Lecanora markjohnstonii (Lecanoraceae, lichenized Ascomycetes), a new sorediate crustose lichen from the southeastern United States

Carly R. Anderson Stewart[1,5], James C. Lendemer[2], Kyle G. Keepers[1], Cloe S. Pogoda[3], Nolan C. Kane[1], Christy M. McCain[1] and Erin A. Tripp[1,4]

[1] *University of Colorado at Boulder, Ecology and Evolutionary Biology Department, Boulder, CO 80309, U.S.A.;* [2] *New York Botanical Garden, City University of New York, New York, NY 10458, U.S.A.;* [3] *University of Colorado at Boulder, Molecular, Cellular, and Developmental Biology Department, Boulder, CO 80309, U.S.A.;* [4] *University of Colorado at Boulder, Museum of Natural History, Herbarium, Boulder, CO 80309, U.S.A.*

Abstract. *Lecanora markjohnstonii* is described as new to science from the southeastern United States, with a primary center of distribution in the southern Appalachian Mountain region. This sterile, sorediate crust is saxicolous on both sandstone and granite and occurs commonly in mixed hardwood-conifer forests with rock outcrops. It is characterized by a gray-green, rimose-areolate thallus, erumpent, raised soralia, and the production of atranorin together with 2-*0*-methylperlatolic acid. Molecular phylogenetic analyses of newly generated rDNA assemblies from a broad sampling of lineages within the Lecanoromycetes and Arthoniomycetes inferred placement of the unknown crust in the Lecanoraceae, specifically within *Lecanora*. Analysis of the *mtSSU* gene region then inferred placement in the *Lecanora subfusca* group. Finally, a fully assembled and annotated mitochondrial genome was compared to other lichenized fungal mitogenomes, including the publicly available *Lecanora strobilina* mitogenome, and showed that the gene region *atp9* was missing as in other members of the Lecanorales.

Keywords. Asexual reproduction, biodiversity hotspot, endemism, genomics, Mark Johnston, natural history collections, new species, phylogenetics, phylogenomics, mitochondrial genome, taxonomic discovery.

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The southern Appalachian Mountains of eastern North America are a hotspot of diversity for many organisms (ATBI 2016), including lichens (Allen & Lendemer 2016; Lendemer & Tripp 2016; Tripp & Lendemer 2019 [in press]). Great Smoky Mountains National Park alone—spanning a mere ca. 830 square miles (ca. 2201 km^2)—hosts nearly 1,000 species of lichens, representing perhaps half of all lichens that occur in the eastern United States (Lendemer et al. 2013; Tripp & Lendemer 2019 [in press]). In recent years, large-scale lichen biodiversity inventories spearheaded by two of the authors (JL & ET) have resulted in the discovery and formal description of many species new to science from this biodiversity hotspot (e.g., Lendemer et al. 2013; Lendemer et al. 2014; Harris et al. 2014; Lendemer et al. 2017; Tripp & Lendemer, in press). Several of the new species uncovered as a result of this work appear to be narrow, range-restricted endemics to the iconic, globally unique, and threatened high-elevation ecosystems of the region (Allen & Lendemer 2015; Lendemer et al. 2017; Tripp & Lendemer 2019 [in press]; Tripp & Lendemer [in press]). These newly discovered species can be considered evidence for reinforcing the ecological importance and biodiversity value of high elevation habitats in the southern Appalachians (Dey 1978; Evans 1947; Lendemer & Allen 2015; Wei & Ahti 2002).

Lichenological study in the southern Appalachians has generally emphasized these charismatic and ecologically relatively intact high elevation ecosystems compared to middle and lower elevations (Degelius 1941; Lendemer & Tripp 2008). Middle and low elevations of the southern Appalachians are

[5] Corresponding author's e-mail: carly.anderson@colorado.edu

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typically characterized by natural landscapes that are comparatively much more fragmented and disturbed (McConnel 2013; White 1984; Wiser et al. 1996). Nonetheless, endemic, rare, or geographically disjunct species are not restricted to the highest elevations of the region: a remarkable diversity of lichens has been documented to occur at low and middle elevations (Lendemer & Tripp 2008, Tripp & Lendemer [in press]; Tripp & Lendemer unpublished data), and these records include a plethora of endemic, rare, and/or disjunct lichen elements (Lendemer et al. Sheard 2014; Muscavitch & Lendemer 2016; Tripp & Lendemer [in press]).

In 2015, authors ET and JT initiated a large-scale, multi-year project aimed at understanding correlates of lichen biodiversity gradients in the southern Appalachian Mountains. This project has involved the establishment of numerous study plots throughout the region, and it was during this work and on numerous occasions that we encountered a chemically and morphologically distinctive sorediate and apparently sterile crustose lichen occurring on sandstone and granite outcrops. A search of the relevant literature on sorediate crustose lichens failed to reveal an existing name for the material (Hodkinson & Lendemer 2012; Lendemer 2010). Although it was readily recognizable even in the field, sexual reproductive structures were unknown in the species, and neither morphology nor chemistry alone or in combination were distinctive enough to assign this unknown entity to a genus or family. Therefore, we used genomic data and associated bioinformatic techniques that we generated and developed for the southern Appalachian lichen project to infer phylogenetic relatedness of the new species to its closest relatives.

First, we generated and assembled rDNA contigs representing diverse lineages of lichen-forming fungi to infer the placement of the focal species within the Lecanoromycetes. Second, building on an inferred placement in the Lecanoraceae, specifically within the genus *Lecanora*, we conducted a finer scale phylogenetic analysis using mtSSU data retrieved from a newly generated, assembled, and annotated mitochondrial genome of the focal species, together with mtSSU data from other species of *Lecanora* retrieved from previously published matrices, to infer placement within *Lecanora*. Finally, we compared mitogenome characteristics of the new species with other lichenized fungal genomes to assess whether the full complement of coding regions was present in the new species. Our results support the hypothesis that this species is new to science and endemic to southeastern United States where it is distributed primarily at low and middle elevations of the southern Appalachian Mountains and foothills. We present the results of these analyses below and formally describe the species as *Lecanora markjohnstonii*.

Materials and Methods

Morphology and chemistry. This study is based on material collected as part of our southern Appalachian lichen project, together with specimens already deposited in herbaria at the University of Colorado (COLO) and New York Botanical Garden (NY). Georeferenced voucher metadata for all collections examined are available online via the C.V. Starr Virtual Herbarium (http://sweetgum.nybg.org/science/vh/), at the COLO internal database (https://botanydb.colorado.edu/index.php), and also on SEINet (http://swbiodiversity.org/seinet/) and iDigBio (https://www.idigbio.org/).

