


Standard Paper

Remarkable cases of parallel evolution of the placodioid thallus growth form in the *Lecanographaceae* (*Arthoniales*) with the description of a new species of *Alyxoria* from Mexico

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Abstract

The new species *Alyxoria sierramadrensis* is described from Mexico where it inhabits limestone rocks. The lichen developing from this fungus is characterized by a placodioid to subfoliose thallus with a white pruinose surface; rounded to shortly elongated ascomata with a black epruinose margin and a widely exposed, white pruinose hymenial disc; hyaline, 3-septate ascospores, 17–25 × 7–9 µm; the presence of anthraquinones rendering the medulla orange. Phylogenetic analyses of nuLSU, mtSSU and *RPB2* sequences place this species in the genus *Alyxoria* (*Lecanographaceae*). This generic affiliation is surprising because all known *Alyxoria* species have a crustose thallus. *Lecanographaceae* mainly includes species without a thallus (lichenicolous taxa) or with a thin crustose thallus, the only exception being *Simonyella variegata* with a fruticose thallus. The new species belongs to the *Alyxoria ochrocheila* subgroup, which includes lichens also frequently known to have anthraquinones, white pruinose hymenial discs and 3-septate ascospores. Phylogenetic analyses further determined the systematic position of the monotypic genus *Phoebus*. This genus, considered as an *Arthoniales* of uncertain family affiliation, is shown to belong to the *Lecanographaceae*. With its placodioid thallus, it is another example of a lichenized fungus with a deviating morphology in thallus structure for the family, increasing the number of remarkable cases of parallel evolution of lichen growth forms within the *Arthoniales*. *Phoebus hydrophobius* is newly recorded for Mexico.

Key words: anthraquinone, biodiversity, lichen, North America, phylogeny, taxonomy

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Introduction

Arthoniales is the third largest order of predominantly lichenized fungi, with c. 1900 species in seven families (e.g. Tehler 1990; Ertz & Tehler 2011; Frisch *et al.* 2014; Lücking *et al.* 2017; Diederich *et al.* 2018). The order also includes non-lichenized, lichenicolous or saprotrophic lineages apparently derived by loss of lichenization (Thiyagaraja *et al.* 2020). During fieldwork in north-eastern Mexico, two species of *Arthoniales*, remarkable within the order by their unusual growth forms, were collected on limestone outcrops. The study area is located in the states of Coahuila and Nuevo León in the north-eastern portion of the country, particularly centred on the metropolitan area of Monterrey, which is located between the Gulf Coastal Plain and the Sierra Madre Oriental (SMO) (INEGI 1986). This part of Mexico has the third least documented lichen fungi diversity in the country, with only 295 species recorded (Herrera-Campos *et al.* 2014),

but recent collections suggest its richness is highly underestimated (A. Huereca, unpublished data). While working on the city's lichen flora inventory, a very striking specimen was documented by a local botanist through the platform of iNaturalist (iNaturalist 2020). This discovery led to the exploration of a saxicolous community inhabiting calcareous rocks with xerophytic vegetation in partially humid conditions (Fig. 1). The limestone outcrops in the region have historically been scarcely sampled and harbour poorly known lichen flora. The two peculiar *Arthoniales* were collected in this habitat. The first has a conspicuous placodioid orange thallus and could be identified as *Phoebus hydrophobius* R. C. Harris & Ladd, a species that was considered endemic to the Ozark Ecoregion in the USA (Harris & Ladd 2007). The second represents a new species that is also striking by having a placodioid to subfoliose thallus. The peculiar growth forms render their systematic placement uncertain within the order *Arthoniales*. The genus *Phoebus* was described as belonging to the family *Roccellaceae* (Harris & Ladd 2007), but was recently placed as '*Arthoniales* genera incertae sedis' due to the lack of molecular data (Lücking *et al.* 2017).

The present study aims to describe the new species in the genus *Alyxoria* and to determine the family affiliation of the genus *Phoebus* with the support of molecular data.

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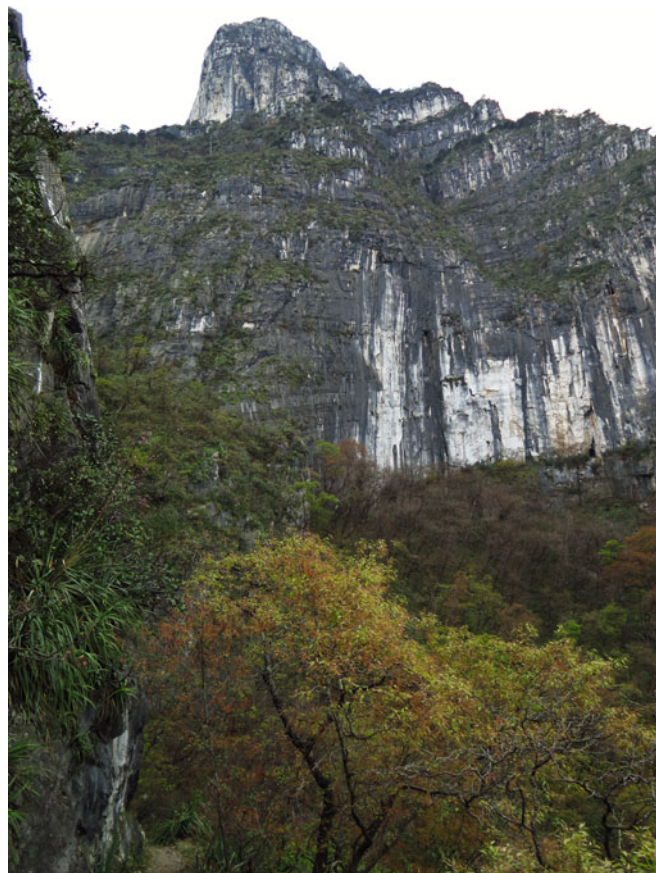


Fig. 1. Massive limestone outcrop at Cerro de las Mitras in Monterrey (Mexico), the type locality of *Alyxoria sierramadrensis*. In colour online.

