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Diversity of *Xanthoparmelia* (Parmeliaceae) species in Mexican xerophytic scrub vegetation, evidenced by molecular, morphological and chemistry data

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Abstract. The genus *Xanthoparmelia* is the largest genus of lichen-forming fungi with about 800 species worldwide. *Xanthoparmelia* is also common in the deserts of central Mexico, but only a few molecular studies exist on its species' diversity in this region. In this study, we sampled 38 *Xanthoparmelia* species from around the world including species from the xerophytic scrubs of central Mexico to assess the diversity using an integrative approach. Molecular phylogenetic analyses were performed using a combination of the ITS, mtSSU and nuLSU genetic markers. We evaluated our phylogenetic results in a context of traditional morphological and chemical characters. The combined evidence of molecular, morphological, and chemical data identified a total of 18 *Xanthoparmelia* species-level lineages occurring in central Mexico. However, numerous traditionally circumscribed species did not form monophyletic groups in the molecular phylogenetic reconstructions. This conflict indicates that taxonomy and species delimitation in the genus *Xanthoparmelia* requires revision and emphasizes the importance of molecular evidence for more robust species delimitations in this genus.

Keywords. Cryptic species, biodiversity, secondary metabolites, lichens, Mexico.

Resumen. *Xanthoparmelia* es el género más grande de hongos liquenizados, con alrededor de 800 especies en todo el mundo. *Xanthoparmelia* es común en los desiertos del centro de México, pero existen pocos estudios moleculares sobre la diversidad de especies en esta región. En este estudio, muestreamos 38 especies de *Xanthoparmelia* de diferentes partes del mundo, incluidas especies de los matorrales xerófilos del centro de México, para evaluar la diversidad usando una aproximación integrativa. Los análisis filogenéticos moleculares se realizaron combinando los marcadores genéticos ITS, mtSSU y nuLSU. Además, evaluamos nuestros resultados filogenéticos en un contexto de caracteres morfológicos y químicos usados en la taxonomía tradicional. Teniendo en cuenta las evidencias obtenidas a partir de caracteres moleculares, morfológicos y químicos se identificaron un total de 18 linajes de *Xanthoparmelia* con categoría de especie que aparecen en el centro de México. Sin embargo, muchas especies tradicionalmente circunscritas no formaron grupos monofiléticos. Este conflicto indica que la taxonomía y delimitación de especies en el género *Xanthoparmelia* requiere revisión y enfatiza la importancia de los datos moleculares para una delimitación más robusta de especies en este género.

Palabras clave. Especies crípticas, biodiversidad, metabolitos secundarios, líquenes, México.

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INTRODUCTION

The diversity of organisms is distributed unequally in the world. Species distributions are influenced by a wide range of factors, including biogeographic history, topography, or climate (Fischer 1960; Brown 2001; Rozzi & al. 2008). These factors can result in species-rich regions, including hotspots of biodiversity where rare and endemic species occur. One of the 25 biodiversity hotspots of the world is the Mesoamerican region that extends from northern Costa Rica and Nicaragua to central Mexico (Myers & al. 2000). The high levels of biodiversity in Mexico have been explained by the country's geographic position between tropical and temperate regions and its importance as a refugial area for temperate species during Pleistocene glaciations (Mittermeier 1988; Ramamoorthy & al. 1993; Myers & al. 2000). Mexico has a wide range of ecosystems from rainforest to dry deserts. We focus on the xerophytic scrub, which is among the most widespread ecosystems in Mexico, covering about 40% of the country and harbouring high biodiversity. This vegetation is adapted to face aridity and mainly consists of low trees, shrubs and succulent plants (Rzedowski 1978). Lichen communities in xerophytic scrub frequently consist of crustose lichens, as well as, members of the lichen-forming fungi family *Parmeliaceae* F.Berchtold & J.Presl. In *Parmeliaceae*, species of the genera *Usnea* Dill. ex Adans., *Parmotrema* A.Massal., *Hypotrachyna* (Vain.) Hale, and *Xanthoparmelia* (Vain.) Hale are particularly common (Lücking & al. 2016).

The genus *Xanthoparmelia* is the most diverse genus of lichen-forming fungi with about 800 species worldwide (Blanco & al. 2004; Thell & al. 2012; Jaklitsch & al. 2016). *Xanthoparmelia* is chemically diverse and the presence or absence of secondary metabolites has been widely used, in conjunction with morphological characters, to delimit species (Hale 1990; Elix 1994; Nash & al. 2016). *Xanthoparmelia* diversified dramatically during the Miocene (Kraichak & al. 2015). Recent studies show that *Xanthoparmelia* could have originated in the African continent during the early Miocene, eventually spreading to Australia and South Africa –both current centers of diversity for this genus. In contrast, the Holarctic has been more recently colonized and diversification happened mainly during the late Miocene and early Pliocene, resulting in a lower diversity of *Xanthoparmelia* in this region (Leavitt & al. 2018). However, in Mexico there exists a high *Xanthoparmelia* diversity and a high chemosyndromic variation has been recognized inside of the genus (Culberson & al. 1979). Due to this, deep studies are needed to clarify many of the phylogenetic clades within the group (Nash & al. 2016). Nevertheless, at the date, there only exists a single molecular study of *Xanthoparmelia* that included five taxa from xerophytic scrub (Barcenas-Peña & al. 2018), despite the fact that this is the most extensive ecosystem in the country and the preferred habitat for *Xanthoparmelia* species. Consequently, the xerophytic scrub is an ecosystem that requires a deep molecular study of *Xanthoparmelia* species (Barcenas-Peña & al. 2018).

In Mexico, *Xanthoparmelia* species are very abundant due to the presence of numerous exposed rocky substrates, and c. 75 species are currently accepted based mainly on phenotypical characters (Nash & al. 2016; Barcenas-Peña & al. 2018). Forty six *Xanthoparmelia* species are recorded from xerophytic scrub vegetation in the country (Lücking & al. 2016; Nash & al. 2016; Barcenas-Peña & al. 2018) mainly from the Sonoran Desert in the north of the country, one of the most studied taxonomically regions (morphological and chemically) (Nash & Elix 2004; Nash & al. 2016). However, the central area of the country still requires attention. In addition, in Mexico there are currently few studies of lichen fungi at the molecular level. For instance, only a work about *Xanthoparmelia mexicana* (Gyeln.) Hale group from xerophytic scrub in Mexico has been done (Barcenas-Peña & al 2018). Here we studied the diversity of *Xanthoparmelia* species in xerophytic scrub vegetation of the central part of Mexico using morphology, secondary chemistry, and molecular sequence data to understand the phylogenetic diversity and relationship among the species.

MATERIAL AND METHODS

Study area

All *Xanthoparmelia* specimens were collected on rocks from different localities throughout arid regions of xerophytic scrub in the central part of Mexico: Zacatecas, Aguas Calientes, San Luis Potosi, Jalisco, Guanajuato, Querétaro, Hidalgo, Estado de Mexico and Mexico City (Fig. 1). Xerophytic scrub occupies approximately 40% of the country's surface and is distributed from the Baja California Peninsula, the Coastal Plain and the lower Sierra de Sonora. Likewise, it is characteristic of the Altiplano from Chihuahua and Coahuila to Jalisco, Guanajuato, Hidalgo and Estado de Mexico, extending to Puebla and Oaxaca. It also constitutes the vegetation of a part of the northeastern Coastal Plain of Coahuila and Tamaulipas. These areas show average temperatures between 12°C and 26°C and an average annual rainfall of 100 ml to 400 ml (Rzedowski 1978).

Anatomical studies

For all specimens collected (about 440, deposited at F and MEXU; Appendix 1) the morphology and chemistry were assessed. Morphological characters, such as, shape and size of the thallus and lobules, isidia shape, lower surface and medulla color were studied according to Hale (1990) and Nash & al. (2016) using a Zeiss Stemi 2000-C stereoscope. Ascomatal anatomy, ascospore, conidia shape and size were studied using a Zeiss Axioscope. Secondary metabolites were identified using spot test with 10% KOH, KC, C, PD and high-performance thin layer chromatography (HPTLC) using solvent system C following established methods (Culberson & Johnson 1982; Arup & al. 1993; Lumbsch 2002; Orange & al. 2010).

