges (**Fig. 15D, H**). A lower surface with these perithecial bulges can be interpreted as verrucose, but we prefer to use this term for non-perithecial verrucae. So defined, a verrucose lower surface exhibits irregular proliferations of cortical cells along the surface, or invaginations of the lower cortex. A verrucose surface in this sense is only found in two species in the Ozarks: *D. dolomiticum* and *D. muhlenbergii*. The latter species is only rarely verrucose but its perithecia frequently produce bulges on the lower surface (**Fig. 15D**). All species can have a smooth lower surface, but a completely smooth lower surface is most common in *D. muhlenbergii* and *D. multifolium*, and is infrequent in *D. dolomiticum*. A slightly wrinkled to foveolate lower surface is most common in *D. arenosaxi*. *Dermatocarpon luridum*, *D. arenosaxi*, *D. muhlenbergii*, *D. dolomiticum* and rarely *D. multifolium* can all have a veined lower surface. Venation is not strictly associated with flowing water; for example, specimens of *D. muhlenbergii* growing in a dry habitat can at times be strongly veined (e.g., *Harris 48190*, NY).

The topography of the lower surface should be used with caution as a taxonomic character because invariably some specimens will be found which do not exhibit the predominate character state. Even rhizinomorphic taxa show some variation. For example a few of the papillae in *D. reticulatum* (*Foster 2303a*; NY) can elongate and develop into rhizinomorphs.

Perithecia and pycnidia. The perithecia and pycnidia in *Dermatocarpon* are immersed in the thallus. In *D. muhlenbergii* both the pycnidia and perithecia are occasionally in areoles (i.e., *Amtoft 1132*, NY). Parasitized or senescent perithecia and pycnidia are frequently deeply sunken in the thallus.

Pycnidia are of little utility for delimiting species. They vary in size within a species and the conidia are for the most part uniform: bacilliform, about 3–5 × 1 μm. In general, pycnidia are less abundant than perithecia. *Dermatocarpon muhlenbergii*, *D. multifolium* and to a lesser degree *D. dolomiticum* may produce abundant pycnidia, sometimes to the exclusion of perithecia, but this condition is not very common.

Perithecium size and shape are rather uniform within species. Smaller or larger than normal perithecia can be found in a thallus but for the most part perithecia are within a typical size range for a species. The perithecia of *D. dolomiticum* are normally quite small (162–320 × 130–360 μm) in comparison to *D. muhlenbergii* whose perithecia range from 335–810 × 245–690 μm. The perithecia of *D. arenosaxi*, *D. tomentulosum* and *D. moulinsii* are

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Figure 6. Cross section of upper thallus of *Dermatocarpon* (stained with lactic fuschin) showing presence or absence of an epinecral layer. **A, B.** *D. muhlenbergii* var. *tenue* (*Wright 187b*, NY). **C, D.** *D. muhlenbergii* (*Muhlenberg 142*, syntype, PH). **E, F.** *D. muhlenbergii* (*Amtoft 1598*, NY). **G, H.** *D. tenue sensu* Heiðmarsson (*Heiðmarsson 1210b*, ASU). Scale bars: A, C, E, G = 10 μm; B, D, F, H = 20 μm. A and E taken with DIC (Differential Interference Contrast).

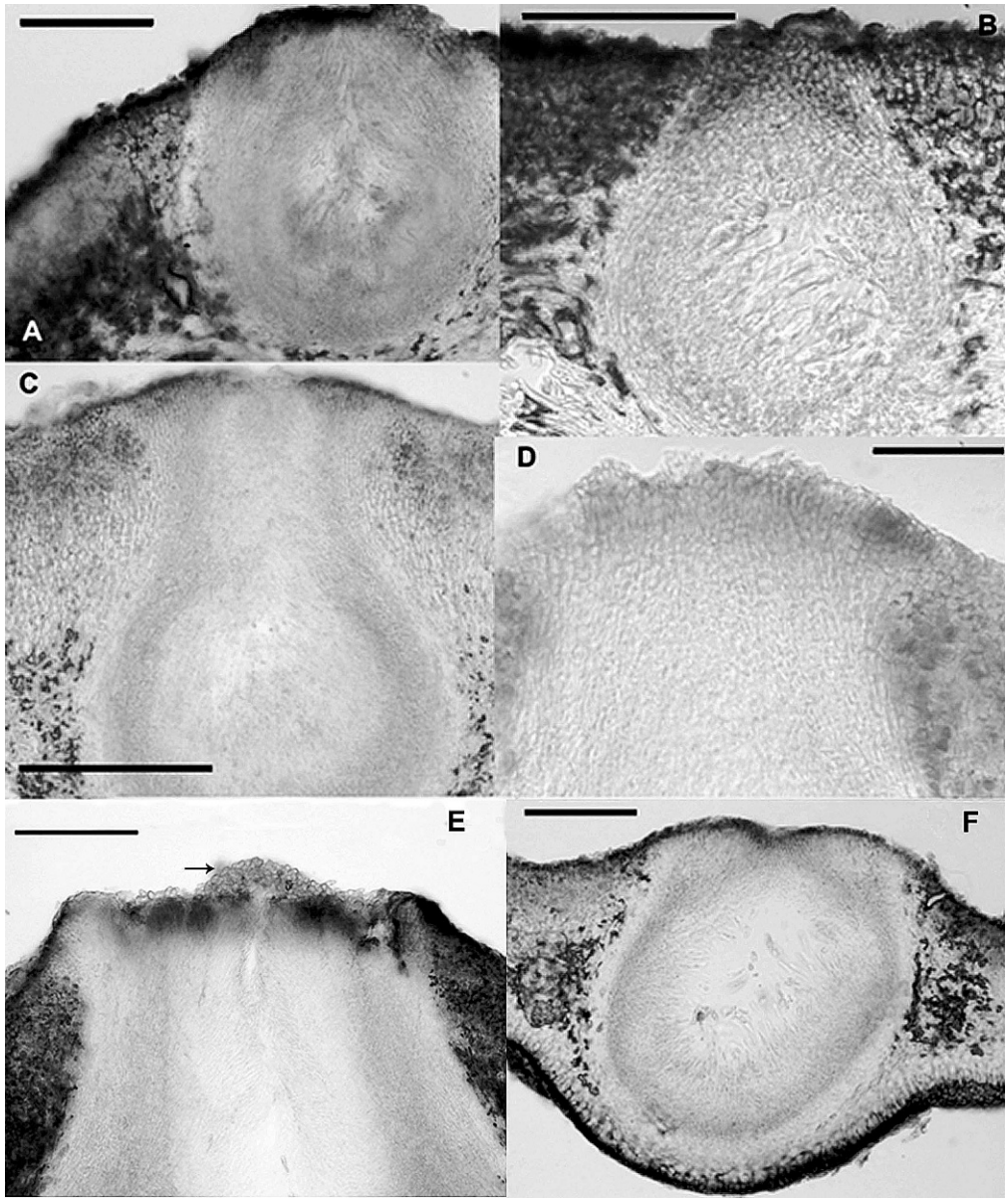

Figure 7. Cross sections of perithecia of *Dermatocarpon*. **A.** Convex ostiole of *D. dolomiticum* (*Harris 25421*, NY). **B.** Flat ostiole of *D. dolomiticum* (*Amtoft 572*, NY). **C.** *D. luridum* var. *xerophilum* with flared, convex ostiole (*Amtoft 603a*, NY). **D.** *D. muhlenbergii* with flared, convex ostiole (*Mattsson 5322*, NY). **E.** *D. muhlenbergii* with extruded ascospores (arrow) (*Amtoft 565*, NY). **F.** Convex ostiole of *D. multifolium* (*Amtoft 1133*, NY). Scale bars: A–F = 100 μm.

similar in size to those of *D. dolomiticum* while those of *D. multifolium* are somewhat larger. The perithecia in the *D. luridum* varieties are relatively large, with ranges in size slightly below that of *D. muhlenbergii*. Perithecial shape (i.e., globose, pyriform, lageniform) is somewhat correlated with size. For instance the perithecia of *D. muhlenbergii* and *D. luridum* var. *xerophilum* are normally pyriform to lageniform (**Figs. 7C, 8F, 15H**) while in the taxa with smaller perithecia the shape is typically pyriform to globose (**Figs. 7A, B, F, 8E, 16F**). The ostiole is usually level with the upper surface of the thallus (**Fig. 8E**) but it can also be slightly or strongly exserted (**Fig. 7C, E**). An exserted ostiole is most common in *D. muhlenbergii*, *D. luridum* var. *xerophilum*, and to a lesser degree in *D. multifolium*. These three taxa

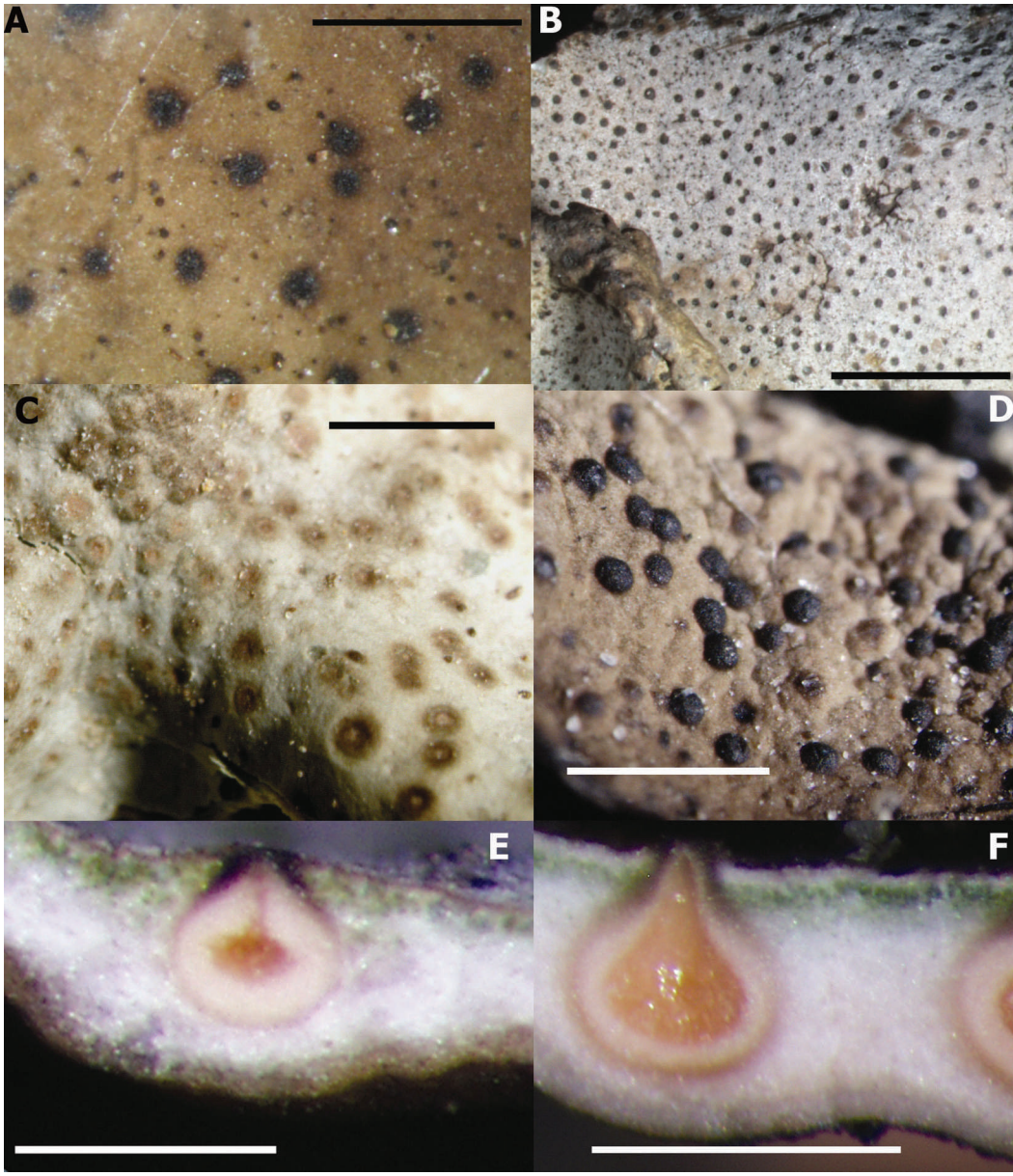

Figure 8. *Dermatocarpon* thalli from above showing perithecia with ostiole flat and level with upper surface (A–C, E), and ostiole convex and extending above upper surface (D, F). **A.** *D. arenosaxi* (*Harris 43473*, NY). **B.** *D. dolomiticum* (*Harris 41413*, NY). **C.** *D. luridum* (*Buck 47683*, NY). **D.** *D. multifolium* (*Amtoft 471*, NY). **E.** *D. dolomiticum* (*Amtoft 1007*, NY). **F.** *D. muhlenbergii* (*Amtoft 497c*, NY). Scale bars: A, C, D = 1 mm; B = 2 mm; E, F = 125 µm.