Materials and Methods

Voucher specimens are deposited in the herbaria MEXU, BR, S, NY and in the private herbarium of Alejandro Huereca. The external morphology was studied and measured using an Olympus SZX12 stereomicroscope. Macroscopic photographs were taken using a Keyence VHX-5000 digital microscope and a VH-Z20R/W/T lens. Hand-cut sections and squash preparations of ascomata and thalli were mounted in water, a 5% aqueous potassium hydroxide solution (K), or in Lugol's iodine solution (1% I₂) without (I) or with K pretreatment (KI) and studied using an Olympus BX51 compound microscope. Sections of thallus were also pretreated in acetone, then mounted in lactophenol cotton blue to determine the type of cortex following Aptroot & Schumm (2011). Measurements refer to dimensions in water. Microscopic images were captured using an Olympus BX51 compound microscope, fitted with an Olympus SC50 digital camera. Colour reactions of the thalli were studied using K, common household bleach (C), K followed by common household bleach (KC), para-phenylenediamine dissolved in ethanol (PD) and long-wave UV (366 nm). Lichen secondary metabolites were identified using thin-layer chromatography (TLC) in solvent B' (Orange *et al.* 2010).

Molecular techniques

Well-preserved and freshly collected specimens lacking any visible symptoms of fungal infection were used for DNA

isolation. Hand-cut sections of ascomata and thalli of *Phoebus hydrophobius* and the new species of *Alyxoria* were used for direct PCR as described in Ertz *et al.* (2015). The lichen material was washed with acetone and then rinsed with water to remove remnants of pigments. The material was placed directly in microtubes with 20 µl H₂O. Amplification reactions were prepared for a 50 µl final volume containing 5 µl 10× DreamTaq buffer (Thermo Scientific, www.thermoscientific.com/onebio), 1.25 µl of each of the 20 µM primers, 5 µl of 2.5 mg ml⁻¹ bovine serum albumin (Thermo Scientific), 4 µl of 2.5 mM each dNTPs (Thermo Scientific), 1.25 U DreamTaq DNA polymerase (Thermo Scientific) and the lichen material. DNA extractions of *Lecanographa* species, viz *L. dimelaenoides* (Egea & Torrente) Egea & Torrente, *L. hypothallina* (Zahlbr.) Egea & Torrente and *L. lyncea* (Sm.) Egea & Torrente s. lat., obtained during a previous study (Ertz & Tehler 2011) were also used for generating missing mtSSU sequences. A targeted fragment of c. 0.8 kb of the mtSSU rDNA was amplified using the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). A fragment of c. 1 kb of the *RPB2* protein-coding gene was amplified using the primers fRPB2-7cF and fRPB2-11aR (Liu *et al.* 1999). The yield of the PCR reactions was verified by running the products on 1% agarose gel using ethidium bromide. Both strands were sequenced by MacroGen® using amplification primers. Sequence fragments were assembled with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'Megablast' searches in GenBank for a preliminary taxonomic assignment.

Taxon selection and phylogenetic analyses

For the phylogenetic analyses, a set of 37 OTUs was used, consisting of taxa representing all major clades currently accepted in the *Lecanographaceae* (Ertz & Tehler 2011; Frisch *et al.* 2014) and three outgroup species, viz. *Dimidiographa longissima* (Müll. Arg.) Ertz & Tehler (*Roccellaceae*), *Lecanactis abietina* (Ach.) Körb. (*Roccellaceae*) and *Opegrapha vulgata* (Ach.) Ach. (*Opegraphaceae*) (Table 1).

The sequences were aligned using MAFFT v.7.402 (Katoh *et al.* 2002) on the CIPRES Web Portal (Miller *et al.* 2010) and manually corrected for errors using Mesquite 3.04 (Maddison & Maddison 2015). Ambiguously aligned regions according to Lutzoni *et al.* (2000) and introns were manually removed and excluded from subsequent analyses.

To examine topological incongruence among data sets, maximum likelihood (ML) analysis was carried out on each of the single-locus data sets. We used RAXML v.8.2.12 (Stamatakis 2014) with 1000 replicates of ML bootstrapping (ML-BS) under the GTRGAMMA model of sequence evolution. Analyses were run on the CIPRES Web Portal (Miller *et al.* 2010). All topological bipartitions were compared for the two loci. A conflict was assumed to be significant when differing topologies for the same set of taxa (one being monophyletic and the other being non-monophyletic) were each supported with bootstrap values ≥ 70 (Mason-Gamer & Kellogg 1996). Based on this criterion, a phylogenetic conflict was detected for *Lecanographa atropunctata* Sparrius *et al.* between the mitochondrial and the *RPB2* genes. Based on the mitochondrial gene mtSSU, *L. atropunctata* (GenBank Accession numbers: KY360244, HQ454548, HQ454688) is the sister species to the genus *Zwackhia* (ML-BS = 77; fig. not shown), while it is the basal taxon of the *Lecanographa* clade (ML-BS = 97; fig. not shown) in the

Table 1. *Lecanographaceae* specimens used in the phylogenetic analyses (Fig. 2) with GenBank Accession numbers and voucher information. Newly generated sequences are in bold; em-dash indicates missing data; * = outgroup.