Morphology was investigated using an Olympus SZX10 stereomicroscope as well as an Olympus BX51 compound epifluorescent microscope. Micromorphology and anatomy were studied using hand sections of the thallus and squash mounts of the propagules in water. All microscopic measurements were obtained using a Retiga 2000R optical imaging system. Chemistry was studied using standard spot test protocols and reagents following Brodo et al. (2001). Thin Layer Chromatography was conducted using Solvents A and C, following Culberson & Kristinsson (1970). The presence of 2-*0*-methylperlatolic acid in the specimens examined was confirmed by comparison to known standards for secondary compounds with similar profiles in TLC. Specifically, reference standards for 2-*0*-methylperlatolic acid (*Loxospora confusa* and *Lecanora pseudistera*), barbatic acid (*Cladonia didyma* var. *didyma*), confluentic acid (*Lecidea tessellata*), divaricatic acid (*Lepraria hodkinsoniana*), perlatolic acid (*Ropalospora viridis*), and sphaerophorin (*Cladonia petrophila*) were used.

Molecular data generation and analyses. Molecular data newly generated for this study were derived entirely from vouchers collected during our southern Appalachian lichen project. We analyzed two sources of molecular data: rDNA contigs from the nuclear genome, and sequence data from the

mtSSU region. A two-step procedure was used to infer phylogenetic placement. First, we examined higher-level phylogenetic relationships of the taxon to other lichens by analyzing newly generated rDNA contigs from a phylogenetically broad set of southern Appalachian taxa (n=74) spanning numerous major lineages within the Lecanoromycetes (n=26 families) in addition to several Arthoniomycetes (**Table 1**). Second, based on results of this analysis that inferred placement in the Lecanoraceae, we examined relationship of the new taxon to other members of Lecanoraceae specifically through analyses of mtSSU sequence data that we retrieved from a newly assembled mitochondrial genome of the new species together with the six-locus dataset published by Zhao et al. (2015).

Laboratory and bioinformatics protocols. Subsamples for DNA extraction were removed from voucher specimens in the field within ~10 hours of initial collection, and subsequently stored at –20°C until return to COLO where they were transferred to a –80°C freezer until extraction. Lichen tissue was pulverized using sterile tungsten carbide beads, and DNA was extracted using Qiagen DNEasy plant kits (Qiagen 2006). To improve lichen DNA concentration, the tissue was subjected to an additional 10 min 65°C incubation step in lysis buffer and washed with pure ethanol prior to elution (Pogoda et al., unpublished data). Whole genome shotgun sequencing libraries were prepared using Nextera XT DNA library kits from Illumina optimized for 1ng of input DNA (Nextera 2017). Sample DNA was quantified using a Qubit 3.0 fluorometer (ThermoFisher Scientific) and then diluted or concentrated to obtain optimal concentration. Nextera adapters i5 and i7 were used as dual-index barcodes to uniquely identify this sample. After quality control assays, libraries were sequenced on the Illumina NextSeq platform for paired-end 150 bp reads. Sequencing was conducted at the COLO BioFrontiers Institute Next-Generation Sequencing Facility in Boulder, Colorado.

Using raw, whole-genome shotgun data, reads were trimmed using Trimmomatic-0.36 (Bolger et al. 2014) with the following parameters: "ILLUMINACLIP:NexteraPE PE.fa:2:20:10MINLEN:140 LEADING:20 TRAILING:20" (Bankevich et al. 2012). The resulting assemblies are composed not only of the primary mycobiont, but also the photobiont and other organisms present at the time of sampling. The primary mycobiont rDNA contig was identified via a command-line BLAST search using *Lecanora cinereofusca* rDNA [NCBI accession KY406736] as a query. The rDNA contig was then web BLASTed against the NCBI non-redundant database to confirm primary mycobiont DNA. The resulting assembly was used for subsequent phylogenetic analyses. All rDNA contigs used in this study have been deposited at NCBI and accession numbers can be found in **Table 1**.

rDNA dataset taxon sampling and assembly. To assess the family-level position of the unknown species, a total of 71 newly-assembled rDNA contigs (in addition to the species in question) were chosen to represent a breadth of species within class Lecanoromycetes. These species included individuals within the Caliciaceae (n=3), Cladoniaceae (n=2), Collemataceae (n=3), Gomphillaceae (n=2), Graphidaceae (n=2), Icmadophilaceae (n=1), Lecanoraceae (n=5). Lecidaceae (n=1), Lobariaceae (n=4), Megalosporaceae (n=1), Nephromataceae (n=2), Ochrolechiaceae (n=1), Pannariaceae (n=3), Parmeliaceae (n=21), Pertusariaceae (n=4), Phlyctidaceae (n=1), Physciaceae (n=3), Pilocarpaceae (n=1), Ramalinaceae (n=2), Rhizocarpaceae (n=1), Ropalosporaceae (n=1), Sarrameanaceae (n=2), Stereocaulaceae (n=3), Teloschistaceae (n=1), Trapeliaceae (n=2), and Umbilicariaceae (n=1). Species of Arthoniaceae (n=2; class Arthoniomycetes) were included as outgroups with which to root the tree.

mtSSU dataset taxon sampling and assembly. In order to place the new species in the context of a recent and well-supported multi-gene tree, the mtSSU region was retrieved from our assembled mitogenome and manually added to a six-locus (mtSSU, nucITS, nucLSU, nucSSU, *RPB1*, *RPB2*) alignment used by Zhao et al. (2015) to infer the phylogeny of Lecanoraceae. The tree was downloaded from TreeBASE(http://purl.org/phylo/treebase/phylows/study/TB2:S15652). Additionally, a BLAST search of the mtSSU region of the new crust revealed high similarity (97%) to *Lecanora orientoafricana* (Kirika & Lumbsch 2012); therefore, this species was also included in the alignment.

Phylogenetic analyses. The rDNA dataset was aligned using MUSCLE-3.7 with default parameters (Edgar 2004) via the CIPRES Science Gateway (Miller et al. 2010). This alignment was then manually curated using Mesquite-3.04 (Maddison & Maddison 2015), with ambiguously aligned

Table 1. Species used in phylogenetic analyses and their corresponding GenBank accession numbers. Refer to Zhao et al. (2015) for all accession numbers of the six-locus *Lecanora* dataset used for the mtSSU phylogenetic analysis in this study. In addition to *L. markjohnstonii, L. orientoafricana* was added to Zhao's original dataset; therefore, that accession is included here.