Taxon sampling

Since a large number of specimens had lichenicolous fungi, only selected specimens of each species were used to obtain DNA. A total of 29 specimens of 18 species were selected for molecular analyses from xerophytic scrub vegetation representing the range of morphological and chemi-

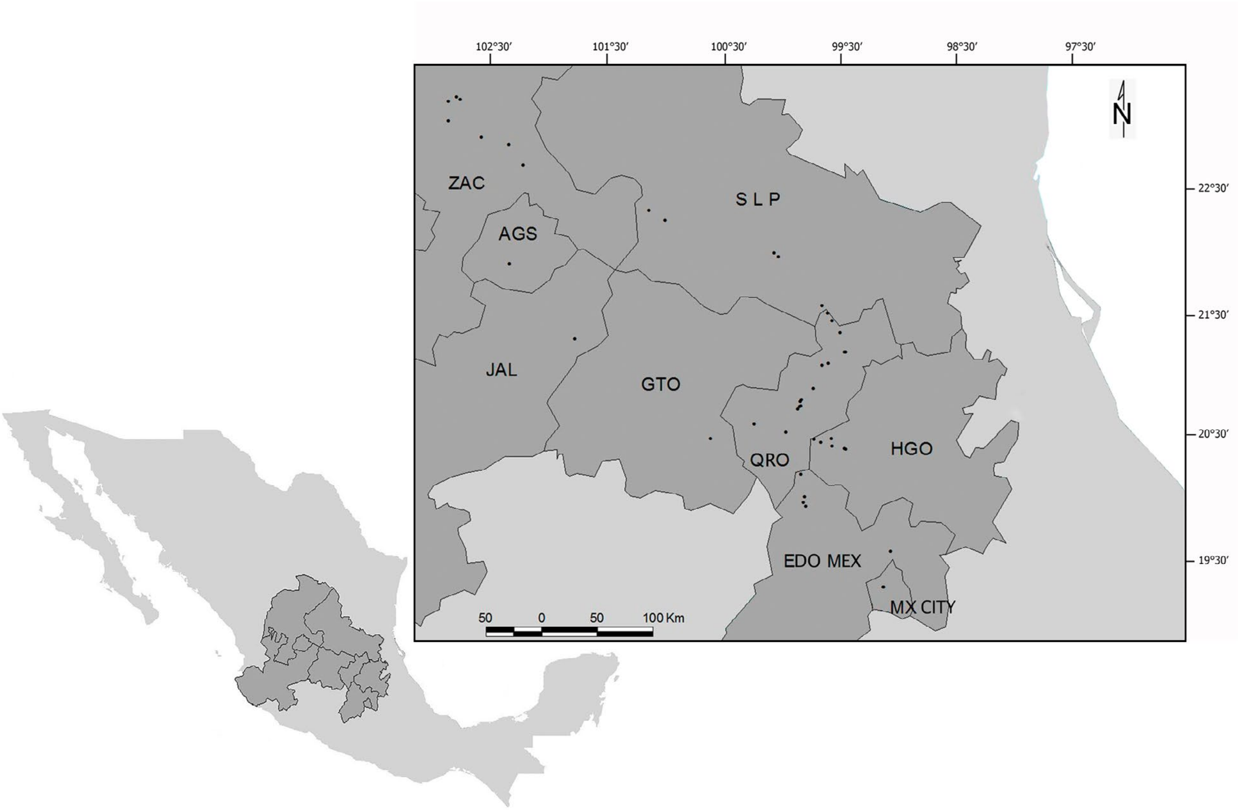

Fig. 1. Location of *Xanthoparmelia* (Vain.) Hale collection sites from arid regions of central Mexico: Mexico City (MX CITY), Estado de México (EDO MEX), Querétaro (QRO), Guanajuato (GTO), Hidalgo (HGO), Aguas Calientes (AGS), Jalisco (JAL), San Luis Potosí (SLP) and Zacatecas (ZAC).

cal variation, and supplemented with sequences of 20 specimens of 4 species from Puebla and Oaxaca (Mexico) of a previous analysis (Barcenas-Peña & al. 2018) (Appendix 2). In addition, sequences of 138 specimens of 27 species from different parts of the world were downloaded from GenBank (Appendix 2). Four species that have previously been shown to be distantly related to the *Xanthoparmelia* species from North America were used as outgroups, including *X. crespoae* Elix, Louwhoff & M.C. Molina, *X. filarszkyana* (Gyeln.) Hale, *X. substrigosa* (Hale) Hale and *X. lithophiloides* (Kurok.) Elix (Leavitt & al. 2018). Altogether, a total of 187 specimens of 38 species were included in this study (Appendix 2).

Molecular methods

From the 29 specimens sampled from Mexico, total genomic DNA was extracted from thallus fragments following the manufacturers' instructions using the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research Corp., Irvine, CA). DNA sequences were generated for three markers using the polymerase chain reaction (PCR): the nuclear ribosomal internal transcribed spacer region (ITS), a region of the mitochondrial small subunit rDNA (mtSSU), and a region of the nuclear large subunit rDNA (nuLSU). PCR reactions contained 6.25 µl of MyTaq™ Red DNA Polymerase (Bioline, Taunton, MA, USA), 5.25 µl of H_2O, 0.25 µl of forward and reverse primers (10 µM), and 0.5 µl of template DNA (10X), for a total reaction volume of 12.5 µl. The ITS region was amplified using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990); mtSSU using primers mrSSU1 and mrSSU3R (Zoller & al. 1999), and nuLSU rDNA using primers AL2R (Mangold & al. 2008) and LR6 (Vilgalys & Hester 1990). PCR products were sequenced using the same primers used for amplification and ABI PRISM 3730 DNA Analyzer (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at The Field Museum, Chicago, Illinois, USA.

Sequence alignment and phylogenetic analysis

ITS, mtSSU and nuLSU sequences were aligned independently using the 'auto' option with FFT-NS-i algorithm, in Mafft v7 (Katoh & Standley 2013), with the remaining

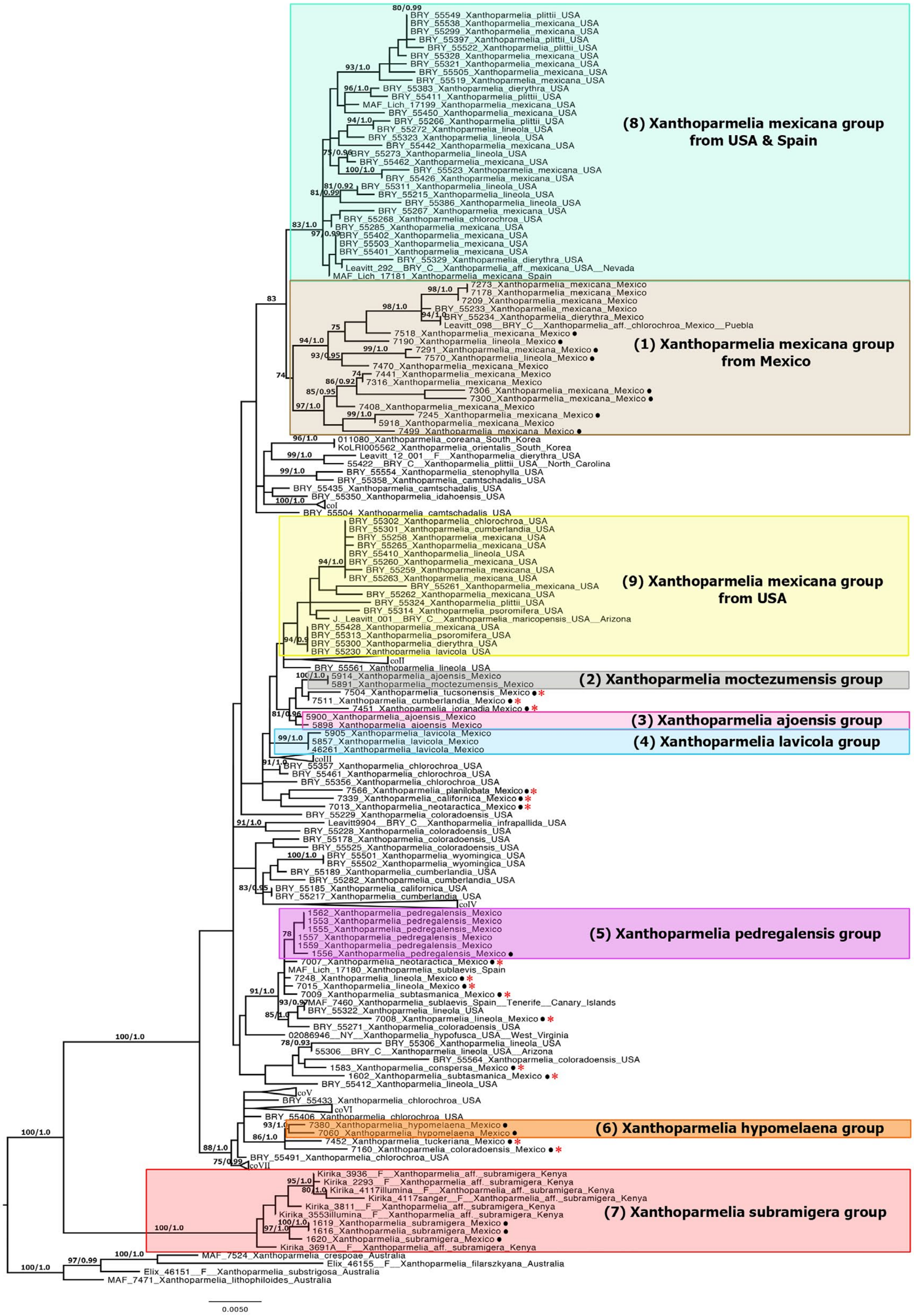

Fig. 2. Phylogenetic relationships of the *Xanthoparmelia* (Vain.) Hale species from Mexican xerophytic scrub based on a concatenated data set of ITS, mtSSU and nuLSU. Topology is based on maximum likelihood (ML) analyses. ML bootstrap values above 75% and Bayesian posterior probability values above 0.95 are indicated on each branch. The 29 specimens samples generated in this study are indicated with a black dot. Nine lineages, including seven major groups of Mexican samples, are indicated with color boxes. A red asterisk indicates Mexican samples falling outside the seven major clades including Mexican specimens (clades 1–7). Collapsed nodes coI–coVII.

