Phylogenetic study and taxonomic revision of the Xanthoparmelia mexicana group, including the description of a new species (Parmeliaceae, Ascomycota)

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Abstract

Xanthoparmelia (Parmeliaceae, Ascomycota) is the most species-rich genus of lichen-forming fungi. Species boundaries are based on morphological and chemical features, varying reproductive strategies and, more recently, molecular sequence data. The isidiate Xanthoparmelia mexicana group is common in arid regions of North and Central America and includes a range of morphological variation and variable secondary metabolites – salazinic or stictic acids mainly. In order to better understand the evolutionary history of this group and potential taxonomic implications, a molecular phylogeny representing 58 ingroup samples was reconstructed using four loci, including ITS, mtSSU, nuLSU rDNA and MCM7. Results indicate the existence of multiple, distinct lineages phenotypically agreeing with X. mexicana. One of these isidiate, salazinic acid-containing lineages is described here as a new species, X. pedregalensis sp. nov., including populations from xerophytic scrub vegetation in Pedregal de San Angel, Mexico City. X. mexicana s. str. is less isidiate than X. pedregalensis and has salazinic and consalazinic acid, occasionally with norstictic acid; whereas X. pedregalensis contains salazinic and norstictic acids and an unknown substance. Samples from the Old World, morphologically agreeing with X. mexicana, are only distantly related to X. mexicana s. str. Our results indicate that X. mexicana is likely less common than previously assumed and ongoing taxonomic revisions are required for isidiate Xanthoparmelia species.
Introduction

The family Parmeliaceae is the largest family of lichenised fungi (Jablonski et al. 2016) currently classified in approximately 70 genera with almost 2,800 species (Lumbsch and Huhndorf 2010, Divakar et al. 2017). Xanthoparmelia, with about 800 described species, is the largest genus of lichen-forming fungi (Lücking et al. 2016), with two centres of distribution in Australia and southern Africa; a smaller number of species occur in the Holarctic (Blanco et al. 2004, Eriksson et al. 2004, Crespo et al. 2010, Thell et al. 2012, Leavitt et al. 2018). To date, 74 species have been reported from Mexico, amongst these species, 27 are isidiate (Nash et al. 2016).

Isidiate Xanthoparmelia species are distributed in boreal, temperate and tropical regions. However, they commonly occur in semi-arid to arid regions worldwide especially on siliceous rocks, such as granite and sandstone. In North and Central America, Xanthoparmelia mexicana (Gyelnik) Hale ranks amongst the most common isidiate species. This taxon is widely distributed and has been reported from western USA, Mexico, Dominican Republic, Argentina, Kenya, Australia, New Zealand, Japan, China and Nepal (Hale 1990, Elix 1994, Nash and Elix 2004). X. mexicana is part of a complex of morphologically similar species, with adnate to slightly attached thalli, cylindrical isidia and a brown lower side of the thalli, which are primarily separated by their secondary metabolites. The species complex also includes X. ajoensis (T. H. Nash) Egan (diffractaic acid), X. dierythra (Hale) Hale (norstictic acid), X. joranadia (T. H. Nash) Hale (lecanoric acid), X. maricopensis T. H. Nash & Elix (norstictic and hyposalazinic acids), X. moctezumensis T. H. Nash (3-α-hydroxybarbatic acid), X. plittii (Gyelnik) Hale (stictic acid), X. schmidtii Hale (barbatic, norstictic and salazinic acids), X. subramigera (Gyelnik) Hale (fumarprotocetraric acid) and X. weberi (Hale) Hale (hypoprotocetraric acid) (Hale 1990, Nash et al. 2016). However, previous studies indicate that current interpretations of morphological features and secondary metabolites likely fail to accurately characterise species-level diversity in isidiate Xanthoparmelia species (Leavitt et al. 2011, 2013).

To better understand the evolutionary history of the Xanthoparmelia mexicana complex and potential taxonomic implications, isidiate Xanthoparmelia specimens were collected from different locations throughout arid regions of Mexico and supplemented with previously available sequence data. The new samples came from xerophytic scrublands in the states Puebla, Oaxaca, San Luis Potosí, Querétaro, Estado de México, Mexico City, Guanajuato, Zacatecas and Hidalgo, all in the central part of Mexico. We focused on sampling Xanthoparmelia populations that were phenotypically similar to X. mexicana, e.g. isidiate specimens containing salazinic acid. X. mexicana was originally described by Gyelnik (1931) as Parmelia mexicana and was later combined into Xanthoparmelia by Hale (1974). The type specimen was collected from San Jerónimo,
in Pedregal de San Angel, Mexico City. The syntype in the Bouly de Lesdain herbarium was destroyed during World War II, whereas the lectotype in the Budapest herbarium (BP) was not available for molecular study. Therefore, we attempted to recollect material at the type locality of *X. mexicana* and other regions throughout Mexico. Based on the results of this study, we formally describe a previously unrecognised species-level lineage comprised of isidiate specimens as new to science.