Species	Voucher	mtSSU	nuLSU	<i>RPB2</i>
<i>Alyxoria bicolor</i>	Rwanda; Ertz 8731 (BR)	EU704062	EU704093	EU704026
<i>A. mougeotii</i>	Great Britain; (L10058)	KJ851007	KJ851078	—
<i>A. ochrocheila</i> 1	Luxembourg; Ertz 7519 (BR)	EU704072	EU704100	EU704036
<i>A. ochrocheila</i> 2	Belgium; Ertz 7500 (BR)	EU704073	EU704101	EU704037
<i>A. aff. ochrocheila</i>	Rwanda; Ertz 8624 (BR)	EU704071	EU704099	EU704035
<i>A. sierramadrensis</i> sp. nov.	Mexico; Huereca AH465-hymenium (MEXU)	—	MT873939	MT878445
<i>A. sierramadrensis</i> sp. nov.	Mexico; Huereca AH465-thallus (MEXU)	—	MT873940	MT878446
<i>A. subelevata</i>	Canary Islands; Ertz 10727 (BR)	—	HQ454575	HQ454715
<i>A. varia</i> 1	France; Ertz 7570 (BR)	EU704075	EU704103	EU704039
<i>A. varia</i> 2	Sweden; Frisch 11/Se1 (UPS)	KJ851006	KJ851027	KJ851147
<i>A. varia</i> 3	Sweden; Thor 26124 (UPS)	KF707642	—	KF707664
<i>Dimidiographa longissima</i> *	Florida; Ertz 9155 (BR)	EU704069	EU704097	EU704033
<i>Heterocyphelium leucampyx</i>	Uganda; Van den Broeck 6326 (BR)	KY360242	—	KY360246
<i>Lecanactis abietina</i> *	Belgium; Ertz 5068 (BR)	AY548813	AY548812	AH013900
<i>Lecanographa amylicata</i>	Sweden; Thor 26176 (UPS)	KF707650	KF707639	KF707659
<i>L. brattiae</i>	USA, California; Ertz 12437 (BR)	—	HQ454571	HQ454711
<i>L. dialeuca</i> 1	Portugal, Porto Santo island; Ertz 10543 (BR)	—	HQ454549	HQ454689
<i>L. dialeuca</i> 2	Portugal, Porto Santo island; Ertz 10569 (BR)	—	HQ454550	HQ454690
<i>L. dimelaenoides</i>	Mexico, Baja California; Tehler 9109 (S)	MT873941	HQ454551	HQ454691
<i>L. aff. farinosa</i>	Canary Islands; Ertz 14053 (BR)	KY360245	HQ454547	HQ454687
<i>L. hypothallina</i> 1	Mexico, Baja California; Tehler 9092 (S)	MT873942	HQ454557	HQ454697
<i>L. hypothallina</i> 2	Mexico, Baja California; Tehler 9108 (S)	MT873943	HQ454558	HQ454698
<i>L. lyncea</i> s. lat.	Mexico, Baja California; Tehler 9084 (S)	MT873944	HQ454559	HQ454699
<i>L. uniseptata</i>	Gabon; Ertz 9859 (BR)	MG845015	HQ454561	HQ454701
' <i>Opegrapha</i> ' <i>brevis</i>	Great Britain; LD: L10094	KJ851005	KJ851077	—
' <i>O.</i> ' <i>celtidicola</i>	Portugal; Diederich 16053 (BR)	EU704066	EU704094	EU704030
<i>O. vulgata</i> *	Belgium; Ertz 7564 (BR)	EU704080	EU704108	EU704044
<i>Phacographa glaucomaria</i>	Sweden; Frisch 11/Se33 (UPS)	KJ851022	KJ851028	KJ851136
<i>P. protoparmeliae</i>	Sweden; Westberg (Frisch 11/Se49, S)	—	KJ851075	KJ851137
<i>P. zwackhii</i>	Sweden; Thor 26283 (UPS)	—	KJ851074	KJ851135
<i>Phoebus hydrophobius</i>	Mexico; Huereca AH464 (BR)	MT873945	—	MT878447
<i>Plectocarpon lichenum</i>	Sweden; Thor 26770 (UPS)	KJ850988	—	KJ851140
<i>P. nephroleum</i>	Sweden; Nordin 5813 (UPS)	KJ851004	—	KJ851139
<i>Simonyella variegata</i>	Socotra; AFTOL-ID 80	AY584631	—	DQ782861
<i>Zwackhia soreidiifera</i>	Sweden; Thor 26210 (UPS)	KJ851024	KJ851055	KJ851142
<i>Z. viridis</i>	Luxembourg; Ertz 7619 (BR)	EU704078	EU704106	EU704042
<i>Z. aff. viridis</i>	Rwanda; Ertz 7807 (BR)	EU704079	EU704107	EU704043

phylogenetic tree based on the nuclear gene *RPB2*. Therefore, *L. atropunctata* was removed from subsequent analyses, although this conflict had no impact on our conclusions regarding the taxonomic affiliation of the newly sequenced taxa. The mtSSU, nuLSU and *RPB2* data sets were then concatenated.

