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MULTILOCUS PHYLOGENY OF THE LICHEN FAMILY MEGASPORACEAE

By

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Thesis

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MULTILOCUS PHYLOGENY OF THE LICHEN FAMILY MEGASPORACEAE

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Abstract:

The lichen symbiosis is one of the oldest studied mutualisms; in fact, Frank and De Bary coined the term "symbiosis" while studying lichens (Frank, 1877; De Bary 1879). The widespread, stable association between the mycobiont and photobiont in lichens offers an ideal system for the study of co-evolution. The recent application of molecular data to lichens has begun to unveil the complexities involved in these associations (Upreti et al. 2015, Spribille et al. 2016). Lichenized fungi make up a huge fraction of fungal diversity (Nash 2008), yet very little is known of their genetic diversity. Fungal taxonomy is notoriously difficult using the standard "barcoding" genes, so the deep relationships between most lichens are poorly resolved (Schoch et al. 2012). Here I apply a combination of Sanger and next-generation sequencing technologies to the cosmopolitan lichen family Megasporaceae to generate a robust phylogenetic tree for the group. The genus *Aspicilia* is highly diverse, poorly collected, common across all continents, and lives on a wide range of substrates and environments (Nordin et al. 2010). *Aspicilia* is principally a genus of crustose lichens inhabiting rocks (saxicolous), although a few species are known to inhabit soil and wood. They are usually tightly attached to the substrate, with a few groups occasionally lifting off the substrate and becoming semi-fruticose (3-dimensional). *Aspicilia* is one of five genera in the recently resurrected family *Megasporaceae* (Nordin et al. 2010) and the most speciose in the family, with over 230 valid names. Phylogenetic analyses confirmed the monophyly of the family Megasporaceae. The new genera *Antidea* and *Arctidea* are described. The recently described *Teuvoa* is subsumed within an expanded *Lobothallia*. The genera *Megaspora* and the recently resurrected *Aspiciliella* are subsumed within *Circinaria.* This study supports the segregation of the Megasporaceae into seven genera, *Antidea*, *Aspilidea, Lobothallia, Arctidea, Sagedia, Circinaria* and *Aspicilia*, describes three new species, proposes nineteen new combinations, and provides a robust phylogeny for the group that will be used as a backbone for future studies in the family.

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Chapter 1 MULTILOCUS PHYLOGENY OF THE LICHEN FAMILY MEGASPORACEAE

Introduction:

Theophrastus (371-284 BC) described a superficial growth on the bark of olive trees using the ancient Greek word λείχω, meaning to lick, possibly because lichens seem to lick the bark of the trees and rocks (Bioetymology, 2017), or because they are efficient at absorbing (licking up) water (Plitt, 1919). The first modern use of the word lichen appears in 1595, from the Greek word leikhēn, meaning "what eats around itself," perhaps in reference to the internal structure of the fungal hyphae enveloping the algae (Dictionary.com, 2017).

Even before the arrival of fungal nomenclature, a few lichens were mentioned in the botanical treatments of Tournefort and placed in the genus *Lichen* (Tournefort 1694,1698). He was the first to classify lichens as their own group of plants. Until Tournefort, lichens were variously classified as algae, fungi, mosses or liverworts.

Linnaeus, although considered the father of modern taxonomy, was no fan of lichens, referring to lichens as the 'rustici pauperrimi' of vegetation, variously translated as the "poor trash' or "poor little peasants" of nature (Jørgensen et al., 1994). However, in his *Species Plantarum*, Linnaeus describes 106 species, placing nearly all of them in the genus *Lichen* (Linnaeus, 1753). Two of these species are relevant to my thesis, *Lichen calcareus* (=*Circinaria calcarea*) and *Lichen cinereus* (= *Aspicilia cinerea*). One of Linnaeus's last students, Erik Acharius, who would later be called the father of lichenology (Nylander 1858; Vitikainen 1976), made the first detailed classification system for lichens. In a series of influential works, *Lichenographiae Suecicae Prodromus* (Acharius 1798), *Methodus* (Acharius 1803), *Lichenographia Universalis* (Acharius 1810), and *Synopsis Methodica Lichenum* (Acharius 1814), he described 40 genera and these works were the basis for modern lichenology. He transferred the few species of Megasporaceae treated in Lichenographia Universalis from the genus *Lichen* to *Urceolaria*. Noteworthy discoveries for my thesis by Acharius include the description of *Sagedia zonata*, and many new combinations, all of which are now synonymized within current genera in the Megasporaceae (Acharius 1810). However, neither Linnaeus nor Acharius imagined that lichens were composed of multiple unrelated organisms. This discovery was not uncovered until the end of the 19th century.

Classification of lichens based mostly on morphology continued throughout the 19th century, and many early lichenologists—(Fée (1824, 1837), De Notatis (1846), Trevisan (1853), Nylander (1854, 1855), Massalongo (1855), and Montagne (1856))—continuously updated the descriptions and relationships of lichens. With the evolving sharpness and clarity of microscopy, they started to include the use of microscopic characters such as spore size, shape, and color. Nylander pioneered the use of chemical reagents in the study of lichens, both in terms of staining (Iodine) and in secondary metabolite detection (KOH and C). He is credited as pioneering the use of the lichen spot test for the quick detection of secondary metabolites. This marked the beginning of the debate over the taxonomic significance of secondary metabolite production in lichens (Nylander, 1853, 1865).

It was during this time that Massalongo (1852) introduced the name *Aspicilia*. However, for the next 100 years, *Aspicilia* was mostly treated as a section in *Lecanora* (Zahlbruckner 1928, Magnusson 1939, 1951, but see Arnold, 1869). In 1858, Nylander classified lichens in a series of forms ranging from mostly algae-like to mostly fungal-like and believed that lichens were an evolutionary link between the two groups (Nylander, 1858). The big breakthrough came with the careful microscopic studies performed by Simon Schwendener in the late 19th century. Initially, Schwendener (1869) viewed lichen-forming fungi as parasites, although "with the wisdom of statesmen,'' and their algal partners as ''helotes,'' the class of slaves in ancient Sparta (Honegger, 2000). At about the same time, De Bary believed that some gelatinous lichens were the mature state of the cyanobacterium *Nostoc*, or "that these organisms are true algae, attacked by some Ascomycetes, whose hyphae penetrate the algae, and form the lichen thallus" (De Bary, 1866). Then in 1877, while studying crustose lichens, Frank introduced the term symbiosis, which was quickly adopted by de Bary (1879). However, a few years later, the British lichenologist, James Mascall Morrison Crombie, stated "it is clear that the Algo-lichen hypothesis rests upon no solid basis whatever, but simply and solely upon imagination..." Crombie continued, "Notwithstanding the laboured arguments by which it has been sought to deprive them of their autonomy and intrude them amongst the Ascomycetes, Lichens still remain a distinct class of plants, intermediate between the Algae and the Fungi" (Crombie, 1884). Seventeen years after Schwendener's discovery, Nylander still wasn't convinced of the duality of lichens, and in agreement with Crombie, held tightly to the idea that lichens "constitute a noble and venerable autonomous class of plants", and said "it is a true saying to-day that the formula

‘Lichens are Fungi living in a symbiosis with Algae,’ is an assertion either of pure fantasy or a slander” (Nylander, 1896). Nylander did not focus on the Megasporaceae, but he did introduce the name *Aspicilia intermutans* (=*Lecanora intermutans*) and *Aspicilia perradiata* (=*Arctidea perradiata*) (Nylander, 1872).

Regardless of the initial unfathomability of the dual hypothesis for many early lichenologists, the concept was eventually adopted. In 1913, the American lichenologist Bruce Fink boldly concluded that “... the lichen is a fungus pure and simple,” and offered the following: “The lichen is a fungus which lives during all or part of its life in parasitic relation with the algal host and also sustains a relation with an organic or an inorganic substratum” (Fink 1913). While it was now agreed that lichens were a consortium of unrelated organisms, the true nature of the relationships between the partners was still far from settled, and the debate still continues today (Spribille et al., 2016, Lucking et al., 2016).

Elias Magnus Fries, considered the founder of modern fungal taxonomy, introduced the names *Parmelia cinerea* var. *aquatica* (=*A. aquatica*), *Parmelia myrinii* (=*Aspilidea myrinii*), and *Lecanora cinerea* var. *obscurata* (=A. obscurata) (Fries, 1831; 1835; 1861). Theodor Magnus Fries, in his *Lichenographia Scandinavica*, introduced the species *A. cupreogrisea* and *A. griseola* (Fries, 1871). Tuckermann, in his *Genera Lichenum: an arrangement of the North American lichens*, placed the majority of *Aspicilia* species in the family Lecanoraceae (Lecanorei) sub family Eulecanorei in the section *Lecanora*. He was acutely aware of the convergent overlapping phenotypes in the group, mentioning that “the section (*Aspicilia*) is indeed, as are other sections, more largely developed in *Lecanora*, and has received proportionate attention; but the difference (depending on the more or less innate apothecium) upon which its distinction is based, is no less a subordinate one in this, than in the other. *Lecanora cinerea* is the well-known type of *Aspicilia*, and exhibits, or at least serves to explain, in mountainous countries, almost the whole circle of variations which distinguishes the group” (Tuckermann, 1872). Even today, we still don’t have a good handle on the phenotypic plasticity in many groups of lichens. In his synopsis of the North American lichens, Part I, Tuckermann treated the species *Lobothallia melanaspis, Circinaria calcarea, Circinaria contorta, Aspicilia cinerea, A. laevata, A. gibbosa,* and *Megaspora verrucosa* all under the genus *Lecanora* (Tuckermann, 1882). Arnold, in a series of publications on the lichens of Italy and Austria, was one of the earliest proponents of *Aspicilia* as its own genus separate from *Lecanora* (Arnold, 1869,1871,1887,1893). Hue, in a 1910 treatment of *Aspicilia*, mainly from Asia, also believed *Aspicilia* was deserved of the rank of genus, and described 58 new species and proposed 56 new combinations. Later, however, his taxonomic eye was seriously questioned by Magnusson and many of his Asian specimens warrant a more detailed inquiry (Hue, 1910; Magnusson 1939). However, most other lichenologists at the time still treated *Aspicilia* as a section of *Lecanora* (Tuckermann, 1882; Hasse, 1913).

At the turn of the 20th century in California, the lichenologist H. E. Hasse, in his treatment of the lichen flora of southern California, treated six species under *Lecanora* section *Aspicilia* (Hasse, 1913). Zahlbruckner, in his ten volumes of *Catalogus lichenum universalis*, placed members of the Megasporaceae in the genus *Lecanora* section *Aspicilia,* and introduced over 30 names, mainly from the Arctic and Asia, including *A. permutata, A. aliena, A. novae-semliae, A. plicigera, A. cingulata, A. hyperboreum, A. lyngei, A. sublapponica, A. heteroplaca, A. mashiginensis, A. disserpens, A. goettweigensis* (Zahlbruckner, 1928a; 1928b). Fink published widely on American lichens from 1890-1930 and is considered America's first great lichen taxonomist. His 1935 Lichen flora of the United States was an influential treatise and was in use well into the 20th century. He treated six species of Megasporaceae, all placed in the genus *Lecanora* (Fink, 1935). Lynge, in a treatment of lichens from Greenland, introduced six new species and treated a total of 21, all placed in *Lecanora* subgenus *Aspicilia* (Lynge, 1937)

The first modern treatment of the Megasporaceae was Magnusson's treatment of the *Aspicilia gibbosa* group, focusing mainly on material from Scandinavia and northern Europe. He described 51 new taxa and treated a total of 114 species (Magnusson 1939). Although Magnusson is considered a "splitter" by modern taxonomists, his treatment is still the most comprehensive work on the genus to date. In his later treatment on the lichens from Torne Lappmark, he described an additional 5 species (Magnusson, 1951). It was not until the 1970's that *Aspicilia* was again treated as a separate genus (Oxner, 1972; Poelt, 1974; Roux, 1977; and Hafellner, 1984). Oxner, working mostly on the lichens of Russia, transferred 48 species from *Lecanora* to *Aspicilia* (Oxner, 1972). In the absence of molecular data, Hafellner (1984) placed the genus *Aspicilia* in the family Hymeneliaceae. The same year, Clauzade and Roux introduced the name *Lobothallia* as a subgenus of *Aspicilia,* and in 1991 Hafellner raised *Lobothallia* to the rank of genus and transferred 4 species to *Lobothallia* (Clauzade and Roux, 1984; Hafellner, 1991). J.C. Wei, in his *Enumeration of lichens from China*, transferred over 30 species described by Zahlbruckner and Magnusson from *Lecanora* to *Aspicilia* (Wei, 1991). Thomson, in a series of papers published on Arctic lichens (1950-1980), ultimately culminating in his book, *American Arctic Lichens: Microlichens*, treats 45 species in *Aspicilia*, many of which appear to be identified with broad species concepts borrowed from European names (Thomson, 1997). I have reviewed many of Thomson's Arctic specimens at the University of Wisconsin herbarium (WIS), and although I was unsuccessful at procuring molecular data, I can conclude that many of his identifications, especially the specimens with European names, need to be revised. His collections warrant a more detailed investigation.

Lumbsch (1994) introduced the family Megasporaceae to accommodate the single species *Megaspora verrucosa*. In the Swedish lichen flora, Foucard treats 73 species all in the genus *Aspicilia* (Foucard, 2001). In 2001, Hafellner introduced the genus *Aspilidea* to accommodate *Aspicilia myrinii*. (Hafellner and Turk 2001). More recently, in a five-locus study of the Lecanoromycetes, Miadlikowska (2006) uncovered a strongly supported sister relationship between *Aspicilia* and *Ochrolechia* and included *Aspicilia* and *Ochrolechia* in the family Pertusariaceae. Schmitt et al. (2006) further divided the family Pertusariaceae and transferred *Aspicilia* and *Lobothallia* into an expanded Megasporaceae. In his *Sonoran Flora*, Owe-Larsson described 19 new species of *Aspicilia*, referencing unpublished ITS data for many of them. He treated a total of 33 species of *Aspicilia*, heavily relying on secondary metabolites and conidial size. His treatment includes the most comprehensive taxonomic key for *Aspicilia* in western North America (Owe-Larsson, 2007). For the lichen flora of Svalbard, Norway, Øvstedal et al. treats 38 species all in the genus *Aspicilia* with a disclaimer that *Aspicilia* is "a difficult genus as little is known of the plasticity of the species. There are probably more species on Svalbard than reported here." They provide an easy-to-use key that works well for the Arctic species of *Aspicilia* (Øvstedal et al., 2009).

In 2010, Nordin et al. published the first phylogenetic analysis focusing on the Megasporaceae (Nordin et al., 2010). The authors analyzed two loci (mitochondrial ribosomal small subunit (mtSSU), nuclear rDNA 28S large subunit (nuLSU)), and resurrected the genera *Sagedia* and *Circinaria*, although they excluded *Aspilidea* from the family. Their analysis had weak support for *Aspicilia* and it was poorly resolved (Nordin et al., 2010). In a 2013 revision of the vagrant "manna group" members of *Circinaria*, Sohrabi (using nuclear rDNA internal transcribed spacer region, ITS1-5.8S-ITS2 (nuITS), nuLSU and mtSSU) recovered a topology that supported the generic delimitations in Nordin et al. (Nordin et al., 2010; Sohrabi et al., 2013a). In 2014, in a broad phylogenetic survey of the Lecanoromycetes, Miadlikowska included *Sagedia* within *Aspicilia*, and *Circinaria*, *Lobothallia*, and *Aspilidea* (with low support) in a highly supported Megasporaceae sister to Ochrolechiaceae within the Pertusariales (Miadlikowska et al., 2014). In his recently published *Microlichens of the Pacific Northwest*, McCune treats the genera *Aspilidea* (1 sp.), *Megaspora* (1 sp.), *Lobothallia* (4 sp.), and *Aspicilia* (48). Awaiting a more comprehensive analysis, however, he chose to include *Sagedia, Circinaria* within *Aspicilia* (McCune, 2017). Other researchers who are currently working on the Megasporaceae include Zakeri (2016, 2017), Paulov (2015, 2016), Sohrabi (2010, 2011, 2012, 2013a, 2013b) Owe-Larsson (2007, 2011), Nordin (2007, 2008, 2009, 2010, 2011, 2013, 2015), and Roux (2011, 2016a, 2016b). Many of the more than 200 published names in *Aspicilia*, especially the early names from Zahlbruckner and Magnusson, lack type specimens, and a more comprehensive monographic treatment is still needed to correctly place the myriad of names they introduced. Currently, the family Megasporaceae is considered to be hyperdiverse, with 248 species in six genera (Lücking et al., 2016).

While lichenologists will continue to debate the importance of the individual components of a lichen thallus, it would be convenient to have a working definition of what a lichen is. The most widely agreed upon definition might be the one put forth by David Hawksworth in 1988. He defined a lichen as, “a stable self-supporting association of a mycobiont and a photobiont in which the mycobiont is the exhabitant” (Hawksworth, 1988). Or a broader definition from Trevor Goward, that lichens “are fungi that have taken up agriculture” (Goward, 1994). While these definitions have worked fairly well describing what lichenologists study, they are by no means complete. In fact, our understanding of what constitutes a lichen is still rapidly evolving. A recent study by Spribille et al. uncovered an additional basidiomycete partner that appears to be integral (at least to the majority of macrolichens) part of the lichen thallus, perhaps even being responsible for initiating the 3-dimensional structure, and also perhaps playing a key role in the production of secondary metabolites (Spribille et al., 2016). It has been known for decades that other organisms, including other bacteria, protists and fungi, inhabit lichens, but whether these players are a requisite to the symbiosis, or are more akin to bio-films remains to be discovered (Grube & Wedin 2016; Grube et al. 2015; Hodkinson et al. 2012). Currently, there is evidence for 20-30 independent lichenization events, spanning 500 million years, and resulting in 19,387 extant species in 985 extant genera (Lücking et al., 2016). There is no doubt that the lichen symbiosis is more complex than previously imagined, and seeing how lichenized fungi span a significant amount of earth's history, arose independently many times, (some lichenized fungal lineages are more distantly related than even birds are to mammals) a single definition may not be that useful.

Recent, broad phylogenetic analyses have solidified some of the family relationships within the Lecanoromycetes, including the relationships between the Megasporaceae, Pertusariaceae and Ochrolechiaceae (Miadlikowska et al. 2006; 2014; Schmitt et al. (2006)). A few smaller studies have focused primarily on the Megasporaceae (Nordin et al. 2010, Sohrabi et al. 2013), and while these studies are generally in agreement, their taxon sampling has been small or narrowly focused, and their use of the two or three standard loci (nuITS, nuLSU, mtSSU) has not provided enough support to definitively uncover the relationships between genera in the Megasporaceae. This uncertainty has lead some authors to accept the recently resurrected genera while others, awaiting more detailed analyses, have continued to use the name *Aspicilia* s.lat. for the majority of species in the Megasporaceae. To clear up these discrepancies, I apply a combination of Sanger and next-generation sequencing technologies to the cosmopolitan lichen family Megasporaceae to generate a robust phylogenetic tree for the group. Specifically, in addition to using the three standard loci (nuITS, nuLSU, mtSSU), I have added a protein-coding gene, the mini chromosome maintenance complex 7 (MCM7), which has significantly increased the resolution of the relationships within the family. I have broadly sampled the Megasporaceae from across the Northern Hemisphere, with a specific focus on *Aspicilia* in Western North America. In Western North America, this family includes many poorly known and undescribed taxa that have been traditionally lumped into a few widespread groups, or overlooked. In addition to providing a well-supported multilocus phylogeny at the generic level, I have focused on updating the North American species concepts used in the Arctic with species concepts used in the American desert southwest. To this end, I describe two new genera, synonymize three genera, describe three new species, and propose nineteen new combinations.

Multilocus phylogeny of the lichen family Megasporaceae

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PREMISE OF THE STUDY: Accurate species delimitation in lichenized fungi remains a major challenge. The mainly saxicolous lichen family Megasporaceae is widely distributed, has diversified into the majority of habitats on earth, contains numerous undescribed taxa, and due to a paucity of discriminating morphological characters, has remained problematic. In Western North America, this family includes many poorly known and undescribed taxa that have been traditionally lumped into a few widespread groups, or overlooked. My aim is to reconstruct a well-supported multilocus phylogeny of the Megasporaceae with an emphasis on the saxicolous members of *Aspicilia* s. str in western North America.