**Material and methods**

**Taxon sampling**

Specimens were studied from the herbaria ASU, BRY, F, MAF and new collections from different localities throughout arid regions from the central part of Mexico (Table 1, Fig. 1). A total of 83 specimens, representing 43 species were included, with an emphasis on isidiate species/populations from Central and North America (all epithets are validly published, with the exception of *X. isidiomontana* nom prov assigned to the clade ‘D2’ from Leavitt et al. 2013). New sequences were generated from 25 specimens and supplemented with 34 sequences from a previous analysis (Leavitt et al. 2018) and 24 additional sequences from GenBank (Table 1). Four species in the genus *Xanthoparmelia* that have previously been shown to be distantly related to *X. mexicana* were used as outgroup – *X. beatricea*, *X. austroafricana*, *X. subramigera* and *X. aff. subramigera* (Leavitt et al. 2018).

![Figure 1. Location of new Xanthoparmelia recollection sites from arid regions from central part of Mexico. Oaxaca (pink), Puebla (green), Mexico City (red), Estado de México (blue), Querétaro (purple), Guanajuato (brown), Hidalgo (grey), Aguas Calientes (yellow), San Luis Potosí (black), Zacatecas (orange).](image-url)
**Table 1.** Collection information for specimens included in the present study: Species, morphological/chemical species identification; DNA code, individual code associated with specimen label in multiple sequence alignments; Species distribution; Voucher information; and GenBank accession numbers for sampled loci in bold text indicates new sequences generated for this study. Specimens sequenced using Illumina technology are indicated by a • with the associated DNA code.