The combined three-locus data set of 37 samples consisted of 2418 unambiguously aligned sites, 583 for mtSSU, 962 for nuLSU and 873 for *RPB2*. A Bayesian analysis was carried out on the concatenated three-locus data set using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method in MrBayes

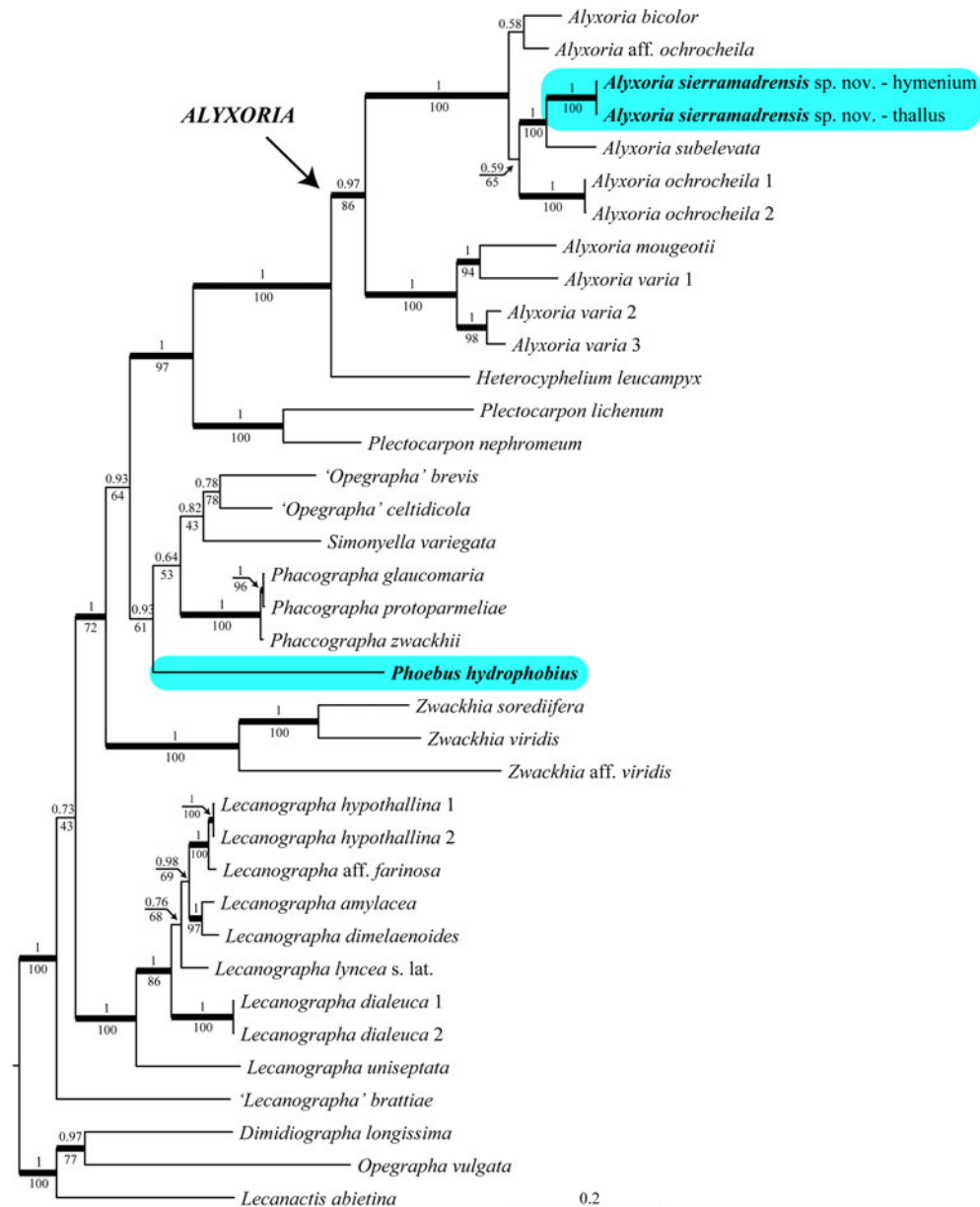


Fig. 2. Three-locus (nuLSU + mtSSU + *RPB2*) Bayesian tree representing the proposed phylogenetic relationships among taxa of *Lecanographaceae*, with three out-group species. MrBayes posterior probabilities are shown above branches, and RAxML bootstrap values are shown below branches. Thicker lines highlight inter-nodes considered strongly supported by both analyses. Names of samples for which sequences were generated in this study are indicated in bold and highlighted with a shaded box. In colour online.

v.3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES Web Portal (Miller *et al.* 2010). Best-fit evolutionary models for each partition were estimated using the Akaike Information Criterion (AIC) as implemented in jModelTest2 (Darriba *et al.* 2012). The GTR + I + G model was selected for the mtSSU as well as for the *RPB2*/1st and *RPB2*/3rd positions, while the TVM + I + G model was selected for the *RPB2*/2nd position and the TrN + I + G model for the nuLSU. Two Bayesian MCMCMC runs were executed in parallel, each using four independent chains and 40 million generations, sampling trees every 1000th generation. Tracer v.1.6.0 (Rambaut *et al.* 2013) was used to ensure that convergence was reached by plotting the log-likelihood values of the sample points against generation time. Convergence between runs was also verified

using the PSRF (Potential Scale Reduction Factor), confirming that values for all parameters were equal to 1.000. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 60 002 post burn-in trees out of the 80 002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. In addition, a maximum likelihood (ML) analysis was performed using RAxML v.8.2.12 (Stamatakis 2014) with 1000 ML bootstrap iterations (ML-BS) and the GTRGAMMA model.