METHODS: To examine the relationships between currently accepted genera and species, phylogenetic analyses were applied to members of the Megasporaceae with a central focus on the genus *Aspicilia*. Morphological, chemical, and substrate preference patterns of 130 specimens were examined and four loci (nuclear rDNA internal transcribed spacer region, ITS1-5.8S-ITS2 (nuITS), nuclear rDNA 28S large subunit (nuLSU), mitochondrial ribosomal small subunit (mtSSU), and the minichromosome maintenance complex component 7 (MCM7) were sequenced.

KEY RESULTS: The monophyly of the family Megasporaceae and the currently accepted genera *Aspilidea*, *Lobothallia*, *Sagedia*, *Circinaria (*including *Megaspora)* were strongly supported. *Aspicilia* as currently circumscribed is paraphyletic and the new genera *Antidea* and *Arctidea* are proposed for the two distinct clades outside of *Aspicilia* s. str. *Aspilidea* is included within Megasporaceae with high support. The recently described *Teuvoa* is subsumed within an expanded *Lobothallia*. The genera *Megaspora* and the recently resurrected *Aspiciliella* are subsumed within *Circinaria*. The relationships among genera are still incompletely resolved. Three new species and nineteen new combinations are proposed.

CONCLUSIONS: Phylogenetic analyses confirmed the monophyly of the family Megasporaceae. The new genera *Antidea* and *Arctidea* are described. This study supports the segregation of the Megasporaceae into seven genera, *Antidea, Aspilidea, Lobothallia, Arctidea, Sagedia, Circinaria* and *Aspicilia*, describes three new species, and provides a robust phylogeny for the group that will be used as a backbone for future studies in the family.

KEY WORDS: *Arctidea*; *Antidea*; Ascomycota; *Aspicilia*; cryptic diversity; Ostropomycetidae; *Teuvoa*; Montana.

Lichens are an obligate consortium of fungal and photobiont partners and offer an ideal system for exploring the evolutionary history of symbiosis. *Aspicilia* is the most speciose of six genera in the family *Megasporaceae* (Lumbsch, 1994, 2007; Nordin et al. 2010), and the most speciose in the family, with over 200 valid names. Widespread in distribution and diverse in ecology, species in *Aspicilia* have overcome many of the environmental challenges that lichens might face. They have successfully colonized many different rock types, from iron-rich and calcium-rich rocks to the cleanest, nutrient poor, siliceous rocks. They are known from a wide range of habitats—from salty sprayzone hypermaritime shorelines to sub-merged in freshwater montane streams and lakes, and from intense UV-blasted desert bottoms to frigid wind-blown alpine peaks.

Aspicilia is a crustose genus characterized by an I- apical dome, sunken apothecia, moniliform paraphyses, large hyaline single-celled ascospores, and bacilliform to acicular conidia. Members of the genus are known to produce a variety of secondary metabolites with aspicilin, norstictic, connorstictic, stictic and substictic acids being common. The majority of the genus is saxicolous, some are terricolous, and a few species colonize hard weathered lignum.

The type species for the genus, *Aspicilia cinerea*, was originally named *Lichen cinereus* by Linnaeus in 1767. Eighty-five years later, Massalongo (1852) introduced the name *Aspicilia*. However, for about the next 100 years, *Aspicilia* was treated as a section in *Lecanora* (Zahlbruckner 1928, Magnusson 1939, 1951), and it was not until the 1970's that *Aspicilia* was again treated as a separate genus (Poelt, 1974, Roux, 1977, and Hafellner, 1984). In the absence of molecular data, Hafellner (1984) placed the genus *Aspicilia* in the family Hymeneliaceae. Lumbsch (1994) introduced the family Megasporaceae to accommodate the single species

Megaspora verrucosa. More recently, in a five locus study of the Lecanoromycetes, Miadlikowska (2006) uncovered a strongly supported sister relationship with *Ochrolechia* and included *Aspicilia* in the family Pertusariaceae. Schmitt et al (2006) further divided the family Pertusariaceae and transferred *Aspicilia* and *Lobothallia* into an expanded Megasporaceae.

In a 2010 study focused on the family Megasporaceae, Nordin et. al., resurrected the genera *Sagedia* and *Circinaria*, although they excluded *Aspilidea* from the family. Nordin's two-locus (nuLSU, mtSSU) analysis had weak support for *Aspicilia* and it was poorly resolved (Nordin et al., 2010). In a 2013 revision of the vagrant "manna group" members of *Circinaria*, Sohrabi recovered a topology that supported the generic delimitations in Nordin et al. (Nordin et al., 2010; Sohrabi et al., 2013a) In 2014, Miadlikowska included *Sagedia* within *Aspicilia,* and *Circinaria, Lobothallia,* and *Aspilidea* (with low support) in a highly supported Megasporaceae sister to Ochrolechiaceae within the Pertusariales (Miadlikowska et al., 2014).

Accurate species delimitation in lichenized fungi remains a major challenge. In Western North America, the lichen family Megasporaceae includes many poorly known and undescribed taxa that have traditionally been lumped within a few widespread species (i.e. *A. cinerea*, *A. caesiocinerea, A. aquatica*), or outright overlooked. Expanding on the recently resurrected genera as in Nordin et al. 2010 and Sohrabi et al. 2013a, 2013b, this analysis uncovered additional deep splits in the family.

No major study of the genus has been undertaken since Magnusson 1939, however, Thomson (1997) broadly surveyed Arctic North American *Aspicilia* and Owe-Larsson et al (2007), as part of the Sonoran Flora Project, treated the Sonoran species, describing 19 new species.

MATERIALS AND METHODS

***Taxon sampling* —**The majority of the specimens used in this study were collected during my fieldwork between 2007-2017, but further specimens were obtained from herbarium loans and collecting efforts of other researchers. Analysis was focused on specimens from the Northern Hemisphere, with a concentrated effort on the group in western North America and the northern Rocky Mountains (specimens from Scandinavia are also included) (Table 1). Additional sequences were downloaded from GenBank (Table 1).

***Morphological study* —** Cross sections of apothecia and pycnidia were done by hand and mounted in water. Presence of norstictic acid and stictic acids were inferred from positive reactions under the microscope with 10% KOH, and para-phenylenediamine respectively, and confirmed with thin layer chromatography in solvent systems A and C (Orange et al., 2010).

***DNA isolation and sequencing* —** Tissue samples for total DNA were extracted from 10-15 healthy apothecia and surrounding tissue. Two 3 mm steel beads were added to the sample tubes and frozen at -80°C for one hour. Samples were then mounted on the TissueLyser II (Qiagen, Germany) and ground in 30 s intervals for 1-2 min at 30/hz., resulting in material that was fine and farinose. DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) following the manufactures instructions except for slight modifications to the first and last steps (Samples were incubated in lysis buffer for one hour and vortexed every 10 min, and were eluted in 50 µl AE buffer twice). DNA quantity was tested on an Implen Nanodrop (Implen, München, Germany).

Standard PCR amplifications were conducted in 25 uL reaction volumes using Ready-To-Go PCR Beads (GE Healthcare, United Kingdom) following manufacturer’s recommendations.

All primers used in this study appear in Table 2.

Amplifications were carried out in an Eppendorf Mastercycler Pro thermal cycler (Eppendorf North America, New York, USA) and performed using the following programs: nuITS: initial denaturation of 2 min at 94 ° C, followed by 35 cycles of the following steps: 94 ° C for 1 min, 54 ° C for 1 min, 72 ° C for 45 s, followed by a final extension at 72 ° C for 7 min. nuLSU: initial denaturation of 4 min at 95 ° C, followed by 35 cycles of the following steps: 94 ° C for 1 min, 54 ° C for 1 min, 72 ° C for 45 s, followed by a final extension at 72 ° C for 5 min. mtSSU: initial denaturation of 4 min at 95 ° C, followed by 35 cycles of the following steps: 94 ° C for 1 min, 54 ° C for 1 min, 72 ° C for 45 s, followed by a final extension at 72 ° C for 5 min. MCM7: initial denaturation of 4 min at 95 ° C, followed by 35 cycles of the following steps: 95 ° C for 30 s, 50 ° C for 40 s, 72 ° C for 1 min, followed by a final extension at 72 ° C for 5 min.

PCR products were cleaned using the Qiagen PCR Purification Kit (Qiagen, Germany) or Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, CA, U.S.A), following

Table II. Primers used in this study

Primers used in this study		
ITS1F	CTT GGT CAT TTA GAG GAA GTA	Gardes and Bruns, 1993. Mol. Ecol. 2:113-118
ITS4	TCC TCC GCT TAT TGA TAT GC	White et al., 1990. pp 315-322
LrlecF	CCT CAG TAA CGG CGA G	Schneider et al, 2015. Lichenologist 47: 269-275.
LROR	ACC CGC TGA ACT TAA GC	Vilgalys unpublished
mtSSU1	AGCAGTGAGGAATATTGGTC	Zoller, S., et al, 1999. Lichenologist 31(5):511-516.
mtSSU3R	ATGTGGCACGTCTATAGCCC	Zoller, S., et al, 1999. Lichenologist 31(5):511-516.
MCM7for	CGTCACTACAAAACAATTCACC	This Study
MCM7forlong	TGG AGT ATG GCA CGC AG	This Study
MCM7Rev	CGC CCA TCT CTT TTG TGA C	This Study
MCM7Revlong	GATTTGCAGCAGCAAGTAT	This Study

manufacturers' instructions, and were visualized on 1% agarose gel stained with ethidium bromide. Sequencing reactions were performed by Eurofins Genomics (Louisville, Kentucky, USA.)

Sequence alignment — Sequences were quality checked and sequence ends were manually trimmed in CODON CODE ALIGNER (Centerville, Massachusetts, USA) or Bioedit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Each sequence was checked against the NCBI nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify that the desired organism was sequenced. An analysis.py python script by Resl (https://github.com/reslp/phylo-scripts, 2015) generated a concatenated alignment and was cropped using the pcrop.py python script, set to 10% (https://github.com/reslp/phylo-scripts, 2015). Alignments were visually checked in AliView (http://www.ormbunkar.se/aliview/). Minor misalignments were manually adjusted and a 250 base pair section towards the 3' end of nuLSU was removed due to a multi-intronic region that was difficult to align. Final alignment used in this analysis is deposited at www.datadryad.org.

Phylogenetic analyses — I reconstructed maximum likelihood trees for each locus and compared the bootstrap support values for each clade. Using a 70% bootstrap value threshold, clades were compared and conflict was assumed to be significant where a monophyletic group was supported with bootstrap values ≥ 70% within one locus and the same group of taxa was supported ≥ 70% as non-monophyletic within another locus (Mason-Gamer and Kellogg EA., 1996). Because no strongly supported conflicts were uncovered between the four loci, downstream relationships and analyses were performed on the concatenated dataset.

I partitioned the alignment into six groups, one each for nuITS, nuLSU and mtSSU, and three for MCM7, corresponding to the first, second and third codon positions. All models available in RAxML were tested using partitionfinder2.1.1 (Lanfear et al., 2012, 2016, Guidon et al., 2010) resulting in a single partition with GTR+I+G as the best-fit substitution model.

I used the raxmlGUI 1.31 (Stamatakis, 2006; Stamatakis et al., 2008; Silvestro & Michalak, 2012) to reconstruct a maximum likelihood (ML) concatenated 4-locus tree. I used *Pertusaria subvelata* as the root and ran 1000 thorough Maximum likelihood bootstraps with model set to GTRGAMMAI.

RESULTS and DISCUSSION

Data Set—

In this study I obtained sequence data from 130 specimens, representing approximately 90 taxa, for a total of 456 new sequences (Table 1). In total, 121 nuITS, 102 nuLSU, 119 mtSSU, and 115 MCM7 sequences were recovered. The final concatenated alignment contained 156 sequences and 4569 positions. Five species of *Ochrolechia*, two *Varicellaria* species, and two *Pertusaria* s. str. species were used as out-groups.

Monophyly of the family Megasporaceae—

This study is the largest comprehensive phylogenetic analysis of the family Megasporaceae. I used a maximum likelihood approach with three nuclear loci and one protein-coding gene. Megasporaceae formed a well-supported monophyletic group composed of seven highly supported clades, corresponding to the genera *Antidea*, *Aspilidea*, *Lobothallia*, *Arctidea*, *Sagedia, Circinaria* and *Aspicilia* (Roman Numerals- Fig. 1). All clades are recovered as monophyletic and highly supported, but their relationships to each other are only weakly to moderately-supported and should still be considered unresolved. This analysis confirms the monophyly of the family placing it with high support as sister to Ochrolechiaceae.

Phylogenetic position of genera within Megasporaceae—

Antidea- The species *Aspicilia brucei*, described in Volume three of the Sonoran Flora, was known to be morphologically and chemically quite similar to *A. cinerea* (Owe-Larsson 2007). However, it differed by having small ascospores and short bacilliform conidia. Molecular data from two specimens of *A. brucei* (Fig. 2 A, B) both from California, including an isotype (Owe-Larsson 9161) were sequenced and analyzed. *A. brucei* was uncovered as a highly supported group basal to the rest of Megasporaceae, and the new genus *Antidea* is described to accommodate this monotypic clade.

Aspilidea- The genus *Aspilidea* is highly supported as sister to the new genus *Antidea* and to the rest of the Megasporaceae, and as in Miadlikowska (2014), but here with high support, is included in the family. The clade included previously sequenced material from Europe as well as new specimens from Newfoundland (McCarthy 2905) and Alaska (Wheeler 3942, 4628, and Talbot/Scholfield 458) that appear to constitute a new species and are described here as *Aspilidea pruinosa* (see below). As a result of this study, two species of *Aspilidea* are now known, *A. myrinii* with sunken black lecidioid-like apothecia and the newly described *A. pruinosa*, with large pruinose elevated apothecia.

Lobothallia- It was known that *Aspicilia determinata* shared many characteristics (short conidia, small spores, and algae below the hypothecium) with *Lobothallia s. str.*, *Aspicilia* subgenus

Pachyothallia sensu Roux, and *Teuvoa* (Owe Larsson, 2007; Sohrabi, 2013). With the addition of molecular data from the *Aspicilia determinata* specimens, in addition to a protein coding gene (MCM7) for *T. junipericola*, the recently described genus *Teuvoa* (also with small spores and bacilliform conidia, and differing by growth on lignin, lack of chemistry, and lack of subhymenial algal layer) is sister to *A. farinosa* and the new *A. determinata* sequences with high support. If *Teuvoa* is to be accepted at the genus level, then two additional genera would have to be described to accommodate both the *A. determinata/A .farinosa* clade and the *A. peltastictoides* group, resulting in a *Lobothallia* clade being split up into four genera. However, it seems more natural to expand *Lobothallia* and subsume *Teuvoa*. *Teuvoa* is therefore synonymized within an expanded highly supported *Lobothallia*. The expanded genus is now characterized by small spores (7-15 µm), short bacilliform conidia (5-10 µm), production of secondary metabolites or not, saxicolous or lignicolous habitat, and as has been shown with other recent additions to *Lobothallia* (Kou et al., 2013; Nordin, 2010; Roux et al., 2016), the extent of lobation is not an informative phylogenetic character for the genus.

Sagedia- *Sagedia* is highly supported but its relationship with other genera is still imperfectly resolved. No specimens of *Sagedia* from North America were available in this analysis, however, four specimens from northern Europe were placed in *Sagedia*. Three specimens (Wheeler 6430, 6434 and 6467) (Fig. 6 a,b) from northern Norway agree with *S. mastrucata* in having a dark papillate thallus and reacting K+red. However, the conidia are slightly longer 10-18 µm and one specimen (Wheeler 6430) reacts strongly PD+ yellow, more closely matching Lynge's description of *A. mastoidea* (Lynge, 1937). Furthermore, these specimens form a well-supported clade (Fig 1. Clade V) separate from the *S. mastrucata* sequences available in Genbank. Nordin et al., however, synonymized *A. mastoidea* under the new name *A. berntii* and placed it with ITS near *A. verrucigera* in the cinerea-group (Nordin et al., 2008), and for now, the identity of these specimens remains unclear. The two other specimens, initially identified as *A. montana (=A.simoensis)* (Nelson S.N.) (Fig. 6c) and *A. grisea* (Wheeler 6288) (Fig. 6d) are here, pending a more detailed analyses of *Sagedia*, placed within *A. simoensis* s. lat.

Circinaria- *Circinaria* (including *Megaspora)* is highly supported as sister to *Aspicilia* s.str. *Megaspora* was described as a subgenus of *Aspicilia* (Clauzade & Cl. Roux, 1984) to house the single species *Aspicilia verrucosa. Megaspora* was later raised to the rank of genus by Hafellner and Wirth (Wirth, 1987). As in previous studies (Nordin 2010; Sohrabi 2012; Sohrabi 2013) *Megaspora* was here uncovered as a highly supported deep split within the *Circinaria* clade. With the four-locus data set and resulting tree topology presented here, it seems natural to retire the name *Megaspora,* synonymizing it with *Circinaria*. However, the name *Megaspora* continues to live on in the family rank.

Recently, Zakieh Zakeri and others (Zakeri et al., 2017) resurrected the genus *Aspiciliella* to accommodate *A. intermutans*, *A. cupreoglauca*, and the newly described *A. portosantana*. This analysis placed *Aspiciliella* with high support as sister to *Megaspora* and *Circinaria* s. str. As with *Megaspora*, *Aspiciliella* is synonymized and included within an expanded *Circinaria*.

Arctidea- *Arctidea* is a highly supported monophyletic clade that contains mainly arctic/alpine species with long conidia, however, its relationship to *Circinaria*, *Sagedia* and *Aspicilia* s.str. is still unresolved. Only specimens with molecular data are transferred to *Arctidea*. Many of Thomson's arctic species concepts and specimens are unresolved. Specimens of his from WISC were sampled for DNA, but as the specimens were 20-40 years old, it is not surprising that they yielded very little usable sequence. In total, twenty-four newly sequenced specimens and seven sequences from Genbank were placed in *Arctidea* (Table 1.). Many of Magnusson's section Orbiculares most likely belong in *Arctidea* (Magnusson, 1939). Further molecular analyses, particularly comparing American arctic species with their relationship to Arctic European and Asian specimens, are needed. While many of the chemical and microscopic characters between *Arctidea* and *Aspicilia* overlap, most specimens can be placed in *Arctidea* owing to their preference for arctic-alpine habitats, tendency to produce radiating determinate thalli, and the long filiform conidia.

Aspicilia- *Aspicilia* contains the majority of species within the family and is the most understudied. *Aspicilia* is currently defined as having and I- apical dome, sunken to sessile apothecia, moniliform paraphyses, medium to large hyaline single celled ascospores, and medium sized bacilliform to acicular conidia (Table 2.). Here, *Aspicilia* was recovered as paraphyletic, with specimens recovered in four clades (I, III, VI, VII in Fig. 1). The first well-supported clade, containing *Aspicilia brucei*, was recovered as basal to the rest of Megasporaceae. The second well-supported clade contained the newly sequenced species *A. determinata* and *A. peltastictiodes*, and was uncovered as sister to *Lobothallia* and *Teuvoa*. The third group contained arctic-alpine specimens sister to *Aspicilia* s. str.

The genus is highly supported but the relationships within *Aspicilia* were poorly resolved. However, with this analysis (including an nuITS tree with a larger taxon sampling not shown), the following seven informal subgroups were uncovered in *Aspicilia* s. str. (Fig 1.)

1) The cinerea-group containing the type for the genus, *A. cinerea*, also including *A. dudinensis*, *A. goettweigensis*, *A. blastidiata*, and *A. subdepressa* (Fig. 10*)*. Currently, the

A. cinerea s.str.-group contains many distinct phenotypes, and further divisions may be necessary.

2) The laevata-group, characterized by species with smooth thalli and deeply sunken apothecia. Usually found close to water (splash-zone of lakes and streams) and is currently represented by *A. laevata*, and *A. indissimilis* (Fig.11*)*.
3) The epiglypta-group containing a group of mostly northern species including *Aspicilia epiglypta*, *A. fluviatilis*, *A. aff intermutans*, *A. granulosa*, *A. prestensis*, and *A. subradians* (Fig. 12).
4) The pacifica-group, comprising *A. aurantiaca*, *A. pacifica*, and *A. santamonicae*, so far only known from coastal states and provinces in Western North America (Fig. 13).
5) The cyanescens-group (but with weak support) containing *Aspicilia cyanescens* and *A. cuprea (*Fig. 13*)*.
6) The nashii-group containing species with dark olive thalli, including *Aspicilia knudsenii*, *A. nashii*, and *A. tenuis,* and the new species *A. bufoides* (Fig. 16, 17, 18).
7) The americana-group comprising *Aspicilia anglica*, *A. fumosa*, *A. arizonica*, *A. boykinii, A. americana* (not included in this study), and *A. olivaceobrunnea* (Fig. 18-22). The specimens of *A. anglica* sequenced here highlight the variable nature of this species (Fig. 21-22), from oddly colored sterile isidiate-phyllidiate individuals to fertile isidiate individuals to nonpropagule producing fertile individuals, and confirms the concepts of Knudsen (Knudsen et al. 2016, Owe-Larsson pers comm. 2017). In western North America this species was variously known as *A. aff. simoensis* or *A. aff. mastrucata*.