parameters set to default values. Ambiguous positions of each alignment were removed using options for a "less stringent" selection on Gblocks 0.91b (Castresana 2000). SequenceMatrix software (Vaidya & al. 2011) was used to concatenate all three alignments. Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian analyses (BA). ML trees were calculated with RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis 2014) on the Cipres Science Gateway (Miller & al. 2010) using GTR+G+I substitution model with 1000 bootstrap pseudoreplicates, with the data partitioned by loci. For the BA, substitution models for each locus were estimated using jModelTest-2.1.9 (Guindon & Gascuel 2003; Darriba & al. 2012), which recommended for ITS locus the TIM2ef+I+G model, for mtSSU locus the F81+I model and for the nuLSU locus the TIM2ef+I+G model. Due the TIM2ef substitution models are not implemented in MrBayes were replaced by the GTR model (Ronquist & Huelsenbeck 2003). The proportion of invariable sites (I) and gamma distributed rates (G) defined in jModeltest were conserved in both cases. Two parallel Markov chain Monte Carlo (MCMC) runs were performed in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), each using 10,000,000 generations which were sampled every 100 steps. A 50% majority rule consensus tree was generated from the combined sampled trees (149,965) of both runs after discarding the first 25% trees as burn-in. Convergence diagnostic as PSRF was reasonably close to 1.0 for all parameters and average deviation of split frequencies was below 0.01 (Gelman & Rubin 1992). Tree files were visualised with FigTree 1.4.2 (Rambaut 2014). The ITS, mtSSU and nuLSU sequences are deposited in GenBank (Appendix 2).

RESULTS AND DISCUSSION

Phylogeny

Our phylogenetic analyses recovered nine well supported clades of *Xanthoparmelia* (Fig. 2). Seven of them (clades 1 to 7) include Mexican specimens of the same species forming monophyletic clusters, the rest of the Mexican specimens are distributed all over the phylogenetic tree. In total our phylogenetic analysis revealed 18 species-level lineages from xerophytic scrublands in Mexico (Fig. 2, Appendix 2). Clades 8 and 9 are *X. mexicana* groups from USA-Spain and USA respectively. The seven major lineages of Mexican *Xanthoparmelia* samples from xerophytic scrublands included: 1) *X. mexicana* s. str., 2) the *X. moctezumensis* T.H.Nash group, 3) the *X. ajoensis* (T.H.Nash) Egan group, 4) the *X. lavicola* (Gyeln.) Hale group, 5) the *X. pedregalensis* Barcenas Peña, Lumbsch & S.D.Leav. group, 6) the *X. hypomelaena* (Hale) Hale group, and 7) the *X. subramigera* (Gyeln.) Hale group.

The 18 species recognized in this analysis have been previously reported in Mexico in morphological and chemical studies (Nash & Elix 2004; Nash & al. 2004; Nash & al. 2016; Barcenas-Peña & al. 2018). However, many of these species did not have sequences available in Genbank including *Xanthoparmelia hypomelaena*, *X. joranadia* (T.H.Nash) Egan, *X. neotaractica* Hale, *X. planilobata* (Gyeln.) Hale, *X. subtasmanica* Elix & T.H.Nash, *X. tuckeriana* Elix & T.H.Nash and *X. tucsonensis* (T.H. Nash) Egan (Appendix 2). The ML and BA phylogeny (Fig. 2) show that the phenotype-based taxonomy in the genus requires revision, with numerous species identified using morphological and chemical characters not forming monophyletic groups. In addition, with exception of *X. pedregalensis* none of these species are type materials from Mexico. In the *X. mexicana* group from the USA and Spain (clade 8) there are not samples from Mexico. As well, within clade 8 samples of *X. dierythra* (Hale) Hale, *X. lineola* (E.C.Berry) Hale, *X. mexicana,* and *X. plittii* (Gyeln.) Hale are included and do not form monophyletic clades. These species are currently distinguished based on their reproduction and secondary chemistry (Hale 1990). However, the substances used as diagnostic characters (norstictic, salazinic, and stictic acids) belong to the same chemosyndrome, which has, in some cases, been interpreted as intraspecific variation (Lumbsch 1998a, 1998b). Further, one sample agreeing with the current circumscription of the vagrant *X. chlorochroa* (Tuck.) Hale clustered in this group. In fact, all phenotypes (=currently accepted species) in this clade also clustered in other clades in the phylogenetic tree. This strongly indicates that the characters used for the circumscription of species in this clade need re-evaluation, considering that a recent study from Australian *Xanthoparmelia* species has observed that closely related samples may exhibit distinct chemical profiles. Additionally, that in the evolution of secondary metabolite composition can be rapid, which may result in convergence between distantly related samples (Autumn & al. 2020). A similar pattern is found in the clade of Mexican *X. mexicana* specimens (clade 1 of Mexican specimens in Fig. 2), in which samples identified as *X. chlorochroa, X. dierythra, X. lineola,* and *X. mexicana* are found and none of them form a monophyletic group. Even though clade 1 is a little below the supported values (bootstrap values above 75%), we still considered it a good example that reflects the need of re-evaluation of species. Additionally, the presence of several well-supported clades within clade 1 suggests that even after the segregation of *X. pedregalensis* (Barcenas-Peña & al. 2018), *X. mexicana* in Mexico is not well understood. However, given that the species was described from central Mexico, we regard clade 1 as *X. mexicana* whereas specimens currently accepted as *X. mexicana* from other parts of the world could belong to other taxa.