The Bayesian tree did not contradict the RAxML tree topology for the strongly supported branches. Therefore, only the Bayesian tree is shown with the PP values added above the internal branches and the ML-BS values added below (Fig. 2). Internodes with ML-BS ≥ 70 and PP ≥ 95 were considered

strongly supported (Alfaro *et al.* 2003; Lutzoni *et al.* 2004). Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results

Phylogenetic analysis

Ten new sequences were obtained for this study and 85 additional sequences were retrieved from GenBank (Table 1). The Bayesian tree obtained from the combined three-locus analysis of the *Lecanographaceae* data set is shown in Fig. 2. The main well-supported lineages are in accordance with the results obtained by Frisch *et al.* (2014) and Van den Broeck *et al.* (2017). The genus *Lecanographa* has a basal position and is recovered as paraphyletic. The generic affiliation of *L. brattiae* (Egea & Ertz) Ertz & Tehler requires further study. The genera *Alyxoria*, *Phacographa*, *Plectocarpon* and *Zwackhia* form well-supported lineages. *Phoebus hydrophobius* has a rather isolated position as sister to a clade including *Phacographa*, two species of *Opegrapha* s. lat. and *Simonyella variegata* J. Steiner but this relationship is weakly supported by both phylogenetic analyses. The genus *Alyxoria* is divided into two fully supported lineages: one containing three specimens of *A. varia* s. lat. (a complex of species in need of revision) and a specimen of *A. mougeotii* (A. Massal.) Ertz *et al.*; a second formed by *A. bicolor* (R. C. Harris & Lendemer) Ertz & Tehler, *A. ochrocheila* (Nyl.) Ertz & Tehler, *A. aff. ochrocheila*, *A. subelevata* (Nyl.) Ertz & Tehler and the new species. This second lineage is referred to hereafter as the '*Alyxoria ochrocheila* subgroup'. The new species is sister to *Alyxoria subelevata* with strong support.

Taxonomy

Alyxoria sierramadrensis Ertz, Huereca, Salcedo-Martínez & Tehler sp. nov.

MycoBank No.: MB 836549

A species of *Alyxoria* characterized by a placodioid to subfoliose thallus, rounded ascomata with a widely exposed, whitish pruinose hymenial disc, an orange, K+ purple medulla and 3-septate ascospores, (17–)19–23(–25) × 7–9 µm.

Type: Mexico, Nuevo León, Monterrey, Cerro de las Mitras, Cara Norte, route to Pico Perico, ascent through Colegio La Salle, 25°43'01.02"N, 100°24'55.65"W, 1314 m elev., Piedmont scrub and Xerophytic scrub ('Matorral submontano' y 'Matorral xerófito') with elements of *Quercus* spp. forest, on limestone rock, 30 March 2019, Alejandro Huereca AH-465 (MEXU—holotype; S—isotype).

(Fig. 3)

Thallus placodioid to subfoliose, epilithic, c. 1–3 cm diam., pale brown, covered by a whitish pruina on most of its surface, smooth, dull, convex, continuous, composed of elongated lobes or areoles 0.8–3 × 0.8–1.5(–2) mm; thallus lobes in section 290–360 µm thick, with a distinct upper cortex; upper cortex hyaline to pale yellow, c. 40–65 µm, of 'Branched type' (*sensu* Aptroot & Schumm 2011: 7), filled with many crystals of calcium oxalate (H₂SO₄!); algal layer c. 40–75 µm; medulla 160–210 µm below the algal layer, including an orange central part c. 100–110 µm thick (anthraquinones), with numerous crystals of calcium oxalate

(H₂SO₄!); lower surface ecorticate, covered by a thin layer (c. 25–40 µm) of black, branched hyphae c. 4–5 µm thick. *Photobiont* trentepohlioid; cells mostly broadly ellipsoid, 12–19 × 9–15 µm.

Ascomata scattered, (0.3–)0.5–1.2 mm diam., rounded, sometimes shortly elongated, sessile, not or slightly constricted at the base; margin c. 0.1–0.2 mm wide, black, smooth, epruinose, slightly raised above the hymenial disc; hymenial disc flat, widely exposed, white pruinose. *Exciple* brown-black, K– or K+ magenta-red supposedly due to presence of an anthraquinone, 75–90 µm thick. *Hypothecium* dark brown, extending down to the substratum, up to c. 300 µm thick. *Hymenium* 125–150 µm tall, hyaline to pale yellowish, clear, I+ red, K/I+ blue becoming slightly greenish blue; *epihymenium* pale brown, I+ persistently blue, K/I+ blue; *subhymenium* 35–50 µm tall, pale yellowish, I+ persistently blue. *Paraphysoids* richly branched and anastomosing, c. 2(–2.5) µm thick, not or only slightly enlarged at apices, up to 3 µm. *Asci* narrowly clavate, (6–)8-spored, 70–80 × 18–21 µm, without or with a tiny ocular chamber; in K/I non-amyloid except for an amyloid (blue) endoascus layer in the upper part, and an amyloid apical ring. *Ascospores* (17–)19–23(–25) × 7–9 µm, 3-septate, oblong to somewhat clavate, not constricted at septa, cells more or less equal in size except one or the two inner cells often slightly broader; over-matured spores becoming brownish with a coating of minutely pigmented granules; perispore distinct, c. 1.5–2 µm thick.

Conidioma seen only once in cross-section, immersed in the thallus, pyriform, 250 × 200 µm; wall paraplectenchymatous, c. 10–20 µm thick, dark brown in the upper half, hyaline below; conidiogenous cells ampulliform, simple, 8–10(–15) × 2 µm; *conidia* bacilliform, simple, hyaline, 5–6 × 1.2–1.5 µm.

Chemistry. Thallus surface C–, K–, KC–, PD–, UV–; medulla C–, K+ purplish, PD–, UV± bright orange. TLC revealed two unidentified anthraquinones of R_f 60 and R_f 63, one unidentified UV+ violet substance after heating, of R_f 55, and one unidentified UV+ reddish substance before heating, of R_f 67 (holotype tested in solvent B').