Species	GenBank accession number	Sequence type
Alectoria fallacina	MH887467	rDNA contig
Anzia colpodes	MH887468	rDNA contig
Arthonia anglica	MH887469	rDNA contig
Arthonia susa	MH887470	rDNA contig
Bryoria bicolor	MH887471	rDNA contig
Bryoria nadvornikiana	MH887472	rDNA contig
Buellia stillingiana	MH887473	rDNA contig
Bulbothrix isidiza	MH887474	rDNA contig
Byssoloma subdiscordans	MH887475	rDNA contig
Caloplaca camptidia	MH887476	rDNA contig
Cetrelia cetrarioides	MH887477	rDNA contig
Cetrelia chicitae	MH887478	rDNA contig
Cladonia coniocraea	MH887479	rDNA contig
Cladonia mateocyatha	MH887480	rDNA contig
Collema subflaccidum	MH887481	rDNA contig
Crocodia aurata	MH887528	rDNA contig
Dimelaena oreina	MH887482	rDNA contig
Fuscopannaria leucosticta	MH887483	rDNA contig
Gomphillus americanus	MH887484	rDNA contig
Gomphillus calycioides	MH887485	rDNA contig
Graphis lineola	MH887486	rDNA contig
Graphis scripta	MH887487	rDNA contig
Herteliana schuyleriana	MH887488	rDNA contig
Heterodermia casarettiana	MH887489	rDNA contig
Heterodermia hypoleuca	MH887490	rDNA contig
Hypogymnia krogiae	MH887491	rDNA contig
Hypogymnia vittata	MH887492	rDNA contig
Hypotrachyna imbricatula	MH887493	rDNA contig
Hypotrachyna virginica	MH887494	rDNA contig
Icmadophila ericetorum	MH887495	rDNA contig
Lasallia pensylvanica	MH887496	rDNA contig
Lecanora albella	MH887497	rDNA contig
Lecanora cinereofusca	MH887498	rDNA contig
Lecanora hybocarpa	MH887499	rDNA contig
Lecanora markjohnstonii	MH887500	rDNA contig
Lecanora markjohnstonii sp. nov	MH221526	Full mitochondrial genome
Lecanora masana	MH887501	rDNA contig
Lecanora orientoafricana	JQ900617	mtSSU
Lecanora rugosella	MH887502	rDNA contig
Lecidea tessellata	MH887503	rDNA contig
Lepra amara	MH887504	rDNA contig
Lepraria caesiella	MH887505	rDNA contig
Lepraria finkii	MH887506	rDNA contig
Leptogium austroamericanum	MH887507	rDNA contig
Leptogium corticola	MH887508	rDNA contig
Lobaria pulmonaria	MH887509	rDNA contig
Loxospora confusa	MH887510	rDNA contig
Loxospora elatina	MH887511	rDNA contig
Megalospora porphyritis	MH887512	rDNA contig
Menegazzia subsimilis	MH887513	rDNA contig
Myelochroa aurulenta	MH887514	rDNA contig
Nephroma helveticum	MH887516	rDNA contig
Nephroma helveticum 2	MH887515	rDNA contig
Ochrolechia trochophora	MH887517	rDNA contig
Parmeliella appalachensis	MH887518	rDNA contig
Parmeliella triptophylla	MH887519	rDNA contig
Parmotrema cetratum	MH887520	rDNA contig
Parmotrema stuppeum	MH887521	rDNA contig
Pertusaria globularis	MH887522	rDNA contig
Phaeophyscia langdoniana	MH887542	rDNA contig
Phlyctis boliviensis	MH887523	rDNA contig
Phyllopsora corallina	MH887524	rDNA contig
Platismatia glauca	MH887525	rDNA contig
Platismatia tuckermanii	MH887526	rDNA contig
Pseudevernia cladonia	MH887527	rDNA contig
Pseudocyphellaria perpetua	MH887529	rDNA contig
Pseudosagedia isidiata	MH887530	rDNA contig
Pseudosagedia rhaphidospermum	MH887531	rDNA contig
Punctelia appalachensis	MH887532	rDNA contig
Pyxine sorediata	MH887533	rDNA contig
Rhizocarpon rubescens	MH887534	rDNA contig
Ropalospora chlorantha	MH887535	rDNA contig
Stereocaulon pileatum	MH887536	rDNA contig
Sticta fuliginosa	MH887537	rDNA contig
Trapelia coarctata	MH887538	rDNA contig
Usnea ceratina	MH887539	rDNA contig
Usnea subfusca	MH887540	rDNA contig
Xanthoparmelia mexicana	MH887541	rDNA contig

regions excluded from the alignment. The six-locus dataset from Zhao et al. (2015) was manually split into its constituent loci in Mesquite. For each locus, terminal gaps and missing sequences were transformed to missing data, while gaps within the locus were left as true gaps. The six datasets were then reassembled in Mesquite and a partition file was created.

For both datasets, we used maximum likelihood (ML) methods implemented in RAxML-8.0 (Stamatakis 2014) to infer phylogenetic relationships among taxa. Default parameters were used and 1000 bootstrap iterations were conducted to assess branch support. Following Zhao et al. (2015), GTRGAMMA was chosen to model rate substitution (gamma rate parameters, GTR rates, and base

frequencies optimized individually for each partition). Resulting trees were visualized in FigTree-1.4.2 (Rambaut 2009) and figures were prepared in Adobe Illustrator CS6-16.0.0 (2012).

Mitochondrial genome assembly. To compare mitogenomic characteristics of the new species with other available lichen mitogenomes, and specifically to assess whether the core energy producing pathway gene *atp9* was present or lacking (see Pogoda et al. 2018), we assembled and annotated a complete mitochondrial genome of the new species. The mitochondrial genome of the primary mycobiont was parsed via a command-line BLAST to the *Lecanora strobilina* mitochondrial reference genome [NCBI accession NC_030051]. It was then circularized from raw reads, oriented to *cox1*, and error-corrected using SAMtools *tview*. Features were annotated using DOGMA (Wyman et al. 2004) and NCBI's Sequin. All alignments and tree datasets used for phylogenetic analysis have been deposited to Zenodo (mtSSU alignment DOI 10.5281/zenodo.1308261 and tree DOI 10.5281/zenodo.1308256; rDNA alignment DOI 10.5281/zenodo.1308259 and tree DOI 10.5281/zenodo.1308252).

Results

We examined a total of 14 specimens collected throughout the southeastern United States that were accessioned as unknown species, other species, or were tentatively assigned to a potentially undescribed lichen in the field. All of the collections were made from exposed sandstone or granite outcrops in relatively shaded forested habitats at low and middle elevations (**Fig. 1**). The specimens were characterized morphologically by having gray-green, rimose-areolate thalli and abundant, erumpent soralia containing fine soredia that were lighter in color than the thallus (**Fig. 2**). All of the specimens examined were also chemically uniform in the production of atranorin together with 2-*0*-methylperlatolic acid.

Our phylogenetic analyses recovered the new species within the Lecanoraceae, embedded in a strongly supported clade (ML bootstrap support BS=87%) of selected species of *Lecanora* belonging to the *L. subfusca* group (*sensu* Brodo 1984) with exception of *L. albella* (Pers.) Ach. that is related to *L. caesiorubella* Ach. (Imshaug & Brodo 1966). Results of phylogenetic analyses of rDNA assemblies compiled from newly generated sequences from diverse lineages within the Lecanoromycetes (**Fig. 3**) were topologically congruent with previously published phylogenies inferred from multi-locus datasets aimed at resolving evolutionary relationships within the Lecanoromycetes (Miadlikowska et al. 2014). Relationships within the clade of *Lecanora* species were not strongly supported; the species in question was recovered in a non-supported (BS=68%) sister relationship with *L. masana* Lendemer & R.C.Harris.