***Taxonomy* —**

Antidea *T.B.Wheeler* ***gen. nov.***

MycoBank no.: MB822149

Similar to *Aspicilia* in general morphology and in producing norstictic acid, but differing in the small ascospores and short bacilliform to filiform conidia.

Typus generis: Antidea brucei (Owe-Larss. & A. Nordin) T.B. Wheeler

Etymology: *Antidea*, early latin meaning to come before, and in the past, a reference to the basal position to all other Megasporaceae.

Notes: This monotypic genus is morphologically very similar to some forms of *A. cinerea*. *A. brucei* differs however in having smaller spores (9-13 x 5-8 µm), shorter conidia (6-10 µm), and the non-moniliform to submoniliform paraphyses.

Arctidea *T.B.Wheeler* ***gen. nov.***

MycoBank no.: MB822150

Similar to *Aspicilia*, in ascospore size, conidial size, and chemistry, but differing in tendency to produce radiating determinate thalli, often with a distinct prothallus, and the arctic-alpine distribution. Differing from all other genera in Megasporaceae by the long filiform conidia.

Typus generis: Arctidea permutata (Zahlbr.) T.B. Wheeler

Etymology: In reference to the northern distribution, and the tendency for members to produce determinate radiating or lobate thalli.

Notes: In their two locus analyses, Nordin et al (2010) and Sohrabi et al. (2013) both uncovered an unsupported split in *Aspicilia s. lat.*. However, neither study specifically focused on *Aspicilia* s. lat. and their taxon sampling reflected that. Here, with an expanded taxon sampling, one additional ribosomal gene, and the addition of a protein-coding gene (MCM7), this split, corresponding to the new genus *Arctidea* is recovered as sister to *Aspicilia s. str.* and *Sagedia* with weak support. Microscopically *Arctidea* can be characterized by medium to long filiform conidia, usually greater than 12um long, and up to 40um in length, and morphologically by the determinate, radiating thalli. Most species display a distinct radiating pattern of cracks between the areoles and end up forming discrete determinate circles, as compared to the majority of the members of *Aspicilia* s. str. which display a more indeterminate growth, with an irregular pattern to the cracks, and are more constrained by the surface upon which they grow (i.e. following cracks and crevices), resulting in less predetermined circular growth. Furthermore, *Arctidea* can be distinguished from the majority of *Aspicilia* s.str. by their arctic-alpine distribution. The majority of the remaining species in *Aspicilia* not sampled in this study with radiating thalli and arctic-alpine distributions likely belong in *Arctidea*.

An argument could be made to recognize only *Antidea*, *Aspilidea* and *Lobothallia* with the rest of the Megasporaceae, including *Arctidea*, *Circinaria* and *Sagedia*, being included in an expanded *Aspicilia*. Even though the differences between *Arctidea* and *Aspicilia* s. str. are few, the differences in conidia size between the other genera are clear (Table 3). I chose to retain smaller genera, which correspond well to these differences in conidia size and shape as opposed to a larger more inclusive *Aspicilia* s. lat.

Aspilidea pruinosa T.B. Wheeler and J.W. McCarthy **sp. nov.**

MycoBank no.: MB822152

Similar to Aspilidea myrinii (Fr.) Hafellner *in the yellowish to grayish-white thallus color, small bacilliform conidia, and producing norstictic acid, but differing by the elevated apothecia with thick, prominent, coarsely pruinose discs with double margins.*

Type: Canada, Newfoundland, Avalon Peninsula, Fairhaven Barrens, Route 203, ca. 8 km southwest of Route 1 (Trans-Canada Highway)-Route 203 intersection, ca. 300 m in from right side of road, 47.55801 ° N -53.84918 ° W, 125m, on argillite, 11 November 2015, *McCarthy 2905* (hb. CANL).

(Fig. 2, C, D, E, F)

Hypothallus thin to thick, usually lacking, but in some specimens gray-black, occasionally visible between areoles and as a thin zone at thallus margin. *Thallus* areolate, contiguous, separated by thin cracks, non-determinate, up to 20 cm in diameter. *Areoles* cream-white to bluish-gray, epruinose to faintly white pruinose, often shiny with a thin epinecral layer, irregular, even to irregularly ridged, 0.5-1.0 mm in diam, 0.1–0.75 mm thick, 1-2 apothecium per areole. *Upper cortex* pseudoparenchymatous, 30–40 μm thick, epinecral layer thin, 3-7 μm thick, indistinct. *Algal layer* thin, discontinuous, irregular, 40–60 μm thick, algal cells chlorococcoid, 8-10 μm in diameter.

Apothecia one or two per areole, lecanorine, round, with thick outer thallus colored margin and a thinner inner darker ring colored like the disc, immature disc briefly sunken, quickly becoming elevated and prominent with a narrowing base, margin smooth, mature disc smooth to slightly roughened, black, blue-grey pruinose, 0.25-1.25 mm in diameter, eventually sessile to raised in thalline ridges. *Exciple* pseudoparenchymatous, cells 4-6 µm, outer layer brownish, inner layer hyaline, 80 to 130 µm thick. *Epihymenium* 20-25 µm tall, dark brown to black. K+ brownish. *Paraphyses* not branching, nonmoniliform, thin, 1 µm thick, apices unexpanded, with pale brown tips. *Hymenium* 80–120 µm, I+blue. *Asci* 8-spored, 50-60 × 15–25 µm, *Aspicilia*-type. *Ascospores* simple, hyaline, (12) 14-17 (19) × 7-9 µm, narrowly ellipsoid. *Hypothecium* hyaline, 80-100 µm, hyaline above, grading into greyish medulla below, algal layer not continuous below hypothecium. *Pycnidia* 150 x 100 µm, rare to common, with a black, slightly elevated ostiole. *Conidia* short bacilliform, (5) 5.5-6.5 (7) x 1-1.5 µm.

Spot tests and chemistry. Medulla K+ yellow to red crystals in section, I (Lugols) ± pale brownish violet. TLC norstictic acid major.

Etymology. Named for the distinctive pruinose discs.

Ecology and distribution. A northern species known from 100-1250 meters, from Alaska and Newfoundland. Has been collected on argillite, schist, phyllite, and quartzite.

Notes. Aspilidea pruinosa is a distinctive species owing to its thick, white to cream colored thallus, and prominent, thick margined apothecia with pruinose discs. North American specimens have been labeled as *Aspicilia arctica*, and *Aspilidea myrinii*. *Aspilidea myrinii* has non-pruinose, black, angular or irregular, immersed apothecia, reminiscent more of a lecideoid than an *Aspicilia*, in contrast to the raised, thick margined apothecia of *A. pruinosa*. *Aspicilia arctica*, has long filiform conidia (15-23 μm) in contrast to the short bacilliform conidia (5-7 μm) of *A. pruinosa*.

Additional Specimens examined. U.S.A., **Alaska**, Gates of the Arctic National Park, Brooks Range, Chimney lake, 67.764651° N 150.500407° W, 740m, on schist, 18 July 2012, *Wheeler 3942* (hb. Wheeler). Upper Nigu River, 67.972488° N 155.376505° W**,** 1115m, on phyllite, 2 August 2012, Wheeler 4628 (hb. Wheeler). Tetlin National Wildlife Refuge, Mount Fairplay, 63.69137° N 142.2432° W, 1250m, on quartzite, 25 July 2001, S.S. Talbot and W.B. Schofield 458 (WIS-L-0103209).

Aspicilia indeterminata T.B. Wheeler **sp. nov.**

MycoBank no.: MB822153

Similar to Aspicilia cinerea (L.) Körb. *group in the immersed apothecia, greyish thallus, blue-green epihymenium and production of norstictic acid, but differing in larger ascospores, submoniliform paraphyses, and bullate areoles.*

Type: U.S.A., Montana, Beaverhead County, Pioneer Mountains, Vipond Park, 45.730623° N -112.863999° W, 2015m, on argillite, 23 June 2014, *Wheeler 7241* (hb. XXX).

(Fig. 12, A, B)

Hypothallus lacking. *Thallus* contiguous, areolate, dense, separated by deep cracks, non-determinate, 4-10 cm in diameter. *Areoles* greyish white to grey, epruinose to slightly white pruinose, angular, 0.2-1 mm in diam, 0.1–0.5 mm thick, one apothecium per areole. *Upper* *cortex* pseudoparenchymatous, 15–30 µm thick, cells to 4-6 µm in diameter. *Algal layer* continuous, undulating, 40–50 µm thick, algal cells chlorococcoid, mostly 10 µm in diameter. *Medulla* obscure, ca. 200 µm tall.

Apothecia one per areole, black, lecanorine, round, with thin to thick margin same color as thallus or slightly darker, disc sunken, concave, margin thin and slightly pruinose, mature disc weakly to densely white pruinose, 0.25-1.25 mm in diameter, eventually raised above thallus. *Exciple* pseudoparenchymatous, cells 4-5 µm, dark gray to hyaline, up to 100 µm thick. *Epihymenium* 10-20 µm tall, blue-green, K+ brown. *Paraphyses* submoniliform, top 2-3 cells rounded and expanded to 4 µm, occasionally branching and anastomosing, 2-3 µm thick. *Hymenium* 100–130 µm tall, I+blue. *Asci* 8-spored, 90× 25 µm, *Aspicilia*-type. *Ascospores* simple, hyaline, (15) 17-20 (22)× 10–13 µm µm, broadly ellipsoid. *Hypothecium* hyaline, 40-60 µm tall, with small oil droplets, algal layer not continuous below hypothecium. *Pycnidia* 150 x 100 µm, rare, inconspicuous. *Conidia* medium, straight, 14-15 x 1-1.5 µm.

Spot tests and chemistry. Spot tests K+ red crystals in the type specimen, but K- in the additional specimen, however norstictic acid was present in both specimens upon analysis with TLC.

Etymology. Named for the indeterminate thalli, and confusion it created when initially discovered.

Ecology and distribution. Forested montane dry sites, from 1500-2015 m, currently only known from Montana. Has been collected on granite, quartz-monzonite, and quartzite.

Notes. Aspicilia indeterminata is an indistinctive, thin, grayish *Aspicilia,* with sunken discs. In the type specimen the disks are weakly pruinose, in contrast to the densely pruinose discs of other specimens. Close to *A. verrucigera* in general morphology, conidia and spore size, but lacking stictic acid and having an epihymenium that is distinctively blue-green as opposed to olive-brown.

Additional Specimens examined. U.S.A., **Montana**, Lewis and Clark Co., Orofino Gulch road, 46.542127° N -112.097662°W, 1525 m, on Tertiary granite boulders, 21 August 2012, *Wheeler 5460,* (hb. Wheeler).

Aspicilia bufoides Owe-Larss. & T.B. Wheeler **sp. nov.**

MycoBank no.: XXX

Similar to Aspicilia nashii Owe-Larss. & A. Nordin *and Aspicilia confusa* Owe-Larss. & A. Nordin*, but differs by having contiguous areolate thallus and by the thallus color. Similar to Aspicilia knudsenii* Owe-Larss. & A. Nordin *in the thick olive-brown areoles, but lacking chemistry and having longer conidia.*

Type: U.S.A., Montana, Lake County, Mission Mountains, East St. Marys Peak Ridge, 47.288438° N -113.905246° W, 2600m, on dolomite, 10 September 2016, *Wheeler 7335* (UPS-Holotype; hb- Wheeler-Isotype *Wheeler 7333*).

(Fig. 17, 18A)

Hypothallus black, fimbriate, well developed. *Thallus* contiguous, areolate, dense, separated by deep cracks, non-determinate, 4–10 cm in diameter. *Areoles* grey with a ±brown tinge or mottled brown, to grey-brown or brown to more commonly dark olive-brown, often white-spotted, warty, irregular, epruinose to slightly white pruinose (especially when growing on calcareous substrates), angular, 0.2–1.2(–2.0) mm in diameter, 0.1–0.6(–1.0) mm thick. *Upper cortex* pseudoparenchymatous, 15–35(–50) µm thick, cells to 4–6 µm in diameter. *Algal layer* continuous, 40–50 µm thick, algal cells chlorococcoid, mostly 7–15 µm in diameter. *Medulla* obscure, ca. 200 µm tall.

Apothecia one or two, rarely three, per areole, often elevated and appearing lecanorine, ±round, with ±thick margin same color as thallus or with pale white margin, disc flat or slightly sunken, mature disc black, sometimes weakly pruinose, (0.2–)0.4–2 mm in diameter, commonly prominently raised above thallus. *Exciple* pseudoparenchymatous, cells 4–6 (–8) µm, dark gray to hyaline, up to 100 µm thick. *Epihymenium* 10–15 µm tall, green to olive-green to olive-brown, K+ golden brown, N+ green to blue-green. *Paraphyses* moniliform, top 3–5(–8) cells rounded and expanded to 4–6 µm, occasionally branching and anastomosing, 2–3 µm thick. *Hymenium* 120–180(–220) µm tall, I+blue. *Asci* 8-spored, (70–)80–120 × (20–)23–32(–40) µm, *Aspicilia*-type. *Ascospores* simple, hyaline, (15–)17–26(–30) × (9–)11–16(–18) µm, broadly ellipsoid. *Hypothecium* hyaline, 40–60(–80) µm tall, with small oil droplets, algal layer not continuous below hypothecium. *Pycnidia* 140–200(–300) x 100–140(–190) µm, common, conspicuous with a black sunken ostiole often surrounded by an elevated white margin. *Conidia* filiform, straight, 12–25(–27) × (0.6–)0.8–1.5 µm.

Spot tests and chemistry. Spot tests K-, PD-, C-. No substances detected with TLC.

Etymology. Named for the latin for toad, bufonem, a nod to the warty, irregular, often white-spotted, olive colored thallus.

Ecology and distribution. Upper montane to alpine, from 500-3300 m, currently known from Montana, Idaho, Utah and California. Some specimens from Arizona (in ASU) also seem to belong to this species, but need further studies, including DNA analysis. The species has been collected on argillite, quartzite, dolomite, limestone, granite, volcanic rock and lignum of dead *Pinus albicaulis* log.

Notes. Aspicilia bufoides is a distinctive, thick, olive-brown to gray-brown *Aspicilia,* with elevated, thick margined apothecia, and conspicuous white spotting on the thallus and apothecial margins. It is similar to *A. nashii*, which however has dispersed, brown convex areoles and a central pale hypothallus. It is also similar to *A. confusa*, which differs by dispersed, gray to gray-brown convex areoles. Further, *A. bufoides* differs from *A. knudsenii* in the lack of chemistry and longer conidia, and from *A. cyanescens* by lacking a distinctive blue-green epihymenium. Microscopically, characters as spore size and conidia length are overlapping in several of the species in the area, i. e. *A. americana, A. bufoides, A. confusa, A. cyanescens*, *A. fumosa, A. nashi,* and *A.* aff *tenuis*, but the species are clearly separated as regards DNA and thallus characters. North American specimens of *A. bufoides* have been misidentified as *A. americana, A. caesiocinerea, A. confusa, A. cyanescens, A. nashii* and *A.* aff *tenuis*.

In the Lichen Flora of the Greater Sonoran Desert Region, *A. confusa* was regarded as a very variable species (Owe-Larsson et al., 2007), hence the name, and at that time DNA (ITS) had been analyzed from only three specimens from Riverside county in California, including the

type. Later on, DNA (ITS) from 3 of the specimens listed as *A. confusa* was analyzed and clearly differed from ITS of *A. confusa* s. str., and it was clear that they belonged to a new species, presently described as *Aspicilia bufoides*. Furthermore, ITS from 4 specimens from Sequoia National Park (Tulare County, California), collected by Judith L. Blakeman (specimens discussed by Owe-Larsson et al (2007), see notes of *A. cyanescens*), was also analyzed at a later timepoint and these specimens were also found to belong to *A. bufoides*, as well as several specimens from Montana, Idaho and Utah analyzed in the present study. In California, *A. bufoides* seems to be one of the most common *Aspicilia* species at higher altitude.
A. confusa seems to have a restricted distribution in southern California, mainly in the counties of Los Angeles, Riverside, Ventura, Tulare and San Benito. *A. nashi* seems to have a local distribution in northern Mexico and southern California.

Additional Specimens examined. **U.S.A., California, Alpine Co.,** Edge of Carson-Iceberg Wilderness: End of Clark Fork Road, on N side of road, 38°25'N, 119°45'W, 2000 m, granite rock, 13 Aug. 1989, *Ryan 24631, 24634, 24648* and *24662* (ASU); **El Dorado Co.,** Trail to Lost Lake from trail through Sugar Pine State Park, about 4 miles WSW of Meeks Bay, 39°01'N, 120°11'W, 2250 m, on granite rocks, with pines, firs, 24 July 1989, *Ryan 23583d* (ASU); **Fresno Co.,** Sierra National Forest, Potter Creek trailhead, FS Road 8S34, T7S, R26E, S34, 37°16'30''N 119°07'30''W, 2600 m, 6 August 1994, *Ryan 32225* (ASU); **Inyo Co.,** Eastern Brook Lakes Watershed, Sierra Nevada Mountains (15 km S of Tom's place, along Rock Creek Road), 37°26'N, 118°44'30''W, 3170 m, on shaded granidiorite rock in Pinus murrayana forest, June 1985, *Ryan 12784* (ASU); Eastern Brook Lakes Watershed, Sierra Nevada Mountains (15 km S of Tom's place, along Rock Creek Road), 37°26'N, 118°44'30''W, 3170 m, on granidiorite, W of small lake W-SW of upper lake, June 1985. *Ryan 12811-b* (ASU); Eastern Brook Lakes Watershed, Sierra Nevada Mountains (15 km S of Tom’s place, along Rock Creek Road), 37°26’N, 118°44’30’’W, 3290 m, on granidiorite, large boulderfield E of upper lake, June 1985, *Ryan 12895* (ASU); **San Bernardino Co.,** San Bernardino Mountains: below Onyx Summit. 34°12.129’N, 116°45.129’W, 8000 feet, substrate: granite, microhabitat: large boulders in sun, habitat: mixed conifer with juniper, note: most common species in Southern California Mts, 16 Sept. 2004, *Knudsen 1714* (UPS); San Gorgonio Wilderness Area, Charleton Peak, 34°06’43.3” N, 116°51’19.5’’W, 3144m, on granite, 6 July 2012, *Knudsen 14707* (UCR); **Siskiyou Co.,** Edge of Marble Mountain Wilderness: To about 1 mi along Shackleford Creek Trail from road 43N21, 41°33’N, 123°03’W, 1325-1450 m, granite rocks, with fir, pine, 22 Aug. 1989, *Ryan 25270* (ASU); Edge of Marble Mountain Wilderness: Trail through Big Meadows, 41°35’N, 123°03’W, 1900-2000 m, granite rocks, 25 Aug. 1989, *Ryan 25424* (ASU); **Tulare Co.,** Kings Canyon National Park, Grant Grove, west of South Boundary Trail service road, directly off of Generals Highway, north side of Sequoia Creek, south facing slope, fir and pine with azalea understory, 21 July 1985, *Blakeman 151* (MIN); Sequoia National Park, Kern Canyon, NW of the south Kern Canyon Ranger Station, bordering Coyote Creek, incense cedar, pine and fir with manzanita, 4 August 1985, *Blakeman 335* (MIN); Sequoia National Park, Kern Canyon, NW of the south Kern Canyon Ranger Station, bordering Coyote Creek, incense cedar, pine and fir with manzanita, 4 August 1985, *Blakeman 342* (MIN); Sequoia National Park, Kern Canyon, NE of the south Kern Canyon Ranger Station, directly north of Soda Spring bordering west bank of the Kern River, incense cedar, pine and fir, 4 August 1985, *Blakeman 369* (MIN); Sequoia National Park, Kern Canyon, 1 mile NE of south Kern Canyon Ranger Station on east bank of Kern River, very rocky and dry, vegetation sparse, 6 August 1985, *Blakeman 399* (MIN); Sequoia National