| Species               | DNA code | Voucher | ITS     | MCM7     | mtSSU     | nuLSU     |
|-----------------------|----------|---------|---------|----------|-----------|-----------|
| *X. aff. chlorochroa*  | 082f     | USA: Utah; Leavitt et al. 55225 (BRY-C) | MG695498 | MG695699 | MG695746 | MG695599 |
| *X. aff. chlorochroa*  | 9866     | USA: Nevada; Leavitt & St. Clair 9866 (BRY-C) | MG695499 | MG695700 | MG695747 | MG695600 |
| *X. aff. coloradoensis* | 135f     | USA: Utah; Leavitt et al. 55255 (BRY-C) | MG695500 | MG695701 | MG695748 | MG695601 |
| *X. aff. protonatrum* | GenBank  | Spain: Zamora; Blanco & Crespo 6216 (MAF-Lich) | –       | –        | –        | –        |
| *X. aff. subramigena* | 9664     | Kenya: Coast, Kirika & Lumbch 4117 (F) | MG695515 | –        | MG695764 | MG695616 |
| *X. ajoensis*         | 14908    | Mexico: Puebla; Barcenas-Peña 5989 (F) | MH580218 | MH686124 | MH699893 | MH699913 |
| *X. ajoensis*         | 14920    | Mexico: Puebla; Barcenas-Peña 5900 (F) | MH580219 | MH686125 | MH699894 | MH699914 |
| *X. ajoensis*         | 14934    | Mexico: Puebla; Barcenas-Peña 5914 (F) | MH580220 | MH580220 | MH699895 | MH699915 |
| *X. angustiphylla*    | GenBank  | USA: Blanco et al. 6768 (MAF) | –       | –        | –        | –        |
| *X. aucticoidea*      | GenBank  | USA: Blanco et al. 6744 (MAF) | –       | –        | –        | –        |
| *X. austroafricanana* | 9549     | Kenya: Coast Prov., Kirika 4485 (F) | MG695542 | –        | –        | MG695644 |
| *X. beatricea*        | E467     | Kenya: E467 (MAF-Lich 17174) | JQ912367 | –        | MG695793 | JQ912462 |
| *X. camtschadalis 1*  | GenBank  | USA: Leavitt et al. 55174 (BRY-C) | HM578630 | –        | –        | HM579042 |
| *X. camtschadalis 2*  | GenBank  | USA: Leavitt et al. 55291 (BRY-C) | HM578744 | –        | –        | HM579156 |
| *X. cf. mexicana*     | 016m     | Pakistan: Tattu; Kahlid, Usman & Khan MKF16 (LAH) | MH580221 | –        | –        | –        |
| *X. cf. mexicana*     | 016m2    | Pakistan: Swar Valley; Khan & Khalid SW-16 (LAH) | MH580222 | –        | –        | –        |
| *X. chlorochroa*      | 536f     | USA: North Dakota; G. Lind 1213 (BRY-C) | HM578887 | HM579688 | KR995372 | HM579298 |
| *X. conpersa*         | GenBank  | Spain: Zamora, Blanco & Crespo s.n. (MAF-Lich 6793) | –       | AF351186 | –        | –        |
| *X. cordillerana*     | E422     | Chile: E422 (MAF-Lich 17198) | JQ912358 | –        | MG695797 | JQ912453 |
| *X. coriana 1*        | GenBank  | South Korea: Hur, J.-S. 005561 | KJ170890 | –        | –        | KJ170890 |
| *X. coriana 2*        | GenBank  | South Korea: Hur, J.-S. 005589 | KJ170883 | –        | –        | KJ170883 |
| *X. coriana 3*        | GenBank  | South Korea: Hur, J.-S. 013905 | KJ170873 | –        | –        | KJ170873 |
| *X. cumberlandia*     | nybg02   | USA: Maine; R. Harris 55563 (NY) | MG695545 | –        | MG695798 | MG695646 |
| *X. dierythra*        | 226f     | USA: Leavitt et al. 55300 (BRY-C) | HM578753 | HM579569 | –        | HM579165 |
| *X. dierythra*        | 439f     | USA: Leavitt et al. 55383 (BRY-C) | HM578833 | –        | –        | HM579245 |
| *X. dierythra*        | 098f     | Mexico: Puebla; Leavitt et al. 55234 (BRY-C) | HM578689 | HM579504 | –        | HM579099 |
| *X. hirodakieniis*    | GenBank  | South Korea: Hur, J.-S. 010532 | KJ170876 | –        | –        | KJ170876 |
| Species           | DNA code | Voucher                          | ITS         | MCM7       | mtSSU       | nuLSU       |
|-------------------|----------|----------------------------------|-------------|------------|-------------|-------------|
| X. hypofusca      | 8837     | USA: West Virginia; Streets (02086946 NY) | MG695550    | MG695717   | MG695803    | MG695651    |
| X. idahoensis 1   | GenBank  | USA: Leavitt et al. 55463 (BRY-C) | HM578915    | HM579708   | –           | HM579323    |
| X. idahoensis 2   | GenBank  | USA: Leavitt et al. 55354 (BRY-C) | HM578805    | HM579620   | –           | HM579216    |
| X. infrapallida   | 9904     | USA: Arizona; Leavitt 9904 (BRY-C) | MG695555    | MG695720   | MG695809    | MG695656    |
| X. isidiovagans   | GenBank  | Spain: 9956 (MAF-Lich)          | AY581094    | JX974718   | AY582330    | AY578960    |
| X. lavicola       | GenBank  | USA: Leavitt et al. 55230 (BRY-C) | HM578685    | HM579500   | –           | –           |
| X. lavicola       | 15489    | Mexico: Morelos; Nash III 46261 (WIS) | MH580227    | MH686131   | –           | MH699920    |
| X. lavicola       | 14894    | Mexico: Puebla; Barcenas-Peña 5857 (F) | MH580223    | MH686127   | MH699896    | MH699916    |
| X. lavicola       | 14905    | Mexico: Puebla; Barcenas-Peña 5884 (F) | MH580224    | MH686128   | MH699897    | MH699917    |
| X. lavicola       | 14906    | Mexico: Oaxaca; Barcenas-Peña 5905 (F) | MH580225    | MH686129   | MH699898    | MH699918    |
| X. lavicola       | 14910    | Mexico: Puebla; Barcenas-Peña 5888 (F) | MH580226    | MH686130   | MH699899    | MH699919    |
| X. lineola        | 245f     | USA: Arizona; EA collection 31–259 (55306 BRY-C) | MG695556    | MG695721   | MG695810    | MG695657    |
| X. maricopensis   | 6698     | USA: Arizona; J. Leavitt 001 (BRY-C) | MG695558    | MG695723   | MG695812    | MG695659    |
| X. mexicana       | 291f     | USA: Leavitt et al. 55328 (BRY-C) | HM578780    | HM579596   | –           | HM579192    |
| X. mexicana       | 786f     | USA: Leavitt et al. 55462 (BRY-C) | HM578914    | HM579707   | –           | HM579322    |
