Xanthosyne (Lecanoraceae), a new genus for *Lecidea varians* and related species in Europe and North America

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ABSTRACT. *Lecidea varians* is among the most common and abundant bark-dwelling crustose lichens in temperate eastern North America. As presently delimited, it is highly variable, including chemical and morphological diversity well beyond that currently accepted for most lichen species. The generic placement of *L. varians* has also been questioned for decades. It has long been recognized as aberrant in *Lecidea* and *Pyrrhospora*, excluded from *Lecidella*, and more recently transferred to *Traponora*. Drawing from the results of extensive chemical, molecular phylogenetic and morphological studies, we show that *L. varians* and its relatives represent a previously unrecognized lineage within the speciose lichen family Lecanoraceae. The lineage appears to occupy an isolated position, distinct from the aforementioned genera, and is newly described as the genus *Xanthosyne* (typified by *L. varians*). The chemical and morphological variation within *L. varians* is mirrored by, but not entirely correlated with, considerable molecular diversity. A new taxonomy is proposed for *L. varians* and its relatives to serve as a framework for future studies. Three species are recognized: *X. varians* (≡ *Lecidea varians*), common and widespread in parts of North America; *X. granularis*, a new species from the Atlantic Coast of eastern North America that differs morphologically from *X. varians* in having a leprose thallus; and *X. sharnoffiorum*, a new species also found mainly along the Atlantic Coast of eastern North America, which has a coarsely granular, non-leprose thallus and produces a unique, unidentified xanthone. Multiple well-supported lineages were recovered within *X. varians* that correlate to varying degrees with chemical and morphological variability, as well as geographic distribution. Eight subspecies are recognized to accommodate the variation within *X. varians*: *X. varians* subsp. *exigua* comb. nov. (≡ *Lecidea exigua*) characterized by the presence of atranorin and a consistent set of three xanthones, is widespread in southern Europe and western North America (coastal California); *X. varians* subsp. *varians* (≡ *Lecidea varians*) is distributed mainly in northeastern North America and produces thuringione and arthothelin; *X. varians* subsp. *morsei* subsp. nov. is morphologically and chemically variable, with one chemotype (thiophanic acid) with a northeastern distribution, and the other with a unique and unidentified xanthone, found mainly in the interior U.S.A.; *X. varians* subsp. *obscura* subsp. nov.

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occurs mainly in the central U.S.A. and North Temperate regions, produces a unique, unidentified xanthone and generally has black apothecia with green epihymenial pigments; *X. varians* subsp. *pseudomorsei* subsp. nov. and *X. varians* subsp. *submorsei* subsp. nov. resemble *X. varians* subsp. *morsei* but differ in molecular sequence characters; *X. varians* subsp. *subtilis* comb. nov. (≡ *Lecidea subtilis*) and *X. varians* subsp. *subexigua* subsp. nov. occur in the Appalachian Mountains, the former producing atranorin and the latter lacking atranorin, both with thiophanic acid with or without other xanthones. In an addendum, *Lecidella subviridis* is discussed with respect to the genus *Xanthosyne.* An identification key is provided for all species and their subspecies within *Xanthosyne*.

KEYWORDS. Ascus type, biogeography, Coastal Plain, cryptic species, *Pyrrhospora quernea*, secondary chemistry, semi-cryptic species.

The historical delimitation of lichen genera based on characters of macroscopic thallus or apothecial morphology and ascospore coloration or septation has long been recognized as highly artificial (e.g., Hertel 1987; Lendemer & Hodkinson 2013; Nordin 2000; Staiger 2005). Considerable progress has been made in recent decades to delimit and segregate monophyletic and morphologically distinct groups from large broadly delimited genera such as *Bacidia* De Not., *Buellia* De Not., *Lecanora* Ach., and *Lecidea* Ach. (e.g., Ekman 1996; Hafellner 1984; Hafellner et al. 1979; Hertel 1987; Vězda 1986). For instance, the lichen families Graphidaceae and Thelotremataceae, whose genus-level taxonomy was widely acknowledged to be artificial (Staiger 2002, 2005), have been extensively studied and revised (e.g., Kraichak et al. 2014; Lumbsch et al. 2014; Rivas Plata et al. 2013; Staiger 2002; Staiger et al. 2006). Nonetheless, many genera have been shown to still comprise heterogeneous elements despite a long period of study (Arup et al. 2013; Ekman 2001; Fryday & Hertel 2014; Gaya et al. 2003; Rodriguez-Flakus & Printzen 2014; Pérez-Ortega et al. 2010; Søchting & Lutzoni 2003; Zhao et al. 2016).

The genus *Lecidea* exemplifies the pattern outlined above wherein, despite decades of study drawing on chemical, morphological and molecular data, common and widespread species that are clearly not closely related to the type, *L. fuscoatra* (L.) Ach., have yet to be transferred elsewhere. Currently, there are 138 taxa of *Lecidea* s.l. reported from North America north of Mexico (Esslinger 2021), and these include many species that should be classified in other families or genera (e.g., Coppins & Fryday 2006; Printzen 1995; Printzen & Tønsberg 1999). The subject of the present study, *L. varians* Ach., described more than two centuries ago based in part on material from eastern North America (Acharius 1814; **Fig. 1** herein), is one such taxon. It is one of the most common and widespread corticolous crustose lichens in temperate eastern North America (Brodo et al. 2001; Lendemer & Harris 2014; Lendemer & Noell 2018; Tripp & Lendemer 2020) and includes a wide range of morphological and chemical diversity.

Lichenologists have recognized for decades that *Lecidea varians* does not belong in the genus *Lecidea* (Hafellner 1993; Hertel & Printzen 2004; Printzen 1995), yet its generic placement remained unresolved. Harris (1985) transferred it to *Pyrrhospora* as a potential solution. It was recognized, however, that the species likely did not belong there based on differences in ascus type and chemistry, among other characters (Hafellner 1993). Thus, it has continued to be treated in *Lecidea* by many authors (e.g., Esslinger 2021; Hertel & Printzen 2004; McCune 2017; Printzen 1995). Remarkably, *L. varians* also has yet to be included in any published phylogeny, either those aimed at resolving the generic placement of species of *Lecidea*

s.l. (Schmull et al. 2011), or broader studies aimed at resolving relationships within the Lecanoraceae and Lecanoromycetediae (e.g., Miadlikowska et al. 2014; Zhao et al. 2016).

Several authors of the present study converged on the need to reassess the delimitation and disposition of *Lecidea varians*. Two of us (JCL and RCH) discovered what appeared to be an undescribed species related to *L. varians* that was common in the Mid-Atlantic Coastal Plain (Lendemer & Noell 2018; Lendemer et al. 2016, both as *Pyrrhospora* sp.). At the same time, IMB and MELS were dealing with Spanish material of what appeared to be *L. varians* but was known to European workers under the name *L. exigua* Chaub. Although Tuckerman (1888) and Fink (1935) recognized *L. exigua* as a synonym of *L. varians*, the earlier name was not used in modern European works (e.g., Aptroot et al. 2009; Clauzade & Roux 1986; Wirth et al. 2013), except for Vězda (1979) who distributed material from the eastern shore of the Black Sea in his exsiccate under the name *L. varians*. In this context, we were inspired to finally resolve the generic placement of *L. varians* and determine whether the species occurred in Europe. After initial results led to the discovery that *L. varians* was much more chemically, morphologically and phylogenetically diverse than previously believed, the project was expanded to include material from throughout the range of *L. varians* and *L. exigua*. The results of this study are presented here.

MATERIALS AND METHODS

Study material. This study is based primarily on specimens deposited in the herbaria of the Canadian Museum of Nature (CANL) and New York Botanical Garden (NY). Fresh material of *L. exigua* was gathered in Portugal and Spain by MELS and from France by Jean-Yves Monnat. Fresh material and numerous herbarium collections from the south central U.S.A. were made available by Caleb Morse (KANU; duplicates at CANL); Jason Dart sent fresh material from California (deposited at CANL); JCL and RTM obtained fresh material from eastern North America (JCL and collaborators deposited at NY; RTM at CANL); Dennis Waters provided material from New Jersey (deposited at NY); Sean Beeching and Malcolm Hodges obtained fresh material from coastal Georgia, U.S.A. (deposited at CANL with duplicates at NY); fresh material from Maine was obtained by Fred Olday and Roger Rittmaster (deposited at CANL); from Nova Scotia by Frances Anderson (deposited at CANL); and from New Brunswick by Stephen Clayden (deposited in NBM). Specimens, including types, from H and UPS were also examined. In all, approximately 420 specimens were examined. Georeferenced voucher data for all NY specimens examined can be accessed via the C.V. Virtual Herbarium at NY (http://sweetgum.nybg.org/science/vh/).

Morphological methods. Specimens studied at CANL were examined with Wild M-5 dissecting microscopes and Leitz or Wild compound microscopes. Sections were cut by hand and observations were initially made in water mounts, usually checked in 10% KOH and sometimes 50% HNO_3. Spore measurements were made in both water and KOH with both giving essentially the same results. Birefringence in the hymenial, epihymenial and excipular tissues was determined using polarizing filters. At NY, specimens were initially studied dry using an Olympus SZ-STB dissecting microscope. Microscopic morphology and anatomy were then studied using an Olympus BX53 compound microscope and sections prepared by hand with a razor blade and mounted in water or iodine.

Chemical methods. Chemistry was initially studied using standard spot tests (K, C, KC, PD, LW UV) following Brodo et al. (2001). This was supplemented by an analysis of over 350 specimens of "*Lecidea varians*" from throughout its range with Thin Layer Chromatography

(TLC) using Solvents A, B′ and C following Culberson & Kristinsson (1970) and Orange et al. (2010). Chromatographs run in solvent C were first exposed to acetic acid fumes for five minutes. Xanthones were determined with reference to pure extracts of arthothelin, thiophaninic acid and thuringione provided by John Elix to IMB, as well as by comparison with standards of *Pertusaria flavicunda* Tuck. for thiophaninic acid and thuringione; *Lecanora confusa* Almb. for the "confusa-unknown" sensu Orange et al (2010) as well as thiophanic and usnic acids; *Pyrrhospora quernea* (Dicks.) Körb. from California for thiophanic acid and arthothelin; *Buellia ocellata* (Flotow) Körber for arthothelin; and *Lecanora expallens* Ach. for thiophanic acid, arthothelin, "confusa-unknown" together with usnic acid and zeorin. The glass plates were examined in long-wave (365 nm) UV light in a UV cabinet against a black background and then photographed using a digital camera or a smartphone. We found that digital photography significantly enhances the fluorescence of many of the xanthones (**Figs. 2–4**) and, because of this, we printed the resulting images and used the photographs for analyzing TLC runs. We used the R_f values and figures published by Orange et al. (2010) and Elix & Crook (1992) as our main references. We found that most xanthones do not develop well using TLC standard acid-charring methods, and so charring was only used after the photography described above on TLC runs mainly with solvent A to visualize other compounds such as atranorin.

Molecular data generation; DNA extraction, amplification and sequencing. Molecular laboratory work was performed at the Canadian Museum of Nature's Laboratory of Molecular Biodiversity. Total genomic DNA was extracted from tissue subsampled from specimens following a silica column purification protocol similar to commercially available DNA extraction kits (modified from Alexander et al. 2007). DNA extraction success was assessed via gel electrophoresis on 1.25% agarose gels stained with ethidium bromide. DNA sequence data were generated for three loci: the ribosomal internal transcribed spacer (ITS) region, (including partial 18S + ITS1 + 5.8S + ITS2 + partial 28S), the nuclear large subunit ribosomal gene (LSU), and the RNA polymerase II largest subunit (RPB1) gene. See **Table 1** for PCR and sequencing primers used. Amplification conditions were the same for the three loci, carried out in a 15 µL volume with 9.05 µL of ddH_20, 3 µL of 5× reaction buffer, 0.3 µL of 10mM dNTP, 0.75 µL of 10 µM each primer, 0.3 U of Q5 DNA Polymerase (New England BioLabs Inc.), and 1 µL of DNA template. An initial denaturation of 98°C for 30 sec was followed by 34 cycles of 98°C for 10 sec, 56°C for 30 sec, 72°C for 30 sec and a final extension step of 72°C for 5 min. DNA template was doubled for difficult samples. Amplification success was assessed via gel electrophoresis in 1.25% agarose gels stained with ethidium bromide.

Sequencing reactions were performed in 10 µL reactions containing 6.2 µL of DNA-grade H_2O, 1.8 µL of 5× reaction buffer, 0.5 µL of primer, 0.5 µL of BigDye Terminator v3.1 Ready Reaction Mix (ThermoFisher Scientific), and 1 µL of diluted PCR products. An initial denaturation of 95°C for 3 min was followed by 30 cycles of 96°C for 30 s and 50°C for 20 s followed by a final step at 60°C for 4 min. Reaction products were purified via an EDTA-NaOH-ethanol precipitation protocol recommended by the sequencing kit manufacturer. Purified DNA pellets were resuspended in HIDI formamide, denatured at 95°C for 5 min, cooled for 2 min, and sequenced via automated capillary electrophoresis on an Applied Biosystems 3500xL Genetic Analyzer (ThermoFisher Scientific).

Molecular data-set assembly and analysis. Sequences were assembled, trimmed, and visually verified to replace questionable designations with nucleotide ambiguity codes and ensure that no stop codons were present in the coding regions of the nuclear protein-coding genes using Geneious 11.1.5 (Kearse et al. 2012).

Based on published studies of morphology (e.g., Hafellner 1993), *Lecidea varians* has long been recognized as a member of the Lecanoraceae. To study its placement and relationships to other morphologically similar genera within that family, we used the six-locus Lecanoraceae dataset published by Zhao et al. (2015) as a framework within which to evaluate our newly generated sequences. The alignment was downloaded from TreeBase (Study ID: 18171) and the concatenated alignment was disassembled into individual single locus alignments using Mesquite 3.70 (Maddison & Maddison 2021). Our newly generated sequences (see **Table 2**) were then manually incorporated into the relevant single locus alignments using Mesquite. The alignments were then manually checked in Mesquite and ambiguously aligned regions defined as part of an exclusion set.

For each single locus alignment, the ambiguously aligned regions were manually removed in Mesquite, uncertainties and polymorphisms were converted to missing data, the terminal gaps were converted to missing data, and the resulting alignment was exported as a PHYLIP formatted file. Each single locus alignment was then subjected to maximum likelihood analyses using IQTree (Minh et al. 2020). A maximum likelihood tree search was performed implementing the models of nucleotide substitution selected by ModelFinder Plus based on the Bayesian information criterion (BIC). Models were selected as follows: ITS: TN+F+I+G4, LSU: TN+F+I+I+R3; MCM7: TN+F+I+G4, RPB1: TIM2e+I+I+R3, RPB2: SYM+R3, SSU: TN+F+R3. Branch support was evaluated with 1000 ultrafast bootstrap replicates (UFBoot; Hoang et al. 2018) and 1000 Shimodaria-Hasegawa approximate likelihood ratio test replicates (SH-aLRT; Guindon et al. 2010). Results were visualized in FigTree to confirm that there was no conflict between individual loci (defined by Mason-Gamer & Kellogg 1996).

The individual single-locus alignments used as inputs for the above analyses were then concatenated in Mesquite to reassemble the six-locus dataset of Zhao et al. (2015) but with our newly generated sequences included. A separate input file was created to partition each locus for the combined multi-locus analyses. The six-locus dataset was then analyzed in the same method as the individual loci, but with partitions implemented.

The six-locus dataset from Zhao et al. (2015) incorporating our newly generated sequences recovered the sequences of *Lecidea varians* and its relatives in a strongly supported clade separate from others in the Lecanoraceae (see Results: Monophyly of the *Lecidea varians* group). To further study relationships within this clade, we carried out additional analyses on a version of the multi-gene dataset that was reduced to the *L. varians* group and with selected sequences of *Lecidella* included as an outgroup based on the results of the six-locus analyses. For this dataset, we reduced the number of loci from six to three due to the large amount of missing data for reference sequences from Zhao et al. (2015), failure of many of our samples to amplify some loci despite repeated attempts in the laboratory which created large gaps in the ingroup dataset, and apparent lack of ingroup sequence variation in some loci based on our initial analysis. The final alignment used to study the *L. varians* group consisted of data from three loci: ITS, nucLSU and RPB1. The alignment was prepared by pruning the larger six-locus dataset to the narrower taxon sampling outlined above, each locus was realigned and manually checked for errors, ambiguously aligned regions reexamined and redefined as an exclusion set, and then partitioned. The dataset was analyzed in the same manner as the six-locus dataset above. Based on BIC scores the following models were implemented: ITS: TN+F+G4, LSU: Tne+I, RPB1: TPM2+G4. The results of all analyses were visualized in FigTree 1.4.4 (Rambaut 2017) and figures were assembled in Adobe Photoshop CS6.

In addition to inferring phylogeny and support values for the three-locus dataset, we generated a pairwise genetic distances matrix for the ITS partition of the dataset. For this analysis, we applied a simple Jukes-Cantor substitution model with no gap penalty in Mesquite. Mean and standard deviations for pairwise distance values were calculated in Excel for within and between the numbered clades recovered in the three-locus alignment.

RESULTS AND DISCUSSION

We analyzed approximately 420 specimens for this study, generating extensive chemical, morphological and molecular data. Collectively, our analyses of these data demonstrate that *Lecidea varians* and its relatives represent a phenotypically distinct group of taxa that are strongly supported as belonging to a distinct, previously unrecognized lineage in the Lecanoraceae. The results outlined below are organized to first present information pertaining to the delimitation of the group within the Lecanoraceae, and then present information about species delimitation of the taxa within the group.