Figure 1. Ecology and habit of *Lecanora markjohnstonii*. **A.** The new species growing on exposed vertical rock face of small boulder, shown with arrow (Little River National Preserve, DeKalb Co., Alabama). **B & C.** A typical rock outcrop habitat in mixed hardwood forest (James D. Martin-Skyline Wildlife Management Area, Jackson Co., Alabama; Rebecca Mountain, Talladega National Forest, Clay Co., Alabama.)

Our analyses of the mtSSU/six-locus dataset recovered the new species in a strongly supported (BS=100%) sister relationship to *Lecanora orientoafricana* (Kirika & Lumbsch, 2012) and embedded within a well-supported clade (BS=82%) comprising members of the *L. subfusca* group, *L. subcarnea* (Sw.) Ach. group, and *L. formosa* (Bagl. & Carestia) Knoph & Leuckert (Knoph & Leuckert 2000; **Fig. 4**).

Based on the results outlined above, the focal species for this study is supported as belonging to the

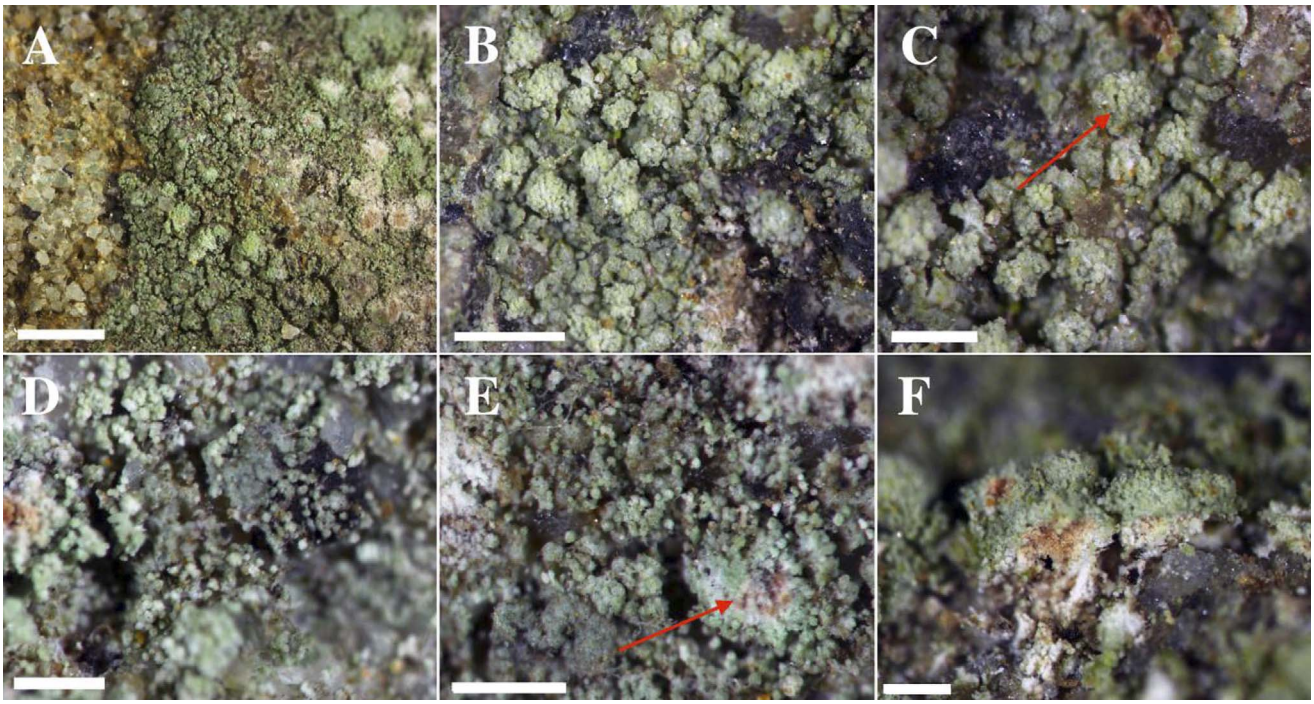

Figure 2. Morphology of *Lecanora markjohnstonii*. **A.** Thallus edge viewed from above showing cracked appearance. **B.** Detail of irregularly shaped soralia. **C.** Detail of erumpent soralium bursting to produce individual granular soredia (arrow shows opening in the soralium). **D.** Detail of thallus surface in early stages of development. **E & F.** Sections through large soralia showing visible white medulla and pigmented hyphae. (A, D and E from *Lendemer 49284*; B, C, and F from *Tripp 8033*) Scales= 2.0 mm in A, 1.0 mm in B, and 0.5 mm in C–F.

Lecanoraceae, and specifically a member of the genus *Lecanora* as presently delimited (e.g., Zhao et al. 2015).

The fully assembled mitochondrial genome (**Fig. 5**) was 62,854 base pairs in length, in contrast to *Lecanora strobilina*, which is 39,842 base pairs in length. The 14 conserved lichen genes found in the mitogenomes of other lichenized fungi (*cob, cox1, cox2, cox3, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, atp6, atp8*, and *rps3*; Pogoda et al. 2018), including *L. strobilina*, were all found to be present in this new crustose lichen. Additionally, the new species was found to lack the protein-coding gene *atp9*, which is similar to other members of the Lecanorales (Pogoda et al. 2018).

After comparing the species to morphologically and chemically similar taxa that have already been described, we concluded that it represents a species new to science and formally describe it below.

Taxonomy

Lecanora markjohnstonii And. Stewart, E.Tripp & Lendemer, *sp. nov.* **Fig. 2**
MycoBank MB 826867

Similar to Lecanora orientoafricana but differing in the production of 2-0-methylperlatolic acid (vs. gangaleoidin) as an accessory to atranorin, saxicolous (vs. corticolous), habitat, and geographical distribution (southeastern United States vs. eastern Africa).

Type: U.S.A. ALABAMA: DeKalb Co., Little River Canyon National Park, E facing slopes above W shore of Little River, Eberhart Trail 0.2 mi S of AL176/Little River Canyon Parkway, 0.2 mi N of jct w/ DeKalb CR148, elev 1123 ft., steep slope with sparse talus, abundant rock outcrops, and mixed hardwood and conifer forest (*Acer saccharum*, *Carya* spp., *Liriodendron tulipifera*, *Nyssa sylvatica*, *Pinus taeda*, *P. virginiana*, *Prunus serotina*), on sandstone, 19 Dec. 2016, *E.A. Tripp 6296 & J.C. Lendemer* (holotype: COLO!; isotype: NY!)