| X. mexicana       | 097f     | Mexico: Leavitt et al. 55233 (BRY-C) | HM578688    | HM579503   | –           | HM579098    |
| X. mexicana       | GenBank  | South Korea: Jang et al. 005486 (KoLRI) | KM250123    | –          | –           | –           |
| X. mexicana       | 15479    | Mexico: San Luis Potosí; Barcenas-Peña 7316 (F) | MH580231    | MH686135   | MH699904    | MH699923    |
| X. mexicana       | 15472    | Mexico: San Luis Potosí; Barcenas-Peña 7408 (F) | MH580229    | MH699932   | –           | MH699922    |
| X. mexicana       | 15466    | Mexico: San Luis Potosí; Barcenas-Peña 7441 (F) | MH686404    | MH686133   | MH699902    | –           |
| X. mexicana       | 15461    | Mexico: Querétaro; Barcenas-Peña 7178 (F) | MH686401    | MH699930   | MH699901    | –           |
| X. mexicana       | 15485    | Mexico: Querétaro; Barcenas-Peña 7209 (MEXU) | MH686402    | MH686136   | MH699905    | –           |
| X. mexicana       | 15471    | Mexico: San Luis Potosí; Barcenas-Peña 7273 (F) | MH686403    | MH699931   | MH699903    | –           |
| X. mexicana       | 15473    | Mexico: Hidalgo; Nash III 45126 (WIS) | MH580230    | MH686134   | –           | –           |
| X. mexicana       | 156f     | USA: Leavitt et al. 55267 (BRY-C) | HM578721    | HM579536   | –           | HM579132    |
| X. mexicana       | 15487    | Mexico: Hidalgo; Barcenas-Peña 7470 (F) | MH580232    | MH686137   | MH699906    | –           |
| X. mexicana       | 14899    | Mexico: Oaxaca; Barcenas-Peña 5918 (F) | MH580228    | MH686132   | MH699900    | MH699921    |
| X. moctezumensis  | 14897    | Mexico: Puebla; Barcenas-Peña 5891(F) | MH580233    | MH686138   | MH699907    | MH699924    |
| Species               | DNA code     | Voucher                                      | ITS     | MCM7    | mtSSU | nuLSU  |
|----------------------|--------------|----------------------------------------------|---------|---------|-------|--------|
| X. norchlorochoroa   | GenBank      | USA: Leavitt et al. 55157 (BRY-C)            | HM578613| HM579432| –     | HM579025|
| X. norchlorochoroa   | GenBank      | USA: Leavitt et al. 55447 (BRY-C)            | HM578899| HM579693| –     | HM579307|
| X. orientalis        | GenBank      | South Korea: Hur, J.-S. 005613 (BRY-C)       | KJ170884| –       | –     | KJ170884|
| X. pedregalensis     | 527          | Mexico: Mexico City; Ruiz-Cazares 1552 (F)   | MHS80238| MHS70735| MHS69912| MHS69929|
| X. pedregalensis     | 526          | Mexico: Mexico City; Ruiz-Cazares 1553 (F)   | MHS80234| MHS70735| MHS69908| MHS69925|
| X. pedregalensis     | 533          | Mexico: Mexico City; Ruiz-Cazares 1557 (F)   | MHS80236| –       | MHS69910| MHS69927|
| X. pedregalensis     | 529          | Mexico: Mexico City; Ruiz-Cazares 1555 (F)   | MHS80235| MHS68613| MHS69909| MHS69926|
| X. pedregalensis     | 531          | Mexico: Mexico City; Ruiz-Cazares 1559 (MEXU)| MHS80237| MHS70735| MHS69911| MHS69928|
| X. plittii           | 498f         | USA: North Carolina; Leavitt et al. (55422 BRY-C)| MG695562| MG695727| –     | MG695664|
| X. psoromifera 1     | GenBank      | USA: Leavitt et al. 55314 (BRY-C)            | HM578766| HM579582| –     | HM579178|
| X. psoromifera 2     | GenBank      | USA: Leavitt et al. 55313 (BRY-C)            | HM578765| HM579581| –     | HM579177|
| X. pulvinaris         | GenBank      | Hungary: Molnar et al. 93943 (BP)            | JQ362484| –       | JQ362485| JQ362486|
| X. isidiomontana nom. prov. | 292f | USA: Nevada; Leavitt (55329 BRY-C) | MG695579| MG695733| MG695834| MG695679|
| X. isidiomontana nom. prov. | E1010 | Spain: E1010 (MAF-Lich 17181) | JQ912354| –     | MG695835| JQ912451|
| X. isidiomontana nom. prov. | E984 | USA: E984 (MAF-Lich 17199) | JQ912386| –     | MG695836| JQ912479|
| X. stenophylla       | 5040         | Kazakhstan: Karkaralinsk; Tshernyshev (BRY-C)| MG695583| MG695737| MG695843| MG695683|
| X. stenophylla       | E708         | Turkey: E708 (MAF-Lich 17196)               | JQ912372| –       | MG695844| JQ912467|
| X. subcumberlandia   | 121f         | USA: Utah; Leavitt et al. (55242 BRY-C)      | MG695584| MG695738| MG695845| MG695684|
| X. subdifluens 1     | GenBank      | Spain: de Paz et al. 17178 (MAF-Lich)       | JQ912381| –       | –     | JQ912474|
| X. subdifluens 2     | GenBank      | Spain: Blanco et al. 9910 (MAF-Lich)        | AY581105| –     | AY582340| AY578973|
| X. sublaevis         | GenBank      | Spain: Tenerife, Canary Islands; Blanco et al. 7460 (MAF) | AY581106| –     | AY582341| AY578974|
| X. subramigera       | 9668         | Kenya: Coast, Kirika 4583 (F)               | MG695525| MG695709| MG695774| MG695626|
| X. tuberculiformis   | GenBank      | South Korea: Jang et al. 012058 (KoLRI)     | KM250131| –     | –     | KM250131|
| X. vicentei          | GenBank      | Spain: Salamanca; Crespo & Molina (7248 MAF-Lich) | AY581112| –     | AY582347| AY578980|
| X. viriduloumbrina1  | GenBank      | USA: Pennsylvania; Lendemer 13314: 1049917 (NY)| HM066945| –     | –     | –     |
| X. viriduloumbrina2  | GenBank      | USA: Pennsylvania; Lendemer 13325: 1049906 (NY)| HM066944| –     | –     | –     |
| X. wyomingica        | 001f         | USA: Utah; Leavitt et al. (55151 BRY-C)     | MG695598| MG695745| MG695864| MG695698|
| X. wyomingica        | 826f         | USA: Wyoming; Leavitt 826 (55501 BRY-C)     | HM578953| HM579746| –     | HM579360|
Morphology and chemistry