Monophyly of the Lecidea varians group. Our reanalysis of the six-locus dataset for Lecanoraceae published by Zhao et al. (2016) was topologically congruent with the results of those authors, and it recovered all the same genus-level clades or species groups, largely with support (**Fig. 5**). The newly generated sequences of *Lecidea varians* and its relatives that were the focus of this study were recovered as monophyletic in a strongly supported clade (SH-aLRT/UFBoot: 99/100) recovered in a strongly supported (98/100) clade with *Lecidella*, the latter of which was poorly supported (45/90). The clades within *Lecidella* were also recovered by Zhao et al. (2015) and Ruprecht et al. (2020). Those with more than one reference sequence were recovered as monophyletic and with strong support ("*Lecidella elaeochroma"* clade: 100/100; "*Lecidella enteroleucella*" clade, represented by one sequence; *Lecidella stigmatea* clade: 100/100). Thus, the lineage comprised of *L. varians* and its relatives is strongly supported as monophyletic and recovered as sister to, but distinct from, members of *Lecidella* s.l. Importantly, this lineage of *Lecidea varians* and its relatives was not recovered as closely related to *Pyrrhospora quernea*, the type of *Pyrrhospora* Ach., which was instead recovered in a strongly supported clade (98/100) with multiple species groups of *Lecanora* s.l. (i.e., *L. formosa, L. subcarnea* group, *L. subfusca* group of Zhao et al. 2016).

Phenotypic delimitation of the Lecidea varians lineage. As has been highlighted in the introduction, the generic placement of *L. varians* has been the subject of considerable disagreement. Although originally described in *Lecidea* more than two centuries ago, nearly every modern author to treat the species has recognized that it is not related to *Lecidea* s.str. (type: *L. fuscoatra*) and instead likely belonged to the Lecanoraceae (Hafellner 1993; Harris 1985; Hertel & Printzen 2004; Printzen 1995). Placement in the genus *Pyrrhospora* by Harris (1985) was also considered untenable, and the species has frequently continued to be treated in *Lecidea* despite the fact it is known not to belong there (e.g., Hertel & Printzen 2004). More recently, Kalb & Kalb (2017) transferred the species to *Traponora* Aptroot. A tabular comparison of morphological and chemical characters illustrates that *L. varians* is morphologically discordant with all of these genera (**Table 3**).

Lecidea varians has most frequently been referred to the genus *Pyrrhospora*, but species of *Pyrrhospora* s.str. (i.e., *P. quernea*) produce K+ purple anthraquinones in the apothecia and have *Lecanora*-type asci (Hafellner 1993; Hitch & Hawksworth 2009; **Fig. 6A** herein). In contrast, we found that all members of the *L. varians* lineage lack anthraquinones and have *Lecidella*-type asci, in agreement with Hertel & Printzen (2004; **Figs. 6B–D** herein). Some

authors have considered *L. varians* to have a *Lecanora*-type ascus (e.g., Kalb & Kalb 2017) perhaps due to the tendency of its asci to stain heavily, which obscures the tholus characters of the *Lecidella*-type ascus with its broad axial mass that is often darkly stained at the edge and almost pierces the tholus (**Fig. 6D**). In *Lecanora*-type asci, the walls of the axial mass do not stain darkly and the axial mass completely pierces the tholus often leaving an indentation at the summit (**Fig. 6A**).

Lecidella is morphologically and chemically similar to *Lecidea varians*, and our phylogenetic analyses recovered the *L. varians* lineage as a well-supported clade sister to *Lecidella*, although the latter was weakly supported. *Lecidella* and *Lecidea varians* share the same ascus type, frequent production of xanthones in the thallus and frequent presence of *cinereorufa*-green pigments in the epihymenium (Knoph et al. 1995; Knoph & Leuckert 1994, 2000, 2004; Leuckert et al. 1990, 1992). Nonetheless, members of the *L. varians* lineage differ from each of the currently recognized *Lecidella* clades in suites of ecological and phenotypic characters. Members of the *Lecidella stigmatea* clade are primarily saxicolous, have a hyaline hypothecium, solitary and relatively large apothecia (0.8–3.0 mm in diameter) and lack xanthones (Hertel & Leuckert 1969; Zhao et al. 2015). Although *L. varians* and its relatives also have a hyaline hypothecium, they differ in having small apothecia (0.1–0.4 mm in diameter) that are frequently clustered, consistently produce one or more xanthones, and are exclusively corticolous or lignicolous. Members of the "*Lecidella elaeochroma* clade" have a brown pigmented hypothecium, solitary and relatively large apothecia (0.4–1.0(–2.0) mm in diameter) and produce xanthones (Knoph & Leuckert 2004; Zhao et al. 2015). *Lecidea varians* and its relatives also produce xanthones but differ in having a hyaline hypothecium and small apothecia (0.1–0.4 mm in diameter) that are frequently clustered. *Lecidella enteroleucella* is closest phenotypically to members of the *L. varians* lineage in that it produces xanthones and has relatively small apothecia (0.2–0.8 mm in diameter) with a hyaline hypothecium (Knoph & Leuckert 2004; Lendemer et al. 2019; Zhao et al. 2015). However, *L. enteroleucella* is strictly saxicolous whereas members of the *L. varians* are corticolous, and the apothecia are solitary and often remain partly immersed in the thallus whereas those of the *L. varians* group are typically clustered and sessile (Hertel 1977; Zhao et al. 2015).

During the final stages of preparation of the current manuscript, Pérez-Ortega et al. (2023) published *Nimisora* Pérez-Ort., M.Svenss. & J.C.Zamora to accommodate an unusual crustose lichen in the Lecanoraceae with biatorine apothecia that they described as *N. iberica* Pérez-Ort., Turégano, M.Svenss. & J.C.Zamora. We were struck by the similar appearance of *N. iberica* to *Lecidea varians*, and the authors even mentioned that their new taxon had been confused with *Lecidea exigua*, which they also stated was synonymous with *Traponora varians* and shared the same ascus type as their new species (referred to as similar to the *Bacidia*-type). Despite the superficial similarities and stated shared ascus type, there are numerous differences between *Nimisora* and *L. varians*. The molecular phylogenetic results presented by Pérez-Ortega et al. (2023) recovered multiple samples of *N. iberica* in a strongly supported clade, on a long branch, sister to *Ramboldia* Kantvilas & Elix, albeit in a much larger clade with weak backbone support and with numerous other genera including *Lecidella*. In contrast, we recovered *Lecidea varians* in a strongly supported clade in a strongly supported sister relationship with *Lecidella*. Phenotypically *N. iberica* differs markedly from *L. varians* in the lack of lichen substances (vs. consistent presence of xanthones and often atranorin), green pigmentation of the exciple (vs. hyaline or brown) and excipular anatomy that consists of broad, radiating hyphae that terminate in expanded round cells (vs. two-parted, inner exciple and outer rim of non-gelatinized

prosoplectenchymous hyphae and lower outer portion of exciple of gelatinized anticlinal branched hyphae).

Based on our analyses and comparisons with other genera in the Lecanoraceae, the *Lecidea varians* lineage occupies an isolated position, sister to *Lecidella*. We initially considered expanding the current delimitation of *Lecidella* to include the *L. varians* lineage. Such a treatment, however, would obscure this distinctive group of crustose lichens within a highly variable and broadly delimited genus. That *Lecidella* appears to be comprised of multiple, discrete, strongly supported evolutionary lineages suggests that it will likely be split into more natural and recognizable groups in the future. We therefore assert that, based on its unique combination of ecology, molecular and phenotypic characters, the *L. varians* lineage merits recognition as a distinct genus, which we introduce in the taxonomic section below under the name *Xanthosyne*.

Characterization of Lecidea varians s.str. As is outlined in the proceeding sections, *Lecidea varians* as presently delimited includes molecular and phenotypic variation well beyond that traditionally recognized as belonging to a single lichen species. To address species delimitation within this lineage, it was first necessary to determine the characteristics of the type. *Lecidea varians* was based on material described as follows: "Habitat in Amer. Septentr. Ad corticem Fraxini Carolineanae & Diospyros Loti; etiam in Gallia" (Acharius 1814; **Fig. 1** herein). The original material for this name corresponds to sheet H-ACH 322 in Acharius' herbarium which hosts five specimens labelled A–E in pencil (**Fig. 7**). Each specimen is annotated with the general location information in ink with Acharius' hand, while additional data appear to have been added in the same pencil as the appended letters in the hand of Theodore Fries. Of these specimens, H-ACH 322A was labelled "Amer. Septentr." by Acharius and then by Fries as having been collected by G.H.E. Muhlenberg, presumably near his home in southeastern Pennsylvania, U.S.A. (Lendemer & Hewitt 2002; Mears 1978). Two specimens were labeled by Fries as having been collected by Pehr Kalm, one in "Carolina amer." (by Acharius) "in fraxino" (by Fries) (H-ACH 322B) and one in "Amer. Boreal." (by Acharius) "in Diospyro Loto." (by Fries) (H-ACH 322C). Strangely, Kalm did not travel to the American colony of Carolina and, as such, the provenance of H-ACH 322B is unclear (Juel 1920; Payne & Newman 2005). The reference to *Disopyros lotus* L. instead of the American *D. virginiana* L. is also perplexing since both species were described by Linnaeus well before Acharius published *L. varians*. The fourth specimen, H-ACH 322D, was labeled as "Lett. No. 24. Pers. Gallica" by Fries and presumably refers to a collection made by C.H. Persoon in France. The final specimen, H-ACH 322E was marked with an asterisk by Acharius that associates it with an additional epithet "β berberidis" in Acharius' hand and was labeled "Suecia" by Acharius and "No. 7. Fries" by Fries. Among these specimens, those labelled A–D appear to be original material of *L. varians*.

Surprisingly, the name *Lecidea varians* does not appear to have been previously typified, despite having been transferred among multiple genera in modern times. Of the specimens that constitute original material, H-ACH 322A and H-ACH 322D consist of a single small bark fragment each, and these are not ideal to serve as a type in the event that it will require repeated future chemical study. H-ACH 322C is a poor choice because although the subsrate corresponds to the protologue, the location ("Amer. Boreal.") does not. Here we select H-ACH 322B as lectotype (**Fig. 7**), the specimen marked as having been collected in "Carol. Amer" by Pier Kalm even though Kalm is not known to have visited this region. It consists of a larger bark fragment with ample material for chemical analysis, and the data do not conflict with the protologue. The specimen could have been collected in the American colony of Carolina by someone other than

Kalm and then either sent directly to Acharius or given to Kalm who then sent it on to Acharius. It is also possible the specimen was collected by Kalm in southern New Jersey, U.S.A. where he was based, or elsewhere in northeastern North America where he travelled (Juel 1920; Payne & Newman 2005). Regardless, the specimen clearly originated in eastern North America; it is just unclear exactly where it was collected and by whom.

The lectotype (**Fig. 7**) is characterized morphologically by a continuous to rimose-areolate, gray-green to yellowish green thallus and pale to dark reddish brown apothecia with a reddish brown to pale brown epihymenium that is birefringent in polarized light (POL+), and chemically by the production of arthothelin and thuringione. While the lectotype is morphologically congruent with many of the collections of *L. varians* that we examined from the region where it was collected, it is chemically anomalous in that most of the material examined from present-day coastal North Carolina and South Carolina, U.S.A., produced thiophanic acid with varying concentrations of confusa-unknown and/or arthothelin. In contrast, the type is chemically more concordant with material from coastal areas of northern New England and the southern Canadian Maritime Provinces where populations that produce arthothelin and thuringione are common. We did, however, document two specimens from Georgia, U.S.A. (*Lendemer 21221-A*, NY; *Brodo 16454C*, CANL) that match the lectotype chemically and morphologically. This indicates that, although rare, this variant does occur in the region where the lectotype is labelled as having been collected.

Morphological variation in the Lecidea varians lineage. As has been discussed above, all of the sequences recovered in the *Lecidea varians* lineage were derived from specimens that shared a unique suite of morphological characters: minute, biatorine apothecia (ca. 0.15–0.35 mm in diameter), small, hyaline, one-celled, relatively thick-walled ascospores (ca. 8–13 × 5–7 µm), an exciple consisting of radiating, conglutinate prosoplectenchyme, usually with some brown pigmentation at least externally, and *Lecidella*-type asci. The sequenced specimens we examined could be placed in one of four morphological groups based on their thallus and apothecial morphology and color. The characteristics of each are summarized in **Table 4** with numbers and percentages given for both the full set of specimens studied in detail (n=332) and the subset of sequenced specimens (n=93). The percentages of the full set and subset are approximately the same.

The first morphological group (Morphotype-A, hereafter) is by far the most common and corresponds to the lectotypes of *Lecidea varians* and *L. exigua*. It is characterized by having a continuous to rimose-areolate, gray-green to yellowish green thallus and pale to dark reddish brown apothecia (**Fig. 8A**) with or without pruina and with a reddish brown to pale brown epihymenium that is usually birefringent in polarized light (POL+).

The second morphological group (Morphotype-B, hereafter) consists of a small number of specimens that have a thin, distinctly leprose, yellow (often dark yellow) thallus (**Fig. 8B**) that is infrequently fertile. The anatomy of the apothecia found in all of the fertile specimens examined corresponds fully with that of Morphotype-A. Hence, the Morphotype-B differs morphologically from Morphotype-A only in the thallus type and coloration.

In the third morphological group (Morphotype-C, hereafter), the thalli vary from smooth and continuous to rimose-areolate. The apothecia are dark brown to black (**Fig. 8C**) with or without pruina and contain varying concentrations of green, HNO_3 + red, epihymenial pigments (i.e., *cinereorufa*-green) that make the epihymenium appear smoky gray-brown, olive or distinctly aeruginose depending on the concentration. The epihymenium is rarely birefringent in polarized light.

The fourth morphological group (Morphotype-D, hereafter) is uncommon. It consists of specimens having thalli that are relatively thick, coarsely granular, and green or gray-green **(Fig. 8D**), never leprose and distinctly yellow as in Morphotype-B. The apothecia are most often red-brown with a red-brown epihymenium but rarely can be dark, even black with green pigments in the epihymenium. They rarely have pruina and almost always lack birefringence (i.e., are POL−).

Chemical variation in the Lecidea varians lineage. From our studies of over 400 specimens using thin layer chromatography (TLC), eleven taxonomically meaningful secondary metabolic substances were detected (**Table 5**), the majority of which were xanthones. The only substances detected in addition to xanthones were atranorin and an unidentified substance that quenches in long-wave UV light prior to acid charring like usnic acid or placodiolic acid (see entry for *Xanthosyne sharnoffiorum* in the taxonomic section). Nine xanthones were detected, five of which have been previously identified in the literature: arthothelin, the "confusa-unknown," thiophanic acid, thiophaninic acid and thuringione. Four of the xanthones we detected do not appear to have been previously reported in the literature and may be unique to *Xanthosyne*.

Arthothelin often occurred as a major or minor accessory together with thiophanic acid and was almost always found to occur in specimens with thuringione. The unnamed substance called "confusa-unknown" by Orange et al. (2010) is, in fact, a major substance in *Lecanora confusa* Almb. Based on our comparative TLC of reference specimens of *L. confusa* and *L. expallens* Ach., this is probably the same substance called "expallens-unknown" by Edwards et al. (2009) as we found it in both taxa. Thiophanic acid was the major substance in most *Xanthosyne* specimens that we examined. Thiophaninic acid was rarely present in *Xanthosyne* and was usually accompanied by other xanthones. Thuringione, which was found in the lectotype of *Lecidea varians,* was infrequent in *Xanthosyne* and almost always was accompanied by arthothelin.

Each of the four unidentified xanthones found in this study does not seem to correspond to any described by Orange et al. (2010) or Elix & Crook (1992). They are discussed individually below. Representative samples of each unidentified xanthone were studied by colleagues at the University of Rennes who concluded that xanthones #2 and #3 appear to be dichloro-O-methylnorlichexanthones of unknown molecular configuration while xanthones #1 and #4 remain entirely unknown (Françoise Lohézic-du-Dévéhat, Solenn Ferron and Phillipe Uriac, unpublished data).

Unknown xanthone #1. This substance fluoresces strong dark yellow or orange in long-wave UV light, reacts C− or C± pale orange, KC−. It runs with thiophaninic acid in solvent A, above it in B′ and just above it in C (**Table 5, Figs. 2–4**). It can occur alone or in the company of other xanthones, especially thiophanic acid. On TLC plates, it runs higher than all the other xanthones except the much rarer xanthone #4 (**Table 5**).

Unknown xanthone #2. Specimens containing this unnamed xanthone fluoresce bright pink-orange in long-wave UV light and react C−, KC−. It runs with thuringione in solvent A, above it in B′ and below it in C (**Table 5, Figs. 2–4**). Xanthone #2 always occurs alone in *Xanthosyne*.

Unknown xanthone #3. Thalli containing this substance are UV+ dark orange or pinkish orange, often faintly so, but the thalli react C+ strong orange, KC+ orange, distinguishing it from specimens containing xanthone #2, which are negative with C. It was found alone in *Xanthosyne* except in two specimens that also contained atranorin (perhaps as a contaminant). Its R_f values

are similar to those of xanthone #2 except in solvent B′ in which it runs significantly lower (**Table 5**).

Unknown xanthone #4. Thalli containing only this compound react UV−, C+ yellow-orange and KC+ yellow-orange. It runs above xanthone #1 in A and C and just below it in B′ (**Table 5**). Xanthone #4 is not common in *Xanthosyne* but is usually found together with thuringione and arthothelin.

Phylogenetic relationships within Xanthosyne. The results of our phylogenetic analyses of three loci (ITS, LSU and RPB1) for samples recovered in the *Xanthosyne* clade (**Fig. 5**) are presented in **Fig. 9**. Eight well-supported clades were recovered, which are numbered 1–8 in the tree. Some of these clades entirely, or in part, conform with morphological groupings, chemical profiles or geographic ranges. Of the eight clades, the most phenotypically distinctive are Clades 7 and 8, suggesting congruence between phenotypic and phylogenetic data. The other clades (Clades 1–6) are less consistent in chemistry and/or morphology although several have strong congruence between the phylogenetic data and morphology, chemistry or geographic ranges (**Fig. 9, right panel**).