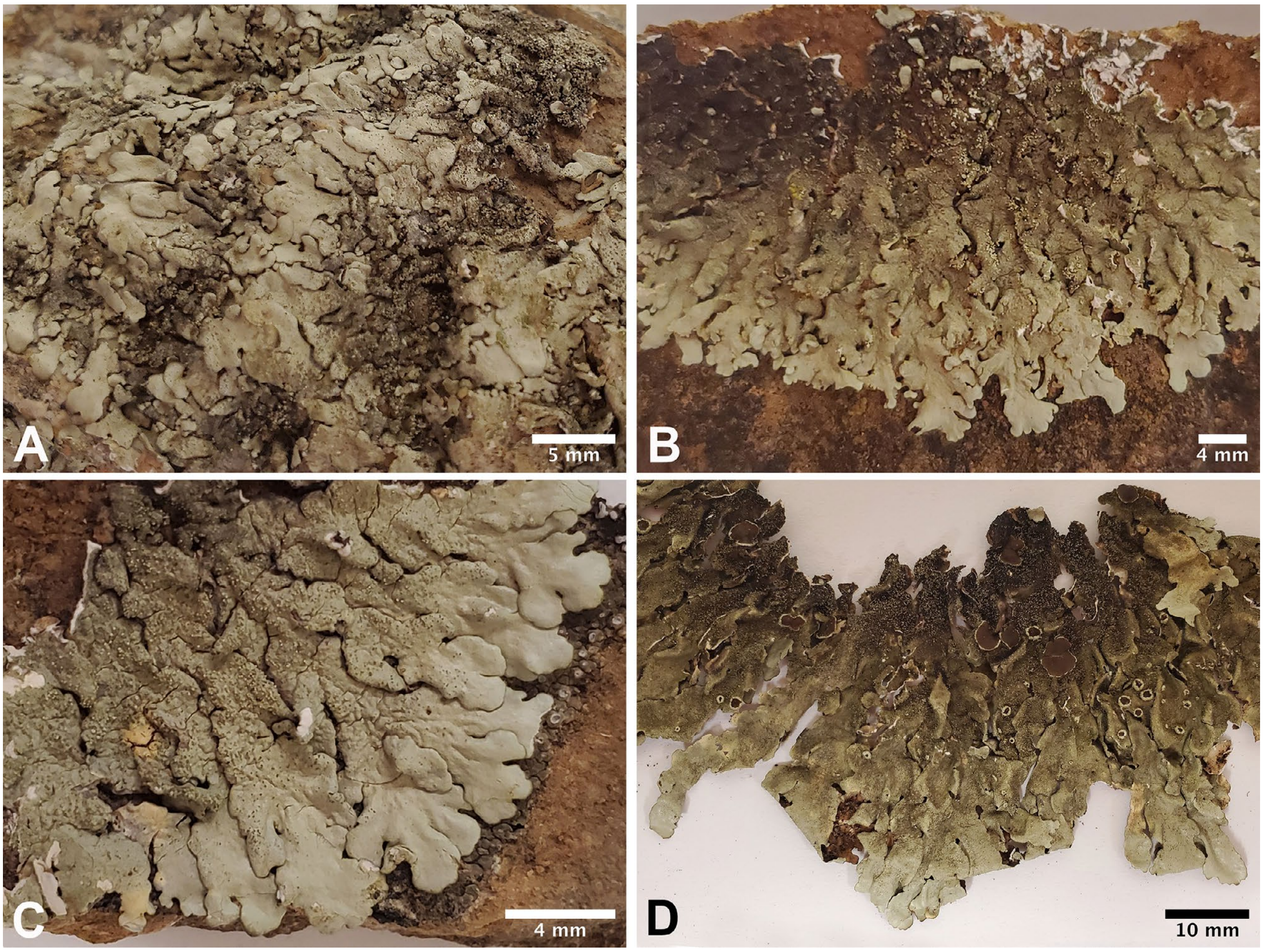

Fig. 3. Some species of *Xanthoparmelia* (Vain.) Hale included in this study: **a,** *Xanthoparmelia ajoensis* (T.H.Nash.) Egan (Barcenas-Peña 5900, F); **b,** *Xanthoparmelia lavicola* (Gyeln.) Hale (Barcenas-Peña 5905, F); **c,** *Xanthoparmelia moctezumensis* T.H.Nash (Barcenas-Peña 5891, F); **d,** *Xanthoparmelia subramigera* (Gyeln.) Hale (Ruiz-Cazares 1620, F).

The *Xanthoparmelia mexicana* group from the USA (clade 9) includes samples of the nonisidiate *X. cumberlandia* (Gyeln.) Hale, *X. maricopensis* T.H.Nash & Elix (also containing hyposalazinic acid), and the chemically different *X. psoromifera* (Hale) Hale (with psoromic acid). Psoromic acid has been found to be inconsistent with monophyletic groups in the distantly related genus *Cladonia* (Pino-Bodas & al. 2012).

The *Xanthoparmelia moctezumensis* clade also includes a sample of *X. ajoensis.* However, the two species contain the closely-related depsides 3-α-hydroxybarbatic or diffractaic acids, respectively. This is consistent with the hypothesis that morphologically identical specimens with closely-related substances often represent variation within a single species (Feige & Lumbsch 1995). However, the majority of specimens phenotypically identified as *X. ajoensis* are found in a separate clade, the *X. ajoensis* group (clade 3) with unresolved relationships to the *X. moctezumensis* group (clade 2). Additional studies with an extended sampling of specimens and genetic markers of these two groups is required to better understand the phylogenetic relationships in this part of the phylogenetic tree.

The *Xanthoparmelia lavicola* group (clade 4) is represented by three samples of this species that was originally described from central Mexico. It was strongly supported as a monophyletic group but its phylogenetic relationships remain unresolved. Another strongly supported clade in the ML analysis was the *X. pedregalensis* group (clade 5) that is closely related to *X. neotaractica*, *X. sublaevis* (Cout.) Hale, *X. lineola* and *X. coloradoensis* (Gyeln.) Hale. *X. pedregalensis* has been treated in detail elsewhere (Barcenas & al. 2018). The *X. hypomelaena* group (clade 6) included two Mexican specimens–here again the relationships remain unresolved. All studied samples of *X. subramigera*

Table 1. Summary of *Xanthoparmelia* (Vain.) Hale species identified in this study, indicating chemistry substances, isidia presence (+) /absence (-) and lower surface colour. Species included in our phylogenetic study indicating those belonging to major groups (in brackets). An asterisk (*) indicates species not included in any of these groups according to Fig. 2.

Species	Chemistry	Isidiate	Lower surface color
X. ajoensis (T.H.Nash) Egan [G3]	Diffractaic and barbatic	+	Pale tan or brown
X. californica Hale *	Norstictic and connorstictic	-	Pale to medium brown
X. coloradoensis (Gyeln.) Hale *	Consalazinic, norstictic, protocetraric and salazinic	-	Brown
X. conspersa (Ehrh. ex Ach.) Hale *	Stictic, constictic, cryptostictic and norstictic	+	Black
X. cumberlandia (Gyeln.) Hale *	Stictic and constictic and norstictic	-	Pale brown or brown
X. hypomelaena (Hale) Hale [G6]	Fumarprotocetraric	-	Black
X. jornadia (T.H.Nash) Hale *	Lecanoric	+	Pale brown
X. lavicola (Gyeln.) Hale [G4]	Psoromic	+	Pale to medium brown
X. lineola (E.C.Berry) Hale *	Salazinic and consalazinic	-	Pale to medium brown
X. mexicana (Gyeln.) Hale s. str. [G1]	Salazinic, consalazinic and norstictic	+	Pale to medium brown
X. moctezumensis T.H.Nash [G2]	3-α-hydroxybarbatic, barbatic, baeomycesic and squamatic	+	Pale brown
X. neoconspersa (Gyeln.) Hale	Stictic, constictic, cryptostictic and norstictic	-	Black
X. neotaractica Hale *	Stictic, norstictic and constictic	-	Pale brown to brown
X. nigropsoromifera (T.H.Nash) Egan	Psoromic, 2'-O-demethylpsoromic	-	Black
X. novomexicana (Gyeln.) Hale	Fumarprotocetraric and protocetraric	-	Pale to medium brown
X. pedregalensis Barcenas Peña, Lumbsch & S.D.Leav. [G5]	Salazinic and norstictic	+	Tan to brown
X. planilobata (Gyeln.) Hale *	Stictic, constictic, cryptostictic and norstictic	-	Black
X. plittii (Gyeln.) Hale	Stictic, constictic, cryptostictic and norstictic	+	Pale to dark brown
X. subramigera (Gyeln.) Hale [G7]	Succinprotocetraric, fumarprotocetraric and protocetraric	+	Pale to medium brown
X. subtasmanica Elix & T.H.Nash *	Salazinic and consalazinic	-	Black
X. tinctina (Maheu & A.Gillet) Hale	Salazinic and consalazinic	+	Black
X. tuckeriana Elix & T.H.Nash *	Fumarprotocetraric and protocetraric	-	Ivory to pale brown
X. tucsonensis (T.H.Nash) Egan *	Diffractaic and barbatic	-	Pale brown

from Mexico and Kenya clustered together (clade 7), indicating that this species indeed has a larger distributional range and is a well-delimited taxon.

A number of Mexican samples did not cluster in one of the seven clades discussed above including *Xanthoparmelia californica* Hale*,* *X. conspersa* (Ehrh. ex Ach) Hale*, X. coloradoensis, X. cumberlandia, X. joranadia, X. planilobata, X. tuckeriana,* and *X. tucsonensis,* as well as samples of *X. lineola, X. neotaractica,* and *X. subtasmanica*, none of them forming monophyletic groups. Samples of some of these species from other localities out of Mexico did not cluster with the Mexican specimens, indicating that the delimitation of these species is poorly understood. Since none of these species' names are based on type materials from Mexico, they might represent distinct lineages that might require formal description. This, however, has to wait until we have gained a better understanding of delimitation of species in Holarctic species of *Xanthoparmelia.* Since the Holarctic species of the genus are hypothesized to have originated and diversified relatively recently, species delimitation with multi-gene data sets have been shown to be difficult (Leavitt & al. 2011, 2013) and hence reduced genomic data sets, such as RADseq might help elucidate species delimitations (Grewe & al. 2017, 2018). In addition, our collection sampling in Mexico will need to be extended, since the described diversity of *Xanthoparmelia* species based on phenotypical characters in the Mexican xerophytic scrub vegetation is higher (44 species) (Nash & al. 2016; Lücking & al. 2009) than found in this work (23 species, 18 included in the phylogenetic analyses).

Taxonomy

We identified 23 species from the Mexican xerophytic scrub based on their morphology, mainly presence of isidia and lower surface colour, as well as secondary substances (Table 1, Fig. 3). Both isidiate and not isidiate species were found in almost the same proportion, while species with pale to brown lower surface were more frequent. Additionally, we see a high variety of secondary substances in species morphologically similar (Table 1). Nevertheless, nearly 44 *Xanthoparmelia* species were found in the Mexican xerophytic scrub mainly from the northern part of Mexico (Nash & Elix 2004; Nash & al. 2004; Nash & al. 2016). While in the present study we collected in the central part of Mexico and the diversity of species found is high (23 putative species-level lineages). Additional studies that can include all of the diversity of the country are necessary. Incorporation of molecular analyses using different genetic markers to the morphological and chemical study towards a better species delimitation is also necessary.

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REFERENCES

Arup U., Ekman S., Lindblom L. & Mattsson J-E. 1993. High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist* 25: 61–71.

Autumn K., Barcenas-Peña A., Kish-Levine S., Huang J-P & Lumbsch T.H. 2020. Repeated colonization between arid and seasonal wet habitats, frequent transition among substrate preferences, and chemical diversity in Western Australian *Xanthoparmelia* lichens. *Frontiers in Ecology and Evolution* 8: 129.