Morphological characters were observed using a Zeiss Stemi 2000-C stereoscope. Ascomatal anatomy, ascospore in addition to conidia shape and size were observed using a Zeiss Axioscope. Secondary metabolites were identified using spot test KOH 10%, KC, C, PD and high-performance thin layer chromatography (HPTLC), using solvent systems C following established methods (Culberson and Johnson 1982, Arup et al. 1993, Lumbsch 2002, Orange et al. 2010).

Molecular methods

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 four markers using polymerase chain reaction (PCR): the nuclear ribosomal internal transcribed spacer region (ITS), a fragment of nuclear large subunit rDNA (nuLSU), the nuclear protein-coding marker minichromosome maintenance complex component 7 (MCM7) and a fragment of the mitochondrial small subunit rDNA (mtSSU). PCR reactions contained 6.25 ml of MyTaq Mix, 25 ml H₂O, 0.25 ml forward and reverse primer and 0.5 ml template DNA, for a total reaction volume of 12.5 ml. The ITS region was amplified using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990); MCM7 using primers MCM7-709f and Mcm7-1348r (Schmitt et al. 2009), mtSSU using primers mrSSU1 and mrSSU3R (Zoller et al. 1999) and nuLSU rDNA using primers AL2R (Mangold et al. 2008) and LR6 (Vilgalys and Hester 1990). PCR products were sequenced using an ABI PRISM 3730 DNA Analyser (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at The Field Museum, Chicago, Illinois, USA. Nine specimens were obtained previously for a global phylogenetic study of the genus and sequenced using next generation sequencing technology as described in Leavitt et al. (2018) (Table 1). In short, metagenomic Nextera libraries (prepared from total DNA extraction) were sequenced on the Nextseq platform at the Core Genomics Facility at the University of Illinois at Chicago, USA. Illumina reads of each specimen were mapped to reference marker sequences downloaded from Genbank (ITS AY581063, nuLSU HM125760, MCM7 HM579689, mtSSU KR995373) using the mapping feature implemented in Geneious v11.0.3 (http://www.geneious.com, Kearse et al. 2012). The consensus sequence of each locus was extracted and added to the data set of Sanger sequences to build a combined alignment.