Clade 7 comprises three samples represented by highly similar sequences that were recovered in a strongly supported clade (SH-aLRT/UFBoot: 100/100) sister to a sequence from a single sample (marked "7?" in **Fig. 9**) that differed significantly in morphology and chemistry. These three samples are all the sequenced specimens characterized by a yellow, leprose thallus (i.e., Morphotype-B) that produce thiophanic acid and confusa-unknown as the major products. The lone sample labeled "7?" is a clearly unconforming specimen (isolate MCM205, Oklahoma, Osage County, Sand Springs, Keystone Ancient Forest Preserve, *C.A. Morse 26224-A*, KANU) with a thin, continuous to rimose thallus (i.e., Morphotype-A) containing an unidentified xanthone. The xanthone was not characterized for this study because it was found in a single otherwise aberrant specimen. As is discussed below, all the specimens belonging to Morphotype-B were collected in the Coastal Plain of Southeastern North America, which is phytogeographically very different from the region where *Morse 26224-A* was collected (Lendemer et al. 2016; and see entry for *Xanthosyne granularis* below). Given the above, *Morse 26224-A* almost certainly represents an additional taxon that requires further study and additional material to resolve.

Clade 8 was strongly supported (99/100) and is entirely composed of specimens with a relatively thick granulose thallus (i.e., Morphotype-D) that produce xanthone #1 as the major substance often accompanied by a UV-quenching unidentified substance. As is discussed below, all the specimens belonging to Morphotype-D were collected in the Coastal Plain of eastern North America (see entry for *Xanthosyne sharnoffiorum* below).

With a single exception (MCM 226), Clades 2 and 5 are uniformly composed of sequences from specimens with Morphotype A, producing mainly thiophanic acid often with accessories, and are widely distributed in eastern North America. They would be recognized by most lichenologists as "typical" *Lecidea varians*, correspond to most published descriptions of this species (e.g., Brodo 1968; Lendemer & Noell 2018), and are represented by many photographs of *L. varians* online. Clades 3 and 4 also include such specimens, intermixed to varying degrees and with varying levels of phylogenetic structure, with sequences of specimens that have Morphotype-C (i.e., with dark apothecia and some green pigmentation in the epihymenium). In the case of Clade 3, all but one sample of "typical" *L. varians* were recovered in a poorly supported subclade (Clade 3A) with the remaining sample of "typical" *L. varians* (MCM352) recovered in a strongly supported (90/93) sister relationship with a sample of

Morphotype-C (MCM213) as part of a polytomy of other samples with Morphotype-C (Clade 3B). Clade 4 consists of many subclades with poorly supported relationships and includes samples of "typical" *L. varians* intermixed among samples with Morphotype-C with xanthone #3.

Clade 3 is perhaps the most perplexing lineage that we studied. One subclade (Clade 3A) was recovered on a very short branch and has specimens with Morphotype-A that always contain thuringione and arthothelin. The lectotype of *Lecidea varians* shares this morphotype and chemotype and would probably belong here if it could be sequenced. In Clade 3B, all but one specimen have Morphotype-C and produce xanthone #2 as their only product. With the exception of that one specimen, all of the samples from Clade 3B were from the interior U.S.A. north to Minnesota as well as the Appalachians. The nonconforming specimen in Clade 3B (MCM352, *F. Olday 4367b*, CANL) is from Maine, has red-brown apothecia and produces thuringione, as do specimens in Clade 3A.

Clade 6 is composed of two specimens that have dark apothecia and contain only xanthone #3, like those in Clade 4 that correspond to "typical" *Lecidea varians*. Both samples are from the same locality in the southern Appalachian Mountains of eastern Tennessee, U.S.A.

All the sequenced specimens containing atranorin, with two exceptions, were recovered in Clade 1, and all have Morphotype-A. The two exceptions were isolates MCM277 and MCM268 which were recovered in Clade 4, and these also had Morphotype-A. There are five subclades within Clade 1, with varying levels of support and labelled Clades 1A-1E in Figure 9. All the samples in Clades 1A and 1B have the same chemistry (atranorin, thiophanic acid, arthothelin and the confusa-unknown), and all were from Europe or California, U.S.A. The type of *Lecidea exigua* has the same morphotype and chemistry. It was collected in France and would likely have been recovered in Clade 1A or 1B if it could have been sequenced. Specimens in Clades 1C, 1D and 1E all were found in the southern Appalachian Mountains and differ slightly in chemistry from those in Clades 1A and 1B. Almost all lack arthothelin as a major product though it sometimes occurs as a trace, but frequently these specimens also contain xanthone #1 as an additional accessory substance.

Genetic distances within Xanthosyne. Considering the results of our molecular phylogenetic analyses and the comparison with various phenotypic characters and biogeography, we also examined pairwise genetic distances between the ITS sequences of our samples. Many studies of lichens have found ITS genetic distance to be a useful tool in developing taxonomies that aim to reconcile the results of analyses of molecular and non-molecular data (e.g., Del-Prado et al. 2010, 2011). We compiled descriptive statistics for the pairwise comparisons of the sequences within and between the numbered clades discussed above (**Fig. 9;** see **Supplementary File S1**). Due to the position of MCM225 on a very long branch separate from the main clade of sequences, we partitioned Clade 8 into two groups (Clade 8A = main clade, Clade 8B = MCM225) when summarizing distances.

All the subclades within Clade 1 were found to have intra- and inter-clade mean distances well below the species-level threshold of 0.0500. While this suggests that Clades 1A–E belong to a single species, the mean distance between Clades 1A and 1B was lower (0.0051 ± 0.0047, n=22) than that for the distances between those two clades and the Clades 1C–E (1A×1C = 0.0133±0.0027, n=22; 1A×1D = 0.0156±0.0036, n=99; 1A×1E = 0.0160±0.0033, n=22; 1B×1C = 0.0146±0.0015, n=4; 1B×1D = 0.0156±0.0024, n=18, 1B×1E = 0.0159±0.023, n=4). Instead, it was closer to the mean distances within clades (e.g., Clade 1A = 0.0046±0.0039, n=55; Clade 1D = 0.0087±0.0039, n=36). This difference in distances supports the recognition of Clades 1A+1B

as distinct from Clades 1C–E, especially given that Clades 1A+1B are from southern Europe and western North America whereas as Clades 1C–E are from temperate eastern North America.

A similar situation to the subclades of Clade 1 was also found in Clades 3A and 3B, wherein the mean distance between the two subclades was below the 0.0500 threshold (0.0355±0.0239, n=37), although in this case, the intra-clade mean distances were greater than in Clades 1A–1E (3A = 0.0103±0.0050, n=20; 3B = 0.0262±0.0246, n=21). With the exception of Clades 1A–E, and Clades 3A and 3B, all other inter-clade mean distances were above the 0.0500 threshold, suggesting that they are distinct and merit recognition at some rank.

It should be noted that the distance between MCM225 and Clade 8A was well above the 0.0500 threshold (0.0828±0.002, n=4). While we included MCM225 in Clade 8A for taxonomic purposes in this study, it likely represents an additional semi-cryptic or cryptic taxon. The situation is different from MCM205 which was recovered as sister to the rest of Clade 7, but on a very short branch.

CONCLUSIONS

Lecidea varians Ach. is shown to belong to a distinct clade within the Lecanoraceae, recognized at the rank of genus and described here as *Xanthosyne*, based on evidence from multiple molecular phylogenetic analyses and as well as ascus morphology and chemistry. *Xanthosyne* is represented by two additional species that are morphologically and chemically distinct and are monophyletic with strong support. They are described as new to science: *X. granularis* (Clade 7, **Fig. 9**) and *X. sharnoffiorum* (Clade 8, **Fig. 9).**

Xanthosyne varians is a wide-ranging species in eastern North America and encompasses a great deal of variation in chemistry and morphology, much of it with geographic definition and relatively strong phylogenetic support. In our conception, *X. varians* comprises Clades 1–6 recovered in our molecular phylogenetic analyses, although we recognize that all these clades include some non-conforming samples (**Fig. 9**). We reconcile the lack of complete congruence between phenotype and phylogeny within the wide-ranging variation of *X. varians* by using infraspecific taxonomy and employing the subspecies rank. This approach mirrors that of other recent studies of taxonomically difficult lichen groups (e.g., Gaya et al 2011; Jørgensen 2019; Magain et al. 2016).

Our treatment of the variation within *Xanthosyne varians* at the rank of subspecies aims to create a taxonomic framework for this group that can be readily adopted by a broad user community and applied to material with varying levels of available data. Although very few existing *Xanthosyne* specimens are associated with sequence data, morphological and chemical study will allow them to be placed in each species as recognized here (i.e., *X. granularis, X. sharnoffiorum, X. varians*). Yet in most cases, molecular sequence data are required to assign material absolutely and unambiguously to a given clade within *X. varians*, even when morphological and chemical data are available. We are aware that genetic distances, monophyly, and the presence of a strong degree of phenotypic and geographic cohesion support the recognition of entities within *X. varians* at the rank of species following the conceptual framework of Lücking et al. (2021). Nonetheless, we contend that recognition of these semi-cryptic taxa at this rank would create a system of parallel taxonomies for material with and without sequence data, and one that moreover would lead to a situation where the average taxonomic user could not confidently apply names at the rank of species. We also assert that the use of infraspecific taxonomy within *X. varians* is appropriate given the high liklihood that there

are additional unsequenced semi-cryptic taxa and phenotypic variants of the already detected semi-cryptic taxa.

Clade 1 includes all but two of the sequenced specimens containing atranorin and comprises multiple lineages (Clades 1A–1E) each of which corresponds to one of two broadly defined phytogeographic regions. Clades 1A and 1B are composed of samples from southern Europe and California, U.S.A., whereas Clades 1C–1E are entirely composed of samples from the Appalachian Mountains of temperate eastern North America. The European-Californian population is distinct chemically from all other taxa in *Xanthosyne* and is entirely allopatric with respect to the Appalachian population. We suspect that the sister relationship between Clade 1C (Appalachian) and Clades 1A+1B (Europe and California), which is poorly supported, would not be recovered in analyses using additional molecular loci. Rather, it is likely that Clade 1C belongs to the same clade as Clades 1D and 1E, which were recovered on a short branch and also poorly supported. Given the striking correlation between chemistry and geographic range within Clade 1, combined with the differences in mean genetic distance among the subclades, we recognize two distinct subspecies.

It seems clear that the type of *Lecidea exigua* belongs to the lineage from southern Europe. It was described from France and entirely conforms to the morphotype and chemotype of our other European and Californian collections recovered in Clades 1A and 1B. Hence, we recognize these clades as *Xanthosyne varians* subsp. *exigua* and apply this name to all *Xanthosyne* from Europe and southwestern North America. The type of *Lecidea subtilis* Degel., described from Great Smoky Mountains National Park and containing atranorin, thiophanic acid and arthothelin, obviously belongs to the population from the Appalachian Mountains represented by Clades 1C–1E. We recognize these as *X. varians* subsp. *subtilis.*

Clade 2 is comprised of specimens that consistently differ phenotypically from those in Clade 1 only in the absence of atranorin. These specimens also differ from many Appalachian samples in Clade 1 by lacking xanthone #1. Given the strong support for this clade and the differences in chemistry from Clade 1, we recognize it as *Xanthosyne varians* subsp. *subexigua.*

Clades 3A and 3B, despite being incompletely resolved, are tentatively recognized taxonomically as two separate subspecies based on their high mean genetic distances, distinctive chemistries, morphologies and geographic distributions. The lineage represented by Clade 3A almost certainly would include the lectotype of *Lecidea varians* based on phenotype and distribution and therefore is treated as *Xanthosyne varians* subsp. v*arians.* Clade 3B, which is very distinctive morphologically, chemically and geographically, is recognized as *X. varians* subsp. *obscura*.

Clade 4, although variable in morphology and chemistry, is strongly supported and recognized here as *Xanthosyne varians* subsp. *morsei*. The clade includes a mixture of "typical" *Lecidea varians* and specimens that produce xanthone #3 with either Morphotype-A or Morphotype-C. Almost all specimens producing xanthone #3, most of them with dark brown apothecia, are included in this subspecies, but some were also recovered in Clade 6 and are treated as a different subspecies below.

Clade 5 is well-supported and represents a phylogenetically distinct grouping of samples that are otherwise indistinguishable phenotypically from *Xanthosyne varians* subsp. *subexigua*. This clade is recognized as *X. varians* subsp. *submorsei*.

Clade 6 is a similar situation to Clade 5, in that it is well-supported and phylogenetically distinct, but comprised of specimens that would be indistinguishable within *Xanthosyne varians*

subsp. *morsei* (Clade 4) if they did not derive from the Appalachian Mountains. We recognize this clade as *X. varians* subsp. *pseudomorsei.*

Below we provide a taxonomic treatment of *Xanthosyne varians* and its relatives, including a key based on phenotypic characters. Summaries of the chemical substances produced by the taxa are presented in **Table 6** and **Fig. 10**.

TAXONOMY

Xanthosyne Lendemer, R.C.Harris, Brodo & McMullin, *gen. nov.*
MYCOBANK MB853067.

A genus of Lecanoraceae differing from Pyrrhospora Körb. in having Lecidella-type asci (vs. Lecanora type asci) and apothecia lacking K+ purple pigments (anthraquinones) in the apothecial tissues (vs. having such pigments); and differing from Lecidella in the structure of the exciple (radiating prosoplechtenchyme, almost always hyaline to brown vs. pseudoparenchyma to radiate prosoplectenchyme, black at the edge, often green or purple in part).

TYPE SPECIES*: Xanthosyne varians* (Ach.) R.C.Harris, Lendemer, Brodo & McMullin

Description. Thallus crustose, continuous to leprose, episubstratal, containing xanthones, ± atranorin and ± unknown substances. Photobiont Trebouxioid, composed of coccoid cells (5.6–)9.0–12.0(–16.5) µm in diameter. Apothecia small, mostly under 0.4 mm diam., biatorine, pale tan or orange-brown to reddish brown, dark brown or black, epruinose or pruinose; epihymenium brown, greenish or hyaline, N– or N+ red, often POL+, with or without granules; hypothecium hyaline; exciple two-parted, inner exciple and outer rim of non-gelatinized prosoplectenchymous hyphae, lower outer portion of exciple of gelatinized anticlinal branched hyphae, usually pale brown, lacking green pigments; asci *Lecidella* type, with a broad axial mass lined with a dark-staining area in K/I, containing eight spores; ascospores one-celled, hyaline, broadly ellipsoid, not halonate, 7.5–12(–15) × 4.5–7.0(–8.5) µm, wall relatively thick and distinct; pycnidia small, inconspicuous; conidia hyaline, filiform, arcuate, 15–18 × 1.0 µm.

Etymology. The epithet combines the Greek "xanthos", meaning yellow, and "–osyne" "indicating a special feature" (Stern 1992). This refers to the often yellowish coloration of the thallus due to the production of xanthones.

Discussion. As has been discussed in the results section, our molecular studies demonstrated that *Xanthoxyne* is sister to *Lecidella* and otherwise distinct from all other genera and genus-level species groups in the Lecanoraceae (**Fig. 5**). A tabular comparison of *Xanthosyne* and morphologically similar genera, as well as with other genera to which *X. varians* has previously been assigned, further reiterates its distinctness (**Table 3**). A close relationship with *Lecidella* would agree particularly with regard to chemistry (e.g., presence of similar xanthones such as thiophanic acid, arthothelin, thuringione in the thallus), apothecial pigmentation (e.g., the frequent presence of green epihymenial pigments) and *Lecidella* ascus type. The type of *Lecidella*, *L. viridans* (Flot.) Körb., a saxicolous species, contains thiophanic acid and arthothelin as well as 4,5-dichloro-norlichexanthone and also has a colorless to yellowish hypothecium (Fletcher et al. 2009).

Xanthosyne granularis R.C.Harris, *sp. nov.* **Fig. 11**
MYCOBANK: MB853068.

Similar to Xanthosyne varians in apothecial morphology, but differing in the thin, leprose thallus (vs. areolate to continuous in X. varians), frequent occurrence on coniferous trees or

palm (vs. typically on hardwoods in X. varians), and apparent restriction to the Coastal Plain of southeastern North America (vs. widespread and common throughout temperate eastern North America in X. varians). Further differing from all other members of Xanthosyne in containing thiophanic acid and confusa-unknown alone as major compounds.

TYPE: U.S.A. GEORGIA. Chatham Co., Skidaway Island State Park, 31.95181, –81.05963, on *Quercus virginiana* wood, 19 Jul. 2019, *S.Q. Beeching 18069A* (NY, holotype; CANL, isotype). [Chemistry: thiophanic acid and confusa-unknown.]

Description. Thallus leprose, esorediate (in the strict sense; see Lendemer 2011), often forming extensive discontinuous patches; prothallus usually evident as a poorly-developed white network of hyphae between the areoles, occasionally also developing a black marginal zone when in contact with thalli of other species; granules ecorticate, ± globose, 25–40(–50) µm in diameter, initially scattered in younger portions of the thallus, occasionally remaining dispersed, but more typically forming piles and becoming densely crowded in center, usually some shade of yellow when fresh (fading in herbarium), rarely almost white, inspersed with small POL+ crystals dissolving in K. Photobiont Trebouxioid, globose (5.6–)7.0–10.5 µm in diameter or becoming somewhat elongate and then 5.6–7.6 × 3.8–5.5 µm. Apothecia single or more often tightly clustered, sessile or slightly immersed among granules, 0.15–0.4 mm in diameter, mostly plane and flat, occasionally becoming slightly swollen, ± constricted at base, commonly brown, often paler buff or tan, rarely grayish brown, sometimes darkening to black, often with multiple color forms even on same thallus, matte or very rarely shiny; epruinose or rarely weakly pruinose; margin often paler than the disc, concolorous or less often ± darker, not raised or weakly raised, sometimes obscured by swelling of the apothecium; epihymenium highly variable in color, from pale brown to pale olivaceous brown or almost colorless, the green pigment K– and HNO_3+ red, usually with POL+ crystals dissolving in K; hymenium hyaline, (45–)60–75 µm thick, lower part inspersed, rarely entirely inspersed, I– (occasionally appearing I+ blue due to crowded I+ asci), POL+; paraphyses mostly unbranched, sometimes weakly branched near apex, of two types 1) apices not expanded, colorless, 1.5–2.0 µm wide, 2) apices ± clavate with brownish sheath, ca. 3.5–4.0 µm across; hypothecium hyaline, inspersed, 20–50 µm thick; exciple biatorine, consisting of two distinct parts, 35–50(–60) µm thick; inner exciple and outer rim of non-gelatinized prosoplectenchymous hyphae, streaked or tinted to varying degrees with various shades of brown, rarely hyaline; lower outer portion of exciple of gelatinized anticlinal branched hyphae, hyaline; asci *Lecidella*-type, clavate, 45–60 × 16.5–19 µm, 8-spored but often with some aborting; ascospores ovoid, sometimes with one end ± acute, in asci with fewer than 8 spores sometimes becoming globose, (8.0–)9.5–10.9(–14.3) × (5.3–)5.8–6.6(–8.1) µm, n=50, L/W ratio 1.3–1.8(–2.2); pycnidia semi-immersed, black, with brown pigmented walls, subglobose, ca. 100–140 µm in diameter by 100 µm high; conidia filiform, curved, ca. 15–18 × 1 µm.