Barcenas-Peña A., Leavitt S.D., Huang J.P., Grewe F. & Lumbsch H.T. 2018. Phylogenetic study and taxonomic revision of the *Xanthoparmelia mexicana* group, including the description of a new species (Parmeliaceae, Ascomycota). *MycoKeys* 40: 13–28.

Blanco O., Crespo A., Elix J.A., Hawksworth D.L. & Lumbsch, H.T. 2004. A molecular phylogeny and a new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (Ascomycota: Lecanorales). *Taxon* 53: 959–975.

Brown J. 2001. Mammals on mountainsides: elevational pattern of diversity. *Global Ecology and Biogeography* 10: 101–109.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552.

Culberson C. & Johnson A. 1982. Substitution of methyl tert.-butyl ether for diethyl ether in standardized thin layer chromatographic method for lichen products. *Journal of Chromatography B* 238: 438–487.

Culberson C., Nash T.H. III & Johnson A. 1979. 3-α-hydroxibarbatic acid, a new depside in chemosyndrome of some Xanthoparmeliae with α-orcinol depsides. *Bryologist* 82: 154–161.

Darriba D., Taboada G.L., Doallo R. & Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Elix J.A. 1994. *Xanthoparmelia*. *Flora of Australia* 55: 201–308.

Feige G.B. & Lumbsch H.T. 1995. Some types of chemical variation in lichens. *Cryptogamic Botany* 5: 31–35.

Fischer A.G. 1960. Latitudinal variations in organic diversity. *Evolution* 14: 64–81.

Gardes M. & Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Gelman A. & Rubin D.B. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–511.

Grewe F., Huang J.P., Leavitt S.D. & Lumbsch H.T. 2017. Reference-based RADseq resolves robust relationships among closely related species of lichen-forming fungi using metagenomic DNA. *Scientific Reports* 7: 9884.

Grewe F., Lagostina E., Wu H., Printzen C. & Lumbsch H.T. 2018. Population genomic analyses of RAD sequences resolves the phylogenetic relationship of the lichen-forming fungal species *Usnea antarctica* and *Usnea aurantiacoatra*. *Mycokeys* 43: 91–113.

Guindon S. & Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704.

Hale Jr. M.E. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae). *Smithsonian Contributions to Botany* 74: 1–250.

Huelsenbeck J.P. & Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Jaklitsch W.M., Baral H.O., Lücking R. & Lumbsch H.T. 2016. Ascomycota. *In* Frey W. (ed.), *Syllabus of Plant Families - Adolf Engler's Syllabus der Pflanzenfamilien*: 1–150. Stuttgart, Gebr. Borntraeger Verlagsbuchhandlung.

Katoh K. & Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kraichak E., Divakar P.K., Crespo A., Leavitt S.D., Nelsen M.P., Lücking R. & Lumbsch H.T. 2015. A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. *Scientific Reports* 5: e10028.

Leavitt S.D., Johnson L.A., Goward T. & St. Clair L.L. 2011. Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (Parmeliaceae) in North America. *Molecular Phylogenetics and Evolution* 60: 317–332.

Leavitt S.D., Kirika P.M., Amo de Paz G., Huang J.P., Hur J.S., Elix J.A., Grewe F., Divakar P.K. & Lumbsch H.T. 2018. Assessing phylogeny and historical biogeography of the largest genus in lichen-forming fungi, *Xanthoparmelia* (Parmeliaceae, Ascomycota). *Lichenologist* 50: 299–312.

Leavitt S.D., Lumbsch H.T., Stenroos S. & St Clair L.L. 2013. Pleistocene speciation in North American lichenized fungi and the impact of alternative species circumscriptions and rates of molecular evolution on divergence estimates. *Plos One* 8: e85240.

Lücking R., Rivas Plata E., Chaves J.L., Umaña L. & Sipman H.J.M. 2009. How many tropical lichens are there. . . really? *Bibliotheca Lichenologica* 100: 399–418.

Lücking R., Villaseñor J.L., Herrera-Campos M.A., Pérez-Pérez R.M., Egan S.R., Esslinger H.L. & Nash III T.H. 2016. Phylogenetic structure of metacommunities in Mexican Parmeliaceae (lichenized Ascomycota: Lecanorales). *Bibliotheca Lichenologica* 110: 27–54.

Lumbsch H.T. 1998a. The use of metabolic data in lichenology at the species and subspecific levels. *Lichenologist* 30: 357–367.

Lumbsch H.T. 1998b. The taxonomic use of metabolic data in lichen-forming fungi. *In* Frisvad J.C., Bridge P.D. & Arora D.K. (eds.), *Chemical Fungal Taxonomy*: 345–387. Marcel Dekker, New York.

Lumbsch H.T. 2002. Analysis of phenolic products in lichens for identification and taxonomy. *In* Kranner I., Beckett R. & Varma A. (eds.), *Protocols in Lichenology Culturing, biochemistry, ecophysiology and use in biomonitoring*: 281–295. Springer, Berlin.

Mangold A., Martín M.P., Lücking R. & Lumbsch H.T. 2008. Molecular phylogeny suggests synonymy of Thelotremataceae within Graphidaceae (Ascomycota: Ostropales). *Taxon* 57: 476–486.

Miller M.A., Pfeifer W. & Schwartz T. 2010. CIPRES Science Gateway survey. Webside: http://www.phylo.org [accessed: 24 Mar. 2020]

Mittermeier R. 1988. Primate diversity and the tropical forest. Case studies from Brazil and Madagascar and the importance of the megadiversity countries. *In* Wilson E.O. (ed.), *Biodiversity*: 145–154. National Academy Press, Washington.

Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B. & Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.

Nash III T.H. & Elix J.A. 2004. *Xanthoparmelia*. *In* Nash III T.H., Ryan B.D., Diederich P., Gries C. & Bungartz F. (eds.), *Lichen Flora of the Greater Sonoran Desert Region* vol. 2: 566–605. Lichens Unlimited, Arizona State University, Tempe.

Nash III T.H., Herrera-Campos M.A. & Elix J.A. 2004. Preliminary treatment of *Xanthoparmelia* for Mexico. *Symbolae Botanicae Upsaliensis* 34: 289–326.

Nash III T.H., Herrera-Campos M.A., Esslinger T.L. & Elix J.A. 2016. *Xanthoparmelia* in Mexico. *Bibliotheca Lichenologica* 110: 621–641.

Orange A., James P.W. & White F.J. 2010. *Microchemical methods for the identification of lichens, second edition with additions and corrections*. British Lichen Society, London.

Pino-Bodas R., Rosa Burgaz A., Martin M.P. & Lumbsch H.T. 2012. Species delimitations in the *Cladonia cariosa* group (Cladoniaceae, Ascomycota). *Lichenologist* 44: 121–135.

Ramamoorthy T.P., Bye R., Lot A. & Fa J. 1993. *Biological Diversity of Mexico: Origins and Distribution*. Oxford University Press, Oxford.

Rambaut A. 2014. FigTree 1.2.2. Webside: http://tree.bio.ed.ac.uk/software/fgtree/ [accessed: 24 Mar. 2020].

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rozzi R., Armesto J.J., Goffinet B., Buck W., Massardo F., Silander J., Arroyo M.K.T., Russell S., Anderson C.B., Cavieres L.A. & Callicott J.B. 2008. Changing lenses to assess biodiversity: patterns of species richness in sub-Antarctic plants and implications for global conservation. *Frontiers in Ecology and the Environment* 6: 131–137.

Rzedowski J. 1978. *Vegetación de México*. Editoral Limusa, México.

Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Thell A., Crespo A., Divakar P.K., Kärnefelt I., Leavitt S.D., Lumbsch H.T. & Seaward M.R.D. 2012. A review of the lichen family Parmeliaceae – history, phylogeny and current taxonomy. *Nordic Journal of Botany* 30: 641–664.

Vaidya G., Lohman D.J. & Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.

Vilgalys R. & Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.

White T.J., Bruns T.D., Lee S. & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds.), *PCR Protocols*: 315–322. Academic Press, San Diego.

Zoller S., Scheidegger C. & Sperisen C. 1999. PCR primers for the amplication of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* 31: 511–516.

Appendix 1. List of *Xanthoparmelia* specimens collected in Mexico and deposited in the F and MEXU herbaria as indicated.