Park, Kern Canyon, 1 mile NE of south Kern Canyon Ranger Station on east side of Kern River, very rocky and dry west facing slope against canyon wall, pine, fir and manzanita, 6 August 1985, *Blakeman 418* (MIN); Sequoia National Park, Kern Canyon, Lower Funston Meadow, west side of trail between the two Lower Funston drift fences on west side of Kern Canyon, pine, fir and some oak on east facing slope, 7 August 1985, *Blakeman 452* (MIN); Sequoia National Park, Kern Canyon, a quarter mile south of Rattlesnake Creek on the west side of the Kern River, east facing slope where canyon floor meets canyon walls, large jumbled granite boulders, 8 August 1985, *Blakeman 495* (MIN); Sequoia National Park, Kern Canyon, a quarter mile north of Rattlesnake Creek on east bank of Kern River, rocky and dry with incense cedar, fir, pine and manzanita, 8 August 1985, *Blakeman 514* (MIN); Sequoia National Park, Kern Canyon, Junction Meadow, 1 mile NW of Junction Meadow on Colby Pass Trail, granite outcrop north of trail, very dry and rocky; vegetation sparse and shrubby, a few willow and manzanita, 12 August 1985, *Blakeman 612* (MIN); Sequoia National Park, Kern Canyon, Junction Meadow, E of Upper Kern Trail, 0.5 mile from junction of Colby Pass Trail at Junction Meadow, SW facing rocky outcrop with mountain mahogany and manzanita, 13 August 1985, *Blakeman 634* (MIN); Sequoia National Forest: Road to Balch Park, 5.4 mi from Hwy 190, 36°13’N, 118°49’W, 2000 feet, 30 Sept. 1989, *Ryan 26674* (ASU); **Tuolumne Co.,** Winter Sports Area, N of Hwy 108, 5 km SW of Fraser Flat Campground, 38°08’N, 119°04’30’’W, 1700 m, on volcanic rock, with pine, oak. 13 Aug. 1989. *Ryan 24362* and *24391* (ASU); Stanislaus National Forest: Herring Creek Road (4N12), 4.5 mi from Hwy 108, 38°13’N, 119°58’W, 2100 m. granite rocks (mostly shaded), fir trees. Aug. 13 1989, *Ryan 24511* (ASU); Edge of Emigrant Wilderness: Above N end of Cherry Lake, 38°02’N, 119°54’45’’W, 1800-1875 m, granite rock, with oak, pine, manzanita, white fir, 16 Aug. 1989, *Ryan 24605* (ASU); **Idaho, Shoshone Co.,** Upper Glidden

Lake Trail, 47.520071° N -115.712539°W, 1935 m, on argillite talus, 22 August 2016, *Wheeler 7313* (hb. Wheeler); **Montana**, **Beaverhead Co.,** Italian Peaks, Upper Nicholia Creek Basin, 44.366330° N -112.843560°W, 2895m, on limestone, 8 July 2016, *Wheeler 7259* (hb. Wheeler); Upper Deadman Creek, 44.372894° N -112.827882°W, 2855m, on *Pinus albicaulis* log, 9 July 2016, *Wheeler 7277* (hb. Wheeler); **Lincoln Co.,** Elk Mtn. Lookout Trail, 48.448129° N -114.913209° W, 1890 m, on quartzite, 10 October 2012, *Wheeler 5865* (hb. Wheeler); **Missoula Co.,** Mission Mtns., North Jocko Peak, 47.270027° N -113.794480° W, 2255 m, on argillite boulder, 4 July 2012, *Wheeler 5399* (hb. Wheeler); **Utah, Summit Co.,** Uinta Mtn. Wilderness, 40.818053° N -110.459951° W, 3292 m, on sandstone, 23 August 2008, *Wheeler 2565* (hb. Wheeler).

New Combinations—

Antidea brucei (Owe-Larss. & A. Nordin) T.B.Wheeler comb. nov.

Basionym: *Aspicilia brucei* Owe-Larss. & A. Nordin, *Lichen Flora of the Greater Sonoran Desert Region* (Tempe) **3**: 73 (2007)

MycoBank no.: MB 822154

Arctidea aliena (Zahlbr.) T.B. Wheeler **comb nov.**

Basionym: *Lecanora aliena* Zahlbr., *Rep. Sci. Res. Norweg. Exped. Novaya Zemlya,* 1921: 7 (1928)

Synonymy: *Aspicilia aliena* (Zahlbr.) Oxner, *Nov. sist. Niz. Rast*. 9: 286 (1972)

MycoBank no.: MB 822155

Arctidea candida (Anzi) T.B. Wheeler **comb. nov.**

Basionym: *Aspicilia polychroma var. candida* Anzi, *Cat. Lich. Sondr.*: 59 (1860)

Synonymy: *Aspicilia candida* (Anzi) Hue, *Nouv. Arch. Mus. Hist. Nat., Paris,* 5 sér. 2: 64 (1912) [1910]

MycoBank no.: MB 822162

Arctidea dendroplaca (H. Magn) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora dendroplaca* H. Magn., *K. svenska Vetensk-Akad. Handl.*, ser. 3 17(no. 5): 156 (1939)

MycoBank no.: MB 822159

Arctidea mashiginensis (Zahlbr.) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora mashiginensis* Zahlbr., *Rep. Sci. Res. Norweg. Exped. Novaya Zemlya*, 1921: 20 (1928)

Synonymy: *Aspicilia mashiginensis* (Zahlbr.) Oxner, *Nov. sist. Niz. Rast.* 9: 289 (1972)

MycoBank no.: MB 822158

Arctidea permutata (Zahlbr.) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora permutata* Zahlbr., *Cat. Lich. Univers.* **5**: 338 (1928)

Synonymy: *Aspicilia permutata* (Zahlbr.) Clauzade & Rondon, *Revta Fac. Ciên. Univ. Lisboa*, Sér. 2, C 14: 17 (1966)

MycoBank no.: MB 822157

Arctidea rivulicola (H. Magn) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora rivulicola* H. Magn.*, Bot. Notiser: 401 (1928)*

Synonymy: *Aspicilia rivulicola* (H. Magn.) Räsänen*, Ann. Acad. Sci. fenn.*, Ser. A 34(no. 4): 73 (1931)

MycoBank no.: MB 822156

Arctidea supertegens (Arnold) T.B. Wheeler **comb. nov.**

Basionym: *Aspicilia supertegens* Arnold, *Verh. zool.-bot. Ges. Wien* 27: 567 (1877)

MycoBank no.: MB 822160

Arctidea virginea (Hue) T.B. Wheeler **comb. nov.**

Basionym: *Aspicilia virginea* Hue*, Nouv. Arch. Mus. Hist. Nat., Paris,* 5 sér. 2: 70 (1912) [1910]

MycoBank no.: MB 822161

Circinaria verrucosa (Ach.) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora verrucosa* Ach., *Lich. univ.*: 354 (1810)

Synonymy: *Megaspora verrucosa (Ach.) Hafellner & V. Wirth, in Wirth, Die Flecht. Baden-Württembergs. Verbreitungsatlas (Stuttgart): 511 (1987)*

MycoBank no.: MB 822163

Circinaria rimisorediata (Valadbeigi & A. Nordin) T.B. Wheeler **comb. nov.**

Basionym: *Megaspora rimisorediata* Valadbeigi & A. Nordin, *Lichenologist* **43**(4): 287 (2011)

MycoBank no.: XXX

Notes: New for North America.

Circinaria cretacea (Gasparyan, Zakeri & Aptroot) T.B. Wheeler **comb. nov.**

Basionym: *Megaspora cretacea* Gasparyan, Zakeri & Aptroot, in Zakeri, Gasparyan & Aptroot, *Willdenowia* 46(2): 248 (2016)

MycoBank no.: MB 822170

Circinaria intermutans (Nyl.) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora intermutans* Nyl., *Flora, Regensburg* 55: 354 (1872)

Synonymy: *Aspiciliella intermutans* (Nyl.) M. Choisy, Cavanillesia 5 (5): 166 (1932)

MycoBank no.: MB 822171

Circinaria cupreoglauca (de Lesd.) T.B. Wheeler **comb. nov.**

Basionym: *Aspicilia cupreoglauca* B. de Lesd., *Bull. Soc. bot. Fr.* 57: 32 (1910)

Synonymy: *Aspiciliella cupreoglauca* (de Lesd.) Zakeri, Divakar & Otte, Herzogia 30 (1): 172 (2017)

MycoBank no.: MB 822172

Circinaria portosantana (Sipman & Zakeri) T.B. Wheeler **comb. nov.**

Basionym*: Aspiciliella portosantana* Sipman & Zakeri, Herzogia 30 (1): 172 (2017)

MycoBank no.: MB 822173

Lobothallia determinata (H. Magn.) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora determinata* H. Magn., Lichens from Central Asia 1: 96 (1940)

Synonymy: *Aspicilia determinata* (H. Magn.) N.S. Golubk., Nov. Syst. Niz. Rast. 9:236 (1972)

MycoBank no.: MB 822174

Lobothallia peltastictoides (Hasse) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora peltastictoides* Hasse., *Bryologist* **17**: 63 (1914)

Synonymy: *Aspicilia peltastictoides* (Hasse) K. Knudsen & Kocourk., *Mycotaxon* 124: 354 (2013)

MycoBank no.: MB 822175

Lobothallia uxoris (Werner) T.B. Wheeler **comb. nov.**

Basionym: *Lecanora uxoris* Werner*, Bull. Soc. Sci. Nat phys. Maroc* 18(2): 130 (1938)

Synonymy: *Aspicilia uxoris* (Werner) V.J. Rico, Aragón & Esnault, *Lichenologist* 39(2): 110 (2007)

MycoBank no.: MB 822176

Lobothallia junipericola (Werner) T.B. Wheeler **comb. nov.**

Basionym: *Teuvoa junipericola* Sohrabi & S. Leav., *Lichenologist* 45(3): 353 (2013)

MycoBank no.: XXX

Lobothallia tibetica (Sohrabi & Owe-Larss.) T.B. Wheeler **comb. nov.**

Basionym: *Aspicilia tibetica* Sohrabi & Owe-Larss., *Mycol. Progr.* 9(4): 492 (2010)

Synonymy: *Teuvoa tibetica* (Sohrabi & Owe-Larss.) Sohrabi, *Lichenologist* 45(3): 357 (2013)

MycoBank no.: MB 822177

Conclusions —

Many recent molecular studies (Leavitt et al., 2016; Schneider et al., 2016; Singh et al., 2015) have uncovered cryptic species hidden within traditional species concepts, or have reorganized relationships at the genus level. Here I show that the family Megasporaceae is no different. I find strong support for recognizing seven genera within the Megasporaceae. The monotypic genus *Antidea*, basal to the rest of the Megasporaceae, is described to accommodate *A. brucei*. *Aspilidea* is confirmed as belonging in the family Megasporaceae with high support. The genus *Lobothallia* is expanded to include the *Teuvoa* and *Pachyothallia* groups. The genus *Circinaria* is also expanded to include *Megaspora* and *Aspiciliella*. *Sagedia* is highly supported as monophyletic, but its relationship to the other genera in Megasporaceae is unresolved. The new genus *Arctidea* is described to accommodate the arctic-alpine species with radiating thalli and long filiform conidia. Although the genera are supported as monophyletic, their relationships to each other are still partly unresolved. Finally, three new species (*Aspilidea pruinosa*, *Aspicilia indeterminata*, and *Aspicilia bufonia*) from North America are described. While the full diversity of the family has yet to be discovered and analyzed with molecular data, this addition to the taxonomy of the Megasporaceae will serve as a backbone for future studies in the group.

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Table 1. Specimens used in this study. Collection information. GenBank accession numbers in bold represent newly obtained sequences.

DNA voucher	Species	Locality and collector number (Herbarium)	nuITS	nuLSU	mtSSU	MCM7
TW328	*Antidea brucei*	USA, California, *Knudsen* 15069 (UCR)				
TW346	*Antidea brucei*	USA, California, Owe-Larsson 9161 (Isotype-ASU)				
TW298	*Arctidea aliena*	USA, Alaska, *Wheeler* 5072 (hb. Wheeler)				
TW291	*A. "aquapermutata"*	USA, Alaska, *Wheeler* 4743 (hb. Wheeler)				
TW280	*A. anseris*	USA, Montana, *Wheeler* 5696 (hb. Wheeler)				
TW252	*A. sp.1*	USA, Alaska, *Wheeler* 4080 (hb. Wheeler)				
TW304	*A. candida*	USA, Alaska, Wheeler 5043 (hb. Wheeler)				
TW287	*A. candida*	USA, Alaska, Wheeler 5257 (hb. Wheeler)				
TW236	*A. cingulata*	USA, Montana, *Wheeler* 7165 (hb. Wheeler)				
TW294	*A. aff. circularis*	USA, Alaska, *Wheeler* 5060 (hb. Wheeler)				
TW307	*A. aff. circularis*	USA, Alaska, *Wheeler* 4170 (hb. Wheeler)				
	A. dendroplaca	Sweden, Torne Lappmark, *Nordin* 5952	HQ259259.1	HM060744.1	HM060706.1	
TW303	*A. disserpens*	USA, Alaska, *Wheeler* 4414 (hb. Wheeler)				
TW312	*A. lesleyana*	USA, Montana, *Wheeler* 7325 (hb. Wheeler)				
	A. mashiginensis	Sweden, Hälsingland, *Nordin* 5790 (UPS)	EU057912.1	HM060732.1	HM060694.1	
	A. permutata	Sweden, Torne Lappmark, *Nordin* 6027 (UPS)	EU057918.1	HM060747.1	HM060709.1	
TW286	*A. permutata*	USA, Alaska, *Wheeler* 4018 (hb. Wheeler)				
TW306	*A. permutata*	USA, Alaska, *Wheeler* 4253 (hb. Wheeler)				
TW292	*A. permutata*	USA, Alaska, *Wheeler* 4318 (hb. Wheeler)				
TW296	*A. permutata*	USA, Alaska, *Wheeler* 4463 (hb. Wheeler)				
TW216	*A. permutata*	USA, Montana, *Wheeler* 5549 (hb. Wheeler)				
TW289	*A. perradiata*	USA, Alaska, Wheeler 3989 (hb. Wheeler)				
	A. rivulicola	Sweden, Torne Lappmark, *Nordin* 5957	EU057922.1	HM060753.1	HM060715.1	
TW305	*A. rivulicola*	USA, Alaska, *Wheeler* 4246 (hb. Wheeler)				
TW297	*A. rivulicola*	USA, Alaska, *Wheeler* 4456 (hb. Wheeler)				
TW295	*A. rivulicola*	USA, Alaska, *Wheeler* 4463 (hb. Wheeler)				
TW259	*A. aff. rosulata*	USA, Alaska, *Wheeler* 5186 (hb. Wheeler)				
	A. supertegens	Norway, Troms, *Owe-Larsson* 9002 (UPS)	EU057936.1	HM060742.1	HM060704.1	
	A. supertegens	Sweden, Torne Lappmark, *Nordin* 6023 (UPS)	EU057938.1	HM060751.1	HM060713.1	

TW237	*A. verruculosa*	Norway, Finnmark, *Wheeler* 6492 (hb. Wheeler)
TW283	*A. virginea*	USA, Montana, *Wheeler* 7272 (hb. Wheeler)
TW240	*A. virginea*	USA, Montana, *Wheeler* 7153 (hb. Wheeler)
TW210	*Aspicilia cinerea*	USA, Montana, *Wheeler* 7214 (hb. Wheeler)
TW246	*A. aff indissimilis*	USA, Montana, *Wheeler* 6830 (hb. Wheeler)
TW284	*A. aff indissimilis*	USA, Montana, *Wheeler* 3870 (hb. Wheeler)
TW212	*A. aff. arizonica*	USA, Montana, *Wheeler* 7212 (hb. Wheeler)
TW317	*A. americana*	Canada, British Columbia, *Goward* 01-1072 (UBC)
TW338	*A. anglica*	USA, California, Knudsen 9996 (UCR)
TW249	*A. anglica*	USA, Montana, *Wheeler* 2954 (hb. Wheeler)
TW250	*A. anglica*	USA, Montana, *Wheeler* 5408 (hb. Wheeler)
TW213	*A. anglica*	USA, Montana, *Wheeler* 5468 (hb. Wheeler)
TW248	*A. anglica*	USA, Montana, *Wheeler* 6036 (hb. Wheeler)
TW247	*A. anglica*	USA, Montana, *Wheeler* 6167 (hb. Wheeler)
TW258	*A. anglica*	USA, Montana, *Wheeler* 7208 (hb. Wheeler)
TW332	*A. anglica*	USA, California, *Knudsen* 17182 (UCR)
TW278	*A. anglica*	USA, Montana, *Wheeler* 3535 (hb. Wheeler)
TW231	*A. arizonica*	USA, Montana, *Wheeler* 7152 (hb. Wheeler)
TW220	*A. aurantiaca*	USA, California, *Wheeler* 7091 (hb. Wheeler)
TW239	*A. berntii*	USA, Montana, *Wheeler* 6883 (hb. Wheeler)
TW266	*A. boykinii*	USA, Montana, *Wheeler* 7249 (hb. Wheeler)
TW282	*A. boykinii*	USA, Montana, *Wheeler* 7273 (hb. Wheeler)
TW313	*A. boykinii*	USA, Montana, *Wheeler* 7263 (hb. Wheeler)
TW277	*A. boykinii*	USA, Montana, *Wheeler* 7274 (hb. Wheeler)
TW311	*A. bufonia*	USA, Idaho, *Wheeler* 7313 (hb. Wheeler)
TW243	*A. bufonia*	USA, Montana, *Wheeler* 5399 (hb. Wheeler)
TW229	*A. bufonia*	USA, Montana, *Wheeler* 5865 (hb. Wheeler)
TW274	*A. bufonia*	USA, Montana, *Wheeler* 7259 (hb. Wheeler)
TW273	*A. bufonia*	USA, Montana, *Wheeler* 7277 (hb. Wheeler)
TW315	*A. bufonia*	USA, Montana, *Wheeler* 7335 (hb. Wheeler)
TW233	*A. bufonia*	USA, Utah, *Wheeler* 2565 (hb. Wheeler)
TW330	*A. bufonia*	USA, California, *Knudsen* 14707 (UCR)
TW333	*A. bufonia*	USA, California, *Knudsen* 14770.2 (UCR)
TW210	*A. cinerea*	USA, Montana, Wheeler 7214 (hb. Wheeler)
TW211	*A. cinerea*	USA, Montana, *Wheeler* 7213 (hb. Wheeler)
TW219	*A. cinerea*	Norway, Finnmark, *Wheeler* 6277 (hb. Wheeler)
TW251	*A. cinerea*	USA, Alaska, *Wheeler* 4890 (hb. Wheeler)

TW285	*A. cinerea*	USA, Alaska, *Wheeler* 7232 (hb. Wheeler)			
TW265	*A. cinerea*	USA, Montana, *Wheeler* 7238 (hb. Wheeler)			
TW314	*A. cinerea "delimitata"*	Canada, Nunavut, *Björk* 31068 (UBC)			
TW347	*A. confusa*	USA, California, Owe-Larrson 9095 (ASU)			
TW337	*A. cuprea*	USA, California, *Knudsen* 13050 (UCR)			
	A. cuprea	USA, California, *Owe-Larsson* 9112 (UPS)	EU057902.1	HM060750.1	HM060712.1
TW331	*A. cuprea*	USA, California, *Knudsen* 16336 (UCR)			
	A. cyanescens	USA, California, *Owe-Larsson* 9151 (UPS)	EU057904.1	HM060745.1	HM060707.1
	A. dudinensis	Sweden, Torne Lappmark, *Nordin* 6036 (UPS)	EU057906.1	HM060748.1	HM060710.1
TW340	*A. epiglypta*	Canada, Labrador, *McCarthy* 1681 (hb. McCarthy)			
	A. epiglypta	Sweden, Västergötland, Nordin 6303 (UPS)	EU057907.1	HM060756.1	HM060718.1
TW224	*A. fumosa*	USA, Montana, *Wheeler* 3844 (hb. Wheeler)			
TW234	*A. fumosa*	USA, Montana, *Wheeler* 5410 (hb. Wheeler)			
TW214	*A. fumosa*	USA, Montana, *Wheeler* 5418 (hb. Wheeler)			
TW281	*A. fumosa*	USA, Montana, *Wheeler* 5934 (hb. Wheeler)			
TW235	*A. fumosa*	USA, Montana, *Wheeler* 6699 (hb. Wheeler)			
TW223	*A. fumosa*	USA, Montana, *Wheeler* 6802 (hb. Wheeler)			
TW228	*A. fumosa*	USA, Montana, *Wheeler* 6820 (hb. Wheeler)			
TW264	*A. fumosa*	USA, Montana, *Wheeler* 7228 (hb. Wheeler)			
TW336	*A. fumosa*	USA, California, *Knudsen* 16514 (UCR)			
TW344	*A. glaucopsina*	USA, Montana, Wheeler 7421			
TW241	*A. indeterminata*	USA, Montana, *Wheeler* 5460 (hb. Wheeler)			
TW272	*A. indeterminata*	USA, Montana, *Wheeler* 7241 (hb. Wheeler)			
	A. indissimilis	Sweden, Torne Lappmark, *Nordin* 5943 (UPS)	EU057909.1	HM060746.1	HM060708.1
TW276	*A. cfr. intermutans*	Iceland, Hoffellsjökull, *Nelson* S.N. (hb. Wheeler)			
TW245	*A. knudsenii*	USA, Montana, *Wheeler* 6798 (hb. Wheeler)			
TW343	*A. knudsenii*	USA, Oregon, Björk 23423 (UBC)			
TW349	*A. knudsenii*	USA, Montana, Wheeler 7496			
	A. laevata	Sweden, Uppland, *Tibell* 23659 (UPS)	EU057910.1	HM060730.1	HM060692.1
TW316	*A. cfr. laevata*	USA, Montana, *Wheeler* 7345 (hb. Wheeler)			
TW263	*A. cfr. laevata*	USA, Montana, *Wheeler* 7226 (hb. Wheeler)			
TW350	*A. nashii*	Mexico, Baja California, Nash 14696-b (Holotype, ASU)			
TW351	*A. nashii*	Mexico, Baja California, Nash 14696-b (Isotype, UPS)			
TW334	*A. pacifica*	USA, California, *Knudsen* 9241 (UCR)			
TW348	*A. phaea*	USA, Montana, Wheeler 7366			
TW230	*A. santamonicae*	USA, California, *Wheeler* 6648 (hb. Wheeler)			
TW293	*A. subradians*	USA, Alaska, *Wheeler* 5044 (hb. Wheeler)			
TW341	*A. subradians*	USA, Alaska, *Wheeler* 5070 (hb. Wheeler)			