Chemistry. Thiophanic acid, confusa-unknown, ± thiophaninic acid. Spot tests (thallus): K−, C+ pale orange, KC+ bright orange to scarlet, persisting, PD−, UV+ dull reddish orange.

Etymology. The epithet "granularis" refers to the granular, leprose thallus that is a very distinctive character of the species and one that readily serves to distinguish it from *X. varians*.

Habitat. *Xanthosyne granularis* most commonly occurs on conifers, and has been collected on the bark, decorticate branches and lignum of *Chamaecyparis, Juniperus*, *Pinus* and *Taxodium*. Less commonly it occurs the on bark and lignum of *Quercus*, and petioles and trunks of *Sabal palmetto*. The species was found twice on branches of the endemic shrub *Ceratiola*

ericoides, once on the old stems of the palm *Serenoa repens* and once on a woody vine of *Vitis*. It is commonly associated with *Amandinea punctata* (Hoffm.) Coppins & Scheid*., Buellia wheeleri* R.C.Harris (≡ *Ciposia wheeleri* (R.C.Harris) Marbach) and *Lecanora strobilina* (Spreng.) Kieffer. Although *X. granularis* is typically not associated with other members of the genus, it has been found growing in close association with *X. sharnoffiorum* and *X. varians* (e.g., *Lendemer 42554* with *X. varians* and *X. granularis* growing together on a *Sabal palmetto* petiole; *Hodges 14775* and *Hodges 14776, X. granularis* with *X. sharnoffiorum* growing together on *Sabal* petioles).

Distribution. This species is common and widespread in forested ecosystems of the Coastal Plain of southeastern North America (**Fig. 12**). It ranges from New Jersey south to Florida and west to at least Alabama. Despite the relatively small number of collections from the Gulf Coastal Plain, we suspect that the species is common there and simply was previously confused with *Xanthosyne varians* or erroneously called *Pyrrhospora quernea* (Lendemer et al. 2016; see comments below). Given its frequency in Florida, it is likely that this species is more widely distributed in the Neotropics and probably occurs at least in the Caribbean.

Discussion. This is the taxon described and illustrated as "*Pyrrhospora* sp." in Lendemer and Noell (2018: 175, 367) and referred to as a "similar undescribed species … in the southeastern Coastal Plain" in a discussion of *Lecidella subviridis* Tønsberg in Lendemer & Harris (2016). The most similar species that occurs with *Xanthosyne granularis* is *X. sharnoffiorum*. In fact, we initially included some material of *X. sharnoffiorum* with extremely granular thalli in our delimitation of *X. granularis* until the chemistry was studied in greater detail. *Xanthosyne sharnoffiorum* differs from *X. granularis* in having a minutely verruculose thallus that becomes granular (compare **Figs. 8B** with **8D**, and **Figs. 11** with. **13**). While extreme forms of the thalli in *X. sharnoffiorum* can approach the gestalt of *X. granularis*, they differ in color (light green to yellowish green in *X. sharnoffiorum* vs. distinctly pale to dark yellow and not greenish in *X. granularis*) and chemistry (xanthone #1 in *X. sharnoffiorum* vs. thiophanic acid and confusa-unknown in *X. granularis*).

Other similar yellowish leprose sterile lichens that contain xanthones and react C+ orange in the thallus include *Lecidella subviridis* (see **Addendum**), *Lecanora expallens* Ach. and *Pyrrhospora quernea.* Although all three species resemble *Xanthosyne granularis* superficially, they all differ at least in chemistry. *Lecidella subviridis* contains atranorin and arthothelin in addition to thiophanic acid and confusa-unknown, has significantly larger spores (15–21 × 7.5–-9 µm vs 8–11(–13) × 5.5–8 µm) and has a northern distribution in North America. *Lecanora expallens* contains usnic acid and zeorin (McCune 2017). Sterile specimens of *P. quernea* can closely resemble those of *X. granularis*, but the latter contains confusa-unknown as a major compound and lacks arthothelin, whereas *P. quernea* has arthothelin (or isoarthothelin in Europe) as a major compound and lacks confusa-unknown, with thiophanic acid as a major or minor accessory (Elix & Tønsberg 2004). In addition, *P. quernea* contains yellow anthraquinones in the apothecia that react K+ deep red to red-purple (Brodo et al. 2001). In fact, we reexamined eastern American specimens named as *Pyrrhospora quernea* (e.g., those reported in CNALH (2020) during the initial writing of this manuscript) and discovered that they all were misidentified, with the majority actually being *X. granularis.* Thus, *P. quernea* does not seem to occur in eastern North America.

Buellia wheeleri is another species with a yellow-pigmented thallus that occurs throughout the southeastern United States and could be confused with *Xanthosyne granularis* (Lendemer & Noell 2018). It occurs on the same substrates as *X. granularis*, but whereas *X.*

granularis is typically associated with conifers, *B. wheeleri* is more frequent on hardwoods (Lendemer & Noell 2018). Although the two species can be confused from a distance, *B. wheeleri* is readily distinguished by the dispersed areolate thallus with discrete soralia, yellow soredia and the production of secalonic acid instead of the xanthones reported for *X. granularis* (see Lendemer & Noell 2018). Some forms of *B. wheeleri* can have very poorly developed areoles, especially thalli growing on decorticate conifer wood, but the soralia remain discrete and do not become confluent so as to resemble a leprose crust (Lendemer & Noell 2018: Fig. 307 H).

Lecanora pyrrhosporoides Bungartz, Elix & Printzen, recently described as a Galapagos Islands endemic (Bungartz et al. 2020), has a very similar morphology, apothecial anatomy and chemistry to species of *Xanthoxyne* and may well be another tropical representative of the genus. Its leprose, granulose thallus closely resembles that of *X. granularis,* but the reported presence of atranorin and somewhat different xanthones in *L. pyrrhosporoides* make it unlikely they are conspecific, even if they turn out to be congeneric. Type or authentic material was requested but was unavailable for study and so the deposition of this species requires additional work.

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).]

U.S.A. ALABAMA: Escambia Co., Conecuh National Forest, Solon Dixon Forestry Education Center, Auburn Center, *J.C. Lendemer 9463* (NY). FLORIDA: Alachua Co., Gainesville, *W.A. Murrill s.n.* (NY); Baker Co., Osceola Wildlife Management Area, Pinhook Unit, *R.C. Harris 39348* (NY); Bradford Co., Lake Butler Wildlife Management Area, Raiford Tract, *W.R. Buck 27344* (NY); Citrus Co., Withlacoochee State Forest, along Florida Trail, *W.R. Buck 24465* (NY); Clay Co., Gold Head Branch State Park, *R.C. Harris 29056* (NY); Dixie Co., Big Bend Wildlife Management Area, Jena Unit, *R.C. Harris 39580* (NY); Duval Co., Big Talbot Island State Park, along trail to "Scrubby Bluff", *R.C. Harris 23891* (NY); Gilchrist Co., Waccasassa Flats, along CR232 ~3 mi E of US129, *W.R. Buck 24403* (NY); Glades Co., Ortona Cemetery, along SR78, *W.R. Buck 34044* (NY); Highlands Co., Archbold Biological Station, on Old SR8, SE Tract, *W.R. Buck 33749* (NY); Lafayette Co., along CR357, 6.4 mi SE of FL51, *W.R. Buck 27299* (NY); Lake Co., Ocala National Forest, along FSR538A off FSR538, *R.C. Harris 23548* (NY); Levy Co., along FL24 ~6 mi NE of jct w/ US Alt. 27 at Bronson, *W.R. Buck 22411* (NY); Marion Co., Ocala National Forest, along CR314 at FSR67, *W.R. Buck 28666* (NY); Martin Co., Jonathan Dickinson State Park, along Florida Trail (Sand Pine Nature Trail), *W.R. Buck 33621* (NY); Nassau Co., Fort Clinch State Park, *R.C. Harris 21144* (NY); Polk Co., Bok Tower Gardens, *R.C. Harris 23760* (NY); St. Johns Co., Guana Rivers State Park, South Beach Use Area, *W.R. Buck 28587* (NY); Seminole Co., Orlando, on campus of Florida Technological University, *Brodo 22706A* (CANL); Sumter Co., Green Swamp Wildlife Management Area, vicinity of gate of SR471, *W.R. Buck 33556* (NY); Taylor Co., Big Bend Wildlife Management Area, Tide Swamp Unit, *W.R. Buck 31078* (NY); Union Co., Lake Butler Wildlife Management Area, along CR231A, *W.R. Buck 27402* (NY); Volusia Co., Orange City, Blue Spring State Park, *W.R. Buck 16865* (NY); Washington Co., Rock Hill, ~7 km due SE of Chipley, *W.R. Buck 27162* (NY). GEORGIA: Bryan Co., Richmond Hill Wildlife Management Area, *M. Hodges 7903* (NY); Camden Co., Cumberland Island, camp forest, *S.Q. Beeching 4183* (NY); Chatham Co., Skidaway Island State Park, *M. Hodges 14776** (CANL); Coffee Co., Broxton Rocks Ecological Preserve, High Point (Baskin Point), *R.C. Harris 38759* (NY); MCIntosh Co., Sapelo Island, Sapelo Island Wildlife Management Area, West Perimeter Rd. 0.5 mi N of Kenan Fields, *J.C. Lendemer 20858* (NY); Washington Co., ~3 mi N of Harrison along CR206/Peacock Rd., *R.C. Harris 36243* (NY). NEW JERSEY: Ocean Co., Island Beach State Park, *I.M. Brodo 29853* (CANL). MARYLAND: Worcester Co., Assateague Island National Seashore, Assateague Island, *J.C.*

Lendemer 31941 & B.P. Hodkinson (NY). NORTH CAROLINA: Bladen Co., Singletary Lake State Park, *W.R. Buck 21470* (NY); Carteret Co., Croatan National Forest, Cedar Point Recreation Area, *J.C. Lendemer et al. 35601* (NY); Dare Co., Cape Hatteras National Seashore, Cape Hatteras Beach Trail 0.5 mi W of Lighthouse Rd., *R.C. Harris 60356* (NY); Alligator River National Wildlife Refuge, W of Whipping Creek Rd., *R.C. Harris 60271* (NY); Buxton Woods Coastal Reserve, SW of terminus of Old Doctor Rd., *J.C. Lendemer 35889* (NY); Gates Co., Chowan Swamp Game Land, E shore of the Chowan River, *R.C. Harris 56999* (NY); Onslow Co., Hammocks Beach State Park, Jones Island, *J.C. Lendemer et al. 38628* (NY); Pender Co., Holly Shelter Game Land, just E of Shaw Highway/SR1523 *R.C. Harris 47143-C* (NY); Tyrrell Co., Pocosin Lakes National Wildlife Refuge, Frying Pan Boating Access, *W.R. Buck 60026* (NY); Washington Co., Bull Neck Swamp, Deep Creek Rd. and Bear Lane, *E. Tripp 4152* (NY). SOUTH CAROLINA: Beaufort Co., Spring Island, NE end, Palmetto Point, *J.C. Lendemer et al. 42554* (NY); Charleston Co., Santee Coastal Reserve Wildlife Management Area, S shore of South Santee River, *J.C. Lendemer et al. 40802* (NY); Francis Marion National Forest, S branch of Guerin Creek, small island along SC98, *W.R. Buck 61970* (NY); Colleton Co., Hutchinson Island, N half of island, *J.C. Lendemer et al. 41973-A* (NY). VIRGINIA: Accomack Co., Chincoteague National Wildlife Refuge, Assateague Island, *B.P. Hodkinson 18603 & J.C. Lendemer* (NY); Northampton Co., Cape Charles Natural Area Preserve, *J.C. Lendemer 31648 & B.P. Hodkinson* (NY).

Xanthosyne sharnoffiorum Brodo, R.C.Harris, Lendemer & McMullin, *sp. nov.* **Fig. 13**
MYCOBANK: MB853069.

Differing from Xanthosyne varians in having a thallus that is minutely verruculose to granular, at least in part, and in producing xanthone #1 often with an unidentified substance (vs. a continuous, smooth and membranous to rimose-areolate thallus; and producing mostly other xanthones; in specimens with xanthone #1, the unidentified substance is absent). Differing from X. granularis in its gray-green color (vs. yellow), the minutely verruculose to granular thallus (vs. leprose) and production of xanthone #1 often with an unidentified substance (vs. thiophanic acid and confusa-unknown).

TYPE. U.S.A. GEORGIA: Chatham County, Skidaway Island State Park, 31.95181°N, 81.05963°W, on petioles of *Sabal palmetto*, *M. Hodges 14775B*, 16 Jul. 2019, (NY, holotype; CANL, isotype). Chemistry: Xanthone #1, unidentified substance absorbing LW UV.

Description. Thallus greenish gray, light green to yellowish green, relatively thick, rimose-areolate to verruculose and finally granulose, the granules mostly corticate, 30–90(–100) µm in diameter; prothallus not detected. Photobiont Trebouxioid, cells more or less globose, (8.1–)9.5–13.3 µm in diameter. Apothecia 0.15–0.35 mm in diameter, red-brown or light to dark brown, or rarely black, matte, epruinose, usually clustered but not fusing; margins thin, even, pale or dark, often becoming excluded; epihymenium red-brown, or brown to gray-brown or olive-brown to greenish (HNO_3+ red), almost always POL−; hymenium hyaline, 50–67(–76) µm high; paraphyses unbranched, with tips either expanded (3.5–5.6 µm diameter) or not (2.9–3.5 µm diameter); hypothecium hyaline, ca. 50 µm thick; exciple radiate prosoplectenchyme, hyaline within, brownish at edge; ascospores broadly ellipsoid (7.4–)8.0–10.0(–11.0) × (4.8–)5.2–6.7(–7.3) µm, L/W ratio (1.1–)1.4–1.8(–1.9). Pycnidia and conidia not seen.

Chemistry. Xanthone #1, usually together with an unidentified substance that strongly absorbs long-wave UV light (like usnic acid), running at R_f classes 5, 5, 5–6 in solvents A, B′

and C, respectively. Spot tests (thallus): K−, C− or rarely C+ pale orange, KC−, PD−, UV− or UV+ dark orange.

Etymology. The epithet honors Stephen and Sylvia Sharnoff, whose consummate skill with a camera and impressive knowledge of lichens, in splendid evidence with their images in *Lichens of North America* (Brodo et al. 2001), have given us some of the finest lichen photographs ever published.

Habitat. This species occurs in wetland forests dominated by *Sabal, Fraxinus, Liquidambar* or *Acer,* or mixed oak-hickory woodlands, rarely pine woods. It grows on the bark of a variety of deciduous trees, especially *Acer* and *Quercus,* as well as the woody petioles of *Sabal palmetto,* rarely conifers (*Pinus, Larix, Taxodium*).

Distribution. *Xanthosyne sharnoffiorum* is widespread, but apparently infrequent, throughout the Coastal Plain of southeastern North America, ranging from New York south to Florida and west to at least Louisiana **(Fig. 14**) with inland occurrences in Kentucky and northern Alabama. The disjunct occurrence in Bullitt County, Kentucky suggests that it may also occur inland up the Mississippi Embayment and adjacent low-elevation ecoregions as is the case for other widely distributed Coastal Plain lichen taxa (see Brodo et al. 2001: 69, Fig 23g; Muscavitch & Lendemer 2016).

Discussion. Considering the breadth of variation assumed for *Xanthosyne varians* s.l., it was surprising to discover that almost all specimens filed under that name that had a granulose thallus consistently produced xanthone #1, usually with an associated unidentified compound. The distinctiveness of this material was supported by our molecular phylogenetic analyses that recovered all of the sequenced samples with this phenotype in a strongly supported clade that was strongly divergent from, and sister to, the rest of *Xanthosyne* (**Fig. 9**) clearly indicating that it was a distinct species, which we named *X. sharnoffiorum*. Only one specimen that could be called granulose lacked these substances (*McMullin 13683*, coastal North Carolina, with thiophanic acid and xanthone #4). It is tentatively being called "*X. varians* s.l. (subsp. *subexigua*, subsp. *submorsei* or subsp. *morsei*)" because, based on its chemistry, it could belong to any of three subspecies: subsp. subexigua, subsp. submorsei or subsp. morsei, which is where it would key out if it were not for the thallus morphology.

In a few specimens of *Xanthosyne sharnoffiorum,* the granules of the thallus were piled up in a way that superficially resembled a leprose morphology as found in *X. granularis*, but in these cases, there were always portions of the thallus that were non-leprose. Chemistry and color also separate them (see Diagnosis above).