Xanthoparmelia ajoensis (T.H.Nash) Egan: Barcenas-Peña 5870, 5872-5874, 5876, 5877, 5880-5882, 5885, 5894, 5899, 5901, 5902, 5907, 5909, 5911, 5917 (F), 5906, 5908, 5913, 5915 (MEXU); ***X. californica*** Hale: Barcenas-Peña 7339, 7475 (F); ***X. coloradoensis*** (Gyeln.) Hale: Barcenas-Peña 7004, 7006, 7011, 7014, 7105, 7119, 7160, 7183, 7202, 7215, 7217, 7225, 7226, 7228, 7230, 7237, 7247, 7250, 7251, 7266, 7276, 7278, 7308, 7312, 7363, 7420, 7440, 7447, 7453, 7458, 7464, 7472, 7492, 7497, 7503, 7510, 7515, 7517, 7522, 7524, 7527, 7529, 7547, 7550 (F), 7074, 7231, 7252, 7305, 7364, 7487 (MEXU), Ruiz-Cazares 1605, 7534, 7537, 7554 (F), 7559 (MEXU); ***X. conspersa*** (Ehrh. ex Ach.) Hale: Barcenas-Peña 7307, 7513 (F), 7442 (MEXU), Ruiz-Cazares 1551, 1583, 1591, 1594, 1597, 1600, 1607, 7538 (F), 1606 (MEXU); ***X. cumberlandia*** (Gyeln.) Hale: Barcenas-Peña 7003, 7068, 7072, 7075, 7097, 7324, 7327, 7328, 7336, 7340, 7343, 7368, 7371, 7429, 7430, 7455, 7462, 7463, 7511 (F), 7332, 7341, 7518 (MEXU), Ruiz-Cazares 7535, 7558, 7567, 7568 (F); ***X. hypomelaena*** (Hale) Hale: Barcenas-Peña 7380 (F), 7060 (MEXU); ***X. joranadia*** (T.H.Nash) Hale: Barcenas-Peña 7411, 7419, 7451 (F), 7298 (MEXU); ***X. lavicola*** (Gyeln.) Hale: Barcenas-Peña 5867-5869, 5875, 5883, 5886, 5897, 7229, 7235, 7239, 7244, 7296, 7329, 7333, 7377, 7383, 7387, 7403, 7406, 7407, 7413, 7415, 7418, 7423-7425, 7456, 7466, 7484, 7496, 7498, 7502, 7520 (F), 5866, 5878, 7416 (MEXU); ***X. lineola*** (E.C.Berry) Hale: Barcenas-Peña 7008, 7015, 7100, 7189, 7190, 7224, 7301, 7309, 7352, 7370, 7388, 7435, 7444, 7448, 7512 (F), 7241, 7248, 7395, 7421 (MEXU), Ruiz-Cazares 7570 (F); ***X. mexicana*** (Gyeln.) Hale: Barcenas-Peña 5889, 5895, 5903, 5904, 5910, 5912, 5916, 7153, 7187, 7198, 7245, 7262, 7263, 7274, 7291, 7300, 7306, 7310, 7348, 7349, 7369, 7372, 7379, 7433, 7434, 7445, 7491, 7505, 7507, 7546 (F), 7356, 7361, 7432, 7499, 7516 (MEXU), Ruiz-Cazares 1578, 1579, 1598 (F), 1581 (MEXU); ***X. moctezumensis*** T.H.Nash: Barcenas-Peña 7264, 7292 (F), 5887, 5890, 5893 (MEXU); ***X. neoconspersa*** (Gyeln.) Hale: Barcenas-Peña 7390, 7393, 7427, 7446, 8000 (F), 7067 (MEXU), Ruiz-Cazares 7562, 7564, 7569 (F), 7555, 7565 (MEXU); ***X. neotaractica*** Hale: Barcenas-Peña 7007, 7013, 7076, 7334, 7359, 7426, 7469 (F), 7069 (MEXU); ***X. nigropsoromifera*** (T.H.Nash) Egan: Barcenas-Peña 7104 (F); ***X. novomexicana*** (Gyeln.) Hale: Barcenas-Peña 7062, 7071, 7221, 7302, 7315, 7318, 7338, 7344, 7353, 7362, 7404, 7405, 7417, 7422, 7454 (F), 7314, 7319, 7357, 7443 (MEXU); ***X. pedregalensis*** Barcenas Peña, Lumbsch & S.D.Leav.: Barcenas-Peña 7477, 7493 (F), 7468 (MEXU), Ruiz-Cazares 1554, 1558, 1577, 1580 (F), 1556 (MEXU); ***X. planilobata*** (Gyeln.) Hale: Barcenas-Peña 7039 (F), Ruiz-Cazares 7536, 7566 (F), 7560 (MEXU); ***X. plittii*** (Gyeln.) Hale: Barcenas-Peña 7506, 7525 (F), Ruiz-Cazares 1586, 1595, 1596, 1608 (F), 1584, 1590 (MEXU); ***X.* sp.**: Barcenas-Peña 5871, 7012, 7023, 7024, 7028, 7031, 7032, 7036-7038, 7040, 7041, 7043, 7044, 7061, 7064, 7079, 7080, 7081, 7083, 7085-7088, 7098, 7099, 7103, 7130, 7135, 7145, 7186, 7191, 7194, 7199, 7203, 7205, 7210-7214, 7216, 7218, 7219, 7220, 7222, 7223, 7246, 7253, 7257, 7261, 7265, 7267, 7269, 7280, 7290, 7320-7323, 7325, 7330, 7331, 7337, 7342, 7346, 7366, 7367, 7373, 7376, 7378, 7382, 7384, 7396, 7397, 7402, 7409, 7428, 7438, 7439, 7450, 7461, 7481-7483, 7485, 7490, 7501, 7521, 7523, 7526, 7528, 7530-7533, 7549 (F), 7070, 7073, 7082, 7084, 7193, 7195, 7196, 7204, 7206, 7208, 7227, 7249, 7282, 7351, 7386, 7394, 7494, 7552, 7553 (MEXU), Ruiz-Cazares 1599, 7539, 7540, 7561, 7563 (F), 1601 (MEXU); ***X. subramigera*** (Gyeln.) Hale: Barcenas-Peña 7260, 7272, 7283, 7284, 7286, 7288, 7289, 7436, 7437 (F), 7281 (MEXU), Ruiz-Cazares 1616, 1618-1620, 7571-7574 (F), 1617 (MEXU); ***X. subtasmanica*** Elix & T.H.Nash: Barcenas-Peña 7009, 7026, 7131, 7201, 7486 (F), Ruiz-Cazares 1576, 1604 (F), 1602 (MEXU); ***X. tinctina*** (Maheu & A.Gillet) Hale: Barcenas-Peña 7255, 7259 (F), 7256 (MEXU); ***X. tuckeriana*** Elix & T.H.Nash: Barcenas-Peña 7000, 7152 (F), 7066, 7452 (MEXU), Ruiz-Cazares 7557 (MEXU); ***X. tucsonensis*** (T.H.Nash) Egan: Barcenas-Peña 7102, 7504 (F), 7136 (MEXU).

Appendix 2. Specimens of *Xanthoparmelia* included in the molecular study: species, country/voucher information, GenBank accession numbers for ITS, mtSSU and nuLSU sequences, respectively. New generated sequences are indicated by an asterisk (*), missing sequences are indicated by a dash (–), and specimens of collapsed nodes for Fig. 2 are marked with their respective clade labels (coI-coVII).