Description. Thallus crustose, sorediate, light green to grayish green when fresh, fading to dusty rose with age in the herbarium, thin, poorly-developed and areolate to thick, with the areoles becoming confluent and then forming a thick, continuous rimose, fissured crust, often forming

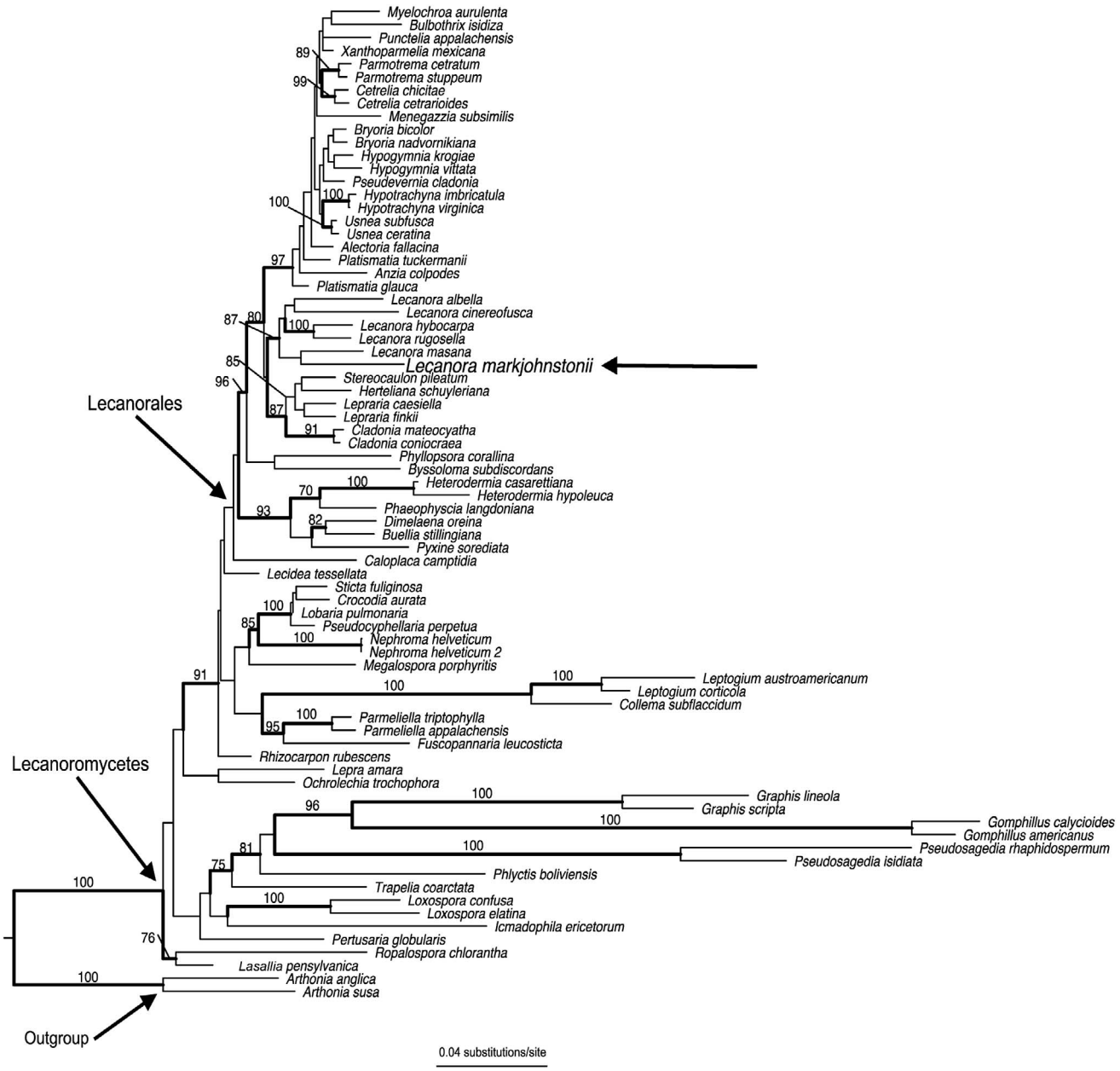

Figure 3. Inferred maximum likelihood phylogenetic tree of newly-generated rDNA contig sequences; representatives from Arthoniomycetes selected as outgroup. Branches greater than or equal to 70% bootstrap are bolded and labeled with values. Nodes corresponding to the Lecanoromycetes and Lecanorales clades are shown.

individual colonies, ±circular and varying from ca. 0.5–10 cm in diameter; prothallus typically not evident, rarely visible as a poorly developed, white, fibrous carpet between the areoles and extending outward from the edge of the thallus; upper surface light green to grayish green, epruinose, dull, uneven to strongly verruculose and then becoming fissured; upper cortex indistinct and poorly developed, medulla creamy white throughout and becoming orangish brown pigmented in core of soralia (the pigment K–); soralia raised, erumpent, plane, up to 1.2 mm in height, circular, laminal, 0.5–1.3 mm in diameter; soredia ±globose, fine, ca. 23–28 μm in diameter, light yellowish green and strikingly lighter than the surrounding thallus, often dispersed across the surface of the thallus and adjacent areas of uncolonized substrate. Apothecia not seen. Pycnidia not seen. Photobiont coccoid (Trebouxioid), with visible pyrenoids, cells globose, ca. 5.4–11.4 μm in diameter.

Figure 4. Inferred maximum likelihood phylogenetic tree of Lecanoraceae inferred from Zhao et al. (2015) 6-locus dataset plus the mtSSU sequence from the new crust and *Lecanora orientoafricana,* with *Protoparmelia* and *Letharia* as outgroups. Branches with support greater or equal to 70% bootstrap are bolded and labeled with values.

Chemistry. Atranorin and 2-*0*-methylperlatolic acid. Spot tests: K+ yellow, C–, KC–, P–, I–, UV+ weak dull orange (cortex) and UV+ faint blue-white (medulla).

Etymology. The epithet "markjohnstonii" honors Mark Johnston. As a priest in the Episcopal Church, he was the Executive Director of Camp McDowell for over 27 years. While there, McDowell became the largest camp in the Episcopal Church, and he started the McDowell Environmental Center, which is the largest environmental center in the southeastern United States. During fieldwork in 2017, Johnston was instrumental in facilitating a canoe trip down remote sections of the Sipsey River, located in Bankhead National Forest in northern-central Alabama, in search of lichens in remnant old-growth forests as part of our southern Appalachian lichen biodiversity investigation. Johnston's lifelong contributions have helped to improve the lives of Alabamians through environmental education and outreach at Camp McDowell. In addition to educating tens of thousands of students over the decades, Mark is a champion of Alabama's wildlands, working to preserve the state's natural heritage and natural resources. Most of his life has been spent actively in the pursuit of social and environmental justice. Mark lives with his wife Maggie, five rescue dogs, chickens, and gardens in a house in the forest that he built himself. He and Maggie have four children and three grandchildren. Besides being passionate about changing the world, Mark is passionate about Alabama's incredible biodiversity and enjoys carpentry, gardening, running, paddling fishing, and hunting.