TW232	*A. verrucigera*	USA, Washington, *Wheeler* 3439 (hb. Wheeler)			
TW320	*Aspilidea myrinii*	Sweden, Jämtland, *Nordin* 7447 (WISC)			
	A. myrinii	Sweden, Nordin 6205 (UPS)		HM060754.1	HM060716.1
TW222	*A. pruinosa*	USA, Alaska, *Wheeler* 4628 (hb. Wheeler)			
TW339	*A. pruinosa*	Canada, Newfoundland, *McCarthy* 2905 (Holotype-CANL)			
TW323	*A. pruinosa*	USA, Alaska, *Talbot/Scholfield* 458 (WISC)			
TW226	*A. pruinosa*	USA, Alaska, *Wheeler* 3942 (hb. Wheeler)			
TW215	*Circinaria arida*	USA, Montana, *Wheeler* 5819 (hb. Wheeler)			
	C. caesiocinerea	Sweden, Uppland, *Tibell* 22612 (UPS)	EU057897.1	HM060731.1	HM060693.1
	C. calcarea	Sweden, Oland, Nordin 5888 (UPS)	EU057898.1	HM060743.1	HM060705.1
TW238	*C. calcarea*	USA, Montana, *Wheeler* 3634 (hb. Wheeler)			
TW335	*C. calcarea*	USA, California, *Knudsen* 11729.3 (UCR)			
	C. contorta	AFTOL-ID 1358 XXX	HQ650638.1	DQ986782.1	DQ986876.1
TW225	*C. contorta*	USA, Montana, *Wheeler* 5765 (hb. Wheeler)			
	C. gibbosa	Sweden, Uppland, *Nordin* 5878 (UPS)	EU057908.1	HM060740.1	HM060702.1
	C. leprosescens	Sweden, Uppland, *Nordin* 5906 (UPS)	EU057911.1	HM060749.1	HM060711.1
TS1821	*C. rimosorediata*	Canada, Alberta, *Spribille* WP642 (hb. Wheeler)			
	C. verrucosa	Slovakia, Schmitt, 24. May 2003 (F)		DQ780307.1	DQ780275.1
	C. verrucosa	Turkey, Corum, *Kinalioglu* 1679 (B)		JQ797497.1	JQ797482.1
	Lobothallia alphoplaca	Iran, East Azerbaijan, Sohrabi 3677 (hb. M. Sohrabi)	JQ797516.1		JQ797480.1
TW227	*L. determinata*	USA, Montana, *Wheeler* 3641 (hb. Wheeler)			
TW242	*L. determinata*	USA, Montana, *Wheeler* 6017 (hb. Wheeler)			
TW279	*L. determinata*	USA, Utah, *Wheeler* 7216 (hb. Wheeler)			
	L. farinosa	France, Roux 25286 (UPS)		HM060761.1	HM060723.1
	L. junipericola	USA, Utah, *St. Clair et al.* 742 (BRY)	JX306744.1		
	L. melanaspis	Sweden, Jämtland, *Nordin* 6622	HQ259272.1	HM060726.1	HM060688.1
TW268	*L. melanaspis*	USA, Montana, *Wheeler* 3905 (hb. Wheeler)			
TW327	*L. peltastictoides*	USA, California, *Knudsen* 14420 (UCR)			
TW270	*L. praeradiosa*	USA, Montana, *Wheeler* 3396 (hb. Wheeler)			
TW269	*L. praeradiosa*	USA, Montana, *Wheeler* 3414 (hb. Wheeler)			
TW271	*L. praeradiosa*	USA, Utah, *Wheeler* 3333 (hb. Wheeler)			
	L. uxoris	Spain, Castilla, *Rico & Pizarro* 3622 (H)	JX306743.1	JX306757.1	
	L. uxoris	Spain, Castilla, *Rico & Pizarro* 765 (BRY)	JX306745.1	JX306759.1	
	Ochrolechia balcanica	Greece, Prov. Drama, *Schmitt* (ESS-20968)	AF329172.1	AF329171.1	AF329170.1

	O. sp.	USA, Alaska, *Spribille* 38907 (GZU)	KR017126.1	KR017190.1	KR017333.1	
	O. subpallescens	USA, *Lumbsch* 19900a (MIN)		GU980985.1	GU980978.1	GU980994.1
	O. subplicans	USA, Alaska, *Spribille* 38350 (GZU)	KR017087.1	KP794974.1	KR017388.1	
	O. upsaliensis	USA, Lumbsch 19916e (MIN)		GU980986.1	GU980979.1	GU980995.1
	Pertusaria albescens	Czech Republic, Bohemia, *Schmitt* (ESS-20967)	AF329177.1	AF329176.1	AF329175.1	
	P. subvelata	USA, Alaska, *Spribille* 39272 (GZU)	KR017110.1	KR017227	KR017389.1	
TW217	*Sagedia mastrucata*	Norway, Finnmark, *Wheeler* 6467 (hb. Wheeler)				
	S. mastrucata	Sweden, Lycksele Lappmark, *Nordin* 5481 (UPS)	EU057914.1	HM060737.1	HM060699.1	
TW221	*S. mastrucata*	Norway, Finnmark, *Wheeler* 6434 (hb. Wheeler)				
TW275	*S. simoensis*	Iceland, Hoffellsjökull, *Nelson* S.N. (hb. Wheeler)				
TW218	*S. simoensis*	Norway, Finnmark, *Wheeler* 6288 (hb. Wheeler)				
	S. simoensis	Norway, Troms, *Owe-Larsson* 9000 (UPS)	EU057926.1	HM060739.1	HM060701.1	
	S. zonata	Norway, Troms, *Owe-Larsson* 8942 (UPS)	EU057946.1	HM060738.1	HM060700.1	
	Varicellaria rhodocarpa	*Bjork* 9653 (UBC)		KJ766675.1	KJ766511.1	
	V. velata	Australia, *Archer* (ESS 21500)		AY300855.1	AY300906.1	

Table III. Comparison between genera accepted in this paper.

	Antidea	*Aspilidea*	*Arctidea*	*Aspicilia*	*Circinaria*	*Lobothallia*	*Sagedia*
Thallus	Indeterminate	Indeterminate	Determinate	Indeterminate to determinate	Indeterminate	Determinate	Indeterminate
Spores/ascus	8	8	8	8	2-6(8)	8	8
Spore shape	ellipsoid to subglobose	ellipsoid	ellipsoid	ellipsoid	broadly ellipsoid to globose	ellipsoid	ellipsoid
Spore size (µm)	9-13 x 5-11	10–20 × 7–11	13.5-19 x 8-11	15-25 x 8-12	18-36 x 12-26	8-18 x 5-12	14-25 x 7-13
Conidia length (µm)	6-10	4-8	12-40	(6)-11-40	6-12	3-8	8-12
Distribution	Southern/Mediterranean	Arctic	Arctic-alpine	Cosmopolitan	Southern	Southern	Northern
Number of species	1	2	20+	±150	~30	~19	3

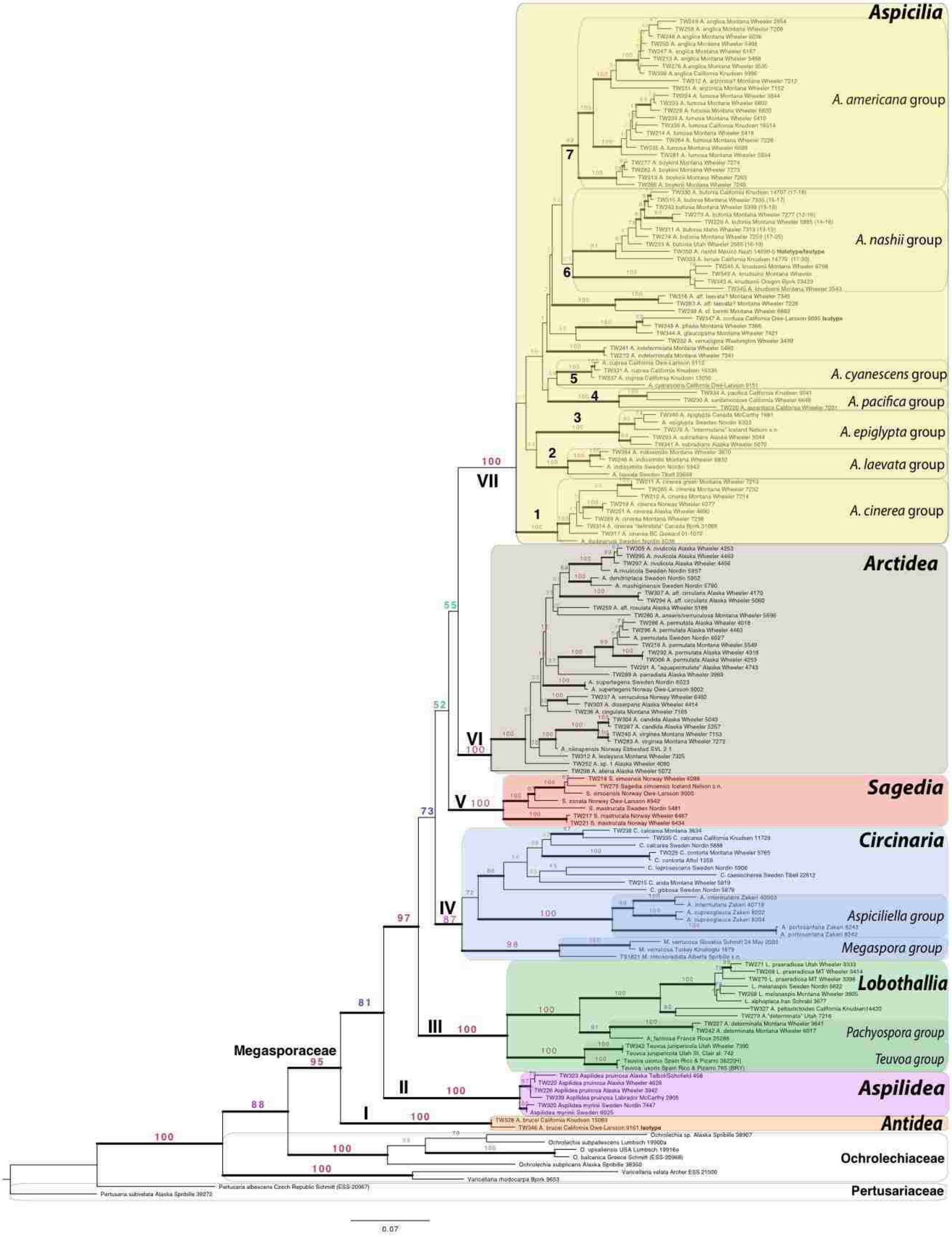

Figure 1. Maximum Likelihood Tree. Bootstrap support values are indicated above branches. Bold indicates highly supported branches (BS ≥ 80%)

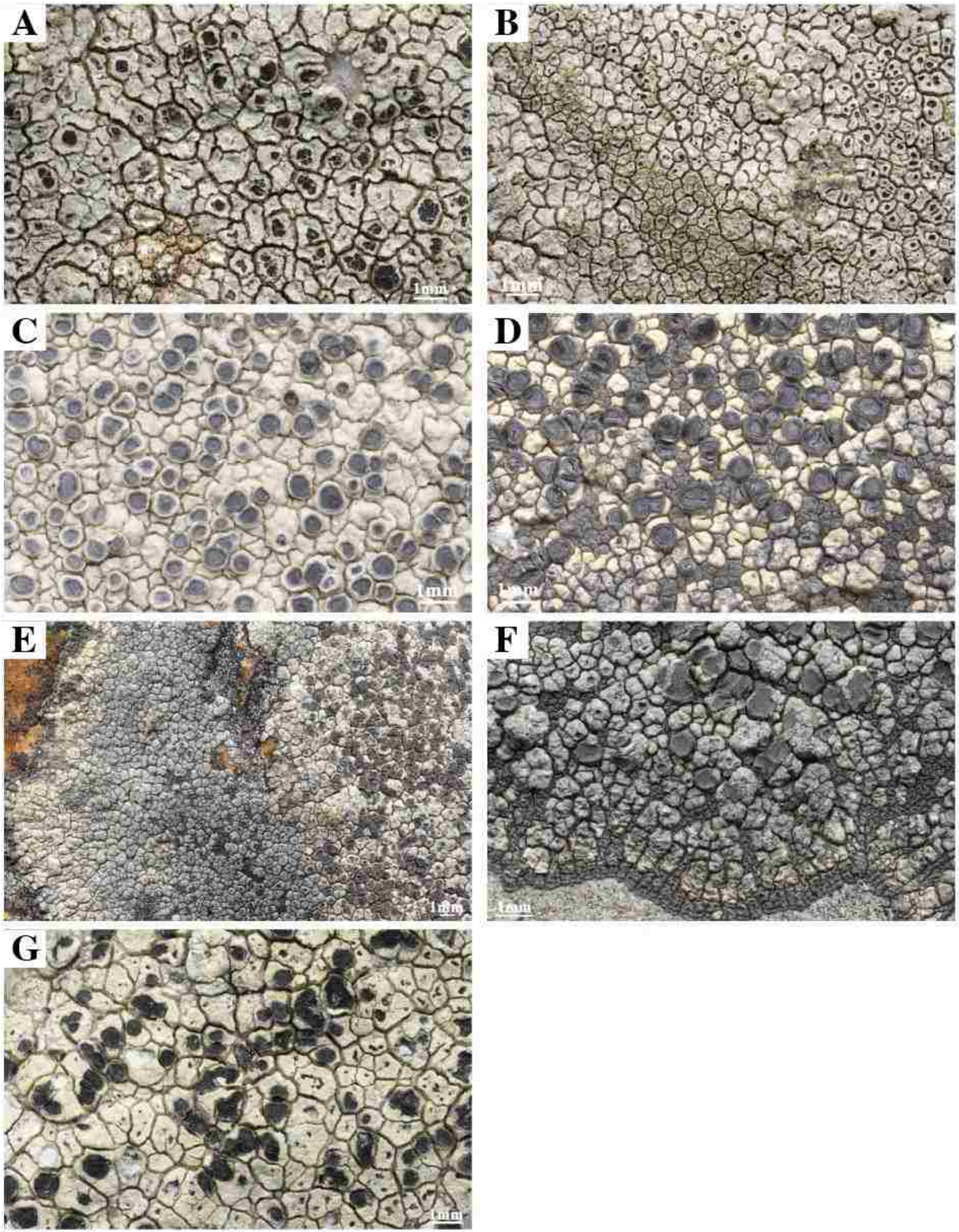

Figure 2. **A.** *Antidea brucei* Knudsen 15069. **B.** *Antidea brucei* Owe-Larsson 9161. **C.** *Aspilidea pruinosa* McCarthy 2905 - (Holotype). **D.** *Aspilidea pruinosa* Wheeler 3942. **E.** *Aspilidea pruinosa* Talbot-Schofield 458. **F.** *Aspilidea pruinosa* Wheeler 4628. **G.** *Aspilidea myrinii* Nordin 7447.

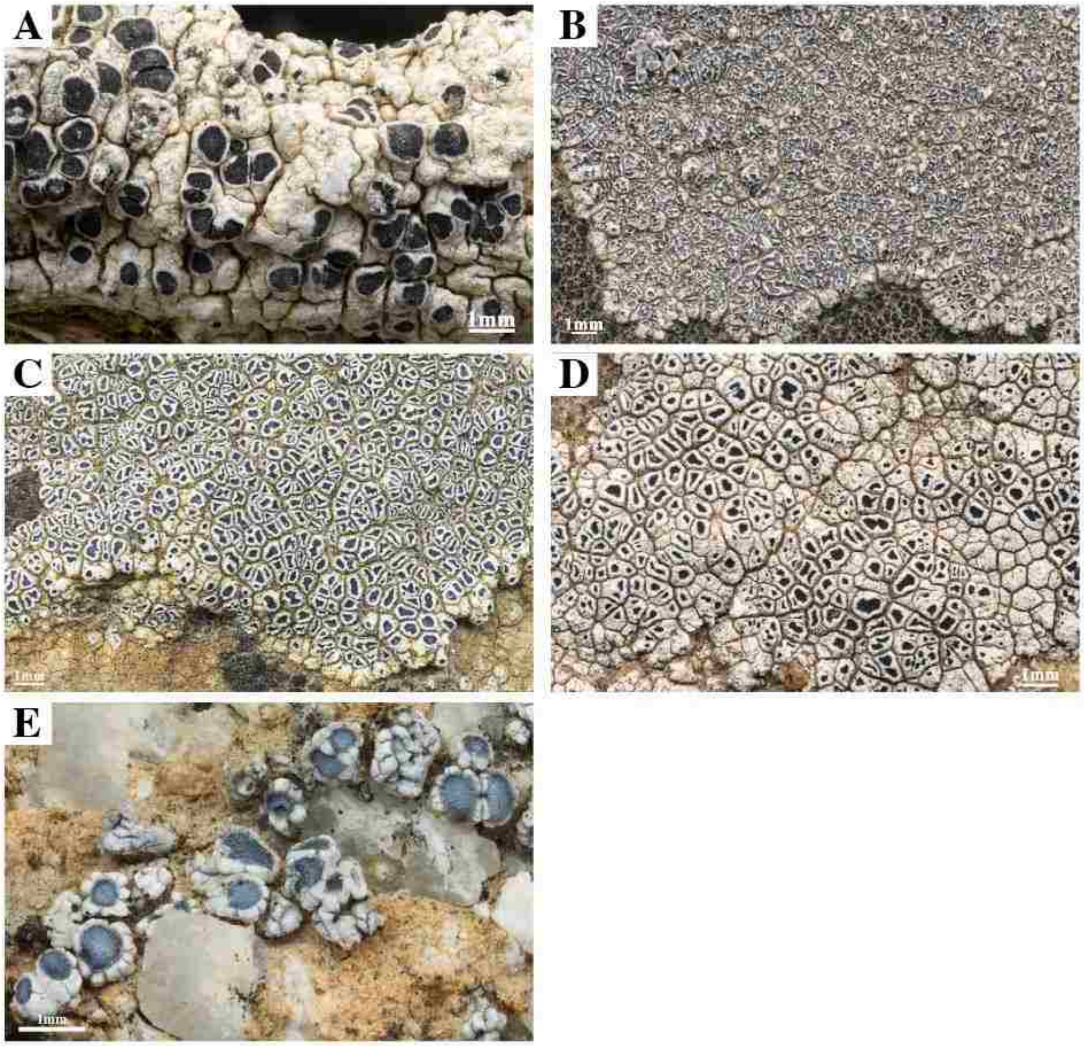

Figure 3. **A.** Lobothallia junipericola Wheeler 7390. **B.** *Lobothallia determinata* Wheeler 6017. **C.** *Lobothallia determinata* Wheeler 3641. **D.** *Lobothallia aff. determinata* Wheeler 7216. **E.** *Lobothallia peltastictoides* Knudsen 14420.