Xanthoparmelia ajoensis (T.H.Nash) Egan, Mexico: Puebla: Barcenas-Peña 5898 (F), MH580218, MH699893, MH699913; ***X. ajoensis***, Mexico: Puebla: Barcenas-Peña 5900 (F), MH580219, MH699894, MH699914; ***X. ajoensis***, Mexico: Puebla: Barcenas-Peña 5914 (F), MH580220, MH699895, MH699915; ***X. atticoides*** (Essl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch, USA: MAF 6744, AY581066, AY582302, AY578929, coII; ***X. californica*** Hale, USA: BRY 55185, HM578641, –, HM579053; ***X. californica***, Mexico: Zacatecas: Barcenas-Peña 7339 (F), MW553769*, –, MW567175*; ***X. camtschadalis*** (Ach.) Hale, USA: BRY 55358, HM578809, –, HM579220; ***X. camtschadalis***, USA: BRY 55360, HM578811, –, HM579222, coI; ***X. camtschadalis***, USA: BRY 55361, HM578812, –, HM579223, coI; ***X. camtschadalis***, USA: BRY 55362, HM578813, –, HM579224, coI; ***X. camtschadalis***, USA: BRY 55435, HM578885, –, HM579296; ***X. camtschadalis***, USA: BRY 55504, HM578956, –, HM579363; ***X.*** aff. ***chlorochroa***, Mexico: Puebla: Leavitt 098 (USA: BRY-C), MG695501, MG695749, MG695602; ***X. chlorochroa*** (Tuck.) Hale, USA: BRY 55231, HM578686, –, HM579096, coV; ***X. chlorochroa***, USA: BRY 55232, HM578687, –, HM579097, coV; ***X. chlorochroa***, USA: BRY 55235, HM578690, –, HM579100, coV; ***X. chlorochroa***, USA: BRY 55251, HM578706, –, HM579116, coIV; ***X. chlorochroa***, USA: BRY 55268, HM578722, –, HM579133; ***X. chlorochroa***, USA: BRY 55279, HM578732, –, HM579144, coVI; ***X. chlorochroa***, USA: BRY 55280, HM578733, –, HM579145, coVI; ***X. chlorochroa***, USA: BRY 55289, HM578742, –, HM579154, coV; ***X. chlorochroa***, USA: BRY 55302, HM578755, –, HM579167; ***X. chlorochroa***, USA: BRY 55340, HM578791, –, HM579203, coVII; ***X. chlorochroa***, USA: BRY 55356, HM578807, –, HM579218; ***X. chlorochroa***, USA: BRY 55357, HM578808, –, HM579219; ***X. chlorochroa***, USA: BRY 55377, HM578827, –, HM579239, coVII; ***X. chlorochroa***, USA: BRY 55403, HM578853, –, HM579265, coII; ***X. chlorochroa***, USA: BRY 55405, HM578855, –, HM579267, coIII; ***X. chlorochroa***, USA: BRY 55406, HM578856, –, HM579268; ***X. chlorochroa***, USA: BRY 55408, HM578858, –, HM579270, coIV; ***X. chlorochroa***, USA: BRY 55409, HM578859, –, HM579271, coVI; ***X. chlorochroa***, USA: BRY 55433, HM578883, –, HM579294; ***X. chlorochroa***, USA: BRY 55461, HM578913, –, HM579321; ***X. chlorochroa***, USA: BRY 55468, HM578920, –, HM579328, coVI; ***X. chlorochroa***, USA: BRY 55469, HM578921, –, HM579329, coVI; ***X. chlorochroa***, USA: BRY 55476, HM578928, –, HM579335, coIII; ***X. chlorochroa***, USA: BRY 55486, HM578938, –, HM579345, coV; ***X. chlorochroa***, USA: BRY 55487, HM578939, –, HM579346, coII; ***X. chlorochroa***, USA: BRY 55490, HM578942, –, HM579349, coII; ***X. chlorochroa***, USA: BRY 55491, HM578943, –, HM579350; ***X. chlorochroa***, USA: BRY 55494, HM578946, –, HM579353, coV; ***X. chlorochroa***, USA: BRY 55499, HM578951, –, HM579358, coIV; ***X. coloradoensis*** (Gyeln.) Hale, USA: BRY 55178, HM578634, –, HM579046; ***X. coloradoensis***, USA: BRY 55228, HM578683, –, HM579093; ***X. coloradoensis***, USA: BRY 55229, HM578684, –, HM579094; ***X. coloradoensis***, USA: BRY 55271, HM578725, –, HM579136; ***X. coloradoensis***, USA: BRY 55525, HM578978, –, HM579384; ***X. coloradoensis***, USA: BRY 55564, HM579017, –, HM579423; ***X. coloradoensis***, Mexico: Estado de México: Barcenas-Peña 7160 (F), MW553770*, MW567198*, MW567176*; ***X. conspersa*** (Ehrh. ex Ach.) Hale, Mexico: Mexico City: Ruiz-Cazares 1583 (F), MW553778*, MW567206*, MW567183*; ***X. coreana*** (Gyeln.) Hale, South Korea: 11080, KJ170870, –, KJ170870; ***X. crespoae*** Elix, Louwhoff & M.C. Molina, Australia: MAF 7524, AY581097, AY582332, AY578963; ***X. cumberlandia*** (Gyeln.) Hale, USA: BRY 55189, HM578645, –, HM579057; ***X. cumberlandia***, USA: BRY 55217, HM578672, –, HM579082; ***X. cumberlandia***, USA: BRY 55282, HM578735, –, HM579147; ***X. cumberlandia***, USA: BRY 55301, HM578754, –, HM579166; ***X. cumberlandia***, USA: BRY 55379, HM578829, –, HM579241, coII; ***X. cumberlandia***, USA: BRY 55391, HM578841, –, HM579253, coII; ***X. cumberlandia***, USA: BRY 55393, HM578843, –, HM579255, coII; ***X. cumberlandia***, USA: BRY 55398, HM578848, –, HM579260, coII; ***X. cumberlandia***, USA: BRY 55399, HM578849, –, HM579261, coIV; ***X. cumberlandia***, USA: BRY 55400, HM578850, –, HM579262, coIV; ***X. cumberlandia***, USA: BRY 55560, HM579013, –, HM579419, coII; ***X. cumberlandia***, Mexico: Querétaro: Barcenas-Peña 7511 (F), MW553766*, MW567197*, MW567172*; ***X. dierythra*** (Hale) Hale, Mexico: BRY 55234, HM578689, –, HM579099; ***X. dierythra***, USA: BRY 55300, HM578753, –, HM579165; ***X. dierythra***, USA: BRY 55329, HM578781, –, HM579193; ***X. dierythra***, USA: BRY 55383, HM578833, –, HM579245; ***X. dierythra***, USA: Leavitt 12-001 (F), KY859524, KY859539, KY859559; ***X. filarszkyana*** (Gyeln.) Hale, Australia: Elix 46155 (F), MG695548, MG695801, MG695649; ***X. hypofusca*** (Gyeln.) B.P.Hodk. & Lendemer, USA: West Virginia: 02086946 (NY), MG695550, MG695803, MG695651; ***X. hypomelaena*** (Hale) Hale, Mexico: Zacatecas: Barcenas-Peña 7380 (F), MW553771*, MW567199* MW567177*; ***X. hypomelaena***, Mexico: Estado de México: Barcenas-Peña 7060 (MEXU), MW553772*, MW567200*, –; ***X. idahoensis*** Hale, USA: BRY 55350, HM578801, –, HM579212; ***X. infrapallida*** (Essl.) O.Blanco, A.Crespo, Elix, D.Hawksw. &