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).] U.S.A. ALABAMA: Morgan Co., Wheeler National Wildlife Refuge, S of Henderson Rd., *J.C. Lendemer 51639** (NY). DELAWARE: New Castle Co., Blackbird State Forest, Cypress Complex Tract, *J.C. Lendemer 35827* (NY). GEORGIA: Chatham Co., Skidaway Island State Park, *M. Hodges 14775-A** (CANL, NY). KENTUCKY: Bullitt Co., Bernheim Arboretum and Research Forest, *D. Ladd 23604* (NY). LOUISIANA: East Baton Rouge Parish, Baton Rouge, *S.C. Tucker 13289* (NY). MARYLAND: Cecil Co., Elk Neck State Forest, Black Hill Tract, *J.C. Lendemer 19986* (NY); Worcester Co., Nassawango Creek TNC Preserve, E shore of Beech Island Run, *J.C. Lendemer 38041 & J. Allen 1566* (NY). NEW JERSEY: Burlington Co, Lebanon State Forest, *S.D. Sharnoff & S. Sharnoff 1513.28* (CANL); Hunterdon Co., West Amwell Township, South County Park, *D. Waters 3382** (NY); Mercer Co., Hopewell Township, St. Michael's Preserve, *D. Waters 2794* (NY); Ocean Co., Island Beach State Park, *I.M. Brodo 29853* (CANL); Readington Township, Echo Hill Park, *D. Waters 4176** (NY). NEW YORK: Putnam Co., Town of

Putnam Valley, Upper Cranberry Pond, *W. Buck 35235* (NY); Westchester Co., Graham Hills County Park, *R.C. Harris 55776* (NY). NORTH CAROLINA: Johnston Co., Newton Grove, Bentonville Battleground, 30 km WSW of Goldsboro, *I.M. Brodo 25036* (CANL). PENNSYLVANIA: Bucks Co., Little Makefield Township, Five Mile Run Preserve, *D. Waters 3123, 3149* (NY); Doylestown, *C.A. Morse 14556, 14557 and A.F. Rhoads* (KANU). Monroe Co., Middle Smithfield Township, Delaware Water Gap National Recreation Area, *W. Buck 49303* (NY). SOUTH CAROLINA: Charleston Co., Francis Marion National Forest, South Branch of Guerin Creek, *W. Buck 61971* (NY), *J.C. Lendemer 41007* (NY).

Xanthosyne varians (Ach.) R.C.Harris, Lendemer, Brodo & McMullin, *comb. nov.* **Fig. 15**
MYCOBANK: MB853070.
≡ *Lecidea varians Ach.,* Syn. Meth Lich. 38. 1814. ≡ *Biatora varians* (Ach.) Eschw., Syst. Lich. 26. 1824. ≡ *Biatora vernalis* var. *varians* (Ach.) Mont., Annls Sci. Nat., Bot., sér. 2 16: 121. 1841. ≡ *Lecidea vernalis* [unranked] *varians* Eschw. *in* Martius, Fl. bras. enum. pl. 1(1): 253. 1833. ≡ *Lecidea vernalis* var. *varians* (Ach.) Zahlbr., Cat. Lich. Univers. 3: 851. 1925. ≡ *Pyrrhospora varians* (Ach.) R.C. Harris, Evansia 2(3): 46. 1985. ≡ *Traponora varian*s (Ach.) J.Kalb & Kalb, Phytotaxa 332(2): 153. 2017.
TYPE: U.S.A., "Carol. amer." (H-ACH 322B!, LECTOTYPE **designated here** [MBT #10019123]). [Chemistry: thuringione and arthothelin.]

Description. Thallus yellowish gray to greenish or greenish gray, thin or thick, smooth and continuous to rimose or rimose-areolate, rarely verruculose; contiguous thalli almost always separated by thick black lines (not a prothallus). Photobiont Trebouxioid, cells more or less globose, (5.7–)8.5–12.5(–16.4) µm in diameter. Apothecia pale to dark orange-brown to red-brown to dark brown or black (often more than one color on a thallus), mat or shiny, mostly epruinose but sometimes with a light to heavy white pruina, round to irregular, scattered or clustered and frequently fusing in part, 0.12–0.35(–0.48) mm in diameter, sessile or rarely sufficiently immersed as to appear lecanoroid, disks flat to slightly convex; margins concolorous with disks or paler, but sometimes darker, thin and even, rarely prominent, usually excluded in mature apothecia; hymenium (37–)50–75(–95) µm high; epihymenium hyaline to yellowish brown or red-brown, or sometimes with olive or greenish pigments that turn red with HNO_3, with or without granules on the surface, frequently birefringent in polarized light (due to walls of asci and/or paraphyses, not to granules, which, if present, are not birefringent), birefringence disappearing in KOH and persistent in HNO_3; paraphyses mostly unbranched, slightly to strongly expanded at the tips, 2.0.–6.1 µm in diameter, not to heavily pigmented; hypothecium hyaline, ca. 20–30 µm high; exciple hyaline or pale orange within, orange-brown at outer edge, composed of fused and radiating somewhat gelatinized hyphae (prosoplectenchyme); ascospores with distinct, relatively thick walls, broadly ellipsoid to ovoid, 7.5–11.5(–13) × 4.5–7.5(–8.5) µm, L/W ratio 1.2–2.5. Pycnidia small and inconspicuous; conidia filiform, curved, 15–18 × 1 µm.

Chemistry. Various combinations of a variety of xanthones including thiophanic acid (most commonly), arthothelin, confusa-unknown, thiophaninic acid, thuringione, xanthone #1, xanthone #2, xanthone #3 and xanthone #4 as well as atranorin in some (see descriptions of subspecies below). Spot tests (thallus): K−, C− or C+ orange, KC− or KC+ orange, PD−, UV−, UV+ dark orange or UV+ pink-orange.

Habitat. *Xanthosyne varians* occurs on the trunks and branches of a wide variety of trees, but it is most frequently on hardwoods and rather rarely on conifers or lignum. It is primarily a

species of well-lighted woodlands and exposed situations and appears to be extremely tolerant of disturbance.

Distribution. In its broad delimitation, *Xanthosyne varians* is widely distributed throughout temperate and subtropical eastern North America and is infrequent in coastal western North America (California and the Pacific Northwest) (**Fig. 16A**), as well as in southern Europe.

Discussion. The variability of "*Lecidea varians"* has long been appreciated and acknowledged, something evidenced even in its epithet. For example, Brodo (2016) suggested that the populations with red-brown apothecia may include several entities. Indeed, even after the exclusion of *Xanthosyne granularis* and *X. sharnoffiorum* based on molecular evidence, *X. varians* remains a species with a considerable amount of diversity. This variability was reflected in the six well-supported lineages revealed in our molecular studies, lineages that were correlated to various degrees with suites of morphological and chemical features. It is unclear exactly why there is not complete congruence between the molecular phylogenetic analyses and phenotypic data, despite many cases where there was a very strong degree of concordance.

One possibility is that this is a group that is undergoing rapid evolution and our results reflect incomplete lineage sorting (e.g., Boluda et al. 2019, 2021; Magain & Sérusiaux 2015; Saag et al. 2014). Another possibility is that these results reflect processes such as hybridization (Keuler et al. 2020), which is a tantalizing explanation given the frequent occurrence of various chemical variants in adjacent thalli (e.g., Culberson & Hale 1973). Given the low degree of resolution and support within the main six otherwise well-supported clades recognized here, it is also possible that additional molecular data, including from more variable markers, would lead to increased levels of resolution and support for subgroups with discrete phenotypic characters, habitats or biogeography. As has been discussed in the Conclusions section above, we have opted to employ infraspecific taxonomy within *Xanthosyne varians* to taxonomically recognize, at the rank of subspecies, the major clades recovered in our phylogeny. It must be stressed that although our sampling is greatly expanded from that which was previously available for this group, molecular studies with additional data and much more intensive sampling across biogeographic regions, chemotypes, and of adjacent thalli is needed to fully address the taxonomy of this group.

Xanthosyne varians *subsp.* varians **Fig. 15**

Description. Thallus thin, rarely thick, continuous, smooth and membranous to rimose-areolate, yellowish white or yellowish gray. Apothecia pale to dark red-brown, rarely black, scattered or clustered, fusing or remaining separate; pruina absent or faint; margins thin to excluded; epihymenium yellowish brown to reddish brown, rarely olive-brown, birefringent in polarized light (POL+); paraphyses mostly unbranched, tips not or barely expanded, 2.6–4.5 µm in diameter; ascospores (7.8–)8.3–11.5(–12.3) × (4.9–)5.5–7.0(–7.9) µm, L/W ratio 1.3–1.9. Pycnidia not seen.

Chemistry. Thuringione and arthothelin, often with xanthone #4 as a minor accessory. Spot tests (thallus): K−, C+ orange, KC+ orange, PD−, UV+ dark orange.

Habitat. On deciduous tree trunks and branches. Substrates include *Quercus, Prunus, Populus* and *Acer.*

Distribution. Based on specimens of *Xanthosyne varians* s.l. containing thuringione and arthothelin, the subspecies is mainly found in the Canadian Maritime Provinces and northeastern United States, with rare occurrences south to Georgia (unconfirmed with DNA), and rare disjuncts in the Ottawa region of Canada (**Fig. 16B**).

Discussion. This subspecies includes the specimens in Clade 3A of **Fig. 9**, all of which contain thuringione and arthothelin as major products. Although we cannot be certain that the Acharian type of *Lecidea varians,* which has the same chemistry, would appear in Clade 3A, because this chemotype appears to be almost monophyletic (a single specimen with the same chemistry appears in the sister Clade 3B), we regard the likelihood to be high.

Clade 3A separates from Clade 3B (subsp. *obscura*) with relatively low support, but there are compelling reasons for recognizing both lineages as separate taxa (see Discussion under subsp. *obscura*).

Specimens examined. [Sequenced specimens are designated by an asterisk (*).] U.S.A. MAINE: Knox Co., Camden, 6 Apr. 2021, *R. Rittmaster a*,b*,c** (CANL); Town of Rockport, Cramer Park, on south bank of Goose River*, F. Olday 4367a*, 4368(1)a** (CANL). NEW JERSEY: Mercer Co., Hopewell Township, Stony Brook Floodplain, *D.P. Waters 2891** (NY).

Xanthosyne varians *subsp.* exigua (Chaub.) Brodo, López de Silanes, Lendemer, R.C.Harris & McMullin, *comb. nov.* **Fig. 17**

MYCOBANK: MB853071.

≡ *Lecidea exigua* Chaub. *in* St.-Amans, Flore Agenaise 478. 1821. ≡ *Biatora exigua* (Chaub.) Fr., Lich. eur. reform. (Lund) 278. 1831. ≡ *Biatora exigua* var. *exigua* (Chaub.) Fr., Lich. eur. reform. (Lund) 278. 1831. ≡ *Lecidea parasema* var. *exigua* (Chaub.) Nyl., Act. Soc. linn. Bordeaux 21(4): 370. 1857. ≡ *Lecidea parasema* subsp. *exigua* (Chaub.) Nyl., Flora, Regensburg 56(5): 72. 1873. ≡ *Lecidea elaeochroma* var. *exigua* (Chaub.) H. Olivier, Fl. Lich. Orne 2: 211. 1884. Type citation: [FRANCE. Lot-et-Garonne Dept:] "Sur l'écorce lisse des chênes et des marronniers, dans les bois. R. R. A. Segougnac, à Cambes, près d'Agen."

TYPE : [FRANCE] "Bonnemairie" [sic; = Bonne Marie in Nomandie?], without date, *L. Chaubard s.n.* (NEOTYPE **designated here**! MBT #10019124, H-NYL 19627!). [Chemistry not determined, see discussion below.]

Description. Thallus greenish gray to pale green or yellowish green, thin to somewhat thick, up to 0.7 mm thick, rimose or continuous, rough or smooth, frequently covered with epiphytic algae; often with a distinct dark gray to black line between adjoining thalli.

Apothecia usually pale, rarely dark yellowish brown to reddish brown, typically epruinose but can be lightly or heavily pruinose, round to irregular in shape, clustered, sometimes merging and fusing with adjoining apothecia, immersed to broadly attached and sessile, 0.15–0.38(–0.45) mm in diameter, margins thin, only discernable in very young apothecia, even with disk, soon excluded; epihymenium pale yellowish brown to red-brown, unchanged in KOH or HNO_3, birefringent in polarized light, but not appearing to be granular, birefringence disappearing after KOH, but persistently birefringent in HNO_3; hymenium clear, 50–62(–70) µm; hypothecium hyaline, thin, ca 20–30 µm; paraphyses not or barely branched, 1.4–1.8 µm thick, tips not or barely expanded, 2.4–4.2(–5.0) µm in diameter, not or slightly pigmented brown; exciple thin, reddish brown, especially at outer edge, consisting of radiating hyphae (prosoplectenchyme), birefringent in polarized light (like epihymenium); ascospores ellipsoid to broadly ellipsoid, (8.4–)9.0–11.5(–12.0) × (4.5–)5.2–6.5(–7.4) µm, L/W ratio 1.4–2.0. Pycnidia not seen.

Chemistry. Thallus K−, C− or + yellow, KC+ pale orange, PD−, UV−; contains atranorin, thiophanic acid, confusa-unknown, arthothelin.

Habitat. In lightly shaded to open woods. Typically on the smooth bark of deciduous trees and shrubs, especially *Quercus* and *Frangula,* but also *Alnus, Castanea, Corylus, Olea, Myrtus, Fagus* and *Betula,* and occasionally on *Pinus*, especially pine cones.

Distribution. In North America, found in southern California and probably Baja California (see Ryan et al. 2004, sub *Lecidea varians*) as well as throughout southern Europe from the U.K. (Aptroot et al. 2009, sub *Lecidea exigua*) to the eastern shore of the Black Sea (**Fig. 18**).

Discussion. Considerable effort was made to locate and study the type material of *Lecidea exigua,* but inquiries to PC and TL where Louis Chaubard's material is deposited (Stafleu & Cowan 1976) were unanswered, perhaps because no original material could be found. Attempts to locate material in other herbaria were similarly unsuccessful. There is a specimen in H-NYL annotated by Christian Printzen in 2003 as the lectotype. While the material is labelled as having come from Chaubard and the information does match that of the protologue (see also, Clos 1893), it cannot unambiguously be identified as original material studied by Chaubard prior to the publication of the name. Nevertheless, we designate this specimen as a neotype as it was the only material definitely associated with the name *Lecidea exigua* and with Chaubard. The morphology of the neotype is in accordance with the other European and western North American material examined. Unfortunately, the chemistry could not be studied because of the small size of the specimen.

Xanthosyne varians subsp. *exigua* comprises Clades 1A and 1B recovered in our molecular phylogenetic analyses (**Fig. 9**) and includes all the sequenced specimens we examined from Europe and western North America. All of the specimens we examined from these regions produced atranorin, thiophanic acid, confusa-unknown and arthothelin as major products and hence we consider this chemical profile combined with the geographic distribution to be characteristic for the taxon.

Xanthosyne varians subsp. *subtilis* (Clades 1C, 1D and 1E) and *X. varians* subsp. *exigua* are the only taxa that consistently produce atranorin as a major substance together with various xanthones. The two subspecies are here recognized as separate despite the lack of complete resolution within Clade 1 (see comments under Conclusions). Subspecies *subtilis* contains only atranorin and thiophanic acid as major products, frequently accompanied by xanthone #1 (which is absent from subsp. *exigua*), although arthothelin and/or confusa-unknown can sometimes be detected as traces. It is only found in the southern Appalachian Mountains. These two subspecies also have some morphological differences worth noting: In subsp. *exigua*, the hymenium is always POL+ (vs. POL+ or POL− in subsp. *subtilis*); the apothecial margins are pale and almost always excluded (vs. frequently persistent, even and black, especially when moist in subsp. *subtilis*), and the apothecia are always clustered, frequently fusing (vs. scattered or clustered, but never fusing in subsp. *subtilis*).

There is one enigmatic specimen of subspecies *exigua* from Europe: *Vězda, Lich. Sel. Exs. 1636*, from the Transcaucasus, Georgia. The packet in CANL contains numerous patches of the lichen, most with Morph A and containing the normal subsp. *exigua* chemistry. A few patches, however, are of Morph C with almost black apothecia, HNO_3 + red pigment, and not birefringent in the epihymenium, and although they contain thiophanic acid, they lack either arthothelin or confusa-unknown. All thalli, however, contain atranorin. The thalli with dark apothecia may well represent another lineage and is worth exploring further.

The presence of temperate European lichens in southern California is not unusual. For example, the distribution of *Lecanora horiza* (Ach.) Lindsay is very similar (Brodo 1984; also

see phytogeographic comments of Brodo et al. 2001: 73–74), so it is not entirely surprising that specimens known from temperate regions of Europe (Clauzade & Roux 1986) nest in the same clades (both in 1A and 1B) as specimens from California.