share an additional perithecial feature which is the tendency for the ostiole to be noticeably convex (**Figs. 7C, D, 8D, 13F, 15E, F**). In *D. luridum* var. *luridum*, *D. arenosaxi*, *D. dolomiticum*, *D. tomentulosum* and *D. moulinsii* the ostiole is most often flat and level with or sunken in the upper surface (**Figs. 7B, 8A–C, E**). In the taxa which typically have a convex ostiole, often taking the form of a cap with the "cap" formed by excipular cells flaring to the left and right of the ostiole and overlying or replacing cells of the upper cortex (**Fig. 7C, D**). This "cap" seems to be absent in convex ostioles of taxa in which the ostiole is typically flat. For example when the ostiole is convex in *D. dolomiticum* (as in some thalli of the type collection) it appears to be the result of an erumpent

perithecium with the upper cortex seemingly retracted (**Fig. 7A**). The pigmentation of the ostiole appears to be both genetically determined and slightly influenced by insolation. *Dermatocarpon muhlenbergii* is the most variable with an ostiole that is brown-black to pale and sometimes reddish. The ostiole in *D. luridum* is often pale brown but it can also be dark brown. *Dermatocarpon dolomiticum* invariably has a black-brown to dark brown ostiole and the same seems to be true for *D. moulinsii* as well. Occasionally the dark brown pigment of the ostiole extends downwards into the exciple (**Figs. 7B, 8E, F**). This regularly occurs in *D. dolomiticum* but is also seen in *D. reticulatum*, *D. moulinsii* and occasionally *D. muhlenbergii* (**Fig. 8F**).

Sometimes the pigment surrounding the incipient ostiole develops very early, even before the ostiole, exciple or hymenium differentiate, and in *D. luridum* var. *xerophilum* this condition produces small dark brown spots on the upper surface (**Fig. 13F**). In *D. muhlenbergii* two perithecia sometimes grow at an angle towards the same single, brown "spot" and it seems that they might fuse to form a double perithecium (**Fig. 15G**) but intermediates have not been observed. This type of double perithecium, which is fairly common in *D. muhlenbergii*, appears to also be caused by invagination of the lower exciple even though the incomplete central wall dividing the hymenium appears to be formed by two excipula. The exciple in all taxa is almost always colorless although it becomes brown in parasitized or senescent perithecia. Very rarely, healthy non-senescent perithecia of *D. luridum* and *D. arenosaxi* have a truly carbonized exciple. The exciple is most often composed of several compact layers of compressed elongate, rectangular to subfusiform cells. However, an exciple composed of loosely arranged and almost subspherical or irregularly rectangular cells is not uncommon in *D. dolomiticum* and occasionally occurs in other taxa as well.

Perithecia may or may not be separated from the lower cortex by medullary hyphae. Often when there is no separation, the perithecia force down the lower cortex and are visible as bulges on the lower surface (**Figs. 7F, 15D, H**). This condition is present in all taxa but is most common in *D. muhlenbergii* and least common in *D. dolomiticum*. The perithecia of *D. dolomiticum* and to a lesser degree of *D. arenosaxi* are often separated from the lower cortex by medullary hyphae (**Fig. 8E**); both these taxa have small perithecia but they also typically have quite thin thalli.

Spores. Spore size in Ozark *Dermatocarpon* is fairly constant but, as with other characters in the genus, not always consistent. For example, *D. luridum* is characterized by spores over 15 μm in length but sometimes produces spores which are only 11–13 μm long (e.g., *Buck 36333*, *45329*, NY). *Dermatocarpon muhlenbergii* usually has spores in the range of 12–15 μm but occasionally produces spores to 19 μm long (e.g., *Amtoft 2013*, NY). *Dermatocarpon arenosaxi* is the most variable in terms in of spore length as well as shape with spores ranging from 8–15 μm which, at maturity, can be globose to narrowly ellipsoid. The spore shape in *Dermatocarpon* is most often ellipsoid. The spores of *D. luridum* can sometimes be narrowly ellipsoid and in *D. luridum* var. *xerophilum* there are almost invariably a few spores which are longitudinally asymmetric. In both *D. luridum* var. *luridum* and var. *xerophilum* the (young?) spores are often surrounded by a gelatinous sheath. Occasionally spores begin to form a germ tube before ejection from the perithecium. This is common in the multiple-lobed species, particularly *D. arenosaxi*, but is also seen in *D. luridum* and *D. luridum* var. *xerophilum*. In *D. arenosaxi* spores may even begin to germinate while still within the ascus. The spores of *D. arenosaxi* frequently have an apiculus at one end; this is the sign of a developing germ tube. Out of more than 400 spores examined, germinating spores were found only twice in *D. muhlenbergii* (*Amtoft 573*, NY; *Wilhelm & Ladd 22061*, MOR) and twice in *D. dolomiticum* (*Amtoft 1002b*, *1377*, NY). The germ tubes in each species were quite different, almost moniliform in *D. dolomiticum*, but composed of cylindrical cells in *D. muhlenbergii*. In *D. arenosaxi*, *D. luridum*, and *D. luridum* var. *xerophilum* the germ tubes are composed of cylindrical cells. The germ tube emerges at the spore apex in all taxa except *D. luridum* and *D. luridum* var. *xerophilum* in which it may emerge subapically. A subapical germ tube is more common and more

strongly subapical in *D. luridum* var. *xerophilum* than in var. *luridum*.

Ecology. Most of the species of *Dermatocarpon* in the Ozarks exhibit such strict ecological preferences that it is possible to predict which species will be present at a particular site if the habitat is known beforehand. Detailed accounts of the ecologies are given under the treatments for each species but some of the basic patterns are discussed here. Of all the species in the Ozarks, only *D. muhlenbergii* is more or less a generalist in terms of habitat and substrate. *Dermatocarpon muhlenbergii* is the most common and widespread species in the Ozarks perhaps because it is able to tolerate both acidic and basic substrates. Its frequency in the Ozarks may also be partly due to the fact that acidic rock can be buffered somewhat by leaching of calcium carbonate from the surrounding limestone and dolomite rocks which are common in the Ozarks. In areas outside the Ozarks (e.g., western North Carolina), where there is little potential for buffering of acidic rock, only a few scattered thalli of *D. muhlenbergii* seem to be present over a large area (Amtoft, pers. observ.).

The species restricted to acidic rock are *D. arenosaxi* and *D. luridum*. *Dermatocarpon arenosaxi* is predictably present in sandstone glades but also grows in gladey areas on chert, rhyolite and granite. It is shade-intolerant and quickly fades out along the shaded periphery of glades. *Dermatocarpon luridum* grows near or along flowing water except for var. *xerophilum* which seems to prefer dry habitats.

Dermatocarpon luridum var. *luridum* is rare in the Ozarks possibly because of its intolerance to alkalinity. Waterways with acidic bedrock in the Ozarks can potentially be alkaline due to the buffering effects of surrounding carbonate rock (D. Ladd, pers. comm.). One of the few places where *D. luridum* is locally abundant in the Ozarks is in Sam A. Baker State Park in the St. Francois Mountains, where there are large amounts of exposed igneous rock. *Dermatocarpon luridum* appears to be most common and abundant where there is little buffering potential, for example in western North Carolina where the bedrock is largely granitic (Amtoft, pers. observ.).

Four species in the Ozarks are for the most part restricted to calcareous rock: *D. dolomiticum*, *D. multifolium*, *D. tomentulosum* and *D. moulinsii*. The last two are rare in the Ozarks with *D. moulinsii* associated with old-growth conditions (Ladd 2002) and *D. tomentulosum* with Ashe juniper forests. *Dermatocarpon dolomiticum* is predictably present in dolomite glades. Unlike the other glade species (*D. arenosaxi*), *D. dolomiticum* is able to tolerate some shade but it thrives in exposed areas. *Dermatocarpon multifolium* prefers and thrives on calcareous rock but is sometimes able to grow on acidic rock, especially of conglomerates perhaps because of the leaching effect of the carbonate rock. Additionally, *D. multifolium* prefers mesic habitats and the horizontal surfaces of rocks.

Taxonomic Treatment of *Dermatocarpon* in the Ozarks

Key to the species of Dermatocarpon *in the Ozarks.*

1. Thallus attached to the substrate by delicate rhizohyphae, no umbilicus or holdfasts present, on soil ... *Clavascidium*/*Placidium*
1. Thallus attached to the substrate by an umbilicus or multiple holdfasts, or with no umbilicus and holdfasts but not attached to soil by rhizohyphae, on rock or soil 2
   2. Lower surface with rhizinomorphs or tomentum 3
      3. Rhizinomorphs present, dark brown to black, composed of ± cylindrical aggregation of cells ..... *D. moulinsii*
      3. Tomentum present, pale brown to brown, composed of moniliform hyphae ..... *D. tomentulosum*
   2. Lower surface without rhizinomorphs or tomentum 4
      4. Thallus composed of a single lobe with a main holdfast, if secondary lobes are present, these lobes without additional holdfasts 5
         5. On calcareous rock 6
            6. Perithecia mostly small, 162–320(–374) µm high × 130–360(–396) µm wide, ostiole brown-black to black, typically sunken or flush with upper surface; upper thallus surface very dark brown to light brown, rarely blue green (no brown pigment), usually partly pruinose; lower surface variable but often verrucose, rarely completely smooth; thalli vagrant or not ..... *D. dolomiticum*
            6. Perithecia mostly large, (335–)420–600(–810) µm high × (245–)355–565(–690) µm wide, ostiole dark to light brown, reddish brown or without pigment, often two-toned, ostiole typically convex; color of upper surface

variable but not very dark brown often pale bluish-green infrequently pruinose; lower surface topography variable but often completely smooth, infrequently verrucose; thalli rarely vagrant ..... *D. muhlenbergii*

5. On acidic rock 7
   7. Thallus not distinctly thin and flexible; single holdfast always present; lower surface tan, pale brown to black, often completely smooth, otherwise veined, wrinkled or verrucose; perithecia large, (335–)420–600(810) × (245–)355–565(690) µm ..... *D. muhlenbergii*
   7. Thallus distinctly thin and somewhat flexible; single holdfast present or absent; lower surface light brown to golden brown, often shiny, foveolate or weakly wrinkled; perithecia small, (148–)200–300(451) × (154–)200–300(–400) µm ..... *D. arenosaxi*