Etymology. The specific epithet refers to the mountain range of the Sierra Madre Oriental (SMO) in the north-eastern part of Mexico where the species has been collected.

Ecology. *Alyxoria sierramadrensis* grows on exposed limestone rocks in semi-humid environments, particularly vertical rock walls with Xerophytic scrub and Piedmont scrub mixed with oaks, sometimes near montane streams or drainages (Fig. 1). It has been found at 800–1500 m above sea level. At the type locality (Fig. 1), the flora found in this habitat belongs to xerophytic scrub with *Agave univittata* Haw., *Astroblepis sinuata* (Lag. ex Sw.) D. M. Benham & Windham, *Callisia repens* (Jacq.) L., *Decatropis bicolor* (Zucc.) Radlk., *Echeveria simulans* Rose, *Echinocereus viereckii* Werderm., *Esenbeckia berlandieri* Baill. ex Hemsl., *Ferocactus hamatacanthus* (Muehlenpf.) Britton & Rose, *Opuntia engelmannii* Salm-Dyck ex Engelm., *Pellaea ovata* (Desv.) Weath., *Sedum palmeri* S. Watson and *Selaginella wrightii* Hieron., among others. These elements are typical for the semi-desert in north-eastern Mexico (Rzedowski 2006). *Alyxoria sierramadrensis* appears to be restricted to exposed rock outcrops, and it was not found on shaded rocks. Associated lichenized fungi include *Bagliettoa calciseda* (DC.) Gueidan & Cl. Roux, *Buellia trachyspora* Vain., *Caloplaca eugyra* (Tuck.) Zahlbr., *C. saxicola* (Hoffm.) Nordin agg., *Lecanora* aff. *marginata* (Schaer.) Hertel



Fig. 3. *Alyxoria sierramadrensis* (holotype). A–C, thallus and ascomata. D, thallus with a section showing the orange medulla due to the presence of anthraquinones. E, ascus in KOH. F, ascus in K/I showing the apical blue ring. G, ascospores in water. Scales: A = 5 mm; B = 2 mm; C & D = 500 µm; E & F = 10 µm; G = 5 µm. In colour online.

& Rambold, *Phoebus hydrophobius*, *Psora pseudorussellii* Timdal, *Speerschneidera euploca* (Tuck.) Trevis., *Squamulea galactophylla* (Tuck.) Arup *et al.*, *Xanthopsorella texana* (W. A. Weber) Kalb & Hafellner and unidentified *Verrucaria*.

Distribution. *Alyxoria sierramadrensis* is rather common in suitable habitats along the physiographic region of the SMO and is currently known from six localities: Coahuila (one) and Nuevo León (five). The locality 'Cañon San Matías' in central Coahuila

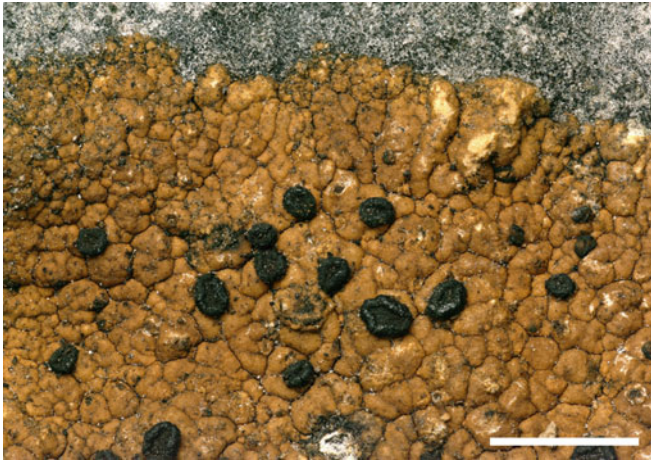


Fig. 4. *Phoebe hydrophobius* (Huereca AH-464). Thallus and ascomata. Scale = 2 mm. In colour online.

is located in the municipality of Nadadores, which belongs to the subprovince of 'Sierras y Llanuras Coahuilenses'. It consists of folded limestone mountain ranges, oriented north-west to south-east, mostly with steep small folds, with a precipitation of 300–400 mm (INEGI 2015). The remaining localities are in Nuevo León, in the subprovince of 'Gran Sierra Plegada', predominantly formed by limestone mountains with scattered hills and valleys, running parallel to the Gulf of Mexico. This mountain range has an average precipitation of 360 mm (INEGI 1986) and creates an orographic barrier with the eastern slopes capturing the moist winds originating in the sea. *Alyxoria sierramadrensis* should be searched for in the state of Texas, especially in the Edwards Plateau and the Ozark Ecoregion, since central and southern Texas and northern Mexico share similar lichen flora and plant communities (Harris & Ladd 2007; McLaughlin 2007; Morse & Lendemer 2019). In Mexico, the neighbouring states of San Luis Potosí and Tamaulipas should be investigated. These states also include portions of the SMO with the same type of rock but are more humid due to the considerably higher precipitation further south in Mexico (900 mm and 491 mm respectively; INEGI 2002, 2017).