Sequence alignment and phylogenetic analysis

Sanger sequences, consensus Illumina reads and sequences available on GenBank were added to an alignment published in Leavitt et al. (2018) using Mafft v7 with the option
ITS, MCM7, mtSSU and nuLSU sequences were aligned independently using the ‘automatic’ option in Mafft v7, with the remaining parameters set to default values. Ambiguous positions of each one-locus alignment were removed using options for a “less stringent” selection on Gblocks 0.91b (Castresana 2000). SequenceMatrix software (Vaidya et al. 2011) was used for the alignment concatenation. Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Analysis (BA). ML trees were calculated with RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis 2014) on the Cipres Science Gateway (Miller et al. 2010) using GTR+G+I substitution model with 1000 bootstrap pseudoreplicates. For the BA, substitution models for each locus were estimated using jModelTest-2.1.9 (Guindon and Gascuel 2003, Darriba et al. 2012): in ITS the TIM2ef+I+G, in MCM7 the K80+G, in mtSSU the TPM2uf+I and in nuLSU the TrN+I were used. Two parallel Markov chain Monte Carlo (MCMC) runs were performed in MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001, Ronquist and 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 of both runs after discarding the first 25% as burn-in. Tree files were visualised with FigTree 1.4.2 (Rambaut 2014). The ITS, MCM7, mtSSU and nuLSU sequences are deposited in GenBank (Table 1).

Results and Discussion

Phylogeny

Results from phylogenetic analyses presented here clearly indicate that the taxonomy in the Xanthoparmelia mexicana group requires revision because different samples assigned to the same species based on phenotypical characters may not form a monophyletic group. Specimens identified as X. mexicana from Asia (Pakistan and South Korea) were distantly related to samples of the species collected in North America and Europe (included in X. isidiomontana nom prov) (Fig. 2). The latter specimens fell into three distinct and well supported clades (clade I-III in Fig. 2). Note that the three distinct and well supported clades did not form a monophyletic group. Clade ‘I’ (=X. isidiomontana’ nom prov, ‘D2’ in Leavitt et al. 2013) included isidiate specimens from North America and Europe and samples identified as X. dierythra, X. mexicana (Figs. 2A and B) and X. plittii, in addition to a number of non-isidiate, fertile specimens. Additional studies will be necessary to better understand the delimitation of X. dierythra, which is also polyphyletic and is currently accommodating specimens with norstictic acid and lacking salazinic acid (Hale 1990). This clade likely represents another species-level lineage lacking formal taxonomic recognition and a formal description of this lineage will be proposed once the phylogenetic placement of X. dierythra s. str. is ascertained.

Clade ‘II’ included specimens collected in the Pedregal, south of Mexico City, which is also the type locality of X. mexicana. However, the new material does not correspond phenotypically with the type specimen of X. mexicana in BP (Fig. 2G). These specimens
Figure 2. Phylogenetic relationships of the *Xanthoparmelia mexicana* group based on a concatenated data set of ITS, mtSSU, nuLSU and MCM7. Topology based on maximum likelihood (ML) analyses. Bootstrap values above 75 and 0.95 posterior probability are indicated on each branch. The clades I, II and III are highlighted in blue, yellow and pink, respectively. Selected specimens representing clades (habit and isidia): I, *X. mexicana* s. lat. (*A*, *B*); II, *X. pedregalensis* (*C*, *D*) and III, *X. mexicana* s. str. (*E*, *F*), a picture of the *X. mexicana* type specimen from BP is included (*G*).

are different from *X. mexicana* specimens (represented by Clade III in phylogenetic analysis) in having less contiguous lobes, densely isidiate thallus, presence of salazinic acid, norstictic acid and an unknown substance. Since clade ‘II’ differs phylogenetically and phenotypically from clade ‘III’ (representing *X. mexicana* s. str. — see below), we describe clade ‘II’ as a species new to science, *X. pedregalensis* (Figs. 2C and D).
Clade ‘III’ includes the majority of samples identified as *X. mexicana* collected in different localities of Mexico (Oaxaca, Puebla, San Luis Potosí, Querétaro, Hidalgo). Specimens recovered in this clade were morphologically and chemically similar to the lectotype of *X. mexicana* in BP (Fig. 2G). Therefore, clade ‘III’ is here recognised as *X. mexicana* s. str. (Gyelnik 1931, Hale 1974) (Figs. 2E and F). So far, we have only been able to confirm the presence of *X. mexicana* s.str. in Mexico. Specimens collected in other areas and previously identified as *X. mexicana* likely represent different species. For example, none of the samples from Asia or those reported in Leavitt et al. (2013) from western United States belongs to *X. mexicana* s. str. Further studies are needed to evaluate the occurrence of this species in other parts of the world, including North America and Europe.