4. Thallus composed of multiple lobes with multiple holdfasts or thallus with multiple holdfasts 8
   8. Thallus sterile, no spores present ..... (see discussion under *D. luridum* and *D. arenosaxi*)
   8. Perithecia and spores present 9
      9. Spores mostly longer than 15 µm; on acidic rock or soil 10
         10. Thalli aquatic to semi-aquatic, growing along permanent or ephemeral waterways or along seepage trails; spores (11–)15–19(–20) × (3–)4–7 µm, usually thin walled ..... *D. luridum*
         10. Thalli not aquatic or semi-aquatic, growing in dry habitats directly on rock or on a thin layer of soil over rock; spores 15.4–21.5 × 5.5–7.7 µm, frequently thick-walled, with walls ≥1 µm ..... *D. luridum* var. *xerophilum*
      9. Spores mostly shorter than 15 µm; on acidic or calcareous rock 11
         11. Substrate known 12
            12. On calcareous rock, usually dolomite ..... *D. multifolium*
            12. On acidic rock, usually sandstone ..... *D. arenosaxi*
         11. Substrate unknown 13
            13. Thallus distinctly cushion-like with erect to sub-erect lobes or thallus mat-forming with elongate ribbon-like lobes ..... *D. arenosaxi*
            13. Thallus not cushion-like, lobes adnate to sub-erect, rounded or if elongate then not mat-forming 14
               14. Upper surface very dark brown to brown-black ..... *D. arenosaxi*
               14. Upper surface not dark-brown to brown black but brown to light brown or grayish brown ... 15
                  15. Lobes with a conspicuous, usually slightly raised, brown margin, lower surface mostly smooth, or slightly foveolate ..... *D. multifolium*
                  15. Lobes without conspicuous, raised brown margin, lower surface smooth, wrinkled or foveolate ..... *D. arenosaxi*

Species treatments.

Dermatocarpon arenosaxi Amtoft, *sp. nov.*

Figs. 9, 10

Thallus multilobatus 1–25 mm diametro, numeroso haptero; pagina supera atrobrunnea ad pallida brunnea ubi humida interdum distincte bicolor viridus et brunnea; pagina inferna pallida brunnea ad pallida auribrunnea vel concolora pagina super, laevis vel foveata; perithecia pusilla (148–)200–300(–451) × (154–)200–300(–400) µm, acscosporae subglobosa vel ellipsoidea. Habitat in expositis rupibus siliceis.

TYPE: U.S.A. MISSOURI. Stone Co.: 2.1 mi S of White River on St. Rd. 5, sec. 20, T.17N., R.11W., 26 Apr 1988, *Harris 21644* (NY, holotype).

Etymology. The specific epithet refers to its predilection for sandstone, especially sandstone glades in the Ozarks.

Description. Thallus attached to rock, or on soil over rock, frequently vagrant, flexible or brittle, mounded or flat and mat-forming, multiple-lobed with multiple holdfasts on the lower surface; holdfasts mostly <1 mm long and wide, scattered or clustered in the center of the thallus, rarely with a single holdfast; lobes 1–25(–30) mm wide, usually thin, 160–280(–380) µm thick, erect or prostrate, undulate or flat, ribbon-like (**Figs. 9C, 10A–C**) or rounded and overlapping (**Fig. 9A**), infrequently broad and rotund; upper side usually somewhat shiny, partly farinose or not, variable in color but often dark brown or brown-black (**Fig. 9C**),

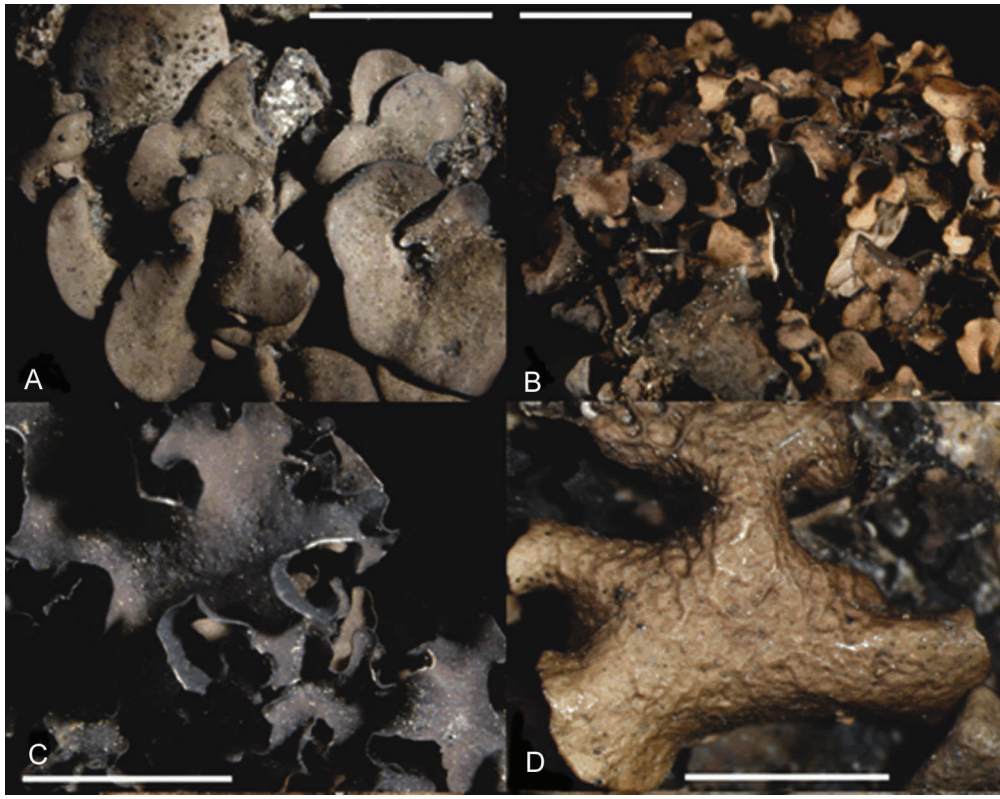

Figure 9. *Dermatocarpon arenosaxi*. **A.** Round-lobed form (*Amtoft 1361*, NY). **B.** Thallus (*Amtoft 1496*, NY). **C.** Ribbon-like lobes (*Harris 21644*, NY). **D.** Foveolate, shiny, lower surface (*Amtoft 1435*, NY). Scale bars: A = 0.5 cm, B = 1 cm, C = 0.5 cm, D = 0.25 cm.

otherwise brown to light brown or tan, sometimes strikingly bicolored green and brown when wet (**Fig. 10B**), rarely with a dark brown margin. Medulla often loose and somewhat glassy but sometimes compact and opaque in thick specimens, hyphae 2.0–4.0(–5.0) µm; lower side light brown to golden light brown and somewhat shiny (**Fig. 9B**), otherwise brown to dark brown, or tan, sometimes fully concolorous with upper side, topography often foveolate (**Fig. 9D**), with shallow wrinkles or veined, but sometimes smooth especially in round lobed forms. Perithecia (148–)200–300(–451) × (154–)200–300(–400) µm; ostiole brown-black, typically flat but occasionally convex, immersed or level with upper surface (**Figs. 8A**), infrequently slightly raised above the upper surface; exciple colorless, very rarely carbonized. Spores ellipsoid or sub-globose, 8.0–15.0 × 5.0–10.5 µm.

Ecology and distribution. This species grows on siliceous acidic rock such as sandstone, chert and granite, and sometimes on a thin layer of soil over acidic rock. In the Ozarks it is predictably present in sandstone glades and thrives in areas with poor drainage or along seepages and seasonal waterways. *Dermatocarpon arenosaxi* prefers exposed gladey areas and flat rocks, and appears to be shade-intolerant since its presence rapidly diminishes along shaded areas. It is locally abundant when present. *Dermatocarpon arenosaxi* has an eastern distribution in North America and has so far been found in the following states: Alabama, Arkansas, Georgia, Illinois, Missouri, North Carolina, South Carolina and Tennessee.

Discussion. The spores in *D. arenosaxi* germinate precociously in the perithecium and remarkably sometimes while still inside the ascus. Spores in the early stages of germination appear minutely apiculate on one end. The germ tube emerges apically and is septate and cylindrical. The tendency for spores to germinate readily and

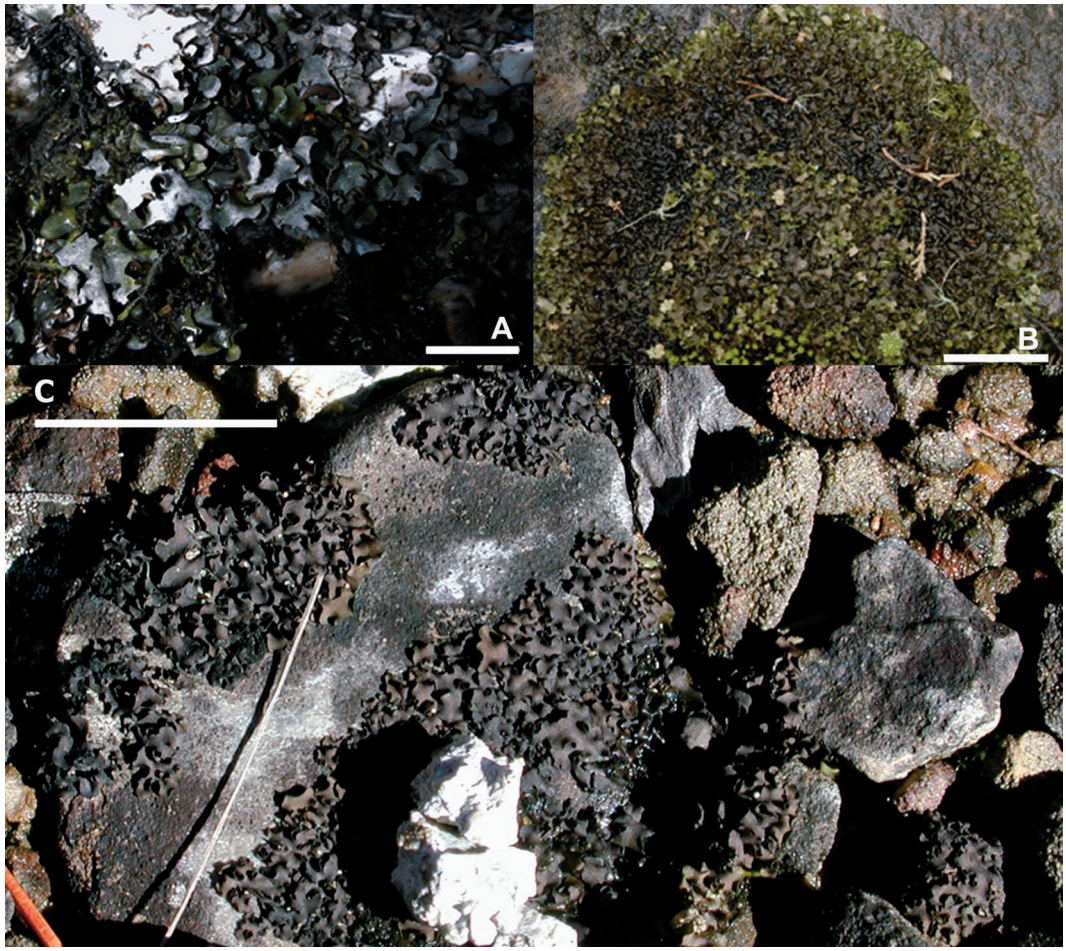

Figure 10. *Dermatocarpon arenosaxi.* **A.** Habit (*Amtoft 556*, NY). **B.** Bicolored, wet thallus (*Amtoft 3073*, NY). **C.** Habit (*Amtoft 596*, NY). Scale bars: A–C = 1 cm.

seemingly prematurely may play a role in the prolific tendency of *D. arenosaxi*. *Dermatocarpon arenosaxi* often forms large mats and can carpet extensive areas. The multiple-lobed thalli may be partly composed of two generations, with the second generation of lobes from spores which have germinated and established on the parent thallus (the first generation). *Dermatocarpon arenosaxi* often detaches from the rock and becomes vagrant. Vagrant thalli are especially common in glades with a thin layer of soil and fragmented rock.