Conservation and threats. Four of the five localities in Nuevo León are found in the metropolitan area of Monterrey in four prominent mountains within the city: Cerro de Chipinque, Cerro de Las Mitras, Cerro de La Silla and Cerro El Topo Chico. All of these are protected nature reserves with different management programmes and protection status (either national or state parks), but they are still under strong anthropogenic pressure, especially urban development (Lazcano *et al.* 2012; Green & Sánchez 2013). Except for the C. de Chipinque, these mountains have become 'artificial sky islands' since the city has encroached on their slopes, disrupting the connectivity between ecosystems, and therefore the exchange of biodiversity (García *et al.* 2013; Lazcano *et al.* 2019). Furthermore, due to the abundance of limestone in the region, mining operations are common even in the city's mountains (Montalvo-Arrieta *et al.* 2010). Unfortunately, despite being designated as a state Protected Natural Area, the type locality of *A. sierramadrensis* includes two limestone quarries on the north-western flanks of the mountain (Lazcano *et al.* 2012, 2019). The greatest threat to the lichen flora in the metropolitan area is the bad air quality, ranked in recent years as one of the

poorest in Mexico and Latin America (Green & Sánchez 2013). This is mainly caused by emissions from the petrochemical and chemical industries as well as from concrete refinement processes, and its vehicle population of almost 2 million units (Molina *et al.* 2019). However, no surveys have been conducted that investigate the impact of air pollution caused by industrial activity on the lichens.

Notes. The new species is unique among the *Lecanographaceae* in the placodioid to subfoliose thallus with a medulla containing large amounts of anthraquinones. The only member of the *Lecanographaceae* known with anthraquinones in the medulla is *Lecanographa subgrumulosa* (Egea *et al.*) Egea & Torrente, a lichenized fungus known from Morocco and south-east Spain that also grows on limestone rocks. That species differs from *Alyxoria sierramadrensis* in having a crustose, ecorticate, white to greyish white, cracked or areolate thallus without lobes at the margin, lirelliform, seldom roundish ascomata, a K+ dark green excipulum, I+ blue hymenium, narrower mature asci (12–15 µm), narrower ascospores (4.5–6 µm) and longer conidia (6–10 µm) (Egea *et al.* 1993). Other *Arthoniales* known to contain anthraquinones in the medulla occur in the genera *Crocellina* and *Roccella*, but these are very different species having lecanorine ascomata and belonging to the *Roccellaceae* (Tehler *et al.* 2010; Ertz *et al.* 2015). Species of *Placolecis* (*Lecanorales*, *Catillariaceae*) are also known from limestone and share a placodioid thallus with a characteristic orange medulla due to the production of anthraquinones, but the ascomata are epruinose, the asci have a prominent amyloid tholus lacking any internal differentiation (*Catillaria*-type) and the ascospores are simple (Yin *et al.* 2019). In our phylogenetic analyses (Fig. 2), the new species is sister to *Alyxoria subelevata*. This latter species also has ascomata with a widely exposed hymenial disc covered by a whitish pruina but it differs from *Alyxoria sierramadrensis* in having a thin crustose thallus, lirelliform ascomata of 0.6–2(–3) × 0.2–0.5 mm and narrower (4–6 µm wide) ascospores (Torrente & Egea 1989).

The thallus is unusual for a species of *Alyxoria* and we wondered whether the ascomata might represent a lichenicolous fungus growing on the thallus of a second species. In order to test this hypothesis, we generated sequences from very tiny fragments of material of both the thallus and the hymenium by direct PCR. We obtained two loci (nuLSU and *RPB2*) for each and included both in the phylogeny. In addition, we also amplified the nuLSU of a second, sterile thallus from the same type specimen. The nuLSU was identical to that of the fertile thallus; this last sequence was not published because it was used only for testing the reliability of our new data. No necrotic areas were found around the ascomata, and the species is known from several localities where no similar lichens are found. Moreover, the new species is part of the *Alyxoria ochrocheila* subgroup, including lichens also known to frequently have anthraquinones and similar ascoma and ascospore types (see Discussion). Thus, the chemistry and morphology also support the phylogenetic placement. For these reasons, we are convinced that the ascomata do not represent a lichenicolous fungus growing on the sterile thallus of another lichen.

Additional specimens examined. **Mexico:** Coahuila: Nadadores, Rancho Salsipuedes 'Cañón San Matías', 27°27'14.2"N, 101°54'58.1"W, 1320 m, Piedmont scrub and Xerophytic scrub ('Matorral submontano' y 'Matorral xerófito') with elements of

Quercus spp. forest, sedimentary rock conglomerate, 2019, *Alejandro Huereca* AH-543, AH-544 (hb. A. Huereca). *Nuevo León*: Monterrey, Cascadas del Cerro de la Silla, 25°37'32.04"N, 100°12'52.78"W, 917 m, Xerophytic scrub with Piedmont scrub elements, limestone rock, 2019, *Alejandro Huereca* AH-466 (BR, MEXU, NY); Santa Catarina, Cañón de Casa Blanca, before the crossing of 'El Paso del Caballero', 25°34'39.34"N, 100°42'38.08"W, 1520 m, ecotone of Piedmont scrub and Xerophytic scrub ('Matorral submontano y Matorral xerófito'), limestone rock, 2019, *Alejandro Huereca* AH-545 (hb. A. Huereca).

Phoebus hydrophobius R. C. Harris & Ladd

Opuscula Philolichenum 4, 64 (2007).

Description and illustration. See Harris & Ladd (2007).

Distribution and habitat. This species was considered endemic to the Ozark Ecoregion in the USA (Harris & Ladd 2007) but is newly recorded for Mexico (Fig. 4). In Mexico, *Phoebus hydrophobius* is found in the same habitat and localities as *Alyxoria sierramadrensis*, but it is relatively more abundant.