Underestimates of species diversity is common amongst under-studied organismal groups (Pawar 2003, Chiarucci et al. 2011, Lücking 2012, Coleman 2015, Troia and McManamay 2016, Troudet et al. 2017), which is particularly evident in lichenised fungi (Crespo and Perez-Ortega 2009, Crespo and Lumbsch 2010, Leavitt et al. 2011, Lumbsch and Leavitt 2011, Leavitt et al. 2013, Leavitt et al. 2016, Lücking et al. 2016, Leavitt et al. 2018). Previous studies concluded that the species delimitation in lichenised ascomycetes with traditional morphological and chemical characters are apparently misleading with respect to species diversity. In the study of Leavitt et al. (2016), several new taxa were described primarily based on evidence from genetic data, but it does not preclude the possibility that additional studies investigating morphological and chemical characters may identify additional independent characters or combinations of characters, supporting the species circumscribed using molecular data. Our results corroborate findings from the previous studies by showing the need of an integrative approach using not only conventional (i.e. morphology and TLC data), but also new sets of data (e.g. DNA sequence data) to better understand the pattern of species diversity. Our study shows that, by incorporating molecular data, the taxonomic status of a conventionally difficult group based on morphology can be resolved: the three main clades belonging to the *X. mexicana* complex do not form a monophyletic group based on our newly reconstructed phylogeny (Fig. 1). In this context, the species diversity in the *X. mexicana* complex is likely under-estimated and morphologically cryptic species may be identified in the future.

**Taxonomy**

*Xanthoparmelia pedregalensis* Barcenas-Peña, Lumbsch & Leavitt, sp. nov.

Mycobank: MB826958

Figs. 2C and D

**Type.** MEXICO. Ciudad de México: Coyoacán, Pedregal de San Angel, 19°19’8.3”N, 99°11’25.93”W, 2321 m elev., xerophytic scrub, on rocks, November, 2017, Ruiz Cazares 1553 (MEXU-holotype), same locality and date Ruiz Cazares 1559 (MEXU-paratype).
**Diagnosis.** Thallus moderately adnate to adnate, imbricate, upper surface yellow-green, lower surface tan-brown, abundant isidia subglobose to cylindrical, simple to branched and medulla containing salazinic and norstictic acids as major compounds and an unknown substance. Differing from the phenotypically similar *X. mexicana* by nucleotide position characters in the ITS sequence as shown in Table 2.

**Etymology.** The taxon name is based on its occurrence in the Pedregal de San Angel region of Mexico.

**Description.** Thallus foliose, moderately adnate to adnate, 2–7 cm in diam., irregularly lobate; lobes subirregular, elongate, plane to subconvex, 1.5–3 mm wide, not lobulate; apices subrotund, smooth, eciliate. Upper surface yellow-green, smooth, shiny, epruinose and emaculate, densely isidiate; isidia initially subglobose, becoming cylindrical to coralloid branched with age, 0.1–0.2 mm in diam., 0.1–0.9 mm tall; tips syncorticate, brown to dark brown, sometimes weakly erumpent; soralia and pustulae absent. Medulla white, with continuous algal layer. Lower surface tan to brown, plane, moderately rhizinate; rhizines pale to dark brown, simple, 0.5–0.9 mm long. Apothecia rare, sessile, 1–2 mm wide, laminal on thallus; disc cinnamon-brown to dark brown; margin smooth, pruina absent; asci: clavate, 8-spored; ascospores hyaline, simple, ellipsoid, 9–10 × 4–5 µm. Pycnidia rare, immersed conidia bifusiform, 5–7 × 1 µm.

**Secondary metabolites.** Upper cortex K–, C–, KC–, P–; medulla K+ yellow then dark red, KC–, C–, P+ yellow-orange. Upper cortex with usnic acid (major); medulla with salazinic (major) and norstictic acids (submajor) and an unknown substance (minor) (Rf 28–30, brown in daylight after heating, UV brown-dark, yellow halo after heating).