Ecology and distribution. *Lecanora markjohnstonii* is known from low and middle elevations (mean elevation 356.55 m) scattered throughout the southern terminus of the Appalachian Mountains and extending into the Piedmont of the eastern United States (**Fig. 6**). This new species can be locally abundant on inclined, exposed, but shaded sandstone and granite rock outcrops located in

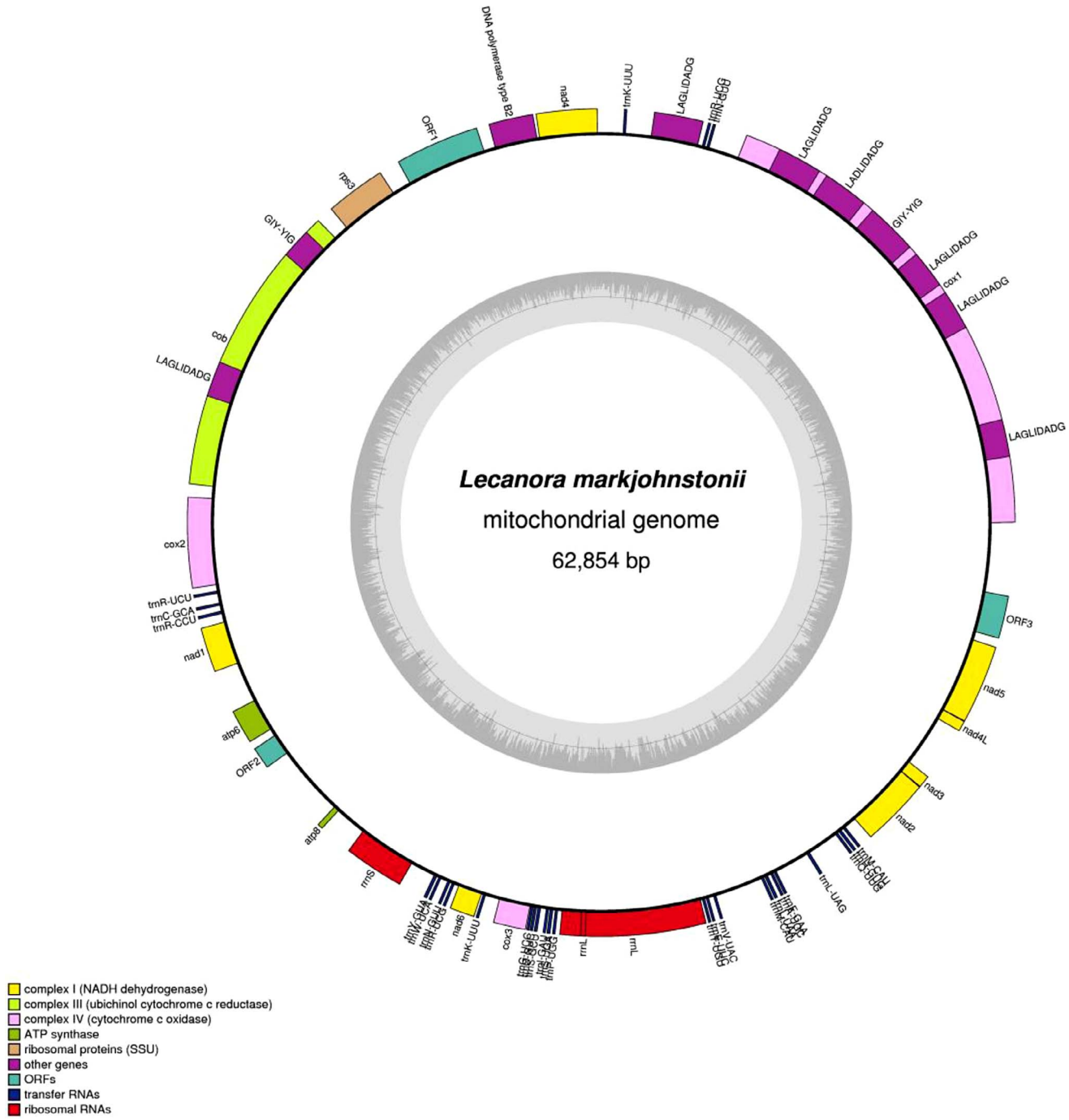

Figure 5. Annotated 62,854 base-pair mitochondrial genome of *Lecanora markjohnstonii*. Specific types of genes or loci are color-coded as denoted in the legend.

intact, forested habitats characterized by mixed native deciduous and conifer trees. Some localities in which the species was collected included both calcareous (e.g., limestone) and non-calcareous rocks (**Fig. 1**), but the new species was found exclusively on the latter at such sites. Mean annual precipitation within the species range is ca. 1444.60 mm, mean temperature is 18.5°C, and net primary productivity (NPP) is 8285 g C m^{-2} yr^{-1}.

Conservation. In the southern Appalachian Mountains, high-elevation lichen communities including the endemics that occur in these ecosystems have been the focus of considerable study from a conservation perspective (Allen & Lendemer 2016;