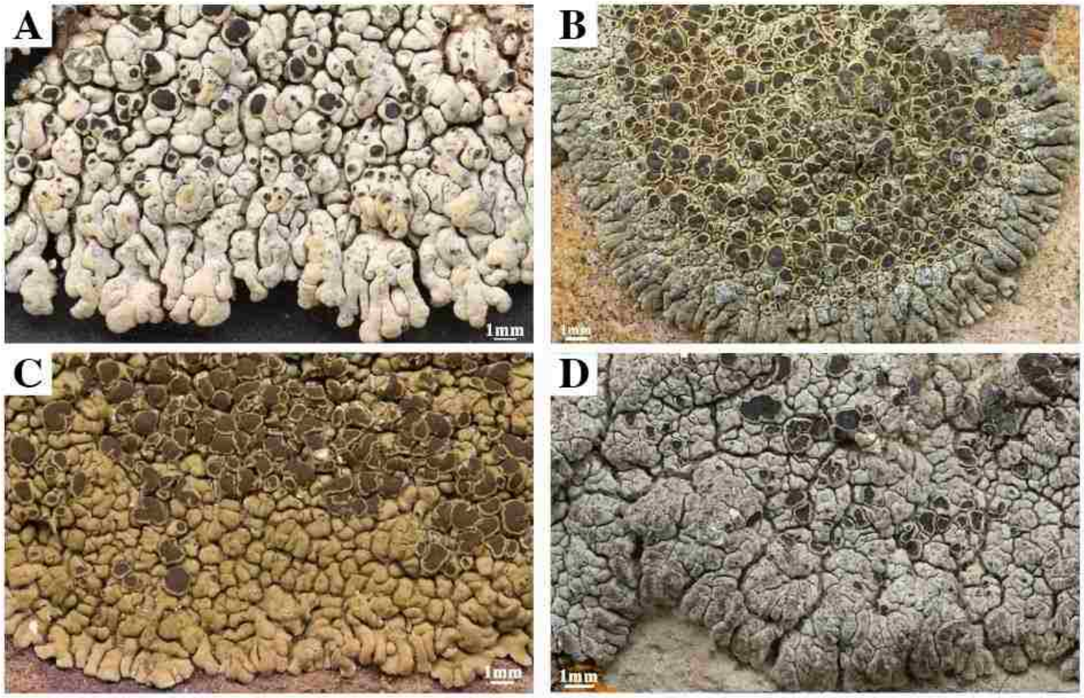

Figure 4. **A.** *Lobothallia melanaspis* Wheeler 3908 **B.** *Lobothallia praeradiosa* Wheeler 3396. **C.** *Lobothallia praeradiosa* Wheeler 3333. **D.** *Lobothallia praeradiosa* Wheeler 3414.

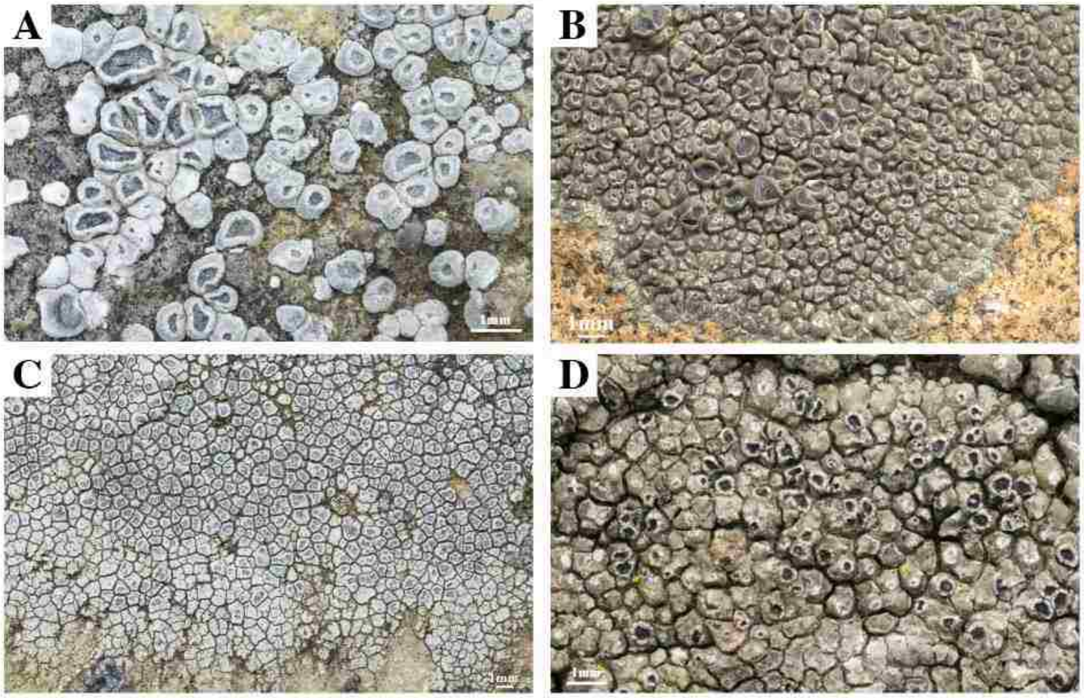

Figure 5. **A.** *Circinaria contorta* Wheeler 5332. **B.** *Circinaria arida* Wheeler 5819. **C.** *Circinaria aff. calcarea* Wheeler 3634. **D.** *Circinaria aff. calcarea* Knudsen 11729.3.

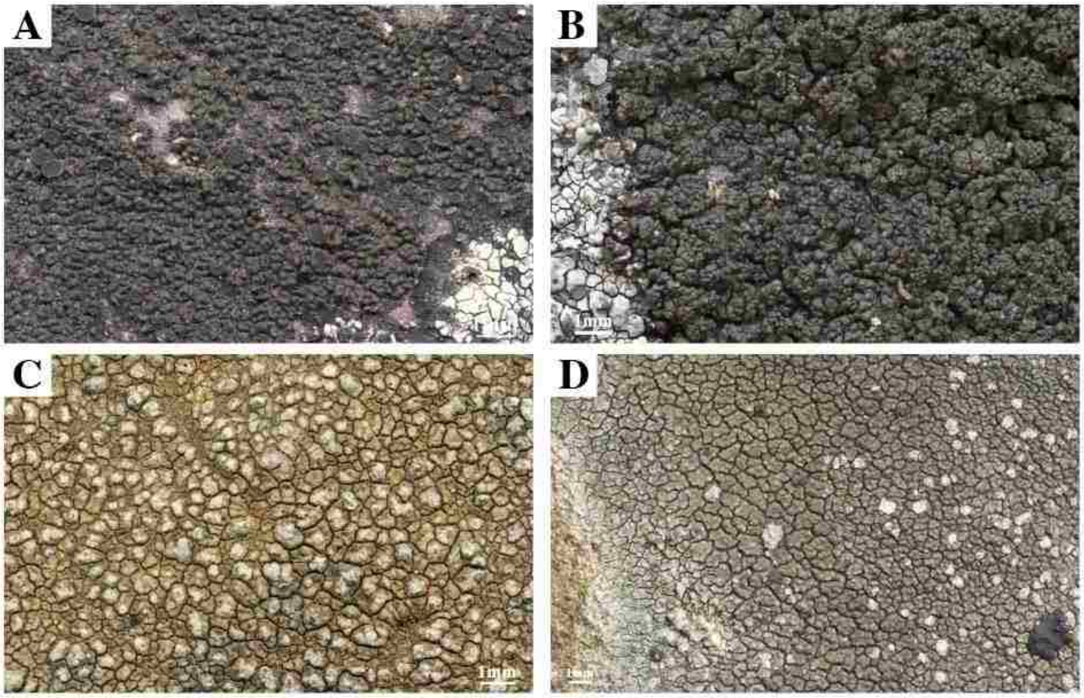

Figure 6. **A.** *Sagedia mastrucata* Wheeler 6467. **B.** *Sagedia mastrucata* Wheeler 6434. **C.** *Sagedia simoensis* Nelson S.N. **D.** *Sagedia simoensis* Wheeler 6288.

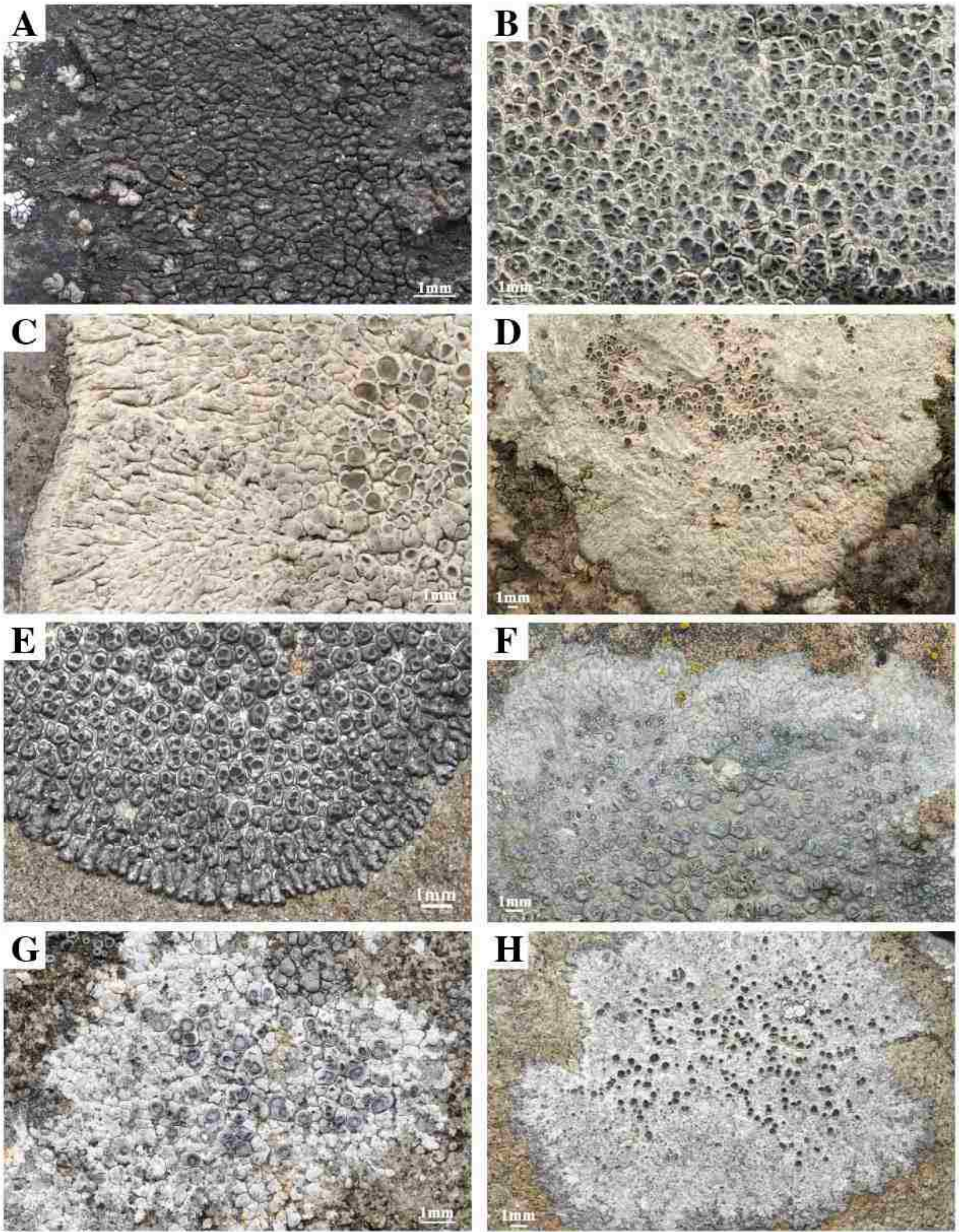

Figure 7. **A.** *Arctidea aliena* Wheeler 5072. **B.** *Arctidea rivulicola* Wheeler 4456. **C.** *Arctidea rivulicola* Wheeler 4463. **D.** *Arctidea rivulicola* Wheeler 4246. **E.** *Arctidea plicigera* Wheeler 5186. **F.** *Arctidea anseris* Wheeler 5696. **G.** *Arctidea sp. 2* Wheeler 5060. **H.** *Arctidea sp. 2* Wheeler 4170.

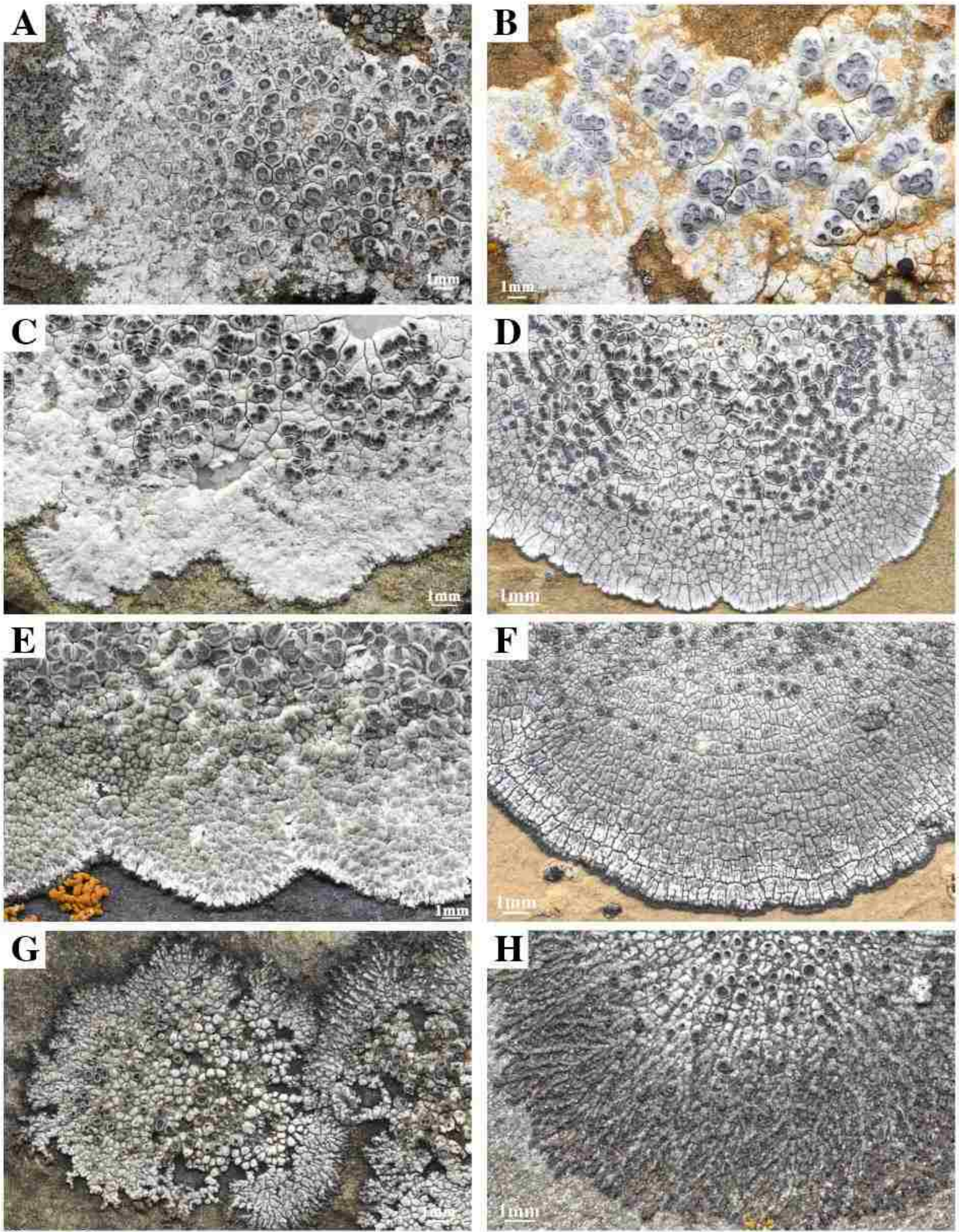

Figure 8. **A.** *Arctidea candida* Wheeler 5043. **B.** *Arctidea candida* Wheeler 5257. **C.** *Arctidea virginea* Wheeler 7272. **D.** *Arctidea virginea* Wheeler 7153. **E.** *Arctidea lesleyana* Wheeler 7325. **F.** *Arctidea cingulata* Wheeler 7165. **G.** *Arctidea disserpens* Wheeler 4414. **H.** *Arctidea verruculosa* Wheeler 6492.

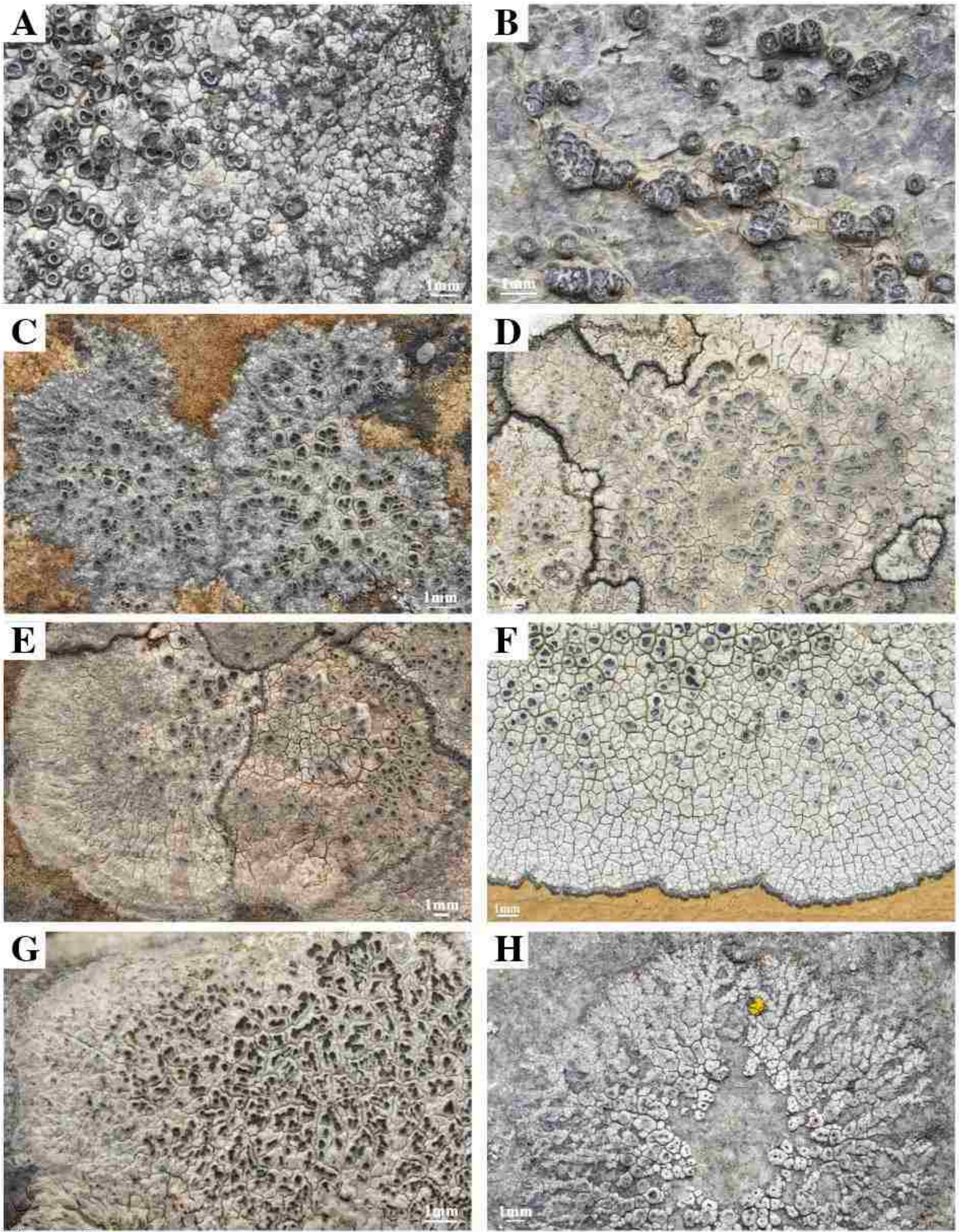

Figure 9. **A.** *Arctidea perradiata* Wheeler 3989. **B.** *Arctidea sp. 1* Wheeler 4080. **C.** *Arctidea “aquapermutata”* Wheeler 4743 **D.** *Arctidea “aquapermutata”* Wheeler 4318. **E.** *Arctidea “aquapermutata”* Wheeler 4253 **F.** *Arctidea permutata* Wheeler 5549. **G.** *Arctidea “aquapermutata”* Wheeler 4463. **H.** *Arctidea permutata* Wheeler 4018.