In the Ozarks *D. arenosaxi* is found growing intermixed with an unnamed species of *Clavascidium* which grows on soil or soil over rock. At first glance the *Clavascidium* sp. superficially resembles *D. arenosaxi*. *Clavascidium* sp. is composed of small, rather friable lobes which are tightly adherent to the substrate unlike *D. arenosaxi*. Additionally, *Clavascidium* sp. bears rhizohyphae on the lower surface and lacks the *Dermatocarpon*-type lower cortex.

Additional collections of *D. arenosaxi* might be found in herbaria as specimens from the southeastern United States identified as *D. intestiniforme* or *D. miniatum* var. *complicatum*. *Dermatocarpon intestiniforme sensu* Purvis et al. (1992) is also multiple-lobed with multiple holdfasts, but differs from *D. arenosaxi* in having a thallus with a contorted intestine-like center, with an often blackish rim along the margin, a consistently Melzer's negative medulla, and a preference for alkaline substrates. Heiðmarsson (2001) synonymized *D. intestiniforme* with *D. miniatum* var. *complicatum* but the characterization and

distribution of these species in North America needs to be investigated further.

Dermatocarpon miniatum var. *complicatum sensu* Heiðmarsson (2001) has a gray, usually "pruinose" upper surface, slightly oblong perithecia and conidia (3.5–)4.0–5.5(–6.0) µm long. North American specimens of *D. miniatum* var. *complicatum* studied (*Merrill 265*, NY; *Jennings s. n.*, NY; *Egan 10257*, Texas, NY; *Buck 39814*, New Mexico, NY; *Heiðmarsson 1350*, SH45, UPS; *Heiðmarsson 1340*, SH3, UPS) differ from *D. arenosaxi* in generally having a thicker thallus, stouter holdfasts (to and exceeding 1 mm in length and width), and an evenly gray farinose upper surface. *Dermatocarpon miniatum* var. *complicatum* does not seem to assume the broad mat-forming habit or have a thallus composed of narrow ribbon-like lobes as is often the case in *D. arenosaxi*.

It is not uncommon for the narrowly elongate, ribbon-like forms of *D. arenosaxi* to be sterile, especially when either the upper surface or both the lower and upper surfaces are dark brown. Sterile specimens of the small, rounded lobed form of *D. arenosaxi* might be confused with *D. luridum*. *Dermatocarpon luridum* is rare throughout the Ozarks and most often fertile; therefore specimens with multiple-lobes and multiple-holdfasts growing on acidic rock are more likely to be *D. arenosaxi* than *D. luridum*. Identification of sterile *D. arenosaxi* from *D. luridum* is discussed further under the treatment for *D. luridum* var. *luridum*.

Additional specimens examined. A total of 78 specimens, 23 of these were non-Ozarkian from Alabama, Georgia, Illinois, New Jersey, North Carolina, South Carolina, Tennessee and the remaining 45 specimens from the following states and counties in the Ozarks. ARKANSAS: Izard Co., Pope Co., Sebastian Co., Stone Co.; MISSOURI: Carter Co., Douglas Co., Greene Co., Iron Co., Jefferson Co., Lawrence Co., Madison Co., Montgomery Co., Newton Co., Reynolds Co., Shannon Co., St. Clair Co., St. Francois, Ste. Genevieve Co., Wayne Co.

Dermatocarpon dolomiticum Amtoft, *sp. nov.*
Fig. 11

Thallus unilobatus 1.5–5.0 cm diametro, saepe dissectus vel rosulatus; pagina supera vulgo atrobrunnea pluries niveo tegmine; pagina inferna atrobrunnea vel pallida brunnea, verrucata vel venosa vel rugosa raro laevis. Perithecia pusilla, pyriforme vel globosum, 162–320(–374) × 130–360(–396) µm; ascosporae ellipsoidea (11.5–)12–13(–15.8) × (4.5–) 5.0–6.5(–7.0) µm. Habitat in expositis rupibus calcaris.
TYPE: U.S.A. MISSOURI. Jefferson Co.: W-facing Ordovician dolomite glade, E of Mammoth Creek Road, on slopes above Ridenour Hollow, sec. 12, T.39N., R.3E., 19 Sep 1990, *Harris 25421* (NY, holotype).

Etymology. The specific epithet refers to its strong preference for dolomitic rock and the predictable occurrence in dolomite glades.

Description. Thallus attached to rock or vagrant, flexible or brittle, broad-lobed, umbilicate; lobes 1.5–5.0 cm wide, often incompletely dissected into several broad lobes (**Fig. 11A–E**), rosette-like or with the appearance of being pinched together in the center (**Fig. 11C–E**), or conduplicate in vagrant forms, margin often erose; upper side usually partly farinose or with a whitish bloom (**Fig. 11A, C, D**), pigmentation typically dark brown or black-brown but sometimes brown, pale brown to grayish light brown (shade form). Medulla thin, loose and somewhat glassy, infrequently thick, dense, and opaque, hyphae (2–)3–5(–7) µm wide with the lumina mostly 1–2× broader than the cell walls, cell walls often cracked; lower side dark brown to black or brown to pale brown, verrucose, verrucose-veined, veined or wrinkled in part, seldom completely smooth. Perithecia small, 162–320(–374) × 130–360(–396) µm, usually weakly pyriform to globose; ostiole black, sometimes dark brown but rarely paler, most often immersed (**Fig. 8B**) or level with upper surface (**Fig. 11F**), but occasionally slightly raised above upper surface; exciple 10–26(–35) µm wide, colorless except around the ostiole with the dark brown to black pigments usually extending slightly downward (**Fig. 11F**). Spores ellipsoid, (11.5–)12–13(–15.8) × (4.5–)5.0–6.5(–7) µm.

Ecology and distribution. This species grows on solid or fragmented calcareous rock with a strong preference for dolomitic rock. Very rarely, it can creep onto non-calcareous rock (*Amtoft 1007*, NY) and then the thalli are noticeably stunted. In the Ozarks it predictably occurs in dry or seepy glades or gladey areas such as along roadsides. It may also be

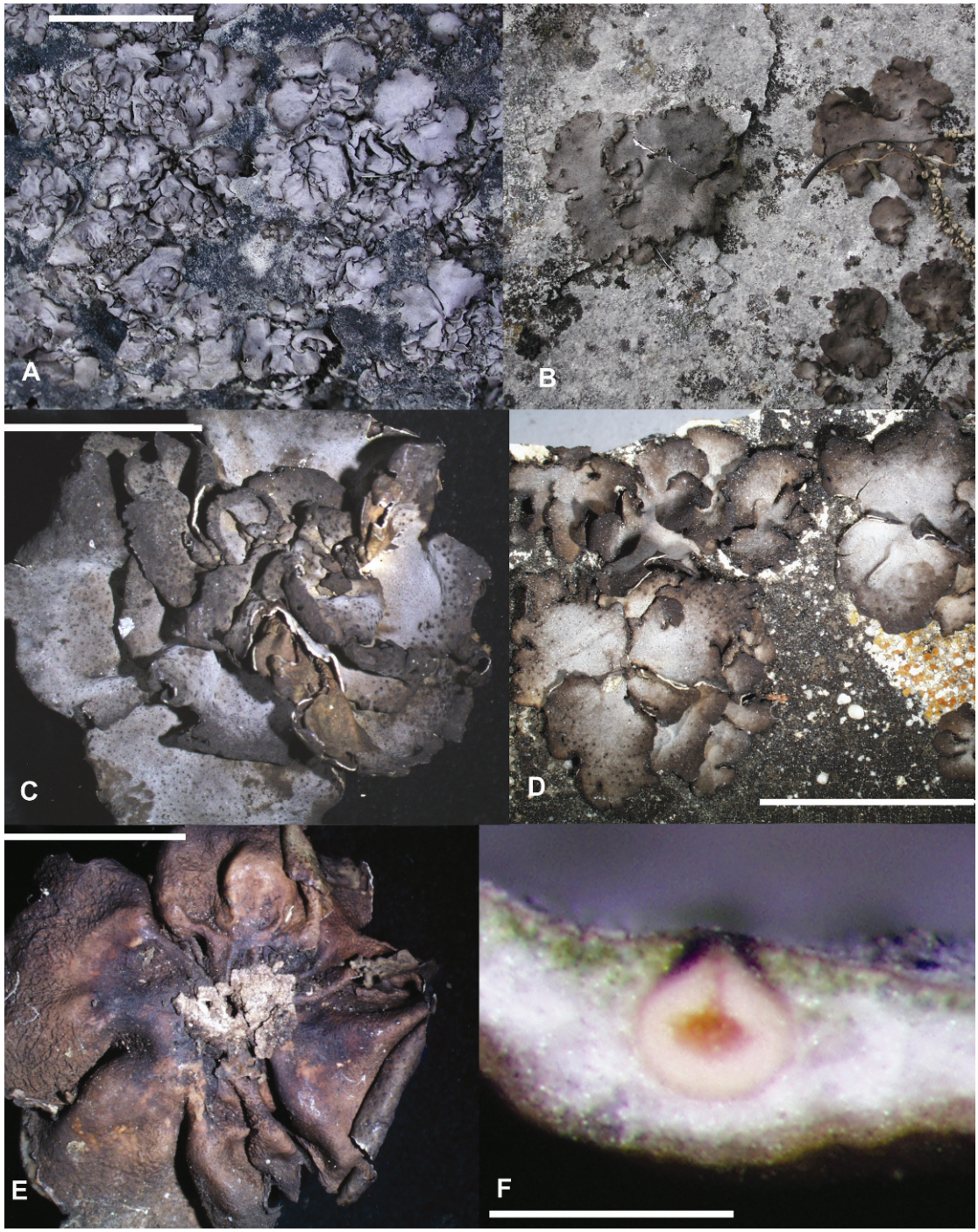

Figure 11. *Dermatocarpon dolomiticum.* **A.** Habit, crowded thalli (*Amtoft 1395*, NY). **B.** Habit (*Amtoft 1016*, NY). **C.** Thallus (*Harris 45690*, NY). **D.** Thallus (*Harris 40506*, NY). **E.** Lower surface and umbilicus (*Harris 45690*, NY). **F.** Perithecium (*Amtoft 1007*, NY). Scale bars: A = 4 cm; B = 3 cm; C, D = 1 cm; E = 2 cm; F = 250 µm.

found in moderately shaded sites, persisting in overgrown remnant glades, or along the periphery of glades. *Dermatocarpon dolomiticum* appears to have an eastern distribution in North America with specimens from Oklahoma, Iowa, Massachusetts, Missouri, New York and Wisconsin.