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).] FRANCE. FINISTERE: Goulien, Penn ar Run Izella, Castanea, 29 Oct. 2018, *J-Y. Monnat s.n.** (CANL). MORBIHAN: Branderion, Boul Sapin, 28 Oct. 2018, *J-Y. Monnat s.n.** (CANL). NORMANDIE: (see Exsiccatae below). Antibes, Bois de la Garoupe, nord immédiat du Sémaphore de la Garoupe, *M. Bertrand 5391a*, 3 (hb Bertrand); Pyrenees-Atlant, 50 km SW of Pau, area ± 0.25 km S to E of St. Engrâce, *P. van den Boom 13547,* (hb. v.d. Boom). JUGOSLAVIA. (see Exsiccatae below). PORTUGAL. Cerca de Valença, en al orilla izquierda del río Miño, *M.E. López de Silanes 12280*, 12281* (SANT-LICH, CANL); Cerca de Vila Nova de Cerveira, en al orilla izquierda del río Miño, *M.E. López de Silanes 12282** (SANT-LICH, CANL). Algarve, Cascata Moinhos de Rocha, *P. & B. van den Boom 35764,* (hb. v.d.Boom); Alentejo, SSW of Évora, SE of alvito, S side of Barragem Odivelas; *P. van den Boom 17190* (hb v.d.Boom); Ribatejo, 7 km E of Tomar, road to Olalhas, *P. van den Boom 19284* (hb v.d.Boom). SPAIN. A Coruña, Teo, Rarís, *M.E. López de Silanes 1a, 2, 12276*, *12277*, 12278** (SANT-LICH, CANL); Caaveiro (Puentedeume), 19 Feb. 1984, *M.E. López de Silanes* s.n. (CANL)**;** Navarra, Valle del Baztán, collado Vendreka, camino de Arregui, 21 July 1993, *O. Breuss s.n.* (MA 5828). SWITZERLAND. Liestal, *J. Hepp s.n.*, sub *Biatora decandollei* Hepp (NY-3723941). U.S.A. CALIFORNIA: [Los Angeles Co.?], Santa Monica Mountains, *H.E. Hasse 362* (NY); San Luis Obispo Co., San Luis Obispo, El Chorro Regional Park, *J. Dart 1094*, 1095** (CANL); San Luis Obispo, Reservoir Canyon, *J. Dart 1137*, 1138*, 1139** (CANL); Santa Barbara Co., "Lotusland" Estate, Ashley Road, Montecito, E of Santa Barbara, *S. Tucker 36443* (CANL); Santa Cruz Island, Cristi Pines, near Sauses Gate, *I.M. Brodo 31942 pp* (CANL).

Exsiccatae. *Vězda, Lichenes Selecti Exsiccati 855,* sub *Lecidea exigua.* JUGOSLAVIA. Dalmatia, insula Korčula: Žrnovska Banja, alt. 30 m s.m., 17 Aug.1969, ad corticem *Pini halepensis.* (CANL).

Vězda, Lichenes Selecti Exsiccati 1636., sub *Lecidea varians*. GEORGIA (ABKHAZIA). Transcaucasus, Colchis, Dist. Suchumi [Sokhumi], in valle angusta rivi supra pagum Mercheuli, alt. 50 m s.m., ad corticem *Alni baubatae O.A.M.* (CANL).

Olivier, Lichenes Exsiccati 429, sub *Lecidea exigua*. France. (NORMANDIE), Tourouvre, Orne, sur les jeunes troncs des taillis (NY).

Kryptogamiae Exsiccatae 2060, sub *Lecidea exigua*. CROATIA. ISTRIA: ad *Quercuum* truncus adviam ferream inter Jurdani et Skalnica, 400-500 m.s.m., *J. Schuler s.n.* (NY).

Xanthosyne varians *subsp.* morsei Brodo, Lendemer & McMullin, *subsp. nov*. **Fig. 19**
MYCOBANK: MB853072.

*Comprising Clade 4 in the phylogeny (**Fig. 9**) and variable in chemistry and morphology including specimens having typical red-brown marginless apothecia with a brown, POL+ epihymenium and containing thiophanic acid, as well as specimens with apothecia that vary from dark red-brown to dark brown to black with greenish epihymenial pigments, usually POL−, and containing xanthone #3 alone. On deciduous trees and shrubs, Ozark and Appalachian regions north to Nova Scotia.*

TYPE: U.S.A. KANSAS: Linn Co., 3 mi W of jct of Kansas Hwys. 7 & 52 in Mound City, Dingus Natural Area, along unnamed tributary to Little Sugar Creek; 38.131578°N, 94.874007°W, 270–314 m, mixed sugar maple-basswood and oak-hickory forest and

scattered prairie openings on E-W-trending ridges and ravines limestone outcrops and scattered sandstone bounders on upper slopes, 2 Apr. 2017, on dead *Quercus* branch, *C.A. Morse 25386[B]*, (KANU, holotype). [Chemistry: xanthone #3 alone.]

Description. Thallus yellowish white to yellowish gray or yellowish green, thin to moderately thick, continuous to rimose-areolate, sometimes becoming verruculose but never granulose, forming small circular patches; prothallus absent or extremely thin and gray, but often forming a black line between adjacent thalli. Apothecia pale to dark red-brown or dark brown, grading into black, epruinose, 0.12–0.35(–0.42) mm in diameter, round, flat to slightly convex, clustered or scattered, sometimes fusing; margins thin, pale, becoming excluded, or even, black (especially on moistened apothecia), persistent or finally excluded; epihymenium pale yellowish brown to reddish brown or smoky gray-brown to olive-brown, rarely greenish, POL+ or POL−; hymenium 55–70(–80) µm high, hyaline; paraphyses free in water mount, unbranched except rarely at tips, capitate or not, (3.1–)3.5–5.2(–5.9) µm in diameter; hypothecium hyaline, thin, up to ca. 50 µm thick; exciple radiating prosoplectenchyme, hyaline within, becoming brown at edge, especially upper parts; ascospores (7.8–)8.3–11.0(–12.2) × (4.2–)5.0–6.5(–7.4) µm, broadly ellipsoid, L/W ratio 1.3–1.9(–2.2). Pycnidia not seen.

Chemistry. Thallus C−, KC− or KC+ pale orange, K± yellow, PD−, UV− or + dark orange, with thiophanic acid and occasionally confusa-unknown as major compounds, ± arthothelin in traces, and rarely atranorin as a minor accessory; or C+ distinct orange, KC+ orange, UV+ dark orange to pale pinkish orange, with xanthone #3 alone.

Etymology. Named in honor of our lichenological colleague, Dr. Caleb A. Morse, of the McGregor Herbarium at the University of Kansas, who provided numerous, beautifully curated and carefully annotated specimens of the *X. varians* group from central U.S.A., many specifically collected for this study, and one of which serves as the holotype of the new subspecies.

Habitat. In well-illuminated open woodlands and glades, often bordering prairie. On the bark of branches and twigs of deciduous trees, especially *Cercis,* but also *Acacia, Carya, Prunus* and *Quercus.* Sometimes also on the wood of fence posts.

Distribution. Our sequenced specimens are mainly in the Ozarks and Appalachians north to New Brunswick (**Fig. 20**). Unsequenced specimens containing xanthone #3 alone have the same basic distribution, but only as far north as southern Pennsylvania (**Fig. 20B**). Note that they may represent either subsp. *morsei* or subsp. *pseudomorsei* (see below).

Discussion. The sequenced specimens that grouped with Clade 4 and constitute subsp. *morsei* are very variable in morphology and chemistry and almost certainly represent separate lineages, but none of them were recovered as monophyletic and there is not strong enough support for them to be recognized taxonomically at this time. The variability of this taxon is underlined by the presence of three morphotypes within the holotype packet alone. Despite differences in the apothecial color (dark red-brown to black), all the thalli contained xanthone #3 and were recovered together in our phylogenetic tree (**Fig. 9**). We are therefore regarding apothecial and epihymenial color as secondarily important compared to the presence of xanthone #3. Nevertheless, specimens containing that compound appear on both Clade 4 and Clade 6 and cannot be assumed to belong to subsp. *morsei.* Furthermore, not all specimens grouped in Clade 4 contain xanthone #3 and one even contains atranorin (MCM 277), like specimens in Clade 1 (subspecies *exigua* and *subtilis*).

It is noteworthy that specimens of subsp. *morsei* containing xanthone #3 tend to have dark brown to black apothecia that contain green pigments and closely resemble typical

specimens of the largely sympatric subsp. *obscura* (described below), which contains xanthone #2. Fortunately, simple spot tests in combination with long-wave ultraviolet light will separate most subsp. *morsei* specimens (C+ bright orange, KC+ bright orange, UV+ dark orange) from similar thalli belonging to subsp. *obscura* (C−, KC−, UV+ bright pink-orange).

Specimens in subsp. *morsei* (i.e., Clade 4) belonging to the thiophanic acid chemotype have red-brown apothecia lacking green pigments (Morphotype A) and therefore resemble specimens with that chemotype in other clades (e.g., in subsp. *subexigua* or subsp. *submorsei*; see below), more than they resemble others in Clade 4 that contain xanthone #3.

Selected specimens examined. (Sequenced specimens in Clade 4, **Fig. 9**)**.** CANADA. NOVA SCOTIA: Cumberland Co., Economy River Wilderness Area, *F. Anderson 3*, 4a*, 4b** (CANL); Halifax Co., Halifax, Point Pleasant Park, *R.T. McMullin 17290** (CANL); Old Annapolis Road Nature Reserve, *R.T. McMullin 17444** (CANL). PRINCE EDWARD ISLAND: Prince Co., Nature Conservancy of Canada's North Enmore Nature Reserve, *R.T. McMullin 14368** (CANL). U.S.A. IOWA: Page Co, Shenandoah, *M.K. Advaita 19825-B** (KANU). KANSAS: Douglas Co., University of Kansas Campus West, Crestline Dr and Clinton Pkwy., *C.A. Morse 25380** (KANU); University of Kansas Ecological Reserves, Fitch Natural History Reservation, *C.A. Morse 25894** (KANU); Linn Co., Dingus Natural Area. along unnamed tributary to Little Sugar Creek, *C.A. Morse 25424-A, 25386-A*, C** (KANU). MAINE: Knox Co., Town of Rockport, Cramer Park, *Olday 4368(1)b*, 4368 (2)** (CANL). MISSOURI: Montgomery Co., W side of Danville Conservation Area in Danville Glade Natural Area, *C.A. Morse 25672** (KANU). OKLAHOMA: Osage Co., Osage Wildlife Management Area, *C.A. Morse 26075c*, 26079-A*, B** (KANU); TNC Joseph H. Williams Tallgrass Prairie Preserve, E-central part, *C.A. Morse 26157b-A** (KANU). PENNSYLVANIA: Bucks Co., Lower Makefield Township, Five Mile Run Preserve, *D. Waters 3126** (NY). TENNESSEE: Greene Co., Cherokee National Forest, Chuckey Mountain, Dry Fork, W of FSR486, *J.C. Lendemer 55375** (NY). VIRGINIA: Albemarle Co., Mount Fair Farm, *N.M. Howe 904** (NY).

Xanthosyne varians *subsp*. obscura Brodo, R.C.Harris, Lendemer & McMullin**,** *subsp. nov.*
Fig. 21

MYCOBANK: MB853073

Similar to X. varians subsp. varians, but with cinereorufa-green pigments (HNO_3+ red) in the epihymenium (vs. infrequently with green pigments), constantly lacking birefringence in the apothecial tissues (vs. frequently birefringent), usually with expanded and capitate tips of the paraphyses (vs. rarely with capitate paraphyses tips), containing xanthone #2 alone (vs. lacking xanthone #2). Mainly East Temperate but avoiding the coastal plain, on the bark of deciduous trees.

TYPE: U.S.A. KANSAS: Bourbon Co., ca 4 mi S, 5.5 mi W of Uniontown, SW side of Bourbon County State Fishing Lake and Wildlife Area, above Wolfpen Creek, 37.78584°N, 95.07510°W, 950–980 ft., disturbed, open, mixed oak-hickory with *Cary ovata, Juniperus virginiana, Quercus muhlenbergii, Quercus rubra, Cercis canadensis, Cornus drummondii, Rhus serotina*, and upland tall grass prairie vegetation on cherty, limestone outcrops, low cliffs and talus of Pennsylvanian Swope Limestone on moderate, S-facing slope, on branches of small *Cercis, C.A. Morse 24947*, 11 Dec. 2015 (KANU, holotype). [Chemistry: xanthone #2.]

Description. Thallus yellowish gray to greenish gray, thin and membranous to rarely thick and rimose-areolate to verruculose, sometimes forming black lines between thalli.

Apothecia black or smoky gray-brown, less commonly dark red-brown, epruinose or infrequently with a coarse bluish pruina, usually clustered, rarely fusing, sessile, 0.10–0.35 mm in diameter, flat to very slightly convex; margins very thin, almost excluded in mature apothecia but usually still visible when moistened with water as a thin black line around a mottled disk, or sometimes paler than disk; epihymenium dull greenish gray to olive or blue-green, less commonly greenish brown, unchanged or darker in KOH and turning red to red-brown in nitric acid (pigment *cinereorufa*-green), not at all birefringent in polarized light (POL−), even on pruinose apothecia; hymenium hyaline, 52–67(–76) µm high; hypothecium hyaline, thin, 20–30 µm thick, irregularly arranged hyphae; paraphyses more or less free in water, entirely free in KOH, 1.0–1.4 µm thick, typically expanding to abruptly capitate tips, (3.0–)4.3–5.8(–6.3) µm in diameter, darkly pigmented in the wall of the tip, but sometimes barely expanded, 2.9–3.5 µm in diameter; exciple biatorine, brown to rarely greenish brown at outer edge and sometimes the outer third, hyaline within, composed of radiating hyphae that sometimes break down and become more or less decomposed prosoplectenchyme, lacking granules; ascospores ellipsoid to broadly ellipsoid, (8.0–)8.4–12.0(–14.1) × 5.4–6.9(–7.4) µm, L/W ratio 1.2–2.0(–2.4). Pycnidia not seen.

Chemistry. Thallus K−, C−, KC−. PD−; UV + bright pinkish-orange. Specimens contain xanthone #2 alone or, very rarely, with thuringione and arthothelin.

Etymology. In reference to the dark, usually black, apothecia (*obscurus* = dark in Latin).

Habitat. On the smooth bark of deciduous trees and shrubs such as *Cercis* and *Amelanchier*, but also others including *Fraxinus, Populus, Quercus* and *Robinia*; rarely on *Juniperus* or *Pinus.*

Distribution. Central United States from Texas to Minnesota and into southern Canada, with rare occurrences in the Appalachians, New Jersey and southwestern British Columbia (**Fig. 22**).

Discussion. Specimens with dark brown to black apothecia that produce xanthone #2 all cluster in Clade 3B, and we refer them to subspecies *obscura*. Within Clade 3, they are separated from specimens with red-brown apothecia containing thuringione and arthothelin (Clade 3A: subsp. *varians*) with low support. A single enigmatic specimen from Maine (MCM 352, *Olday 4367b*) of this latter chemotype and having red-brown apothecia lacking green pigments (**Fig. 22**, square symbol) appears in Clade 3B based on its ITS sequence (the LSU and RPB1 sequences were unobtainable). The LSU and RPB1 sequences might have resolved this anomaly, but we do not regard this as sufficient reason to refrain from recognizing Clades 3A and 3B at the subspecies level based on their very different chemistry, morphology and geographic ranges.

Although the presence of expanded, capitate paraphyses tips is strongly correlated with the presence of xanthone #2, it is not without exceptions, and capitate paraphyses tips are also frequently found in subsp. *morsei* and sometimes in subsp. *varians.* Even under a broad circumscription of subsp. *obscura* (i.e., including some with red-brown apothecia), the epihymenium and exciple of all specimens except MCN 352 (discussed above) were POL−.

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).] CANADA. BRITISH COLUMBIA: Hastings, *J. Macoun 2710B* (CANL). ONTARIO: Belleville, *J. Macoun 2715* (CANL); Ottawa, *J. Macoun 198* (CANL); Wooler, *J. Macoun 160A* (*2711*). QUEBEC: Aylmer, *J. Macoun 291B* (CANL). U.S.A. ALABAMA: Jackson Co., James D Martin Skyline Wildlife Management Area, Turkey Creek, *E.A. Tripp 6476* (NY). IOWA: Fayette Co., 1894, *B. Fink* (CANL); Winneshiek Co., Highlandville, *C.C. Freeman 22084* (KANU). KANSAS: Anderson Co., Paintbrush Prairie, along S side of SW 800 Rd, W of intersection with US Hwy

59/KS Hwy 31, *C.A. Morse 25402** (KANU); University of Kansas Ecological Reserves, Prairie Preserve (Welda Prairie), *C.A. Morse 24357** (KANU); Bourbon Co., Hollister Wildlife Area: just SW junction of 165th St & Hackberry Rd., *C.A. Morse 25820** (KANU); Chautauqua Co., Chautauqua Springs Park, *M.K. Advaita 1978* (KANU); Cherokee Co., Hallowell, *C.A. Morse 11783* (KANU); Douglas Co., Baldwin City, *C.A. Morse 24396a* (KANU); Cowley Co., Arkansas City, Chaplin Nature Center, *C.A. Morse 10728* (KANU); Elk Co., Elk City Falls and Bridge, *M.K. Advaita 2608* (KANU); Labette Co., Big Hill Lake, 4.5 mi E of Cherryville, *M.K. Advaita 2852* (KANU); Linn Co., Dingus Natural Area along unnamed tributary to Little Sugar Creek, *C.A.Morse 25424-B*,C** (KANU); Montgomery Co., W side of Elk City Lake, along Elk River Hiking Trail, *C.A. Morse 25862* (KANU); Osage Co., Osage State Fishing Lake and Wildlife Area, *M.K. Advaita 1506* (KANU); Wilson Co., Wilson State Fishing Lake, Buffalo, *M.K. Advaita 2552* (KANU). MAINE: Knox Co., Town of Rockport, Cramer Park, on south bank of Goose River*, F. Olday 4367 b** (CANL). MINNESOTA: [no specific locality on label], 1896, *B. Fink* (CANL 5537); Pine Co., St. Croix State Forest, south of Rock Lake, *J.P. Schuster 1117* (CANL). MISSOURI: Shannon Co., Cardareva State Forest along north side of Current River, *T. Chadwell 94* (KANU). NEBRASKA: (See Exsiccatae below). NORTH CAROLINA: Swain Co., Great Smoky Mountains National Park, White Oak Branch Trail, *J.C. Lendemer 46001* (NY). OHIO: Scioto Co, *J. Wolfe 788C* (NY). OKLAHOMA: Murray Co., Chickasaw National Recreation Area, *C.A. Morse 10691b* (KANU); Ottawa Co., Miami, *E.F. Smith s.n.* (KANU); Osage Co., TNC Joseph H. Williams Tallgrass Prairie Preserve, E-central part, *C.A. Morse 26157b-B** (KANU); Tulsa Co., Tulsa, Oxley Nature Center, *M.K. Advaita 10739* (KANU); Washington Co., Copan, at Copan Lake, *M.K. Advaita 10659a* (KANU). TEXAS: Mason Co., Mason Mountain Wildlife Area, *C.A. Morse 23473b* (KANU); Montague Co., Sunset, *C.A. Morse 18695a* (KANU). WEST VIRGINIA: Mercer Co., Bluefield, *I.M. Brodo 4609* (CANL).