Specimens examined. **Mexico:** *Coahuila*: Nadadores, Rancho Salsipuedes 'Cañón de los Cedros', 27°20'49.93"N, 101°51'36.32"W, 1090 m, Piedmont scrub ('Matorral submontano') with elements of *Quercus* spp. forest, on limestone rock, 2018, *Alejandro Huereca* AH-254 (hb. A. Huereca); Nadadores, Rancho Salsipuedes 'Cañón San Matías', 27°27'14.2"N, 101°54'58.1"W, 1320 m, Piedmont scrub and Xerophytic scrub ('Matorral submontano' y 'Matorral xerófito') with elements of *Quercus* spp. forest, sedimentary rock conglomerate, 2019, *Alejandro Huereca* AH-542 (MEXU). *Nuevo León*: Monterrey, Cascadas del Cerro de la Silla, 25°37'32.04"N, 100°12'52.78"W, 917 m, Xerophytic scrub and Piedmont scrub ('Matorral xerófito y Matorral submontano'), with elements of *Quercus* spp. forest, limestone rock, 2019, *Alejandro Huereca* AH-175 (hb. A. Huereca); Monterrey, Cerro de las Mitras, Cara Norte, route to Pico Perico, ascent through Colegio La Salle, 25°43'01.02"N, 100°24'55.65"W, 1314 m, Xerophytic scrub and Piedmont scrub ('Matorral xerófito y Matorral submontano') with elements of *Quercus* spp. forest, limestone rock, 2019, *Alejandro Huereca* AH-464 (BR); Santa Catarina, Cañón de Casa Blanca, before the crossing of 'El Paso del Caballero', 25°34'39.34"N, 100°42'38.08"W, 1520 m, ecotone of Piedmont scrub and Xerophytic scrub ('Matorral submontano y Matorral xerófito'), limestone rock, 2019, *Alejandro Huereca* AH-545 (hb. A. Huereca).

Discussion


Besides the discovery of a new species, the most important result obtained from the present study is the placement of two lichenized fungi developing placodioid thalli within two separate lineages of the *Lecanographaceae*. This family was first recognized by Ertz & Tehler (2011) before it received its formal status by Frisch *et al.* (2014). Seven genera (*Alyxoria*, *Heterocyphelium*, *Lecanographa*, *Phacographa*, *Plectocarpon*, *Simonyella*, *Zwackhia*) were previously accepted in the *Lecanographaceae* (Ertz & Tehler 2011; Frisch *et al.* 2014; Van den Broeck *et al.* 2017) and all are represented in our phylogenetic analysis (Fig. 2).

Phoebus is thus the eighth genus confirmed in this lineage. The *Lecanographaceae* include non-lichenized (lichenicolous) fungi, lichenized fungi with crustose, predominantly thin thalli and one fruticose species, *Simonyella variegata*. *Alyxoria sierramadrensis* and *Phoebus hydrophobius* are therefore the first species with a placodioid thallus recognized as belonging to the *Lecanographaceae*. Similar growth forms in the *Arthoniales* were known only in the genus *Roccellina*. This latter genus of *Roccellaceae* is remarkably diverse in terms of thallus type, ranging from crustose to fruticose, and including placodioid to sub-foliose species. The placodioid thallus led Harris & Ladd (2007) to compare the genus *Phoebus* with *Roccellina*, despite the authors admitting that it does not seem closely related to that genus due to the unique thallus pigmentation and anatomy. The specimens of *Alyxoria sierramadrensis* were also annotated first as 'Roccellina' before our molecular investigation. Historically, thallus growth forms played an important role at the family and generic systematic level in the *Arthoniales*, but recent evidence from molecular studies showed that it has often been overestimated, even for generic delimitation. Multiple evolution of the fruticose growth form for instance is now well documented in the families *Opegraphaceae* and *Roccellaceae*, even within genera such as *Dendrographa*, *Pentagenella* and *Roccellina* (Tehler & Irestedt 2007; Ertz & Tehler 2011). Within *Lecanographaceae*, the fruticose lichen *Simonyella variegata* is surprisingly related to the crustose lichen '*Opegrapha celtidicola* (Jatta) Jatta' and lichenicolous species of *Opegrapha* s. lat. and *Phacographa* (Van den Broeck *et al.* 2017; fig. 2), but the generic delimitation within this lineage is in need of further investigation. While the growth forms of *Alyxoria sierramadrensis* and *Phoebus hydrophobius* might have pointed to a relationship with the genus *Roccellina* or at least to a placement in the family *Roccellaceae*, the ascocata, asci and ascospore types clearly support a placement in the family *Lecanographaceae*. Some characters of our new species also support the placement in the genus *Alyxoria*, particularly the combination of a prominent and epruinose black margin with a widely exposed and whitish pruinose hymenial disc (at least shared by some species in the genus such as *A. subelevata*, the sister taxon of *A. sierramadrensis*), and short few-septate ascospores with a distinct gelatinous sheath. The new species is part of the *Alyxoria ochrocheila* subgroup, which includes lichens also known to frequently produce anthraquinones. As in *Alyxoria ochrocheila*, the epihymenium and subhymenium of *A. sierramadrensis* are I+ persistently blue while the hymenium is I+ red, a very rare combination in the *Arthoniales*, perhaps restricted to the *A. ochrocheila* subgroup (Ertz 2009).

Alyxoria sierramadrensis and *Phoebus hydrophobius* are two remarkable examples of parallel evolution of the placodioid growth form within the *Lecanographaceae*; within *Arthoniales*, this was previously known only from the genus *Roccellina* in the *Roccellaceae*. The two species further highlight the high degree of morphological plasticity in the *Arthoniales* and provide additional new proof that generic delimitation cannot always rely on thallus morphology. Much remains to be done to improve our understanding of the evolution within *Arthoniales*, for which molecular data are still limited despite important progress in the last decade.

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