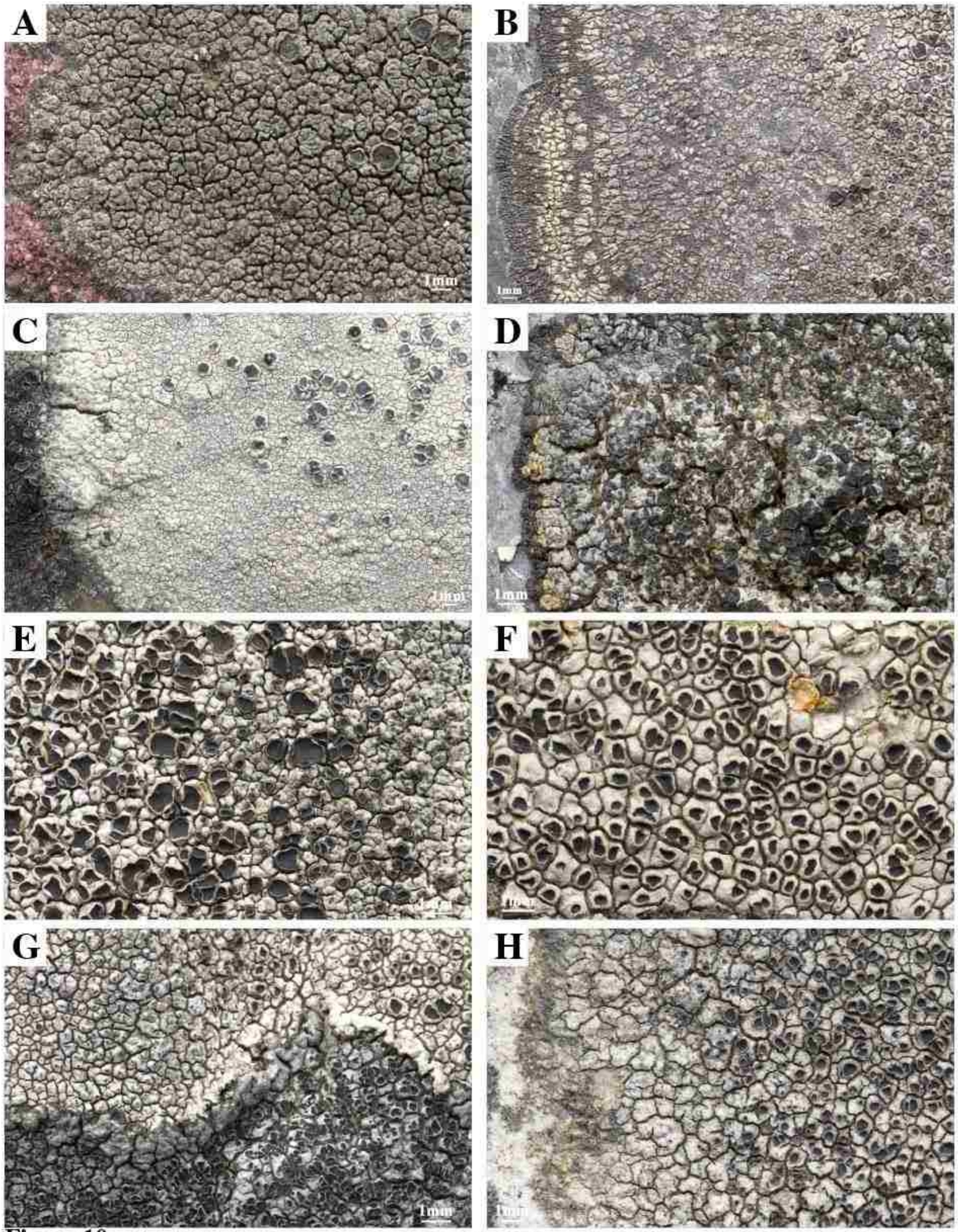

Figure 10. **A.** *Aspicilia cinerea* Goward 01-1072. **B.** *Aspicilia cinerea* Bjork 31068. **C.** *Aspicilia cinerea* Wheeler 7238 **D.** *Aspicilia cinerea* Wheeler 4890. **E.** *Aspicilia cinerea* Wheeler 6277 **F.** *Aspicilia cinerea* Wheeler 7214. **G.** *Aspicilia cinerea* Wheeler 7213. **H.** *Aspicilia cinerea* Wheeler 7232.

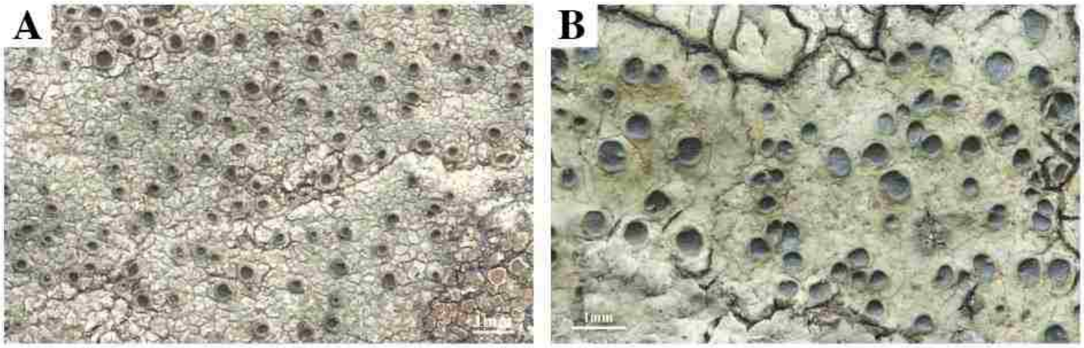

Figure 11. **A.** *Aspicilia indissimilis* Wheeler 6830. **B.** *Aspicilia indissimilis* Wheeler 3870.

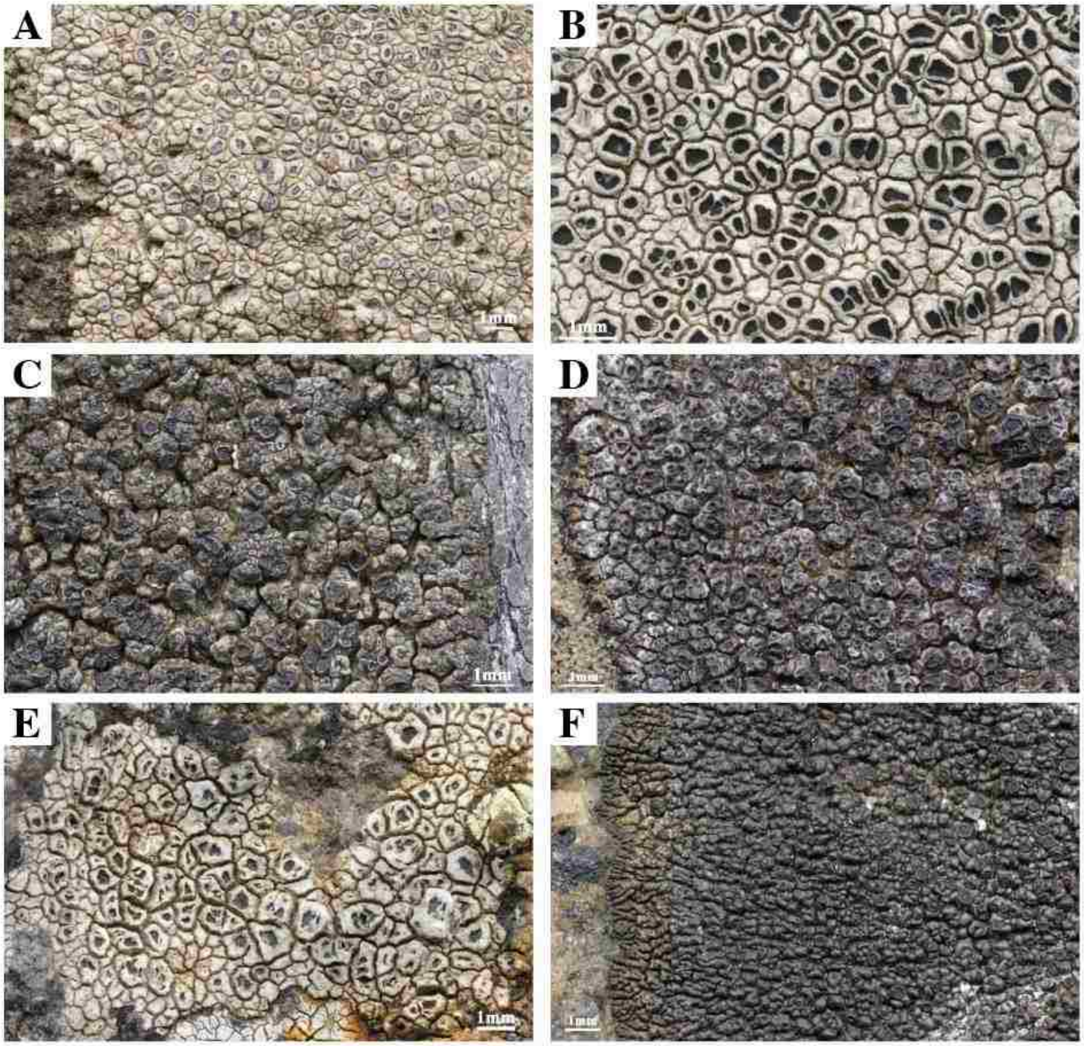

Figure 12. **A.** *Aspicilia indeterminata* Wheeler 5460. **B.** *Aspicilia indeterminata* Wheeler 7241- Holotype. **C.** *Aspicilia subradians* Wheeler 5044. **D.** *Aspicilia subradians* Wheeler 5070. **E.** *Aspicilia aff. epiglypta* Nelson S.N. **F.** *Aspicilia epiglypta* McCarthy 1681.

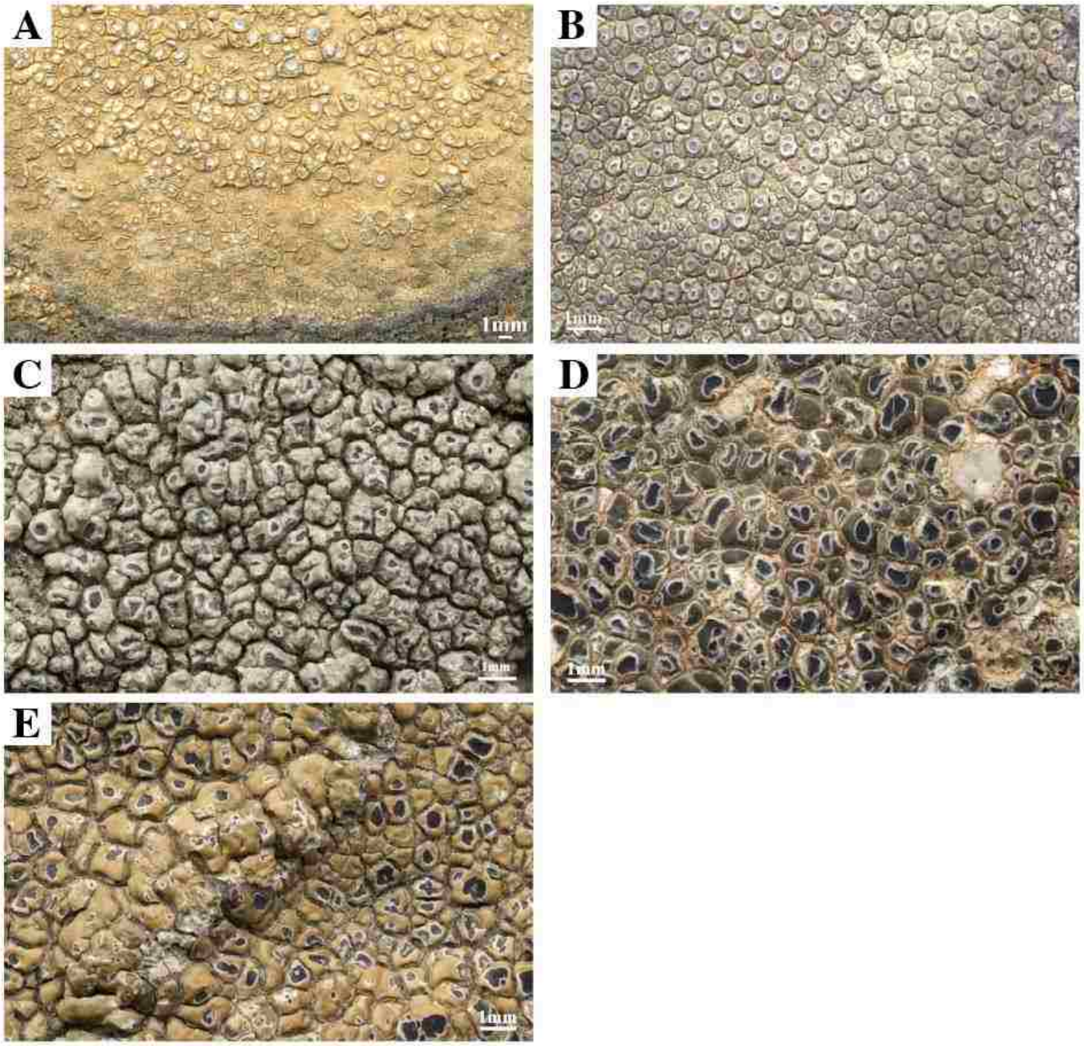

Figure 13. **A.** *Aspicilia aurantiaca* Wheeler 7091. **B.** *Aspicilia santamonicae* Wheeler 6648. **C.** *Aspicilia pacifica* Knudsen 9241. **D.** *Aspicilia cuprea* Knudsen 13050. **E.** *Aspicilia cuprea* Knudsen 16336.

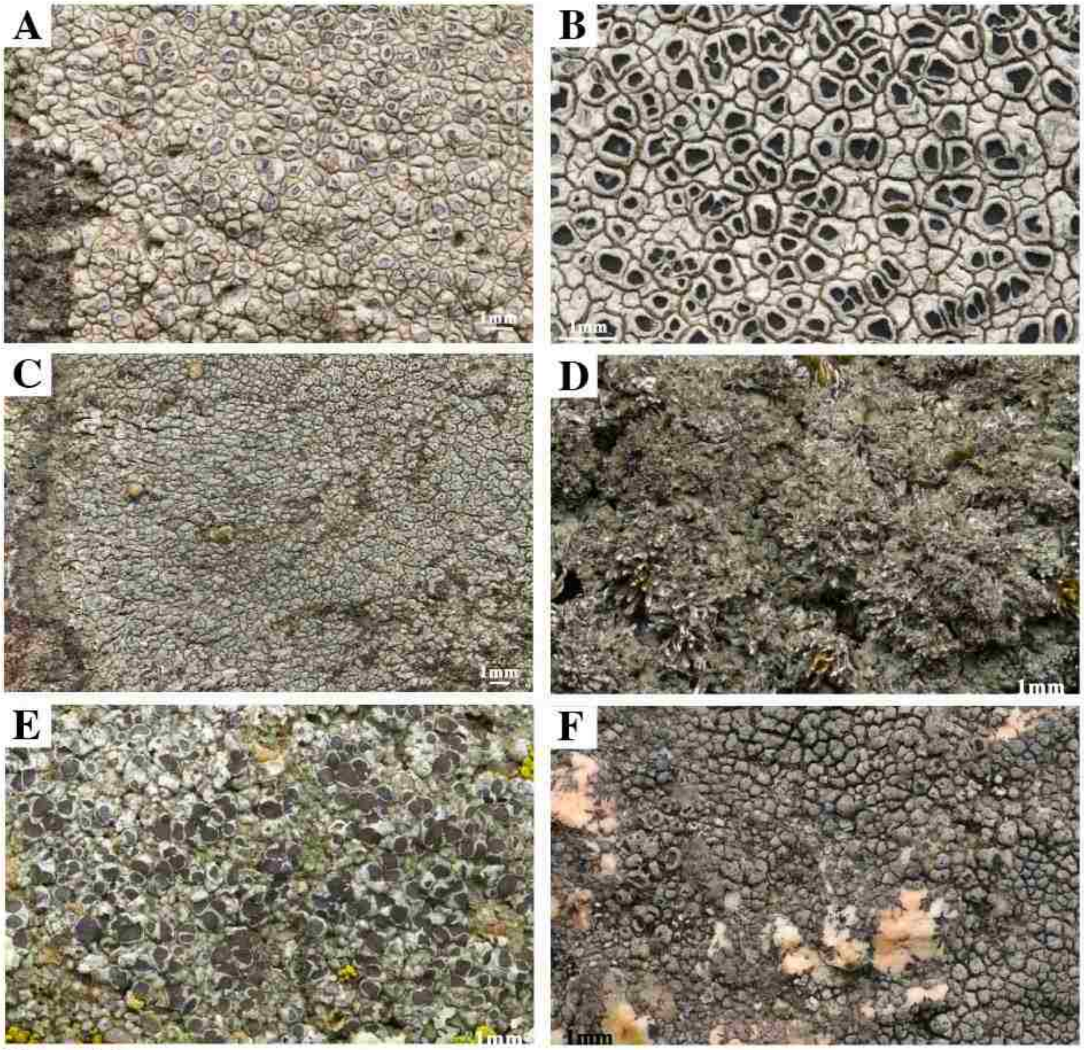

Figure 14. **A.** *Aspicilia indeterminata* Wheeler 5460. **B.** *Aspicilia indeterminata* Wheeler 7241- Holotype. **C.** *Aspicilia verrucigera* Wheeler 3439. **D.** *Aspicilia glaucopsina* Wheeler 7421. **E.** *Aspicilia phaea* Wheeler 7366. **F.** *Aspicilia confusa* Owe-Larsson 9095-**Isotype.**

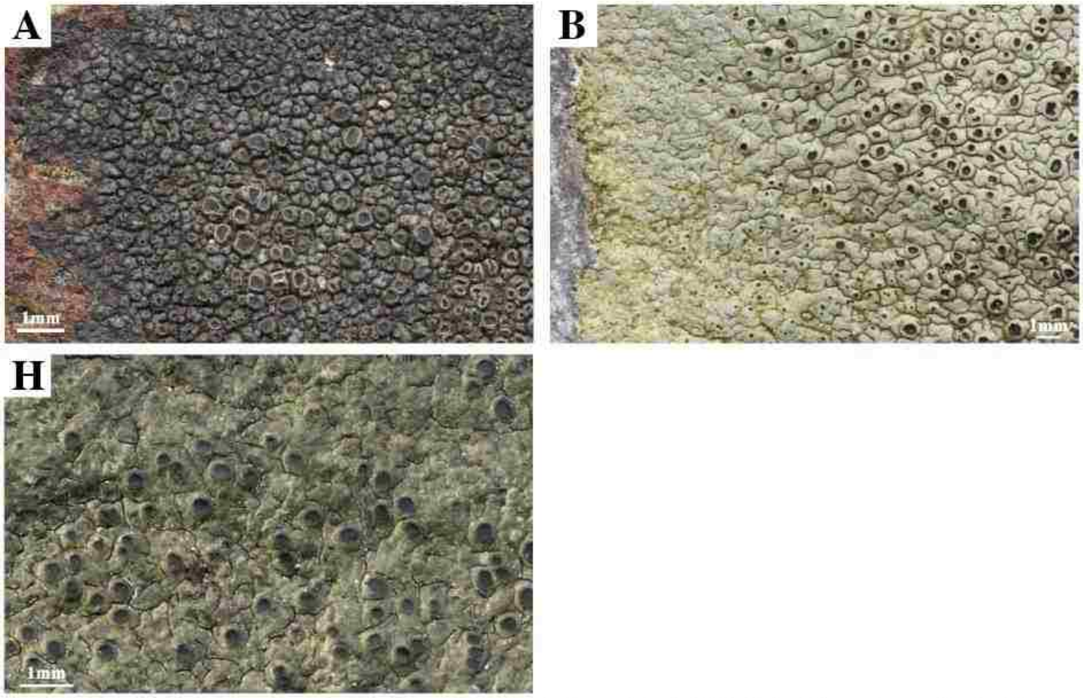

Figure 15. **A.** *Aspicilia aff. berntii* Wheeler 6883. **B.** *Aspicilia aff. laevata* Wheeler 7226. **C.** *Aspicilia aff. laevata* Wheeler 7345.