Lumbsch, USA: Leavitt 9904 (BRY-C), MG695555, MG695809, MG695656; ***X. joranadia*** (T.H.Nash) Hale, Mexico: Jalisco: Barcenas-Peña 7451 (F), MW553768*, –, MW567174*; ***X. lavicola*** (Gyeln.) Hale, USA: BRY 55230, HM578685, –, HM579095; ***X. lavicola***, Mexico: Morelos; Nash III 46261 (WIS), MH580227, –, MH699920; ***X. lavicola***, Mexico: Puebla; Barcenas-Peña 5857 (F), MH580223, MH699896, MH699916; ***X. lavicola***, Mexico: Oaxaca; Barcenas-Peña 5905 (F), MH580225, MH699898, MH699918; ***X. lineola*** (E.C.Berry) Hale, USA: Arizona: 55306 (BRY-C), MG695556, MG695810, MG695657; ***X. lineola***, USA: BRY 55215, HM578670, –, HM579080; ***X. lineola***, USA: BRY 55272, HM578726, –, HM579137; ***X. lineola***, USA: BRY 55273, HM578727, –, HM579138; ***X. lineola***, USA: BRY 55306, HM578759, MG695810, HM579171; ***X. lineola***, USA: BRY 55311, HM578763, –, HM579175; ***X. lineola***, USA: BRY 55322, HM578774, –, HM579186; ***X. lineola***, USA: BRY 55323, HM578775, –, HM579187; ***X. lineola***, USA: BRY 55386, HM578836, –, HM579248; ***X. lineola***, USA: BRY 55410, HM578860, –, HM579272; ***X. lineola***, USA: BRY 55412, HM578862, –, HM579274; ***X. lineola***, USA: BRY 55561, HM579014, –, HM579420; ***X. lineola***, Mexico: Estado de México: Barcenas-Peña 7015 (F), MW553761*, **–**, MW567167*; ***X. lineola***, Mexico: Estado de México: Ruiz-Cazares 7570 (F), MW553776*, MW567204*, MW567181*; ***X. lineola***, Mexico: Estado de México: Barcenas-Peña 7008 (F), MW553760*, MW567192*, MW567166*; ***X. lineola***, Mexico: Querétaro: Barcenas-Peña 7190 (F), MW553774*, MW567202*, MW567179*; ***X. lineola***, Mexico: Querétaro: Barcenas-Peña 7248 (MEXU), MW553763*, MW567194*, MW567169*; ***X. lithophiloides*** (Kurok.) Elix, Australia: MAF 7471, AY581078, AY582314, AY578942; ***X. maricopensis*** T.H.Nash & Elix, USA: J. Leavitt 001 (BRY-C), MG695558, MG695812, MG695659; ***X. aff. mexicana***, USA: Nevada: Leavitt 292 (BRY-C), MG695579, MG695834, MG695679; ***X. mexicana*** (Gyeln.) Hale, Mexico: San Luis Potosí: Barcenas-Peña 7300 (F), MW553783*, MW567211*, MW567188*; ***X. mexicana***, Mexico: Querétaro: Barcenas-Peña 7518 (MEXU), MW553775*, MW567203*, MW567180*; ***X. mexicana***, Mexico: BRY 55233, HM578688, –, HM579098; ***X. mexicana***, USA: BRY 55258, HM578713, –, HM579123; ***X. mexicana***, USA: BRY 55259, HM578714, –, HM579124; ***X. mexicana***, USA: BRY 55260, HM578715, –, HM579125; ***X. mexicana***, USA: BRY 55261, HM578716, –, HM579126; ***X. mexicana***, USA: BRY 55262, HM578717, –, HM579127; ***X. mexicana***, USA: BRY 55263, HM578718, –, HM579128; ***X. mexicana***, USA: BRY 55265, HM578719, –, HM579130; ***X. mexicana***, USA: BRY 55267, HM578721, –, HM579132; ***X. mexicana***, USA: BRY 55285, HM578738, –, HM579150; ***X. mexicana***, USA: BRY 55299, HM578752, –, HM579164; ***X. mexicana***, USA: BRY 55321, HM578773, –, HM579185; ***X. mexicana***, USA: BRY 55328, HM578780, –, HM579192; ***X. mexicana***, USA: BRY 55401, HM578851, –, HM579263; ***X. mexicana***, USA: BRY 55402, HM578852, –, HM579264; ***X. mexicana***, USA: BRY 55426, HM578876, –, HM579287; ***X. mexicana***, USA: BRY 55428, HM578878, –, HM579289; ***X. mexicana***, USA: BRY 55442, HM578894, –, HM579303; ***X. mexicana***, USA: BRY 55450, HM578902, –, HM579310; ***X. mexicana***, USA: BRY 55462, HM578914, –, HM579322; ***X. mexicana***, USA: BRY 55503, HM578955, –, HM579362; ***X. mexicana***, USA: BRY 55505, HM578957, –, HM579364; ***X. mexicana***, USA: BRY 55519, HM578972, –, HM579379; ***X. mexicana***, USA: BRY 55523, HM578976, –, – ; ***X. mexicana***, USA: BRY 55538, HM578991, –, HM579397; ***X. mexicana***, Spain: MAF-Lich 17181, JQ912354, MG695835, JQ912451; ***X. mexicana***, USA: MAF-Lich 17199, JQ912386, MG695836, JQ912479; ***X. mexicana***, Mexico: San Luis Potosí; Barcenas-Peña 7316 (F), MH580231, MH699904, MH699923; ***X. mexicana***, Mexico: San Luis Potosí; Barcenas-Peña 7408 (F), MH580229, –, MH699922; ***X. mexicana***, Mexico: San Luis Potosí; Barcenas-Peña 7441 (F), MH686404, MH699902, –; ***X. mexicana***, Mexico: Querétaro; Barcenas-Peña 7178 (F), MH686401, MH699901, –; ***X. mexicana***, Mexico: Querétaro; Barcenas-Peña 7209 (MEXU), MH686402, MH699905, –; ***X. mexicana***, Mexico: San Luis Potosí; Barcenas-Peña 7273 (F), MH686403, MH699903, –; ***X. mexicana***, Mexico: Hidalgo; Barcenas-Peña 7470 (F), MH580232, MH699906, –; ***X. mexicana***, Mexico: Oaxaca; Barcenas-Peña 5918 (F), MH580228, MH699900, MH699921; ***X. mexicana***, Mexico: San Luis Potosí: Barcenas-Peña 7291 (F), MW553777*, MW567205*, MW567182*; ***X. mexicana***, Mexico: Querétaro: Barcenas-Peña 7245 (F), MW553785*, MW567213*, –; ***X. mexicana***, Mexico: San Luis Potosí: Barcenas-Peña 7306 (F), MW553782*, MW567210*, MW567187*; ***X. mexicana***, Mexico: Guanajuato: Barcenas-Peña 7499 (MEXU), MW553759*, MW567191*, MW567165*; ***X. moctezumensis*** T.H.Nash, Mexico: Puebla: Barcenas-Peña 5891(F), MH580233, MH699907, MH699924; ***X. neochlorochroa*** Hale, USA: BRY 55366, HM578817, –, HM579228, coIV; ***X. neotaractica*** Hale, Mexico: Estado de México: Barcenas-Peña 7013 (F), MW553784*, MW567212*, –; ***X. neotaractica*** Hale, Mexico: Estado de México: Barcenas-Peña 7007 (F), MW553764*, MW567195*, MW567170*; ***X. norchlorochroa*** Hale, USA: BRY 55367, HM578818, –, HM579229, coII; ***X. orientalis*** Kurok., South Korea: KoLRI005562, KM250136, –, KM250136; ***X. pedregalensis*** Barcenas Peña, Lumbsch & S.D. Leav., Mexico: Mexico City: Ruiz-Cazares 1556 (MEXU), MW553757*, MW567189*, MW567163*; ***X. pedregalensis***, Mexico: Mexico City; Ruiz-Cazares 1552 (F), MH580238, MH699912, MH699929; ***X. pedregalensis***, Mexico: Mexico City; Ruiz-Cazares 1553 (MEXU) Type, MH580234, MH699908, MH699925; ***X. pedregalensis***, Mexico: Mexico City; Ruiz-Cazares 1557 (F), MH580236, MH699910, MH699927; ***X. pedregalensis***, Mexico: Mexico City; Ruiz-Cazares 1555 (F), MH580235, MH699909, MH699926; ***X. pedregalensis***, Mexico: Mexico City; Ruiz-Cazares 1559 (MEXU), MH580237, MH699911, MH699928; ***X. planilobata*** (Gyeln.) Hale, Mexico: Estado de México: Ruiz-Cazares 7566 (F), MW553767*, –, MW567173*; ***X. plittii*** (Gyeln.) Hale, USA: North Carolina: 55422 (BRY-C), MG695562, –, MG695664; ***X. plittii***, USA: BRY 55266, HM578720, –, HM579131; ***X. plittii***, USA: BRY 55324, HM578776, –, HM579188; ***X. plittii***, USA: BRY 55397, HM578847, –, HM579259; ***X. plittii***, USA: BRY 55411, HM578861, –, HM579273; ***X. plittii***, USA: BRY 55522, HM578975, –, HM579382; ***X. plittii***, USA: BRY 55549, HM579002, –, HM579408; ***X. psoromifera*** (Hale) Hale, USA: BRY 55313, HM578765, –, HM579177; ***X. psoromifera***, USA: BRY 55314, HM578766, –, HM579178; ***X. stenophylla*** (Ach.) Ahti & D. Hawksw., USA: BRY 55554, HM579007, –, HM579413; ***X. sublaevis*** (Cout.) Hale, Spain: Tenerife, Canary Islands: MAF 7460, AY581106, AY582341, AY578974; ***X. sublaevis***, Spain: MAF-Lich 17180, JQ912356, MG695848, JQ912452; ***X. aff. subramigera***, Kenya: Kirika 2293 (F), MG695513, MG695762, MG695614; ***X. aff. subramigera***, Kenya: Kirika 3553illumina (F), MG695510, MG695758, MG695610; ***X. aff. subramigera***, Kenya: Kirika 3691A (F), MG695511, MG695760, MG695612; ***X. aff. subramigera***, Kenya: Kirika 3811 (F), MG695521, MG695770, MG695621; ***X. aff. subramigera***, Kenya: Kirika 3936, MG695512, MG695761, MG695613; ***X. aff. subramigera***, Kenya: Kirika 4117illumina (F), MG695514, MG695763, MG695615; ***X. aff. subramigera***, Kenya: Kirika 4117sanger (F), MG695515, MG695764, MG695616; ***X. subramigera*** (Gyeln.) Hale, Mexico: Mexico City: Ruiz-Cazares 1619 (F), MW553780*, MW567208*, MW567185*; ***X. subramigera***, Mexico: Mexico City: Ruiz-Cazares 1616 (F), MW553779*, MW567207*, MW567184*; ***X. subramigera***, Mexico: Mexico City: Ruiz-Cazares 1620 (F), MW553781*, MW567209*, MW567186*; ***X. substrigosa***, Australia: Elix 46151 (F), MG695586, MG695850, MG695686; ***X. subtasmanica*** Elix & T.H.Nash, Mexico: Estado de México: Barcenas-Peña 7009 (F), MW553762*, MW567193*, MW567168*; ***X. subtasmanica***, Mexico: Mexico City: Ruiz-Cazares 1602 (MEXU), MW553758*, MW567190*, MW567164*; ***X. tuckeriana*** Elix & T.H.Nash, Mexico: Jalisco: Barcenas-Peña 7452 (MEXU), MW553773*, MW567201*, MW567178*; ***X. tucsonensis*** (T.H.Nash) Egan, Mexico: Querétaro: Barcenas-Peña 7504 (F), MW553765*, MW567196*, MW567171*; ***X. wyomingica*** (Gyeln.) Hale, USA: BRY 55449, HM578901, –, HM579309, coIII; ***X. wyomingica***, USA: BRY 55498, HM578950, –, HM579357, coII; ***X. wyomingica***, USA: BRY 55501, HM578953, KY859540, HM579360; ***X. wyomingica***, USA: BRY 55502, HM578954, –, HM579361; ***X. wyomingica***, USA: BRY 55528, HM578981, –, HM579387, coIII; ***X. wyomingica***, USA: BRY 55529, HM578982, –, HM579388, coIII.