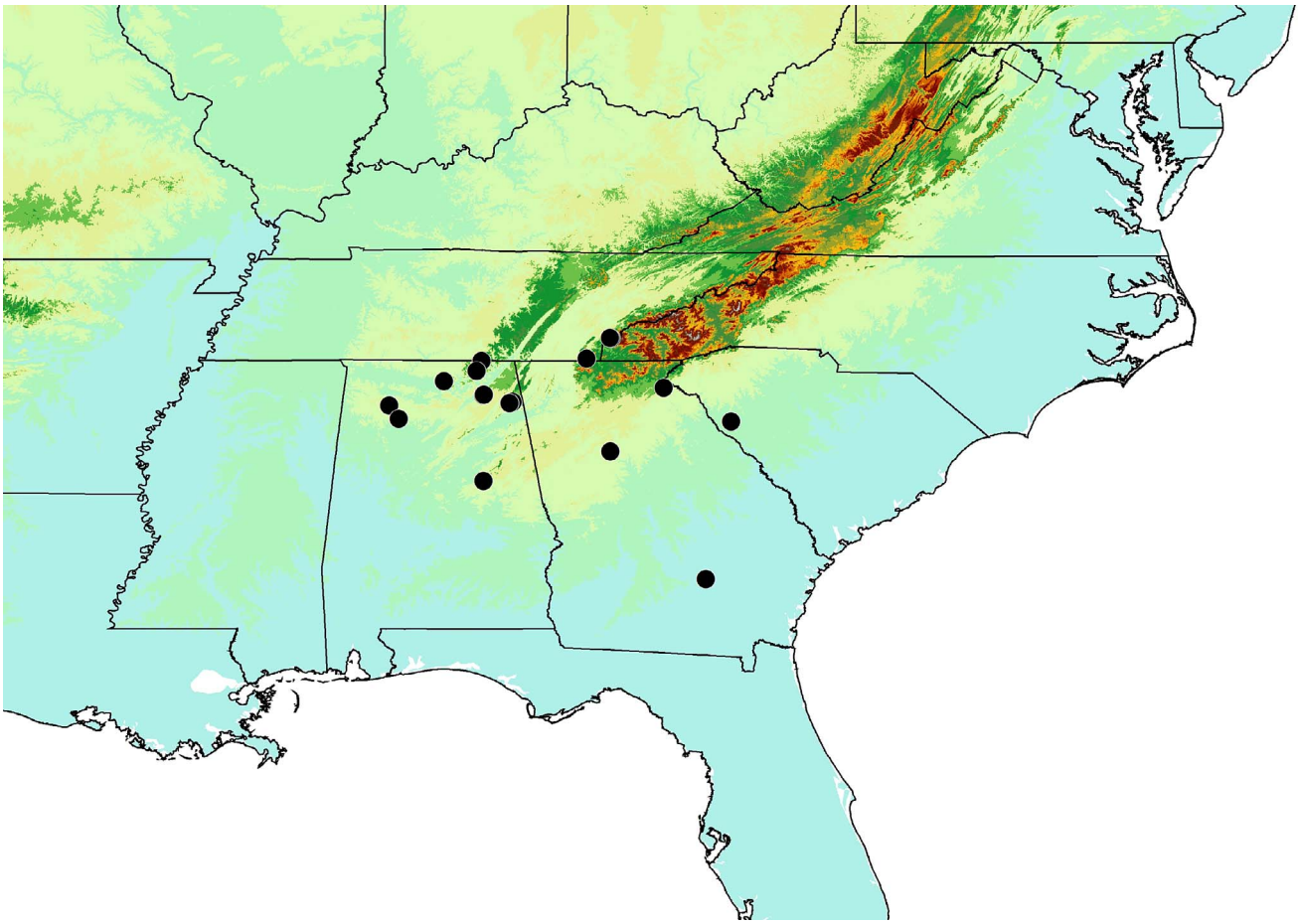

Figure 6. Geographical distribution of *Lecanora markjohnstonii* based on specimens examined for this study. Color variances show an additional 100m elevational gain per color change.

Lendemer et al. 2013). Our description of a unique, highly distinctive species that appears to be endemic to low- and middle-elevation non-calcareous rock outcrops in the southeastern United States highlights the potential for discovery in these environments. Although the southeastern United States is known to host numerous rare and endemic vascular plant species (Baskin & Baskin 1988; Murdock 1994; Noss et al. 2014), such habitats are comparably much less studied from a lichenological standpoint (but see Allen & Lendemer 2016). Further study of rock outcrop lichen communities in the southeastern United States is urgently needed to understand more fully the distributions of endemic lichens and other unusual elements that likely occur there, as well as to document their population sizes and assess potential conservation needs.

Notes. *Lecanora* is the most diverse genus of lichens in North America, containing >235 species (Esslinger 2018). It is also the most diverse genus in the southern Appalachians, where ca. 40 species are known from Great Smoky Mountains National Park alone (Lendemer et al. 2013; Tripp & Lendemer, 2019 [in press], Tripp & Lendemer [in press]) and at least nine are known from the small confines of Mount Mitchell State Park (Lendemer et al. 2017). Although phylogenetic relationships within Lecanoraceae are not fully resolved and *Lecanora* itself is polyphyletic (Miadlikowska et al. 2014; Zhao et al. 2015), our analyses suggest strongly that *L. markjohnstonii* should be assigned to this genus as presently delimited. However, owing to incomplete taxon sampling across highly species-rich groups within *Lecanora* including the *L. subfusca* group, it is not possible to infer with confidence the relationship of this species to its closest sister species.

Based on our molecular phylogenetic analyses, *Lecanora markjohnstonii* is likely closely related to *L. masana* and *L. orientoafricana*. However, it differs from both of those species in multiple characters.

Lecanora masana is a corticolous species narrowly endemic to high elevation habitats in the southern Appalachians (Allen & Lendemer 2015) and thus differs from *L. markjohnstonii* in both substrate and geographic distribution. It also differs morphologically from the new species in being fertile and having an esorediate thallus, and chemically in the production of usnic acid together with arthothelin as accessories to atranorin and 2-*0*-methylperlatolic acid (Lendemer et al. 2013). *Lecanora orientoafricana* is similar to *L. markjohnstonii* in having a sorediate thallus; however, it differs in occurring on bark rather than rock, the production of gangaleoidin rather than 2-*0*-methylperlatolic acid as an accessory to atranorin, and is known only from eastern Africa (Kirika et al. 2012).

Lecanora masana and *L. orientoafricana* (the latter only known from Kenya) are not the only members of *Lecanora* known to produce 2-*0*-methylperlatolic acid. In fact, a number of such species have been described from temperate and tropical regions worldwide (Brodo 1984; Guderley 1999; Lumbsch 1994). The vast majority of such taxa differ from *L. markjohnstonii* in being corticolous or lignicolous and in having esorediate thalli. Indeed, this is the case for *L. dispersogranulata* Szatala, *L. epirhoda* Vain., *L. helva* Stizenb., *L. iseana* Räsänen, *L. labiosa* Stizenb., and *L. toroyensis* Zahlbr. (Elix & Lumbsch 1996; Guderley 1999; Lumbsch 1994; Miyawaki 1988; Stizenberger 1890; Zahlbruckner 1933). Several additional corticolous, esorediate species from various geographical regions differ further from *L. markjohnstonii* in the production of accessory substances such as psoromic acid (*L. paramerae* I.Martínez, Aragón & Lumbsch), usnic acid (*L. achroa* Nyl.) and xanthones (*L. mikuraensis* Miyaw., *L. pangerangoensis* Zahlbr.; Elix & Lumbsch 1996; Lumbsch 1994; Martínez et al. 1999). One chemically similar corticolous species, *L. novaeguineae* Lumbsch, is also sorediate; however, it differs from *L. markjohnstonii* in the additional production of norstictic acid (Lumbsch 1994). *Lecanora appalachensis* Lendemer & R.C.Harris, *L. nothocaesiella* Lendemer & R.C.Harris, *L. layana* Lendemer and *L. thysanophora* R.C.Harris are four additional sorediate species that can occur in the southern Appalachians and produce atranorin; however, all are corticolous and produce zeorin or usnic acid as accessories to atranorin (Harris et al. 2000; Lendemer et al. 2013; Lendemer 2015). Additionally, *L. thysanophora* produces a well-developed white prothallus (Harris et al. 2000).