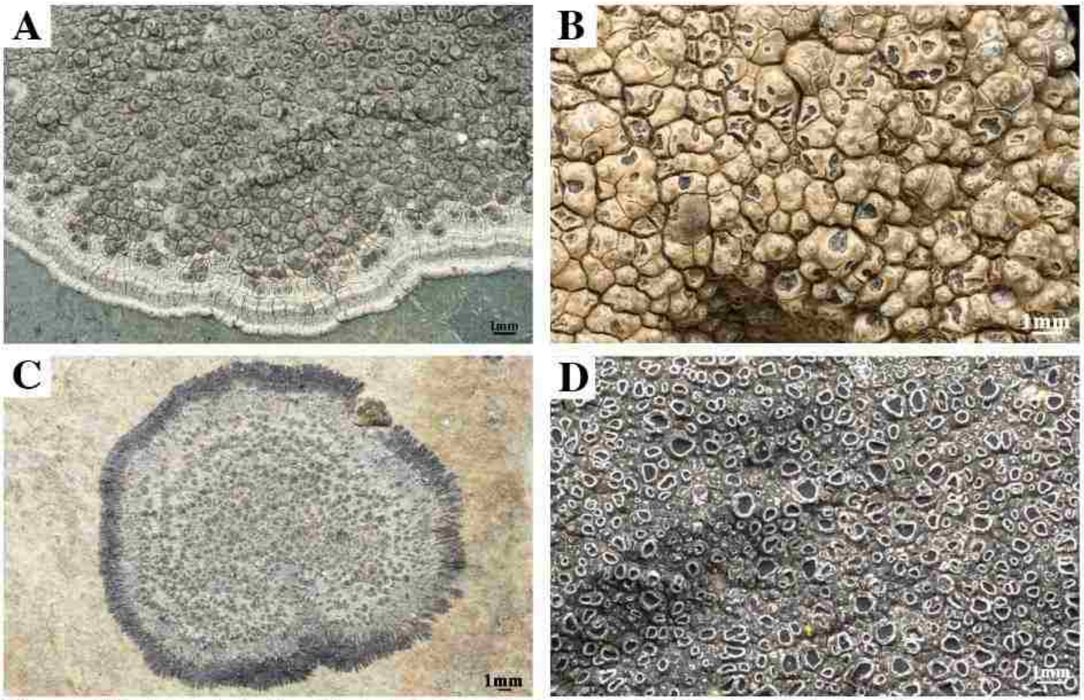

Figure 16. **A.** *Aspicilia knudsenii* Wheeler 3543. **B.** *Aspicilia knudsenii* Bjork 23423. **C.** *Aspicilia knudsenii* Wheeler 7497. **D.** *Aspicilia knudsenii* Wheeler 6798.

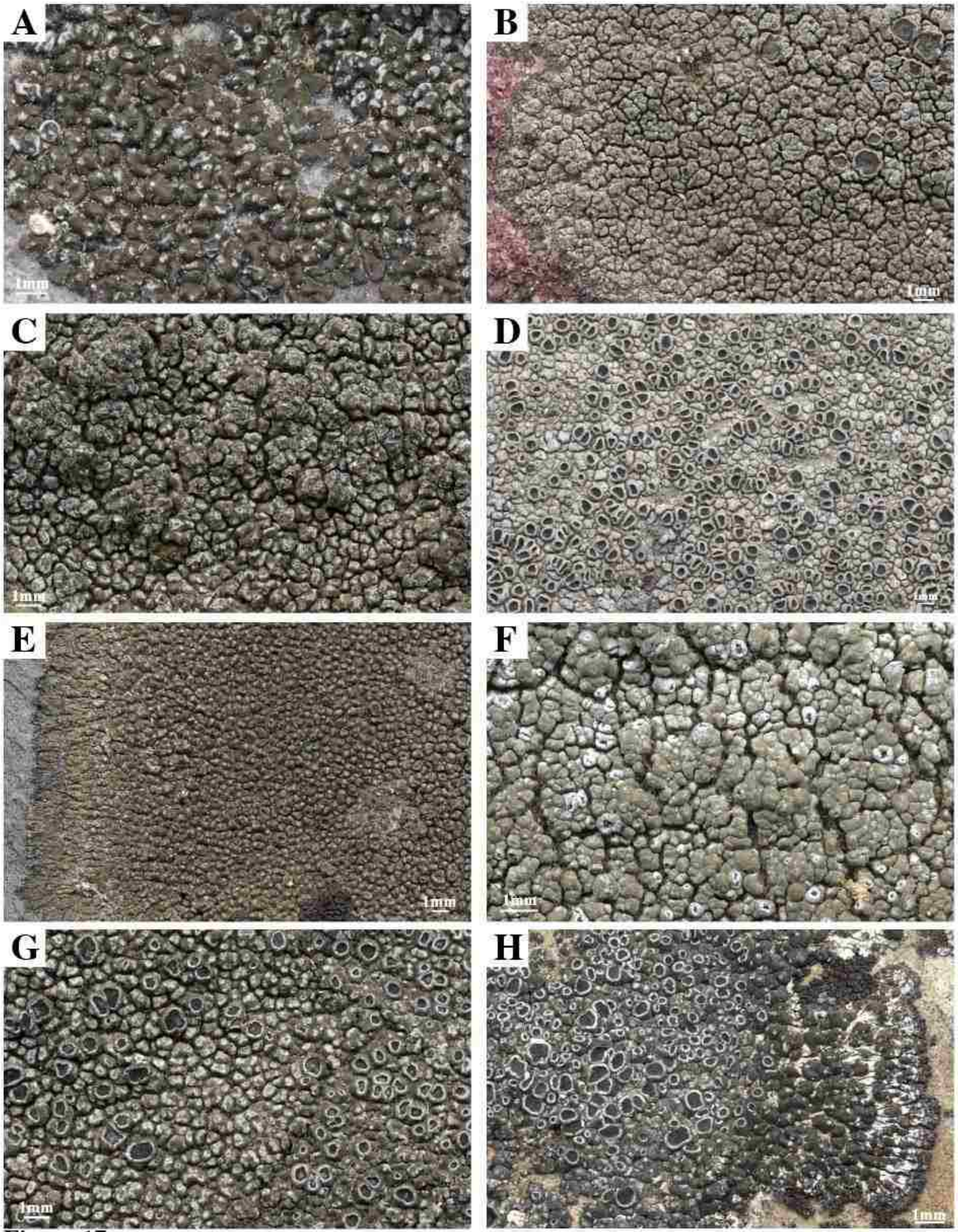

Figure 17. **A.** *Aspicilia tenuis* Knudsen 14770.2. **B.** *Aspicilia bufoides* Wheeler 2565 **C.** *Aspicilia bufoides* Wheeler 7259. **D.** *Aspicilia bufoides* Wheeler 7313 **E.** *Aspicilia bufoides* Wheeler 5865. **F.** *Aspicilia bufoides* Wheeler 7277. **G.** *Aspicilia bufoides* Wheeler 5399. **H.** *Aspicilia bufoides* Wheeler 7335.

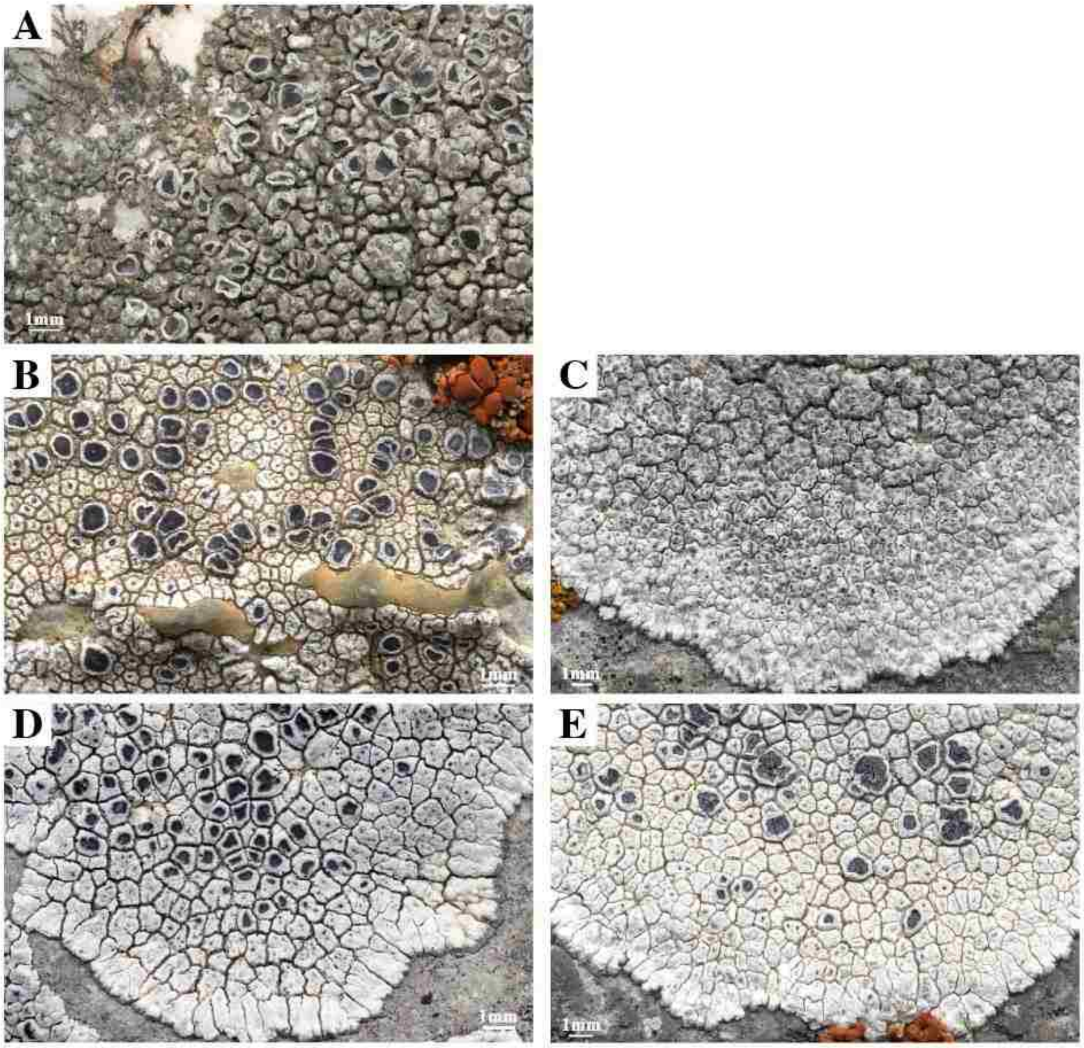

Figure 18. **A.** *Aspicilia bufoides* Knudsen 14707. **B.** *Aspicilia boykini* Wheeler 7249 **C.** *Aspicilia boykini* Wheeler 7274. **D.** *Aspicilia boykini* Wheeler 7273. **E.** *Aspicilia boykini* Wheeler 7263.

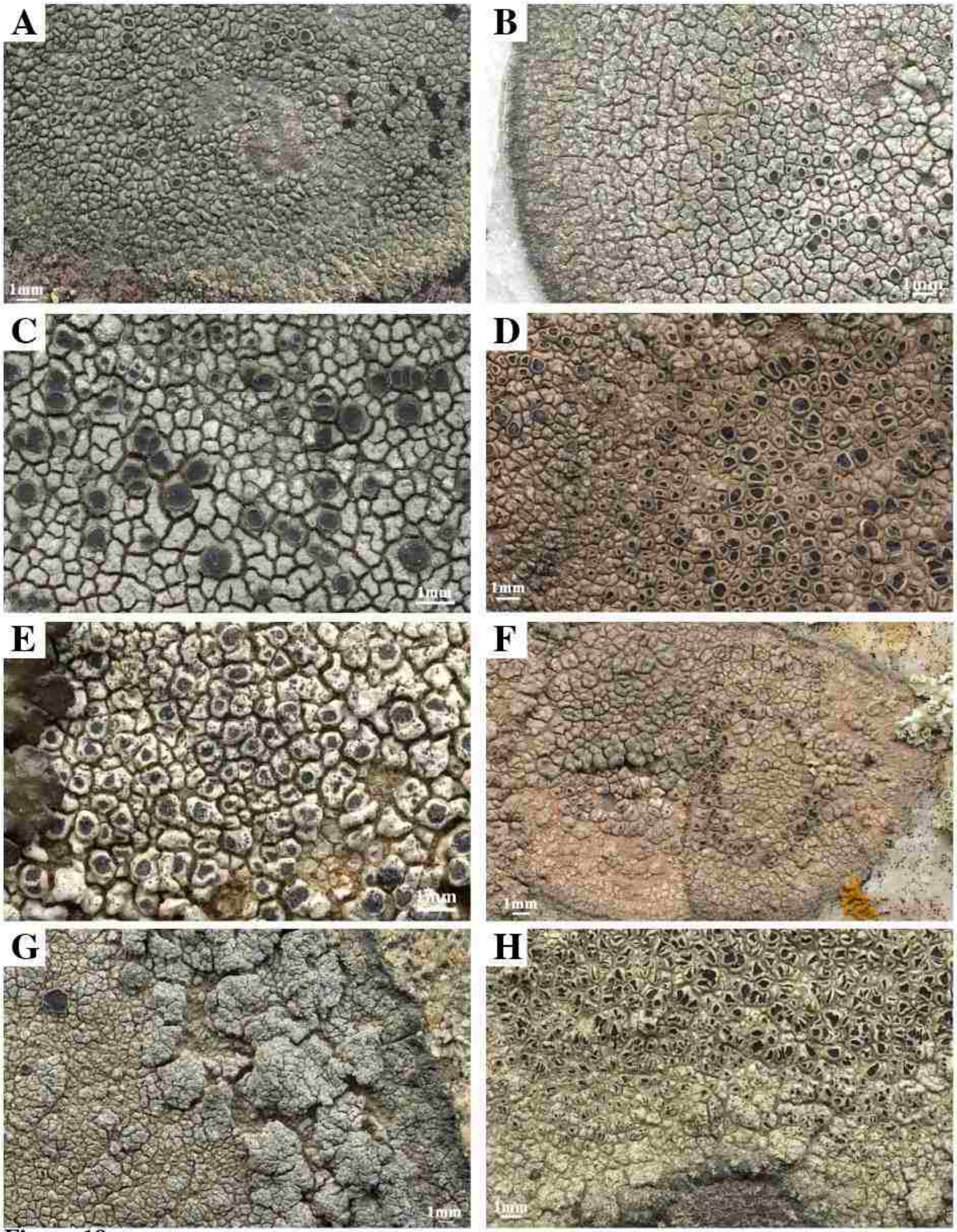

Figure 19. **A.** *Aspicilia fumosa* Wheeler 5934. **B.** *Aspicilia fumosa* Wheeler 6699. **C.** *Aspicilia fumosa* Wheeler 7228. **D.** *Aspicilia fumosa* Wheeler 5418. **E.** *Aspicilia fumosa* Knudsen 16514. **F.** *Aspicilia fumosa* Wheeler 5410. **G.** *Aspicilia fumosa* Wheeler 6820. **H.** *Aspicilia fumosa* Wheeler 6802.

Figure 20. A. *Aspicilia fumosa* Wheeler 3844.

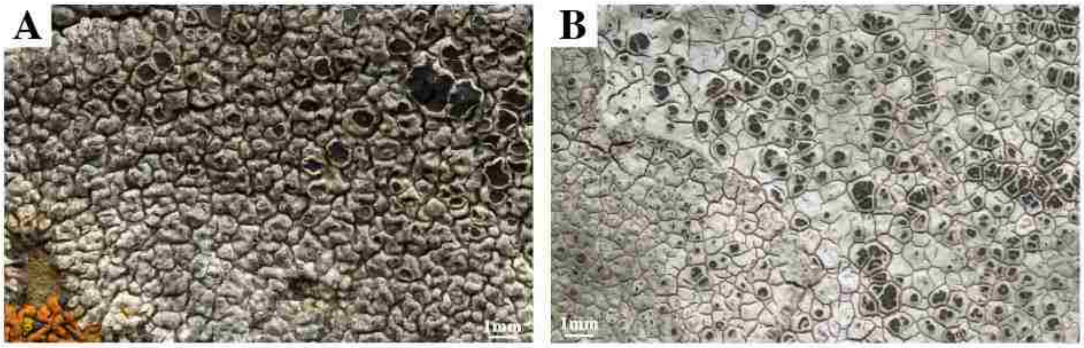

Figure 21. **A.** *Aspicilia arizonica* Wheeler 7152. **B.** *Aspicilia arizonica* Wheeler 7212.

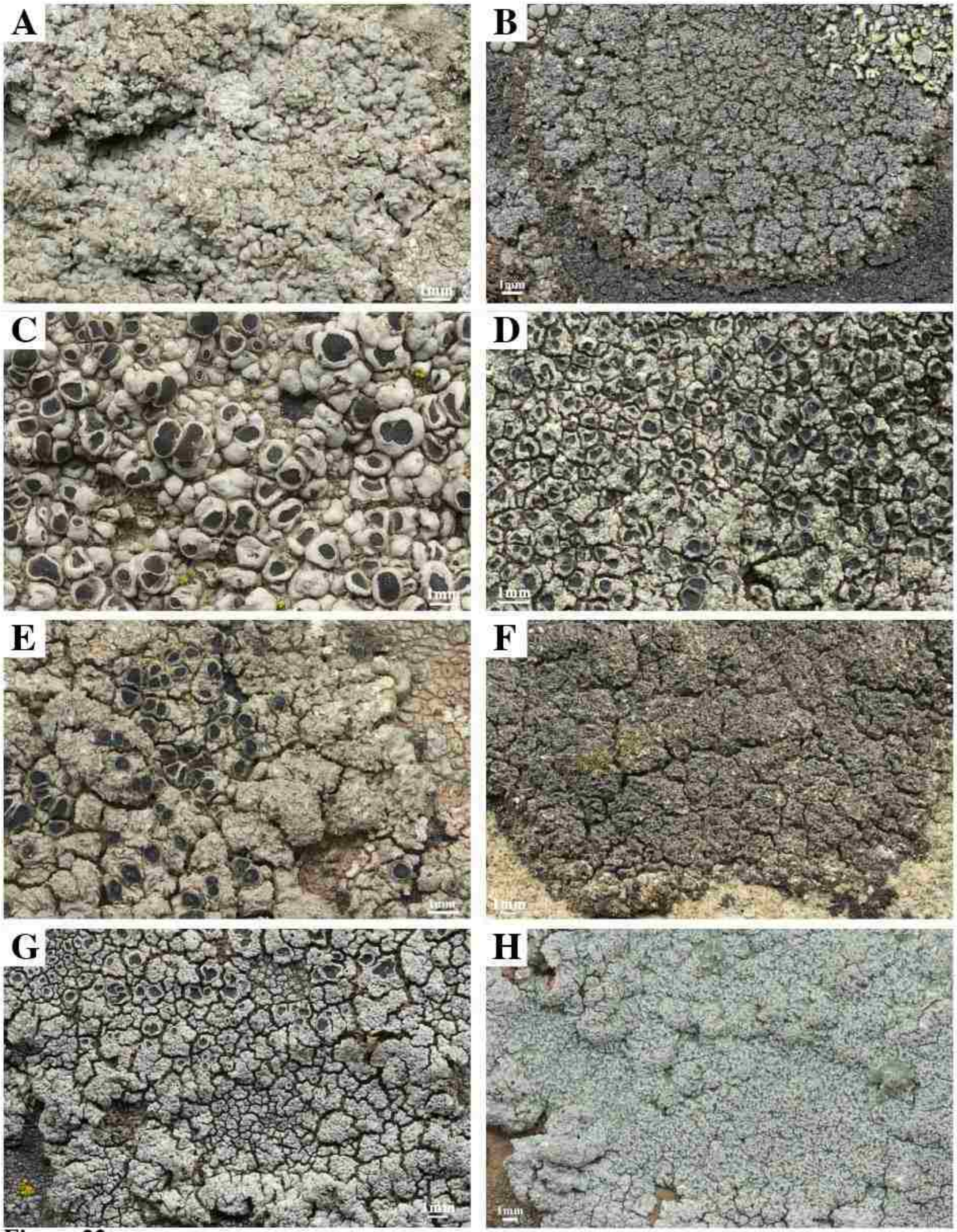

Figure 22. **A.** *Aspicilia anglica* Knudsen 9996. **B.** *Aspicilia anglica* Wheeler 3535. **C.** *Aspicilia anglica* Wheeler 5468. **D.** *Aspicilia anglica* Wheeler 6167. **E.** *Aspicilia anglica* Wheeler 5408. **F.** *Aspicilia anglica* Wheeler 6036. **G.** *Aspicilia anglica* Wheeler 7208. **H.** *Aspicilia anglica* Wheeler 2954.

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