Exsiccatae. *Cummings, Decades of North American Lichens 191*, sub *Biatora varians.* U.S.A. NEBRASKA: Weeping Water, *T.A. Williams*, 29 Nov. 1895 (CANL)

Xanthosyne varians *subsp.* pseudomorsei Brodo, Lendemer & McMullin**,** *subsp. nov.*

Fig. 23

MYCOBANK: MB853074

Identical in morphology and chemistry to the dark-fruited Xanthosyne varians subsp. morsei chemotype that produces only xanthone #3 but belonging to a separate genetic lineage.

TYPE: U.S.A. TENNESSEE: Monroe Co., Cherokee National Forest, Unicoi Mountains, upper slopes of McIntyre Lead of Sassafras Ridge, 35°20′43″N, 84°03′52″W, 3935 ft., mixed hardwood (*Acer saccharum, Betula lenta, Quercus rubra, Q. prinus*) forest with *Kalmia-Rhododendron* understory and large conglomerate rock outcrops, on *Acer saccharum,* 18 Dec. 2017, *J.C. Lendemer 54856 & E. Tripp* (NY, holotype). [Chemistry: xanthone #3 alone.]

Description. Thallus yellowish white, very thin, continuous to rimose-areolate. Apothecia dark brown to black, epruinose, with thin, dark margins that become excluded, 0.2-0.33 mm in diameter, scattered to clustered, not fusing; epihymenium greenish brown (HNO_3+ red), POL−; tips of paraphyses not, or barely, expanded, 2.5–3,5(–4.5) µm in diameter; ascospores 8.7–11.1 × (5.7–)6.5–7.7 µm, L/W ratio (1.1–)1.5–1.8. Pycnidia not seen.

Chemistry. Thallus C+ distinct orange, KC+ orange, UV+ dark orange to pale pinkish orange; contains xanthone #3 alone.

Etymology. The epithet "*pseudomorsei"* (false morsei) is used because those specimens were identical to some of those in clade 4 (i.e., subsp. *morsei,* identical morphologically as well as containing the unique xanthone #3) but were on a separate well-supported lineage.

Habitat. Found in mixed hardwood forests on *Acer saccharum* and presumably other deciduous trees.

Distribution. The Unicoi Mountains of Tennessee (**Fig. 20**).

Discussion. This subspecies, distinguishable from the xanthone #3 chemotype of subsp. *morsei* only when sequenced, is found in the mountains of east Tennessee. It therefore differs in distribution from other specimens of subsp. *morsei* containing xanthone #3 **(Fig. 20**). It appears in a well-supported clade (Clade 6) basal to subspecies *submorsei* and *morsei* in the phylogeny.

Additional specimen examined. (Sequenced) U.S.A. TENNESSEE: Monroe Co., Cherokee National Forest, Unicoi Mountains, upper slopes of McIntyre Lead of Sassafras Ridge, *J.C. Lendemer 54857** with *E. Tripp* (NY).

Xanthosyne varians *subsp*. subexigua Brodo, Lendemer & McMullin, *subsp. nov.*
Fig. 24

MYCOBANK: MB853075.

A cryptic subspecies (not distinguishable from subspecies morsei and submorsei without sequencing) sister to X. varians subsp. exigua, with apothecia varying from red-brown (common) to black (rare), with or without green pigments in the epihymenium, and containing thiophanic acid usually with traces of confusa-unknown. Found in the southern Appalachian Mountains.

TYPE: U.S.A. South Carolina. Oconee Co., Sumter National Forest, E-facing slopes above Hell Hole Creek, FSR765 ~1 mi S of jct w/ Charlie Cobb Rd., 34.8138, –83.2104, 536 m., remnant hardwoods (*Acer, Carya, Quercus alba, Q. prinus, Q. rubra*) in recent timber harvest area, on fallen *Acer rubrum* branch, 16 Apr. 2019, *J.C. Lendemer 58552* (NY, holotype). [Chemistry: thiophanic acid and a trace of confusa-unknown.]

Description. Thallus greenish gray, thin or thick, continuous and smooth to rimose-areolate to verruculose. Apothecia red-brown, brown or black, rarely pruinose, scattered to clustered, fusing or not, 0.13–0.3(–0.4) mm in diameter; margins thin, even, often black, finally excluded; epihymenium brown, gray or green, POL− or, rarely, POL+; tips of paraphyses not or barely capitate, 2.1–3.0(–5.0) µm in diameter; ascospores 8.4–10.6(–12.6) × (4.8–)5.6–8.0 µm; L/W ratio 1.3–1.6. Pycnidia not seen.

Etymology. In reference to its phylogenetic position as sister to subsp. *exigua* (and subsp. *subtilis*).

Chemistry. Thallus C−, KC− or KC+ pale orange, K± yellow, PD−, UV− or + dark orange; contains thiophanic acid ± traces of confusa-unknown.

Habitat. In hardwood stands, on deciduous trees including *Acer saccharum* and *Quercus rubra.*

Distribution. Southern Appalachian Mountains (Alabama, Georgia, South Carolina, Tennessee) (**Fig. 25**).

Discussion. The specimens on Clade 2 constitute subsp. *subexigua.* They are morphologically similar to specimens of subspecies *subtilis* and *exigua* but lack atranorin and arthothelin, even in traces. They are sympatric with the former and allopatric with the latter, and, despite the 100% support for their genetic distinctness, are probably very closely related to those

subspecies. Its distinctions from the very similar subspecies *submorsei* are mentioned in the Discussion of the latter.

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).] U.S.A. ALABAMA: Lawrence Co., Bankhead National Forest, Sipsey Wilderness Area, ridge above headwaters of West Bee Branch, *J.C. Lendemer 50102** (NY); Bankhead National Forest, Sipsey Wilderness Area, slopes above E shore of West Bee Branch, 0.5 mi N of confluence of West Bee Branch and East Bee Branch, *E. Tripp 6658B** (NY). GEORGIA: Union Co., Chattahoochee National Forest, Coosa Bald Scenic Area, W-facing slopes of Duncan Ridge, *E. Tripp 9542** (NY); Chattahoochee National Forest, E-facing slopes between Chestnut Knob and Fisher Knob, *J.C. Lendemer 58155** (NY). SOUTH CAROLINA: Oconee Co., Sumter National Forest, S-facing slopes of Poor Mountain, *J.C. Lendemer 58627** (NY). TENNESSEE: Monroe Co., Cherokee National Forest, E-facing slopes of Baker Grave Mountain above Quarry Creek, *J.C. Lendemer 53550** (NY).

Xanthosyne varians *subsp.* submorsei Brodo, Lendemer & McMullin**,** *subsp. nov.*
Fig. 26

MYCOBANK: MB853076.

An almost cryptic subspecies sister to Xanthosyne varians subsp. morsei forming a well-supported lineage (Clade 5). It is morphologically similar to subsp. varians and subsp. exigua but tends to have pale apothecia and is frequently pruinose, sometimes heavily so, whereas other thiophanic-containing subspecies usually have darker red-brown apothecia without, or with very light, pruina. Found in scattered localities in southeastern U.S.A.

TYPE: U.S.A. TENNESSEE. Monroe Co., Cherokee National Forest, Unicoi Mountains, Bob Bald, Benton MacKeye Trail/ Bob Bald Connector/Trail54A, ~2.6 mi N of Cherohala Skyway/TN165 at Beech Gap, 35°22′20″N, 84°00′30″W, 5088 ft., mature northern hardwood forest (*Aesculus, Betula alleghaniensis, Fagus* dominant) with *Rhododendron* and sparse *Tsuga*, on *Aesculus*, 18 Dec. 2018, *J.C. Lendemer 54702 & E. Tripp* (NY, holotype). [Chemistry: thiophanic acid, xanthone #1, trace of arthothelin.]

Description. Thallus yellowish to greenish gray, thin or thick, rimose-areolate to verruculose. Apothecia pinkish brown to beige or sometimes red-brown, often heavily pruinose, 0.19–0.35(–0.42) mm in diameter, scattered or clustered, usually not fusing; epihymenium yellowish to pale brown or reddish brown, POL+ or rarely POL−; ascospores broadly ellipsoid, (9.0–)10.0–12.9(–13.5) × 5.1–7.7(–9.0) µm; L/W ratio 1.2–1.8(–2.2). Pycnidia not seen.

Etymology. In reference to its phylogenetic position (clade 5) as sister to subsp. *morsei* (clade 4).

Chemistry. Thiophanic acid, frequently with xanthone #1, ± traces of arthothelin. Spot tests (thallus): K± yellow, C−, KC− or KC+ pale orange, PD−, UV− or UV+ dark orange.

Habitat. In mixed hardwood forests, on deciduous trees, especially *Acer*, but also others including *Aesculus* and *Fagus*.

Distribution. Southeastern United States from southern Pennsylvania and New Jersey to Georgia, North Carolina and Tennessee including both montane and coastal localities (**Fig. 27**).

Discussion. This subspecies, although much like other *Xanthosyne varians* populations with red-brown apothecia, does have some distinguishing features. It resembles *X. varians* subsp. *subexigua* but usually has paler, pinkish apothecia (vs. red-brown to black), pale brown epihymenium with no green pigments (vs. brown to gray-green) and is almost always POL+ (vs.

mostly POL−). The ascospores are also slightly larger (9–)10–13.5 × 5–7.7(–9) µm (vs. 8–10.5(–12.5) × (5–)5.6–8 µm). Although both produce thiophanic acid, their accessory substances are different: traces of arthothelin and occasionally with xanthone #1 in subsp. *submorsei* vs. traces of confusa-unknown and lacking xanthone #1 in subsp. *subexigua.*

Specimens examined. [Sequenced specimens are designated by an asterisk (*).] U.S.A. GEORGIA: Union Co., Chattahoochee National Forest, slopes above W shore of tributary to Cooper Creek, *J.C. Lendemer 57967** (NY). NEW JERSEY: Hunterdon Co., West Amwell Township, South County Park, Delago Section, *D. Waters 3392** (NY). NORTH CAROLINA: Dare Co., Alleghany River National Wildlife Refuge, *R.T. McMullin 13544** (CANL); Washington Co., Bull Neck Swamp, *R.T. McMullin 13580** (CANL). PENNSYLVANIA: Bucks Co., Lower Makefield Township, Five Mile Run Preserve, *D.P. Waters 3131** (NY). TENNESSEE: Monroe Co., Cherokee National Forest, Unicoi Mountains, Bob Bald, Benton MacKeye Trail/Bob Bald Connector/Trail 54A, *J.C. Lendemer 54702*, 54773** (NY).

Xanthosyne varians *subsp.* subtilis (Degel.) Brodo, Lendemer & McMullin, *comb. nov.*

Fig. 28

MYCOBANK: MB853077.

≡ *Lecidea subtilis* Degel., Ark. f. Bot. 30A(3): 40. 1942.

TYPE: U.S.A. TENNESSEE: Sevier Co., Great Smoky Mountains National Park, Cherokee Orchard, on *Tilia* in deciduous forest, 760 m., 11 Sept. 1939, *G. Degelius s.n.* (UPS!, holotype). [Chemistry: atranorin, thiophanic acid, arthothelin.]

Description. Thallus yellowish to greenish gray, thin to somewhat thick, rimose-areolate or continuous and membranous. Apothecia pale to dark reddish brown, rarely black, typically epruinose but can be lightly pruinose, clustered or scattered, never fusing with adjoining apothecia, 0.20–0.36(–0.55) mm in diameter, margins usually thin to excluded, occasionally even and dark; epihymenium brown, rarely olive-gray, unchanged in KOH or HNO_3, POL+ or POL−; hymenium 55–73 µm high, hyaline; paraphyses tips barely or somewhat expanded, 2.3–(–4.5)–5.0 µm in diameter, not or slightly pigmented brown; exciple thin, reddish brown, especially at outer edge, consisting of radiating hyphae; ascospores ellipsoid to broadly ellipsoid, 8.7–11.6(–12.9) × 5.1–6.6(–7.5) µm, L/W ratio (1.3–)1.5–2.3. Pycnidia not seen.

Chemistry. Atranorin, thiophanic acid and frequently xanthone #1 as major compounds, ± traces of confusa-unknown and/or arthothelin. Spot tests (thallus): K−, C− or C+ yellow, KC+ pale orange, PD−, UV−.

Habitat. In lightly shaded to open woods. Typically on the smooth bark of deciduous trees and shrubs, most commonly *Acer saccharum* and *Betula alleghaniensis*, but also including *Acer rubrum, Hamamelis, Magnolia, Prunus* and *Tilia*.

Distribution. In the southern Appalachian Mountains north to Virginia (but see discussion below) (**Fig. 29**).

Discussion. All sequenced specimens from the southern Appalachians that contain atranorin, with one exception, belong to this subspecies. The exception is *Lendemer 55375*, which nests with Clade 4, subsp. *morsei*. All eastern specimens with significant amounts of atranorin are in the southern Appalachians with the exception of *Vězda Exs. 1608* from Louisiana, which has atranorin but only traces of thiophanic acid and xanthone unknown #1. It probably does not belong to subsp. *subtilis*. Also see Discussion under *Xanthosyne varians* subsp. *exigua.*

Selected specimens examined. [Sequenced specimens are designated by an asterisk (*).] U.S.A. ALABAMA: Lawrence Co., Bankhead National Forest, Sipsey Wilderness Area, slopes above E shore of West Bee Branch, *E. Tripp 6658A** (NY); Bankhead National Forest, Sipsey Wilderness Area, slopes above N/W shore of Borden Creek, *J.C. Lendemer 50279** (NY); Jackson Co., James D. Martin-Skyline Wildlife Management Area, S shore of Little Coon Creek, *E. Tripp 6393A* (NY). GEORGIA: Gilmer Co., Chattahoochee National Forest, SE slopes of Big Bald Mountain, *J.C. Lendemer 57318**, *57348, 57357** (NY). NORTH CAROLINA: Graham Co., Nantahala National Forest, Oak Knob, *R.C. Harris 61049** (NY). TENNESSEE: Blount Co., Great Smoky Mountains National Park, Rich Gap, jct of Long Hungry Ridge Trail and Gregory Ridge Trail, *J.C. Lendemer 56725** (NY); Great Smoky Mountains National Park, Rocky Top, Appalachian Trail ~0.2 mi SW of Thunderhead Mountain, *J.C. Lendemer 54948** (NY); Carter Co., Cherokee National Forest, N-slopes of Roan Mountain, Ash Gap, Appalachian Trail 0.7 mi N of Toll House Gap, *E. Tripp 8948*, J.C. Lendemer 56023*, 56089** (NY); Cocke Co., Cherokee National Forest, Lamb Mountains, Lamb Gulf, Cold Springs Creek, *J.C. Lendemer 57228* (NY); Greene Co., Cherokee National Forest, Bald Mountains, Bald Mountain Ridge Scenic Area, Poplar Cove*, J.C. Lendemer 55255*, 55243* (NY); Monroe Co., Cherokee National Forest, Unicoi Mountains, Bob Bald, Benton MacKeye Trail, *J.C. Lendemer 54760* (NY); Polk Co., Cherokee National Forest, Big Frog Wilderness Area, E-slopes of Bark Legging Lead of Big Frog Mountain, *J.C. Lendemer 54455* (NY); Sevier Co., Great Smoky Mountains National Park, Silers Bald, Appalachian Trail above Silers Lead, *J.C. Lendemer 56911** (NY); Great Smoky Mountains National Park, Mount LeConte, N-facing slopes above Trillium Gap Trail, *J.C. Lendemer 57140* (NY); Unicoi Co., Cherokee National Forest, Unaka Mountain, N-facing slopes above Unaka Mountain Rd./FSR230, *J.C. Lendemer 55804*, 55926** (NY).