Discussion. *Dermatocarpon dolomiticum* is characterized by the combination of small perithecia, a black ostiole that is flush with or sunken in the upper surface, a brown-black to brown or grayish brown upper surface that is partly farinose or with a whitish bloom, a partly verrucose or verrucose-veined lower surface, an often rosette-like thallus with erose margins, a seemingly obligate preference for calcareous rock, and predictable presence in dolomite glades. Thalli may be found growing individually or in colonies tightly packed together. *Dermatocarpon dolomiticum* is closely related to *D. muhlenbergii*, *D. moulinsii* and *D. tomentulosum*. *Dermatocarpon muhlenbergii* has broader habitat and substrate preferences; the thallus is more often entire rather than deeply dissected or rosette-like, and the upper surface is normally brown to blue-green or grayish, not often brown-black or farinose or with a whitish bloom as in *D. dolomiticum*; the perithecia are larger with a different shape and pigmentation. The perithecia in *D. muhlenbergii* frequently produce bulges on the lower surface; this also occurs in *D. dolomiticum*, but it is not as frequent and characteristic as in *D. muhlenbergii*. *Dermatocarpon moulinsii*, *D. tomentulosum* and *D. reticulatum* differ in bearing rhizinomorphs, a tomentum, or papillae but have similar perithecia: small, with the ostiole usually flat, black and typically immersed or level with the upper surface.

Dermatocarpon dolomiticum is variable but the form found in open glades is unmistakable. The typical glade form (**Fig. 11A, C, D**) (e.g., *Harris 25421*, holotype, NY) can be recognized by a rosette-like thallus and a dark brown or brown-black upper surface with an obvious whitish bloom. *Dermatocarpon dolomiticum* is one of two *Dermatocarpon* species in the Ozarks with a tendency to become vagrant. Vagrant forms are generally found in areas of poor drainage such as on flat rocks which are level with the ground.

Additional specimens examined. A total of 110 specimens, nine non-Ozarkian specimens from Iowa, Massachusetts, New York, Wisconsin, and Iowa and the remaining 101 specimens from the following states and counties in the Ozarks. OKLAHOMA: Cherokee Co.; MISSOURI: Boone Co., Christian Co., Dallas Co., Dent Co., Franklin Co., Gasconade Co., Greene Co., Jefferson Co., Laclede Co., McDonald Co., Maries Co., Montgomery Co., Oregon Co., Ozark Co., Phelps Co., Stone Co., St. Francois Co., Taney Co., Texas Co., Washington Co., Webster Co.

Dermatocarpon luridum (With.) J.R. Laundon var. **luridum**, Lichenologist 16: 222. 1984; *Lichen luridus* With., Bot. Arr. Veg. Gr. Brit. 2: 720. 1776. HOLOTYPE: Dillenius, Hist. Musc., tab. 30, fig. 128. 1742 (reprinted in Laundon 1984), [from rivulet stones in Wales], epitype, OXF (non vidi). **Fig. 12**

Description. Thallus attached to rock, flexible, rigid or brittle, often mat-forming, multiple-lobed, rarely single-lobed, lower surface with multiple holdfasts; holdfasts mostly narrowly cylindrical, often to 1 mm or more in length or width (**Fig. 12C**), scattered, invariably a few marginal (**Fig. 12D**); lobes 145–450 μm thick, (0.4–)0.8–2.8 cm wide, flat, undulate or conduplicate (**Fig. 12A, B**), rotund or elongate, ribbon-like or not, flexible; upper side often blue-green with little to no melanin (**Fig. 12A**), or if growing in exposed areas brown to dark brown. Medulla mostly opaque, hyphae narrow, 2–3(–5) μm wide, often easily separating, lumen usually ca. 1–2× broader than cell wall; lower side most often pinkish tan to light pale brown, but sometimes also dark brown, completely smooth or veined, but not verrucose. Perithecia 270–520 × 250–462 μm, pyriform to sub-globose, frequently pushing down the lower cortex and forming bulges on the lower surface; ostiole brown-black to pale tan, brown pigment often extending into the upper cortex, level with or sunken in upper surface (**Fig. 8C**), seldom slightly exserted; exciple colorless, 27–52 μm wide. Spores ellipsoid to narrowly ellipsoid, simple, rarely 1–2-septate, (11–)15–19(–20) × (3–)4–7 μm, with or without a gelatinous sheath, occasionally extruded, sometimes germinating inside the perithecium; germ tube subapical to apical, septate and composed of rectangular cells.

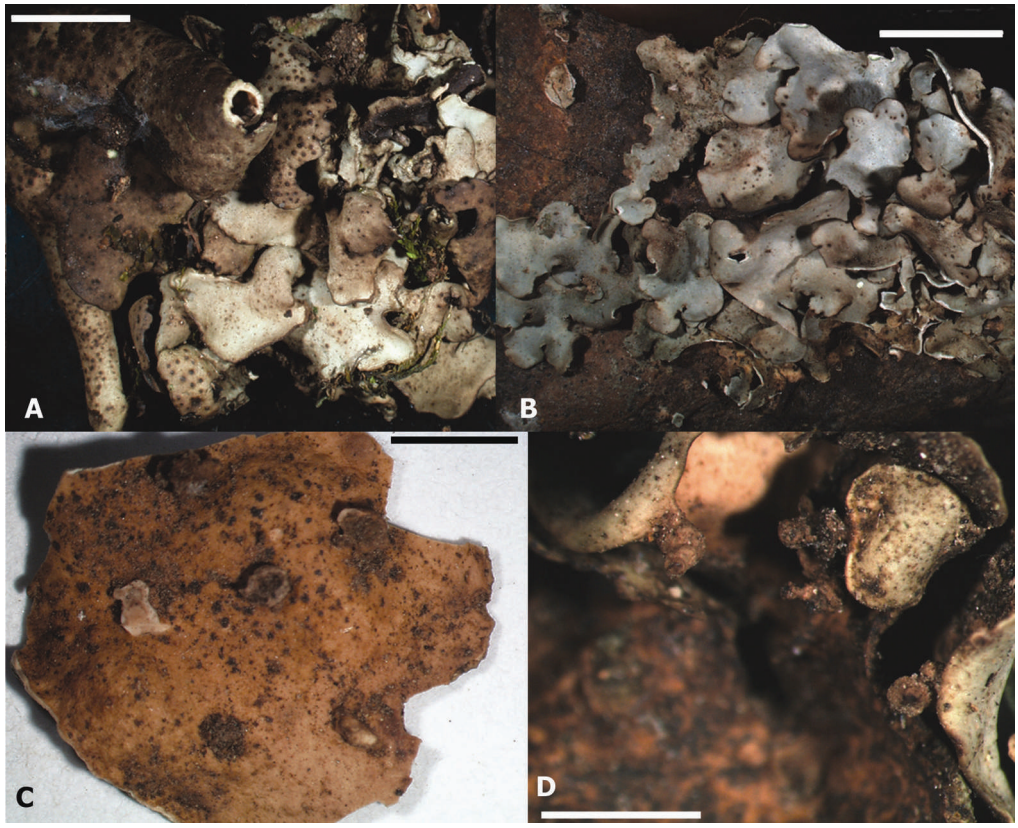

Figure 12. *Dermatocarpon luridum*. **A.** Habit (*Buck 45329*, NY). **B.** Habit (*Amtoft 1500*, NY). **C.** Lower surface showing holdfasts (*Amtoft 1500*, NY). **D.** Marginal holdfasts (*Buck 45329*, NY). Scale bars: A = 5 mm; B = 0.5 cm; C, D = 2 mm.

Ecology and distribution. *Dermatocarpon luridum* is restricted to acidic rock and grows in, along, or near flowing water such as rivers, creeks and permanent seepages but it can withstand periods of drying out. It is generally absent from waterways with heavy silt deposition. *Dermatocarpon luridum* is widespread and transcontinental in North America but is rare throughout the Ozarks with a southerly distribution.

Discussion. *Dermatocarpon luridum* is characterized by a multiple-lobed thallus with scattered multiple holdfasts which are often present along the margin and reach 1 mm in length, long spores, and an obligate preference for aquatic or semi-aquatic habitats. The often pinkish tan or pale tan lower surface is also characteristic. A dark brown lower surface and dark brown upper surface is sometimes due to an acellular brown granular deposit (e.g., *Harris 51430*, *Amtoft 2048*, NY).

In the Ozarks *D. luridum* is the only species with multiple lobes and multiple holdfasts growing in aquatic to semi-aquatic habitats that can have a completely melanin-free (blue-green) upper surface and pale tan to pinkish tan lower surface.

Dermatocarpon luridum often has elongate cells extending from near the lower part of the upper cortex into the algal layer (**Fig. 5E**). This has been observed by other authors (e.g., Stevens 1941) as well. These elongate cells extending into the algal layer are not always present but appear to be unique to *D. luridum* (also sometimes occurring in var. *xerophilum*) among the Ozark taxa.

Sterile specimens of *D. luridum* can be distinguished from *D. arenosaxi* by a generally thicker, broader thallus and ability to grow in shaded habitats. In the Ozarks, sterile, multiple-lobed specimens with a blue-green surface, growing in semi-aquatic to aquatic shaded habitats belong to *D. luridum*. The lobes of *D. luridum* can be narrowly elongate and ribbon-like as in *D. arenosaxi* but this condition is not common. Instead, the lobes of *D. luridum* tend to be larger and broad and rotund. The

lower surface of *D. luridum* may be pinkish tan, a color which has so far not been observed in *D. arenosaxi*. The holdfasts of *D. luridum* tend to be rather long and stout, often at least 1 mm in length with at least a few present along the lobe margin, whereas in *D. arenosaxi* they are usually much smaller and often not present along the lobe margin. See also *D. arenosaxi* discussion under *D. luridum* var. *xerophilum*.

Additional specimens examined. A total of 47 specimens, 42 non-Ozarkian from Alabama, Connecticut, Illinois, Kentucky, Maine, New Jersey, North Carolina, South Carolina, Tennessee, Vermont and 7 Ozarkian specimens from the following states and counties. ARKANSAS: Franklin Co., OKLAHOMA: Cherokee Co., Sequoyah Co.; MISSOURI: Wayne Co.; ILLINOIS: Randolph Co.

Dermatocarpon luridum var. **xerophilum**

Amtoft, *var. nov.* **Fig. 13**

Thallus multilobatus, numeroso haptero, margo pluries fuscobrunneus et incrassatus, ascosporae ellipsoidea vel subfusiforma 15.4–21.5 × 5.5–7.7 µm pluries pachyparies. Habitat xeric vel mesic super solum vel rupes maximam partem siliceae.

TYPE: U.S.A. ARKANSAS. Pope Co.: Ozark Nat'l. Forest, Kings Bluff, S of AR 16, 6 mi E of AR7 at Sand Gap, 2002, *Amtoft 603a* (holotype, NY).

Description. Thallus attached to rock or on soil over rock, 215–650 µm thick, frequently rigid, somewhat flat to strongly convex (**Fig. 13A, B**), multiple-lobed with multiple holdfasts; holdfasts stout and well developed, scattered and marginal; lobes 5.0–20(–30) mm wide, usually overlapping, shingle-like, rotund, frequently with a brown margin (**Fig. 13C**); upper side light brown to dark brown, partly blue-green or not, often two-toned. Medulla typically compact, hyphae 2.2–4.4(–5.5) µm wide, often conglutinate; lower side pale tannish brown or brown, smooth or veined. Perithecia 350–643 × 270–517 µm, lageniform to globose; ostiole usually convex and often flared (**Fig. 9C**), often slightly exserted, and somewhat gaping or sunken in with a donut-like appearance when viewed from above (**Fig. 13E, F**), neck sometimes tubular (**Fig. 7C**); exciple colorless. Spores ellipsoid to subfusiform, sometimes with one side flat, 15.4–21.5 × 5.5–7.7 µm, occasionally ejected in a cirrus, frequently thick-walled, wall ≥ 1 µm, often with a gelatinous sheath, germinating inside the perithecium or not; germ tube subapical or apical, composed of rectangular cells.