**Distribution and ecology.** The new species was found in xerophytic scrub vegetation, in Pedregal de San Angel south of Mexico City, growing on volcanic rocks. It is currently known only from the type locality.

**Notes.** *Xanthoparmelia pedregalensis* is morphological and chemically similar to *X. mexicana*. However, the latter has more contiguous lobes and is less isidiate than *X. pedregalensis*. In addition *X. mexicana* has salazinic (major) and consalazinic acid (minor) and usually norstictic and protocetraric acids (trace) in the medulla, whereas *X. pedregalensis* contains salazinic (major) and norstictic acids (submajor) and an unknown substance. Distinguishing the two species is supported by molecular data.

**Additional specimens examined.** Mexico. Ciudad de México: Coyoacán, Pedregal de San Angel, 19°19'8.3”N, 99°11'25.93”W, 2321 m elev., xerophytic scrub, on rocks, November, 2017, Ruiz Cazares 1552 (MEXU); 19°19'15.19”N, 99°11'15.22”W, 2311 m, Ruiz Cazares 1555, 1557 (F).

| Table 2. Differences of nucleotide positions in the ITS marker between *X. mexicana* and *X. pedregalensis.* |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species         | 36 | 115 | 379 | 425 | 450 | 466 | 488 | 496 |
| *X. mexicana*    | G  | C  | A  | C  | T  | C/T | G  | A  |
| *X. pedregalensis* | A  | T  | G  | G  | C  | A  | C  | G  |
New state records

*Xanthoparmelia ajoensis* (Nash) Egan, 1975: 217.

*Xanthoparmelia ajoensis* Nash, 1974: 234. [Type collection: Organ Pipe Cactus National Monument, Pima Co., Arizona, USA, Nash 5999 (ASU, holotype; DUKE, US, isotypes).] New to Oaxaca, *X. ajoensis* is distributed across western USA and Mexico where it has previously been reported from Baja California Sur, Durango, Morelos, Puebla, Sinaloa and Sonora on acidic rocks, often in open, arid habitats at relatively low elevations (Hale 1990, Nash and Elix 2004, Nash et al. 2016).

**Specimens Examined:** Mexico. Oaxaca: Quiotepec, 17°54'18.9"N, 96°58'01.8"W, 696 m elev., xerophytic scrub, on rock, October, 2016, Barcenas-Peña 5906, 5908, 5913, 5915 (MEXU).

*Xanthoparmelia moctezumensis* Nash in C. Culberson, Nash & Johnson, 1979: 155. [Type collection: 28 km E of Moctezuma, Sonora, Mexico, Nash 12548 (ASU, holotype; DUKE, US, isotypes).]

New to Puebla. *Xanthoparmelia moctezumensis* is distributed throughout south-western USA and Mexico where it has been reported from Baja California Sur, Durango, Sinaloa and Sonora on acidic rocks, often in open, arid to woodland habitats (Nash and Elix 2004, Nash et al. 2016).

**Specimens Examined:** Mexico. Puebla: San Rafael Coxcatlán, 18°13'16.6"N, 97°07'22.4"W, 1148 m elev., xerophytic scrub, on rock, October, 2016, Barcenas-Peña 5887, 5890, 5891, 5893 (MEXU).

*Xanthoparmelia mexicana* (Gyelnik) Hale, 1974: 488.

New to Querétaro, San Luis Potosí and Zacatecas. *Xanthoparmelia mexicana* has been reported from Baja California, Baja California Sur, Chihuahua, Coahuila, Distrito Federal, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Nuevo León, Oaxaca, Puebla, Sonora and Veracruz, on acidic rocks, often on soil near the coast in open, arid habitats (Nash et al. 2004, 2016).

**Specimens Examined:** Mexico: Querétaro. Téquisquiapan, Rancho Las Fuentes, 20°33'51.0"N, 100°01'54.6"W, 1942 m elev., xerophytic scrub, on rock, August, 2017, Barcenas-Peña 7516; San Luis Potosí, Mexquitic de Carmona, La Campana, 22°15'28.9"N, 101°05'26.8"W, 2012 m elev., xerophytic scrub, on rock, August, 2017, Barcenas-Peña 7441; Zacatecas, Fresnillo, El Poleo, 23°06'16.4"N, 102°54'24.3"W, 2227 m elev., xerophytic scrub, on rock, August, 2017, Barcenas-Peña 7356 (all MEXU).
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