KEY TO SPECIES OF *XANTHOSYNE*

1. Thallus sorediate to leprose, granules ecorticate, 20-40(–50) µm in diameter……..….. 2
1. Thallus continuous, thin to thick, membranous to rimose-areolate or granulose, but not sorediate or leprose, yellowish white, yellowish gray or greenish gray, C+ orange or C−.....3

2. Contains thiophanic acid and confusa-unknown and frequently thiophaninic acid, lacking atranorin and arthothelin; spores 8–11(–13) x 5.5–8 µm); southeastern coastal plain; thallus pale to dark yellow; apothecia infrequent, dark red-brown ……***X. granularis*** [clade 7]
2. Contains atranorin, thiophanic acid, arthothelin and confusa-unknown; spores 11–17(–21) x 5–9 µm; northeastern forests in North America, mostly northern in Europe; thallus yellowish gray to pale yellow; apothecia common in North America, pale to dark brown …….. ***Lecidella subviridis*** [see Addendum]

3. Thallus well developed, rimose-areolate and verruculose becoming granulose, granules mostly corticate, 30–90(–100) µm in diameter, C− or C± pale orange, KC−, UV− or UV+ dark orange; contains xanthone #1 as the only xanthone, usually together with an unidentified substance that strongly absorbs LW UV light; apothecia red-brown to dark brown, rarely black; epihymenium almost always POL−; eastern U.S., New York to Alabama ..... ***X. sharnoffiorum*** [clade 8]
3. Thallus relatively thin, often membranous, becoming rimose when thicker, but not granulose-verruculose; apothecia pale to dark brown, red-brown or black; epihymenium POL+ or –; xanthone #1 infrequent, and then always with another xanthone .....….4

4. Thallus contains xanthone #2 alone, C−, KC−, UV+ bright pinkish orange; apothecia black to dark gray-brown, rarely red-brown; epihymenium green to olive, HNO_3+ red, POL−; northern and central states, Appalachians, rare in northeast.....***X. varians* subsp. *obscura*** [clade 3B]
4. Thallus lacks xanthone #2 but contains other xanthones, C+ or –, KC+ or –, UV+ or –; apothecia pale to dark orange- or red-brown, dark brown or sometimes black; epihymenium red-brown and HNO_3– to smoky gray-brown or olive to greenish, and then HNO_3+ pink, POL− or POL+.....…….. 5

5. Thallus contains xanthone #3 alone, C+ and KC+ strong orange, UV± dark orange to pinkish orange; apothecia usually dark brown, less frequently dark red-brown or black, round with thin, even margins that appear to be black when the apothecia are moistened; epihymenium smoky gray-brown to olive, HNO_3+ pink; mostly in the Ozarks and adjacent interior states as well as the Appalachians, rare elsewhere in east 6
5. Thallus lacks xanthone #3, C+ orange or C−, KC+ orange or KC−, UV+ dark orange or UV−, apothecia pale to dark reddish brown, margins even to excluded, not usually black when wet; epihymenium brown to reddish brown, HNO_3–, most frequently POL+ 7

6. Mostly east-central U.S.A., common……..... ***X. varians subsp. morsei*** [clade 4]
6. Tennessee mountains, rare…….***X. varians* subsp. *pseudomorsei*** [clade 6]

7. Thallus contains atranorin …….. 8
7. Thallus lacks atranorin ..…..... 9

8. Thallus contains thiophanic acid, arthothelin and confusa-unknown as major products, lacks xanthone #1; epihymenium always POL+; California and southern Europe ..... ……..……..…….. ***X. varians* subsp. *exigua*** [clades 1A, 1B]
8. Thallus contains thiophanic acid frequently with xanthone #1, often with arthothelin and/or confusa-unknown, but almost never all four compounds together, and then, contains xanthone #1; Appalachians …… ***X. varians* subsp. *subtilis*** [clades 1C, 1D, 1E]

9. Thallus contains thuringione and arthothelin both as major compounds, C+ orange, KC+ orange; Maritime Provinces to South Carolina, more or less coastal; rare elsewhere in northeast.....……...…..***X. varians* s.l. [*X. varians* subsp. *varians.*** clade 3A, OR rarely **subsp. *obscura***, clade 3B**]**
9. Thallus lacks thuringione but contains thiophanic acid with or without confusa-unknown or arthothelin; East Temperate …..... ***X. varians* s.l. [*X. varians* subsp. *subexigua,* subsp. *morsei*** OR **subsp. *submorsei.*** clades 2, 4, 5**]**

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Acharian type of *Lecidea varians* several decades ago, still useful to us now. Erin Tripp is thanked for her initial collaboration with JCL to produce a phylogeny for *L. varians* using metagenomic data. While these data and analyses were ultimately not included as the study evolved, they helped provide early molecular evidence that *L. varians* was distinct from *Pyrrhospora*. We also thank the curators of H, GB and UPS for the loan of critical types, as well as the curators of the following herbaria for lending us important European material of *Xanthosyne* for study: B, BCN, BG, LEB-LICH, MACB, MAF, MA-LICH, SANT-LICH and the private herbaria of Michel Bertrand, J. L. Farou and Pieter van den Boom. We are especially grateful to Drs. Françoise Lohézic-du-Dévéhat, Solenn Ferron and Phillipe Uriac at the University of Rennes for devoting a great deal of time in an attempt to characterize and name our unknown xanthones. Colin Freebury and Fenja Brodo gave the near-final draft a thorough read, and we much appreciate their useful comments and corrections. We also thank the two reviewers for their useful and important comments and suggestions.

AUTHOR CONTRIBUTIONS

IMB, RTM and JCL conceived and designed the study, and were responsible for development of the final taxonomic framework. RCH was involved in early stages of the work, carried out microscopy and study of generic delimitation, and recognized *X. granularis* as distinct. RB, MP and AG generated all molecular data used in the study. JCL analyzed the molecular data and prepared corresponding figures with IMB. IMB and NM developed and performed chemical analyses and prepared the figures and tables dealing with chemistry, with refinements to the techniques devised by NM. RTM carried out microscopy, prepared figures and distribution maps. MELS collected key European material and aided in locating type material in herbaria. IMB led the writing of the manuscript.

ADDENDUM

After this manuscript was submitted, the similarities of *Lecidella subviridis* Tønsberg to species of *Xanthosyne,* especially *X. granularis,* were brought to our attention (see Discussion under *X. granularis*). *Lecidella subviridis* is mainly a northern species normally found sterile in Norway where it was discovered and described (Tønsberg 1992). Its chemical profile is identical with that of *X. varians* subsp. *exigua*, but it has a thin dispersed sorediate to subleprose or entirely leprose thallus and significantly larger spores than that subspecies or any other *Xanthosyne* that we studied for this revision. The species was first reported from North America by Coppins & Fryday (2006) based on collections from Michigan, and its distribution was expanded and discussed by Lendemer & Harris (2016). We examined specimens of *L. subviridis* from Norway as well as the holotype and found Tønsberg's description of the thallus variation to be basically accurate and thorough. (We found the spores in the holotype not quite as large as given in the protologue; see Key above.) Although it was too late to collect or sequence fresh material of *L. subviridis*, small subunit ribosomal RNA sequences based on material from the Ukrainian Carpathian Mountains in GenBank (Vondrák et al. 2018) place the species extremely close to *X. varians* subsp. *exigua* in accordance with their identical chemical profiles, rather than *X. granularis,* despite their close morphological resemblance. It seems clear that the species should be transferred to *Xanthosyne,* but since we have not examined the GenBank voucher nor incorporated the species into our phylogeny, we will refrain from making the new combination here. That should be done in a future contribution in the context of a full review of *Lecidella* together with other species that might be included within *Xanthosyne.*

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Supplementary documents online:

Supplementary File S1. Phylogeny with support values not included in Figure 9, and descriptive statistics for pairwise genetic distances of ITS sequences of *Xanthosyne* taxa recognized.

Supplementary File S2. Specimens examined that were unsequenced and therefore of uncertain placement, including exsiccatae.

Figure 1. Protologue of *Lecidea varians* reproduced from Acharius (1814: 38).

Figure 2. Exemplar thin layer chromatography (TLC) of secondary metabolite profiles for *Xanthosyne* taxa including standards for comparison and performed in solvent A. The upper half of the picture shows a fresh, untreated plate viewed in long-wave ultraviolet light (LW UV) and the lower half is the same plate viewed in short wave ultraviolet light (SW UV). Both pictures were photographed with the digital camera in an iPhone. Note that many bluish white fluorescent compounds that appear in LW UV (most of them presumably terpenes or inorganics from the substrate) do not appear in SW UV. Abbreviations for compounds are as follows: ABS, absorbing spot; AR, arthothelin; AT, atranorin; CFU, confusa-unknown; N, norstictic acid; TH, thiophanic acid; THU, thuringione; US, usnic acid; X1, X2, X3, X4, xanthones 1, 2, 3 and 4, respectively. Numbered lanes correspond to the following: 1, 8, 19 = atranorin and norstictic acid standards; 2 = *X. varians* subsp. *exigua* (*López de Silanes 22280*, SANT-LICH, CANL), AT, TH, AR, CFU; 3 = *X. varians* subsp. *subtilis* (*Lendemer 56911*, NY), AT, TH, AR, CFU, X1; 4 = *X. varians* subsp. *subexigua* (*Lendemer 58627*, NY), TH, trace of CFU; 5 = *X. varians* subsp. *varians* (*Olday 4368(1)a*, CANL), THU, AR, X4; 6 = *X. varians* subsp. *obscura* (*Macoun s.n.*, CANL 5278), X2; 7 = *X. varians* subsp. *morsei* chemotype 2 (*Morse 20251b*, KANU), X3; 9 = *X. varians* subsp. *submorsei* (*Waters 3392*, NY), TH, ABS (high); 10 = *X. varians* subsp. *pseudomorsei* (*Lendemer 54856*, NY), X3; 11 = *X. granularis* (*Beeching 18069*, CANL), TH, CFU; 12 = *X. sharnoffiorum* (*Hodges 14775*, CANL), X1, ABS (low); 13 = *Lecanora expallens* standard (*Brodo 10022*, CANL), US, TH, AR, CFU; 14 = *X. varians* s.l. [subsp. v*arians or obscura*] (*G.K. Merrill s.n. = Lich. Exs. 206*, CANL), THU, AR, X4; 15 = *Lecanora confusa* standard (*Lamb 733*, CANL), US, TH, AR (trace), CFU; 16 = thuringione pure standard; 17 = arthothelin pure standard. Compare with Fig. 10.

Figure 3. Exemplar thin layer chromatography (TLC) of secondary metabolite profiles for *Xanthosyne* taxa including standards for comparison and performed in solvent B′. Data for each numbered lane is provided in the caption for Figure 2.

Figure 4. Exemplar thin layer chromatography (TLC) of secondary metabolite profiles for *Xanthosyne* taxa including standards for comparison and performed in solvent C. Data for each numbered lane is provided in the caption for Figure 2.

Figure 5. Phylogeny of Lecanoraceae inferred from the six-locus dataset (ITS, nucLSU, mtSSU, MCM7, RPB1, RPB2) of Zhao et al. (2016) and incorporating newly generated sequences of the *Lecidea varians* lineages (labelled as *Xanthosyne*). Presented as the most likely tree. Support

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values ≥50 are displayed as Shimodaria-Hasegawa approximate likelihood ratio test (SH-aLRT) / IQ-Tree ultrafast bootstraps (UFBoot), with both values rounded to the nearest integer, values of 100 denoted with an asterisk "*" and values below 50 denoted with a hyphen "-". Branches with support ≥80 SH-aLRT and ≥95 UFBoot are thickened. Clade nomenclature and outgroup selection follows Zhao et al. (2016), except *Lecidella* which follows Zhao et al. (2015).

Figure 6. Comparison of ascus types in *Pyrrhospora* s. str. and *Xanthosyne*. A, *Lecanora*-type ascus in *P. quernea* (*Buck 30348*, NY). B, *Lecidella*-type ascus in *X. varians* subsp. *exigua* (*Olivier 429*, NY). C, *Lecidella*-type ascus in *X. granularis* (*Lendemer 20858*, NY). D, *Lecidella*-type ascus in *X. varians* s.l. (*Lendemer 32151*, NY). All from preparations in K/I. Scales = 20 µm.

Figure 7. *Lecidea varians* in Acharius herbarium, H-ACH. **A.** Sheet of H-ACH 322. **B.** H-ACH *322B*, lectotype, with fragment from micropacket in upper left corner.

Figure 8. Morphotypes in *Xanthosyne* lineage. **A.** Morphotype A, from *X. varians* subsp. *morsei* (*Waters 3126*, Pennsylvania, NY), scale = 1 mm. **B.** Morphotype B, from *X. granularis* (*Beeching 18069A*, Georgia, NY), scale = 1 mm. **C.** Morphotype C from *X. varians* subsp. *obscura* (*Morse 25402*, Kansas, KANU), scale = 1 mm. **D.** Morphotype D, from *X. sharnoffiorum* (*Waters 4176*, New Jersey, NY), scale = 1 mm.

Figure 9. *Xanthosyne* phylogeny inferred from the three-locus dataset (nrITS, nucLSU, mtSSU, MCM7, RPB1, RPB2) pruned from Zhao et al. (2016), incorporating newly generated sequences. Presented as the most likely tree. Branches with Shimodaria-Hasegawa approximate likelihood ratio test (SH-aLRT) support ≥80 and IQ-Tree ultrafast bootstraps (UFBoot) support ≥95 are thickened. A version of the tree with all support values mapped is included in the Supplementary File S1. Color-coded panel on right depicts phenotypic characters and geographic origin of samples as follows: Chemical products: absent (dark gray}, trace (white), present (colored). Thallus: leprose (yellow), granular (green), continuous (light gray). Apothecium color: red-brown (red), dark-brown (brown), black (black). Pruina: Present (lilac), absent (dark gray). Epihymenium: POL+ (blue), POL− (dark gray). Epihymenium green pigments: present-dense (dark green), present-sparse (light green), absent (gray). Morphotype category: A (gray), B (yellow), C black), D (green). Geography: Appalachian (green), Canadian Maritimes/New England (dark blue), Mid-Atlantic Coastal Plain (red), Southern Coastal Plain (light blue), Interior/Central Uplands (orange), Western North America (purple-pink), Europe (yellow). An "X" indicates apothecia were lacking.

Figure 10. Schematic representation of TLC runs in solvents A, B′ and C, for *Xanthosyne* taxa and standards. **A.** Solvent A. **B.** Solvent B′. **C.** Solvent C. Abbreviations in spots are the same as those in Figure 2.

Figure 11. *Xanthosyne granularis*. **A-B.** Thallus and apothecia, scale = 1 mm. **A.** *Beeching 18069A*, Georgia, holotype (NY). **B.** *McMullin 13683,* North Carolina (CANL). **C–G.** *Lendemer 20858*, Georgia (NY). **C.** Apothecia section, scale = 65 µm. **D.** Pycnidium and conidia, scale = 50 µm. **E.** Ascospores in an ascus stained with phloxine, scale = 20 µm. **F.** Asci tips at 100x, stained with K/I. **G.** Exciple stained with phloxine, scale = 20 µm.

Figure 12. Global distribution of *Xanthosyne granularis.* Circles = sequenced; triangles = unsequenced specimens identified by morphology and chemistry.

Figure 13. *Xanthosyne sharnoffiorum*. **A–E.** *Hodges 14775,* Georgia, holotype (CANL). **A.** Thallus and apothecia, scale = 1 mm. **B.** Apothecial section, scale = 50 µm. **C.** Ascus tip in K/I at 400x. **D.** Ascospores, scale = 12 µm. **E.** Paraphyses, scale = 12 µm. **F.** Thallus and apothecia, *Brodo 25036,* North Carolina (CANL), scale = 1 mm.

Figure 14. Global distribution of *Xanthosyne sharnoffiorum.* Circles = sequenced; triangles = unsequenced specimens identified by morphology and chemistry.

Figure 15. A–B. *Xanthosyne varians* subsp. *varians*, thallus and apothecia, scale = 1 mm. **A.** H-ACH 322B, lectotype. **B.** *Olday 4367,* Maine (CANL). **C–E.** *Xanthosyne varians* s.l. **C.** Apothecial section in phloxine, scale = 50 µm, *Lendemer 33752,* Delaware (NY). **D.** Ascus tips in K/I at 400x, *Lendemer 32151,* Delaware (NY). **E.** Conidia, scale = 20 µm, *Harris 57909,* Maryland (NY).

Figure 16. North American distribution of *Xanthosyne varians*. **A.** *Xanthosyne varians* in the broadest sense (all subspecies as well as those annotated as "s.l."; see also **Supplementary File S2**). **B.** *Xanthosyne varians* subsp. *varians.* Circle = sequenced; triangle = unsequenced specimens containing thuringione and arthothelin (most likely subsp. *varians*, but possibly subsp. *obscura*; see Discussion under subsp. *obscura*).

Figure 17. *Xanthosyne varians* subsp. *exigua.* **A.** Thallus and apothecia, *L. Chaubard s.n.,* France, lectotype (H-NYL *19627*), scale = 1 mm. **B–D.** *Monnat 2,* France (CANL), scale = 1 mm. **B.** Thallus and apothecia. Greenish color due to epiphytic algae commonly seen especially in European material. **C.** Apothecial section, scale = 50 µm. **D.** Asci tips at 400×, stained with K/I. **E.** Thallus and apothecia, *Dart 1139,* California (CANL), scale = 1 mm.

Figure 18. Distribution of *Xanthosyne varians* subsp. *exigua.* **A.** North American distribution. **B.** European distribution

Figure 19. *Xanthosyne varians* subsp. *morsei, Morse 25386(B)*, Kansas, holotype (KANU), scale = 1 mm.

Figure 20. A. Global distributions of *Xanthosyne varians* subsp. *morsei* (dots, and white star for holotype) and subsp. *pseudomorsei* (black star) based on sequenced specimens. **B.** Distribution of *X. varians* s.l. mapped by chemotype. White dots and triangles indicate chemotype with xanthone #3, sequenced and unsequenced, respectively. For comparison, squares indicate specimens within clade 4 (subsp. *morsei*) with thiophanic acid (all sequenced).

Figure 21. A–E. *Xanthosyne varians* subsp. *obscura, Morse 24947,* Kansas, holotype (KANU). **A.** Thallus and apothecia, scale = 1 mm. **B.** Apothecial section, scale = 55 µm. **C.** Asci tips, scale = 12 µm. **D.** Hymenium showing paraphyses with expanded, pigmented tips, scale = 12 µm. **E.** Ascospores, scale = 12 µm.

Figure 22. Global distribution of. *Xanthosyne varians* subsp. *obscura.* Round dot = sequenced, with xanthone #2; triangle = unsequenced, with xanthone #2; square = sequenced specimen with thuringione and arthothelin (MCM 352); star = holotype.

Figure 23. *Xanthosyne varians* subsp. *pseudomorsei, Lendemer 54856*, Tennessee, holotype (NY), scale = 1 mm.

Figure 24. *Xanthosyne varians* subsp. *subexigua, Lendemer 58552*, South Carolina, holotype (NY), scale = 1 mm.

Figure 25. Global distribution of. *Xanthosyne varians* subsp. *subexigua.* (all sequenced); star = holotype.

Figure 26. *Xanthosyne varians* subsp. *submorsei, Lendemer 54702*, Tennessee, holotype (NY), scale = 1 mm.

Figure 27. Global distribution of. *Xanthosyne varians* subsp. *submorsei.* (all sequenced); star = holotype.

Figure 28. *Xanthosyne varians* subsp. *subtilis, Degelius s.n*., Tennessee, holotype (UPS), scale = 1 mm.

Figure 29. Global distribution of *Xanthosyne varians* subsp. *subtilis.* Dots = sequenced; star = holotype; triangles = unsequenced specimens of *X. varians* s.l. in eastern North America containing atranorin and thiophanic acid alone, or with xanthone #1, as major compounds, considered here to be subsp. *subtilis.*