Discussion. *Dermatocarpon luridum* var. *xerophilum* is characterized by a multiple-lobed thallus with multiple holdfasts, most often an upper surface that is bicolored brown and bluish green or two-toned dark brown and light brown, usually a brown margin and long spores to 22 µm in length. Unlike *D. luridum* it is not obligately hydrophilous and instead is found in xeric to mesic habitats on soil over rock or directly on rock. In the Ozarks this species is restricted to acidic rock but there are specimens (e.g., *Harris 44090*, NY) from outside the Ozarks collected on calcareous rock. The thallus can become semi-vagrant, convex and mound-forming, and remarkably rigid; none of these characteristics is seen in *D. luridum* var. *luridum*. *Dermatocarpon luridum* var. *xerophilum* also tends to have longer spores than var. *luridum*. Since *D. luridum* var. *xerophilum* does not grow in aquatic to semi-aquatic habitats, herbarium specimens of this lichen are sometimes found under the name *D. miniatum* (e.g., *Wilhelm & Ladd 22697*, MOR). *Dermatocarpon luridum* var. *xerophilum* is distinctive in habitat and sometimes in gross morphology (*Amtoft 589*, *607a*; *Buck 38431*, *37317*, NY) but there are specimens which could not have confidently been placed (in or out of *D. luridum* var. *luridum* or as long-spored forms of *D. miniatum*) in the absence of molecular or habitat data. The sister relationship between *D. luridum* var. *xerophilum* and var. *luridum* is well supported in both the ITS and combined analyses (**Figs. 2, 3**). The combined analysis nests var. *xerophilum* within var. *luridum* but there is no statistical support for this relationship (**Fig. 3**). The morphological distinction between the two is based only a few available specimens of var. *xerophilum*. With more specimens it should be possible to fully account for the variation within var. *xerophilum* and determine if species status is perhaps warranted. The description of this lichen as a variety will hopefully prevent multiple-lobed, long-spored specimens growing in dry habitats from being filed under a multiple-lobed variety of *D. miniatum*. The

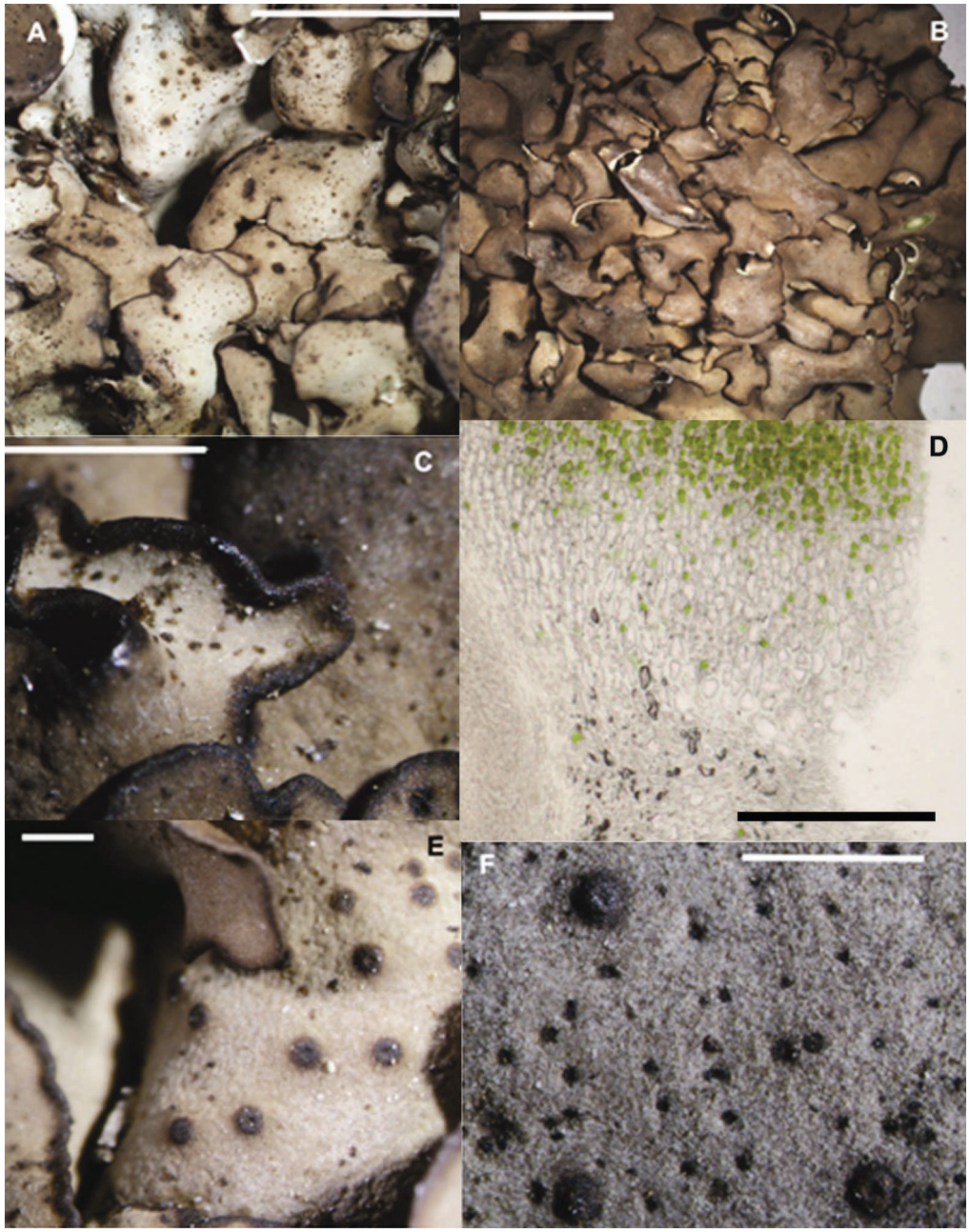

Figure 13. *Dermatocarpon luridum* var. *xerophilum*. **A.** Two-toned thallus (*Amtoft 603a*, NY). **B.** Convex thallus of overlapping lobes (*Amtoft 603a*, NY). **C.** Close-up of lobe with brown raised margin (*Amtoft 603a*, NY). **D.** Cross section of thallus with well-developed layer of fungal cells between algal layer and medulla (*Amtoft 603a*, NY). **E.** Upper surface showing perithecia with gaping ostioles (*Amtoft 603a*, NY). **F.** Upper surface showing perithecia with convex ostioles (three), and developing perithecia (small black dots) (*Wilhelm 22697*, MOR). Scale bars: A, B = 0.5 cm; C = 0.75 mm; D = 100 µm; E, F = 0.5 mm.

recognition of this ecological variety emphasizes the importance of recording habitat data which is frequently lacking in herbarium specimens of *Dermatocarpon*. Herbarium specimens from eastern North America filed under *D. miniatum* with the following combination of characters are likely referable to the new variety of *D. luridum*: thallus multiple-lobed with multiple holdfasts, lobes with or without a raised brown margin, long spores with a gelatinous sheath or

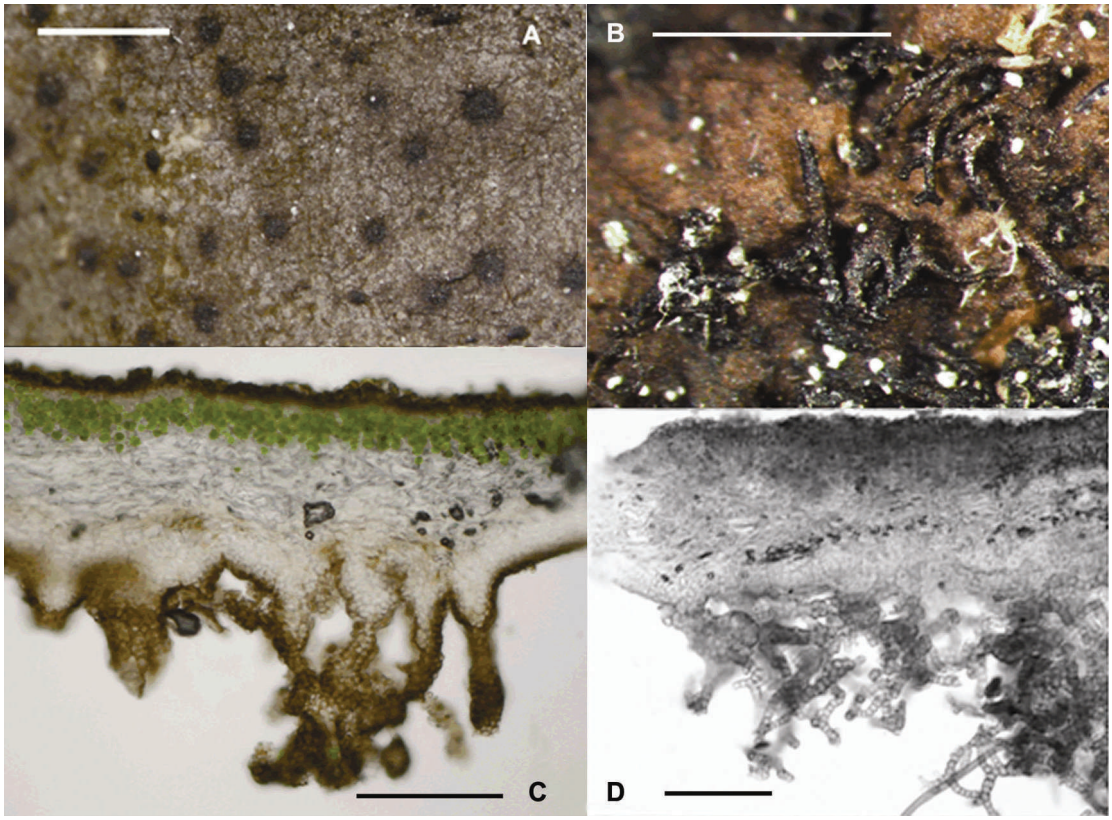

Figure 14. A. *Dermatocarpon moulinsii* upper surface with ostioles level with upper surface (*Amtoft 1038*, NY). **B.** *D. moulinsii* lower surface showing rhizinomorphs (*Amtoft 931*, NY). **C.** *D. moulinsii* cross section of thallus showing cylindrical rhizinomorphs many cells wide (*Amtoft 1038*, NY). **D.** *D. tomentulosum* cross section of thallus showing moniliform hyphae of tomentum (*Ferguson s.n.*, NY). Scale bars: A= 2 mm, B = 1 cm, C = 100 µm. D = 50 µm.

thick wall ca. 1 µm wide, and growing in a dry habitat.

Dermatocarpon luridum var. *xerophilum* appears to have a southern distribution with sequenced specimens from southern Missouri, Oklahoma and Arkansas.

Sterile specimens of *D. luridum* var. *xerophilum* for which the substrate is not known can be distinguished from *D. multifolium* by the larger lobes and most often a noticeably rigid thallus. *Dermatocarpon multifolium* is found in rather mesic habitats while *D. luridum* var. *xerophilum* appears to grow in dry habitats. The thallus of var. *xerophilum* can also be mound-like; this growth form does not occur in *D. multifolium*.

Additional specimens examined. Eight specimens total, all Ozarkian from the following states and counties. ARKANSAS: Franklin Co., Pope Co., Stone Co.; OKLAHOMA: Cherokee Co.; MISSOURI: Ste. Genevieve Co.