In contrast to the above, a smaller number of *Lecanora* species that produce atranorin and 2-*0*-methylperlatolic acid are saxicolous (e.g., *L. cenisioides* Lumbsch, *L. gongesiana* Miyaw., *L. neosonorensis* Lumbsch & T.H.Nash, *L. plumosa* Müll.Arg., *L. puniceofusca* Bagl. and *L. rhodi* Szatala). Nonetheless all of those species differ from *L. markjohnstonii* in having esorediate thalli (Dickhäuser et al. 1995; Elix & Lumbsch 1996; Lumbsch 1994; Lumbsch & Nash 1995; Miyawaki 1988).

As opposed to other members of the genus *Lecanora*, *L. markjohnstonii* is perhaps most likely to be confused with sorediate and typically sterile crustose lichens from the southeastern United States, particularly *Biatora chrysantha, Herteliana schuyleriana* Lendemer, *Loxospora confusa* and *Vainionora americana*. In particular, *Vainionora americana* Kalb, Tønsberg & Elix is morphologically similar in thallus morphology and color, and also produces atranorin. While that species is typically corticolous, it occasionally also occurs on non-calcareous rock (Lendemer, unpublished data). Nonetheless, unlike *L. markjohnstonii*, *V. americana* produces xanthones as accessories to atranorin, and is sometimes found fertile (Kalb 2004). *Herteliana schuyleriana* is similar to the new species in that it produces atranorin, grows on non-calcareous rocks, and has a greenish-gray thallus. However, the former produces roccellic acid instead of 2-*0*-methylperlatolic acid, and has a well-developed blastidiate thallus (Lendemer 2016). *Biatora chrysantha* (Zahlbr.) Printzen is another saxicolous sorediate crustose lichen that occurs in the Appalachian Mountains and is superficially similar to *L. markjohnstonii* in having yellowish soralia and a poorly developed areolate thallus (Brodo et al. 2013). However, *B. chrysantha* is easily distinguished by the production of gyrophoric acid rather than atranorin and 2-*0*-methylperlatolic acid. Like *Lecanora markjohnstonii, Loxospora confusa* Lendemer produces 2-*0*-methylperlatolic acid and has a greenish-gray thallus (Lendemer 2013). However, that species does not produce atranorin, is corticolous, and produces fragile isidia rather than soralia.

Caloplaca yuchiorum Lendemer & C.A.Morse is an additional species that produces atranorin, is saxicolous, and is morphologically similar to the new species in having a thin, sorediate thallus (Lendemer & Morse 2010). However, *C. yuchiorum* does not

produce 2-*O*-methylperlatolic acid and is known to produce apothecia, unlike the new species. Similarly, *Fuscidea recensa* (Stirt.) Hertel, V.Wirth & Vězda could be confused with the new species, but it differs in chemistry (divaricatic acid) and the presence of a dark prothallus (Fryday 2008; Oberhollenzer & Wirth 1990).

Other specimens examined. U.S.A. ALABAMA: Cherokee Co., Little River National Preserve, S of Canyon View Forest Rd., 17 Dec. 2016, on sandstone. *J.C. Lendemer 49284 & E.A. Tripp* (NY). Clay Co., Talladega National Forest, Hollins Wildlife Management Area, Rebecca Mountain, S of AL148 at jct w/ FSR607 & Pinhoti Trail, 28 Mar. 2017, on rock, *J.C. Lendemer 50460* (NY). DeKalb Co., Little River Canyon National Park, E facing slopes above W shore of Little River, Eberhart Trail 0.2 mi S of AL176/Little River Canyon Parkway, 19 Dec. 2016, on rock, *J.C. Lendemer 49464 & E.A. Tripp* (NY). Jackson Co., Buck's Pocket State Park, along South Sauty Creek, 3 Oct. 1998, on rock, *R.C. Harris 42413* (NY); Buck's Pocket State Park, S facing slopes above N shore of South Sauty Creek, 0.2 mi W of Jackson CR452/DeKalb CR173, 15 Dec. 2016, on rock, *E.A. Tripp 6109 & J.C. Lendemer* (COLO, NY); James D. Martin-Skyline Wildlife Management Area, slopes above E shore of Hurricane Creek, along Walls of Jericho Trail 0.5 mi W of AL79, 24 Dec. 2016, on sandstone, *E.A. Tripp 6487 & J.C. Lendemer* (COLO, NY), *E.A. Tripp 6520 & J.C. Lendemer* (COLO, NY). Lawrence Co., Bankhead National Forest, Sipsey Wilderness Area, slopes above N shore of Sipsey River, 0.3 mi SW of confluence w/ Thompson Creek & 1.8 mi NE of confluence w/ Bee Branch, 7 Mar. 2017, on sandstone, *J.C. Lendemer et al. 51471* (NY). Madison Co., Red Stone Arsenal, ca. 0.25 mi SE from summit of Weedin Mountain, 25 May 2017, on sandstone, *C.J. Hansen 6994* (NY). Winston Co., Bankhead National Forest, Corinth Glade, W of CR57 1.6 mi S of AL74/US278, 13 May 2017, on sandstone, *J.C. Lendemer et al. 52019* (COLO, NY). GEORGIA: Coffee Co., Broxton Rock Ecological Preserve, vicinity of Rock Falls along Rocky Creek, 9 mi NE of Broxton, 3 Feb. 2003, on sandstone, *R.C. Harris 47038* (NY). Rockdale Co., Panola Mountain State Park, middle-low slopes of Panola Mountain, ca. 3 mi SW of Klondike, 17 Apr. 2007, on granite, *J.C. Lendemer 8985* (NY). Stephens Co., Chattahoochee National Forest, ca. 2 km above Toccoa Falls, along Toccoa Creek, 21 Sept. 1992, on rock, *R.C. Harris 28022* (NY). SOUTH CAROLINA: Abbeville Co., Sumter National Forest, Parsons Mountain, from jct. of FSR515 and 515B to summit, 17 Mar. 1997, on sandstone, *W.R. Buck 31741* (NY), *R.C. Harris 40309* (NY).

Conclusions

The southern Appalachian Mountains are a recognized hotspot for lichen biodiversity. The description of the present species adds to an already surprising number of typically sterile, asexually reproducing, crustose lichens that have been described from the region. At the same time, this new report contributes to the growing recognition of a substantial diversity of endemic lichens in the southeastern United States, and highlights the scale and scope of biodiversity that remains to be documented in this region. Despite the presence and frequency of herbarium specimens of *Lecanora markjohnstonii* collected more than two decades ago, its wide geographical distribution, and its distinctive morphology, this species is only now formally described. This situation is common in lichenology and highlights the need for more taxonomic and biodiversity studies of lichens in southeastern North America.

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