Dermatocarpon moulinsii (Mont.) Zahlbr., Nat. Pflanzenfam. 1(1*): 60. 1903; *Endocarpon moulinsii* Mont., Ann. Sci. Nat. Bot. II, 20: 358. 1843. TYPE: FRANCE, 1842, *Moulins s.n.*

Fig. 14A–C

Description. Thallus ca. 2–3.5 cm wide, attached to rock, brittle, single-lobed, broad, dissected or not, umbilicate; upper side grayish brown, light brown to dark brown, partly farinose, with or without a pruinose appearance. Medulla loose, seldom conglutinate, hyphae (2–)3–5 µm wide, hyphal wall sometimes cracked; lower side dark brown to black or partially light brown, melanin mostly restricted to basal most cells, rhizinomorphs present(**Fig. 14B, C**); rhizinomorphs ca. 30–80 µm wide and to 200 µm

long, brown to brown-black, cylindrical with no central medulla, branched or not, composed of cortical cells (**Fig. 14C**); cells subspherical, angular or not, ca. 3–8 μm wide. Perithecia ca. 215–386 × 230–357 μm, globose or pyriform; ostiole immersed or even with upper surface (**Fig. 14C**), infrequently exserted, black, sometimes brown, flat or convex; spores ellipsoid to sub-globose, 8.8–11.1 × 5.5–6.6 μm.

Habitat and ecology. On dolomite bluffs with old-growth conditions (Ladd 1997), and on small ledges in the periphery of dolomite glades. *Dermatocarpon moulinsii* is rare in the Ozarks.

Additional specimens examined. A total of ten specimens, three non-Ozarkian from Colorado and Utah, seven Ozarkian from the following states and counties. MISSOURI: Douglas Co., Ozark Co., Stone Co.

Dermatocarpon muhlenbergii (Ach.) Müll. Arg., Bot. Jahrb. Syst. 6: 377. 1885; *Endocarpon muhlenbergii* Ach., Syn. Meth. Lich. 101. 1814. TYPE: U.S.A. PENNSYLVANIA, *Muhlenberg 142* (isotype, PH!). **Fig. 15**

Description. Thallus to 7.5 cm wide (Arkansas, 1954, *Hale s.n.*, NY), attached directly to rock, very rarely vagrant, rigid or flexible, single-lobed, entire, slightly dissected or with a few secondary lobes (**Fig. 15B, C**), umbilicate, rarely with a few secondary holdfasts; upper side pale grayish-brown, light brown to brown, bluish-green (shade form), or less often pinkish light brown, smooth or minutely wrinkled, occasionally farinose. Medulla often of dense, wormy conglutinate hyphae, with thick or less frequently thin-walled hyphae 3–4(–6) μm, lumen often <2× broader than cell wall; lower side tan, pale brown to black, completely smooth, veined or wrinkled in part, seldom verrucose. Perithecia large, (335–)420–600 (–810) × (245–)355–565(–690) μm, pyriform or lageniform, occasionally sub-globose, often pushing down the lower cortex and forming bulges on the lower surface, sometimes ''double'' (**Fig. 15G**); ostiole slightly to strongly exserted, infrequently immersed in upper surface, most often convex with a ''cap'' (**Fig. 15E, F**), cap pale to dark brown, sometimes paler in the center than along margin or with a pale brown margin and dark brown center (**Fig. 15E**), immature or senesced perithecia usually lacking a convex cap and are sunken or flush with upper surface; exciple (20–)26–48(–65) μm wide, colorless, rarely brown throughout, seldom with dark brown pigment extending downward. Spores ellipsoid, (9–)12–15(–19) × (4–)5–7(–9) μm, sometimes extruding from the ostiole in a cirrus (**Fig. 7E**). Pycnidia common, pyriform, immersed in the thallus or infrequently in areolae (*Buck 37416*, NY) and then the ostiole often noticeably broad.

Distribution and ecology. In the Ozarks *D. muhlenbergii* is widespread on calcareous rock but may also occur on acidic rock. *Dermatocarpon muhlenbergii* grows in a wide variety of habitats from xeric to semi-aquatic and in shaded areas such as under ledges to highly exposed bluff faces. *Dermatocarpon muhlenbergii* appears to be widespread in eastern North America.

Discussion. *Dermatocarpon muhlenbergii* is characterized by a broad-lobed, umbilicate thallus bearing rather large perithecia with a convex ostiole mouth. *Dermatocarpon muhlenbergii* is the most widespread and common *Dermatocarpon* species in the Ozarks where it is also the only broad-lobed, umbilicate species that consistently occurs on both calcareous and acidic rocks.

The name *D. muhlenbergii* is not currently in use and is here resurrected. *Dermatocarpon muhlenbergii* is the correct name for specimens from Missouri which are referred in Heiðmarsson (2003) to *D. americanum*. In his molecular study, Heiðmarsson (2003) concluded that two specimens, SH32 (*Mattsson 5322*, UPS) and SH33 (*Heiðmarsson 1056*, UPS), falling in the same clade could possibly represent different taxa: SH33 from Arizona was attributed to *D. americanum sensu strictu*, and SH32 from Missouri, bearing a black lower surface was posited as a potential new species. Heiðmarsson did not refer the Missouri specimen to *D. muhlenbergii* because it has a medulla that stains red with Melzer's reagent and the medulla of the type of *D. muhlenbergii* does not stain red. The ITS sequences of SH32 and SH33 were obtained from GenBank and included in my ITS analysis. The position of SH33, *D. americanum*, was not totally resolved but it was close to *D. dolomiticum* and *D. muhlenbergii* (**Fig. 2**). SH32 (Missouri) is included in a well-supported

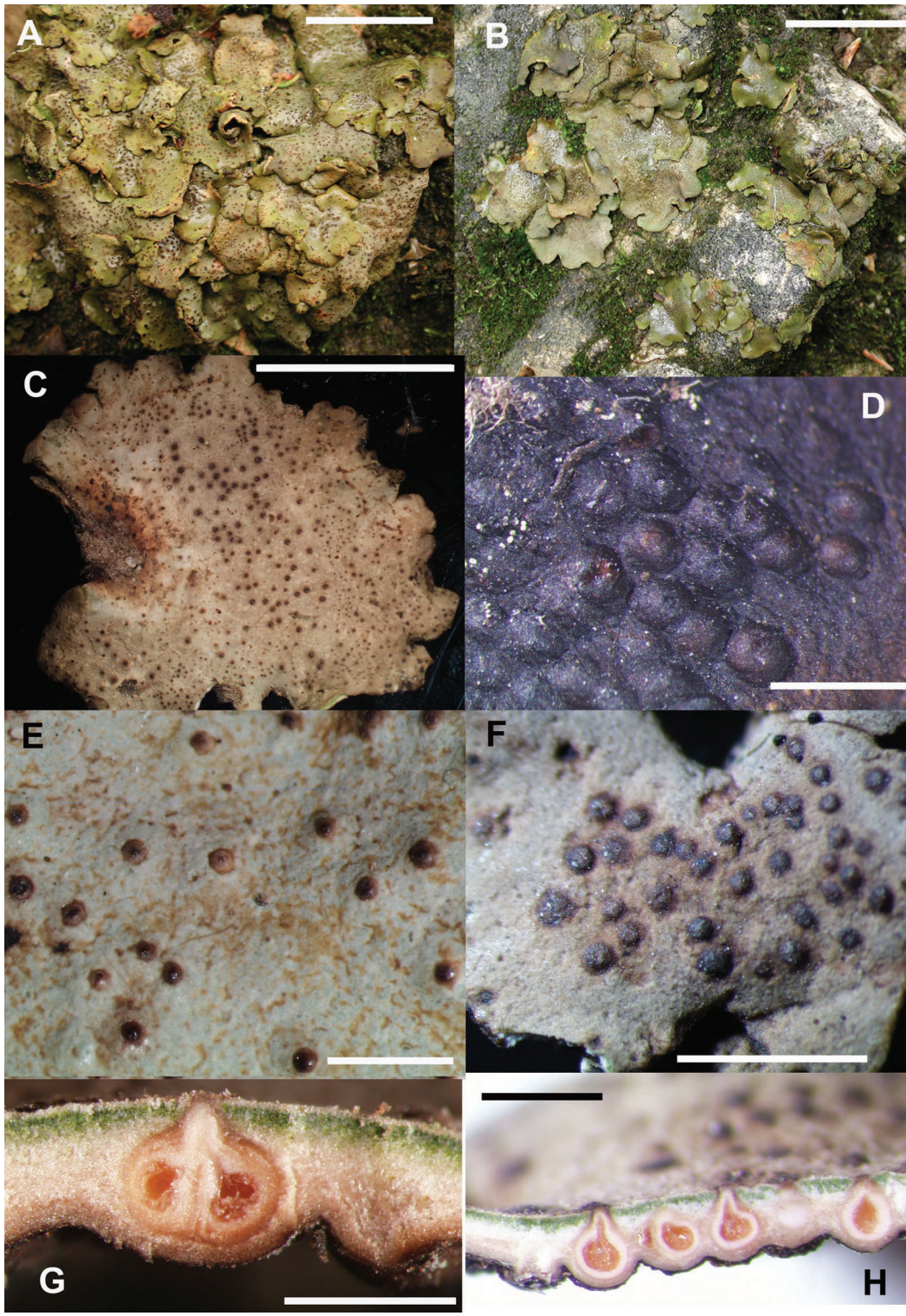

Figure 15. *Dermatocarpon muhlenbergii*. **A.** Habit, crowded thalli (*Amtoft 919*, NY). **B.** Habit (*Amtoft 919*, NY). **C.** Thallus (*Amtoft 3356a*, NY). **D.** Lower surface with bulges from perithecia (*Amtoft 583*, NY). **E.** Upper surface with (some two-toned) convex ostioles (*Amtoft 402a*, NY). **F.** Upper surface with convex cap-like ostioles (*Amtoft 583*, NY). **G.** "Double" perithecium (*Amtoft 493b*, NY). **H.** Pyriform perithecia (*Amtoft 583*, NY). Scale bars: A = 1.5 cm, B = 2 cm, C = 1 cm, D–F = 1 mm, G = 550 µm, H = 1 mm.

clade which we are attributing to *D. muhlenbergii*. Morphologically and anatomically SH32 agrees with *D. muhlenbergii*. Specimens of *D. muhlenbergii* react negatively, positively or ambiguously to Melzer's reagent (**Fig. 4**) so this character cannot be used to exclude SH32 from *D. muhlenbergii*. This is additionally supported by the fact that Heiðmarsson found the type of *D. muhlenbergii* to react negatively to Melzer's reagent while we found an isotype (PH) to react positively to Melzer's reagent. For these reasons, the reaction of the medulla to Melzer's reagent is not a reliable character to use to distinguish between *D. muhlenbergii*, *D. americanum* and *D. miniatum*. *Dermatocarpon americanum* specimens from Arizona (ASU) differ in having perithecia with black ostioles mouths that are immersed or level with the upper surface.

Additional specimens examined. A total of 133 specimens, 23 non-Ozarkian from Cuba, Haiti, Puerto Rico, U.S.A. (Alabama, Connecticut, Illinois, Iowa, Ohio, Tennessee, Pennsylvania, Vermont, Virginia, West Virginia) and 110 specimens from the following states and counties in the Ozarks. ARKANSAS: Carroll Co., Crawford Co.; ILLINOIS: Jackson Co., Monroe Co., Union Co.; MISSOURI: Barry Co., Benton Co., Boone Co., Camden Co., Cape Girardeau Co., Carter Co., Cedar Co., Christian Co., Crawford Co., Dallas Co., Dent Co., Douglas Co., Franklin Co., Howell Co., Iron Co., Laclede Co., Lincoln Co., McDonald Co., Maries Co., McDonald Co., Morgan Co., Newton Co., Oregon Co., Ozark Co., Perry Co., Polk Co., Pulaski Co., Shannon Co., Ripley Co., Stone Co., St. Charles Co., St. Francois Co., Ste. Genevieve Co., St. Louis Co., Taney Co., Texas Co., Warren Co., Washington Co., Wayne Co., Wright Co.; OKLAHOMA: Cherokee Co., Muskogee Co.

Dermatocarpon multifolium Amtoft, *sp. nov.* **Fig. 16**

Thallus multilobatus 0.5–2.0(–2.5) cm diametro, lobi typice ≤ 1 cm diametro, numeroso haptero; margo pluries fuscobrunneus et incrassatus; pagina inferna atrobrunnea vel pallida brunnea, laevis raro venosa vel rugosa. Perithecia pyriforme vel globosum, (182–)289–484 × (181–)266–473 μm. Habitat in mesic rupibus calcaris.

TYPE: U.S.A. MISSOURI. Texas Co.: Gist Ranch Conservation Area, N of Ranch Road, 2.5 mi E of MO 137, on Nagle Drive (Ranch Road), just E of Peters Creek, 37°10′41″N, 91°48′19″W, T.29N., R.8W., sec. 22, NE¼, on dolomite boulder in forest, 4 Nov 2004, *Amtoft 3295* (NY, holotype).

Description. Thallus (0.5–)0.6–2.0(–2.5) cm wide, composed of a few to many small lobes with multiple holdfasts; holdfasts ± short, < 0.5 mm long, usually somewhat flattened, mostly central, and often clustered together, sometimes with one umbilicus-type holdfast (of fused holdfasts?) and a few inconspicuous smaller holdfasts (**Fig. 16E**); lobes 0.2–0.9(–1.3) cm wide, rounded (**Fig. 16A, B, D**), rarely narrow and elongate (**Fig. 16C**) and then the thallus usually sterile or with pycnidia only; lobes flat or partly concave and cup-like or convex, margin dark brown and raised (**Fig. 16C, D**); upper side pale gray brown to dark brown sometimes partly bluish-green, damp thalli often appearing slightly two-toned. Medulla compact or loose, hyphae 2–4 (–5) μm wide, conglutinate or not, with the lumen usually wider than the hyphal wall; lower side dark brown-black to light brown, rarely pale tan, smooth, rarely veined or wrinkled, not verrucose. Perithecia globose or pyriform (**Fig. 7F**), (192–)289–484 × (181–)266–473 μm; ostiole brown-black to brown, strongly convex or level with the upper surface (**Figs. 7F, 8D**), if convex then often with a broad "cap" composed of brown, thick walled, irregular round cells; exciple (11–)21.1–31.1(–46.1) μm, colorless. Spores ellipsoid 9.7–14 × 4.5–6 μm.

Distribution and ecology. This species is occasional in the Ozarks but often locally abundant where present. *Dermatocarpon multifolium* grows in mesic habitats on shaded calcareous boulders or bluffs with a preference for the horizontal surfaces and dolomite. It also grows on calcareous sandstone (*Harris 21604*, NY) and concrete (*Amtoft 3757*, NY) and very rarely occurs on weakly calcareous (*Harris 49285*, NY) or non-calcareous rock.

Discussion. *Dermatocarpon multifolium* is usually present in many small colonies. Thalli are typically crowded together (**Fig. 16A, B**), difficult to separate and generally mat-forming. Individual lobes or thalli are normally ≤1 cm in diameter, rarely reaching 2.5 cm. The lobes are often partly concave or cup-like and have a brown margin that is slightly

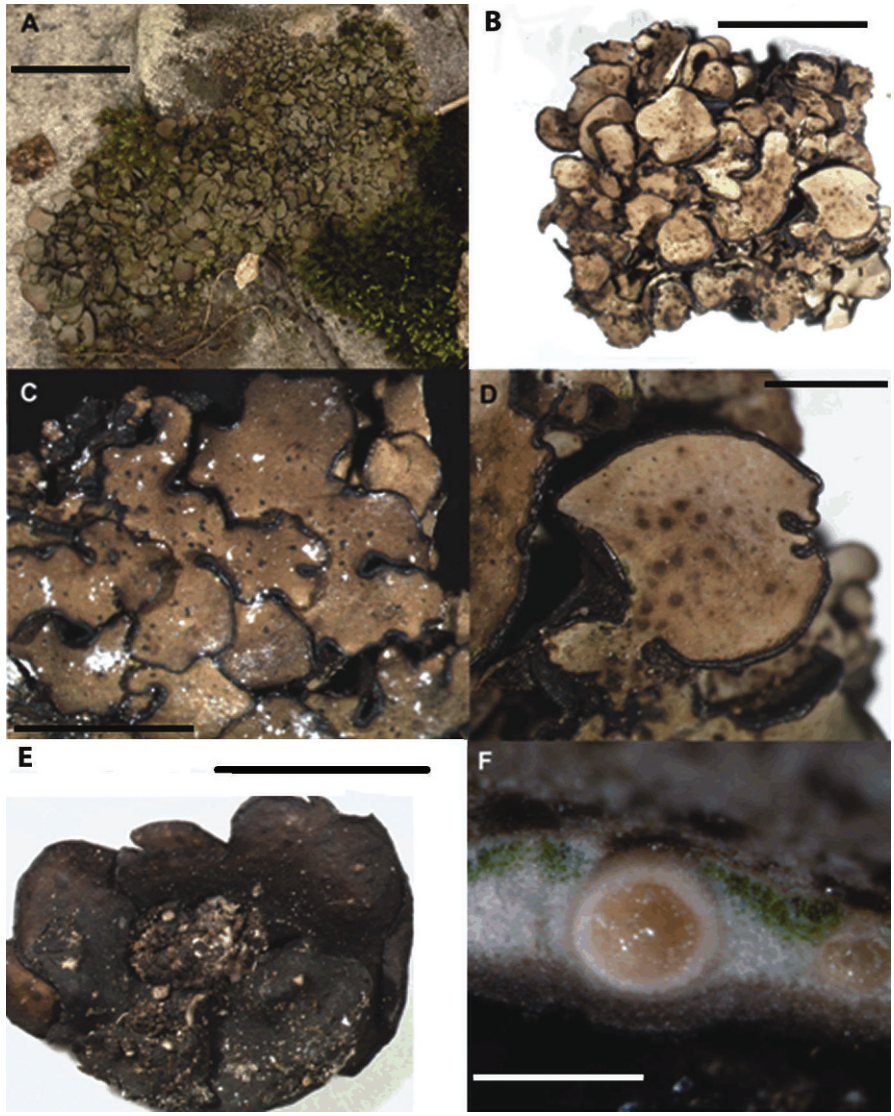

Figure 16. *Dermatocarpon multifolium*. **A.** Habit (*Amtoft 1083*, NY). **B.** Habit (*Buck 42784*, NY). **C.** Rare, narrow elongate form (*Amtoft 3299*, NY). **D.** Lobe showing brown, raised margin (*Buck 42784*, NY). **E.** Lower surface with main central holdfasts and smaller secondary holdfasts (*Buck 42784*, NY). **F.** Section of sub-globose perithecium (*Amtoft 471*, NY). Scale bars: A= 1.6 cm, B = 1 cm, C, D = 1 mm, E = 0.5 cm, F = 0.3 mm.

to strongly raised. The ostiole may be strongly convex or flat but usually there are at least a few perithecia with a convex mouth. In addition the ostiole tends to become quite broad.

Dermatocarpon multifolium often grows alongside *D. muhlenbergii*. It is difficult to separate small or immature thalli of *D. muhlenbergii* from aberrant single-lobed or immature(?) specimens of *D. multifolium* which occasionally lacks or has only rudimentary secondary holdfasts. Young thalli of *D. muhlenbergii* sometimes have a brown margin thus making the problem worse. The young thalli of *D. muhlenbergii* are more flat and not cup-like with uplifted margins as *D. multifolium* sometimes is. The perithecia of *D. muhlenbergii* are typically narrowly-pyriform or lageniform and not frequently globose as in *D. multifolium*. Small sterile thalli with no pycnidia and a single holdfast probably belong to *D. muhlenbergii* since *D. multifolium* usually produces either perithecia or pycnidia. Thalli with abundant

pycnidia are not uncommon and are occasionally rosette-like rather than mat-forming.

Additional specimens examined. A total of 36 specimens, three non-Ozarkian from Missouri and 33 Ozarkian from the following states and counties. ARKANSAS: Crawford Co., Franklin Co., Stone Co.; MISSOURI: Barry Co., Christian Co., Crawford Co., Dent Co., Douglas Co., Franklin Co., Ozark Co., Maries Co., Shannon Co., Stone Co., Taney Co., Washington Co., Texas Co., Wright Co.

Dermatocarpon tomentulosum Amtoft, The Bryologist 109: 182. 2006. **Fig. 14D**

TYPE: U.S.A. TEXAS. Hayes Co.: E side of Shade Road, S of CR 1492, 2 mi S of Wiberley on SR 12, 29°58'44.3″N, 98°06'26.4″W, *Juniperus ashei* forest on shaded calcareous rock, 17 Aug 2005, *Amtoft 3600* (NY, holotype).

Description. Thallus single-lobed, 0.75–4.5 cm wide, thin, ca. 200–380 μm thick, with a single, marginal or central umbilicus; margin even to slightly lobulate in older thalli; upper side pale grayish or greenish brown to brown, often with a slightly pruinose appearance or farinose surface. Medulla loose and somewhat glassy, of hyphae 3–5 μm wide; lower side minutely tomentose with infrequent bald (tomentum-free) areas; tomentum dark brown, composed of basal cortical cells proliferating to form moniliform hyphae (**Fig. 14D**); hyphae 44.0–187.0 × (4.4–)6.6–12.1 μm, unbranched or branched, concolorous with the brown pigment deposited on the surface cortical cells, arranged in a single plane, one to several cells wide at the base, sometimes more than one cell wide above the base. Perithecia common, 220–262 × 209–220 μm; ostiole dark to light brown around the mouth and level with or slightly immersed in the upper surface; exciple hyaline. Spores (8.8–)11.0–13.2 × 4.4–5.5(–6.6) μm.

Distribution and ecology. *Dermatocarpon tomentulosum* is a rare species known from only few localities in North America. It has been found in Missouri and Texas in the United States, and Cat Island and New Providence in the Bahamas. Despite extensive collecting of *Dermatocarpon* in the Ozarks this species has been found in only one locality there. As discussed in Amtoft (2006) specimens filed as *D. moulinsii* or *D. miniatum* in other herbaria may uncover more collections of this new species. It appears that *D. tomentulosum* prefers calcareous rock, moderately shaded habitats and *Juniperus ashei* forests.

Discussion. *Dermatocarpon tomentulosum* is characterized by a single-lobed thallus and a lower surface which bears a minute tomentum. Macroscopically the tomentum in *D. tomentulosum* forms a delicate carpet over the lower surface. This tomentum (**Fig. 14D**) is distinct anatomically from the rhizinomorphs (**Fig. 14C**) in *D. moulinsii* (Amtoft 2006). Based on both morphological and molecular data *D. tomentulosum* is closely related to *D. moulinsii* and *D. reticulatum*.

Additional specimens examined. A total of six specimens, four non-Ozarkian from Bahamas, U.S.A. (Texas) and two Ozarkian specimens. MISSOURI: Stone Co.

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