A new species of *Reichlingia* (Arthoniaceae) from the grasslands of central North America

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Abstract. *Reichlingia americana*, a new saxicolous species, is described from Oklahoma, U.S.A., representing the first records of the genus in North America. The species is distinguished from other members of the genus by a combination of its epilithic, rimose thallus; submuriform ascospores; presence of 2′-*O*-methylperlatolic acid; and unique habitat on sheltered sandstone faces. Distinctions from related species are discussed and a key to members of the genus is provided.

Keywords. *Arthonia*, *Arthothelium*, biogeography, Crosstimbers, Great Plains, lichen taxonomy, Oklahoma, 2′-*O*-methylperlatolic acid, sandstone.

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Reichlingia Diederich & Scheid. was introduced to accommodate a single species, *R. leopoldii* Diederich & Scheid., initially thought by the authors to represent a sporodochial lichenicolous hyphomycete producing brown, verrucose conidia (Diederich & Scheidegger 1996). The authors proposed that the host was an unknown, sterile, sorediate lichen with a trentepohlioid photobiont. Subsequent studies revealed the putative host of *R. leopoldii* was actually its thallus (Diederich & Coppins 2009), and phylogenetic analyses by Frisch et al. (2014b, 2020) and Ertz et al. (2020) revised and expanded the genus to include additional, fertile species. Members of *Reichlingia* are united by similar morphology, septate or submuriform ascospores, and the presence of 2′-*O*-methylperlatolic or perlatolic acid; notably, only the type species bears sporodochia. The genus currently comprises six taxa variously distributed in Africa and western Europe (Cannon et al. 2020; Ertz et al. 2020; Frisch et al. 2014b, 2020). Van den Broeck et al. (2018) and Ertz et al. (2020) showed *Reichlingia* and unassigned members of *Arthonia* Ach. s.l. to be sister to *Synarthonia* Müll.Arg, an exclusively corticolous, mostly tropical genus comprising 20 taxa that produce evernic and psoromic acids, parietin, and unidentified xanthones. Collectively, *Reichlingia* and *Synarthonia* are sister to *Coniocarpon* DC. This clade, which has been recovered in several recent studies, has been called the *Coniocarpon-Reichlingia* clade (Ertz et al. 2020; Frisch et al. 2014a; Van den Broeck et al. 2018).

During fieldwork in the Crosstimbers region of the southeast Great Plains, we discovered an unusual member of the Arthoniaceae, whose morphology and chemistry are indicative of a new species of *Reichlingia*. We describe the species here.

Materials and Methods

Specimens were studied dry using dissecting microscopes. Water mounts were hand sectioned with razor blades. Microscopic characters were observed in water, 10% KOH (K), 10% HNO_3, 10% H_2SO_4, Lugol's iodine (I) or K followed by I (KI) and images were captured and measured to the nearest 0.1 µm. Measurements are presented as a simple range, or, where sufficient material allowed, as the average ($\bar{x}$) ± one standard deviation (SD), bounded by the smallest and largest observed values, and followed by the sample size [n], i.e.: (smallest observed–)$\bar{x}$-SD–$\bar{x}$+SD(–largest observed) [n]. Specimens were analyzed using standard spot tests (reagents are abbreviated following Brodo et al. 2001) and thin layer chromatography (TLC). TLC

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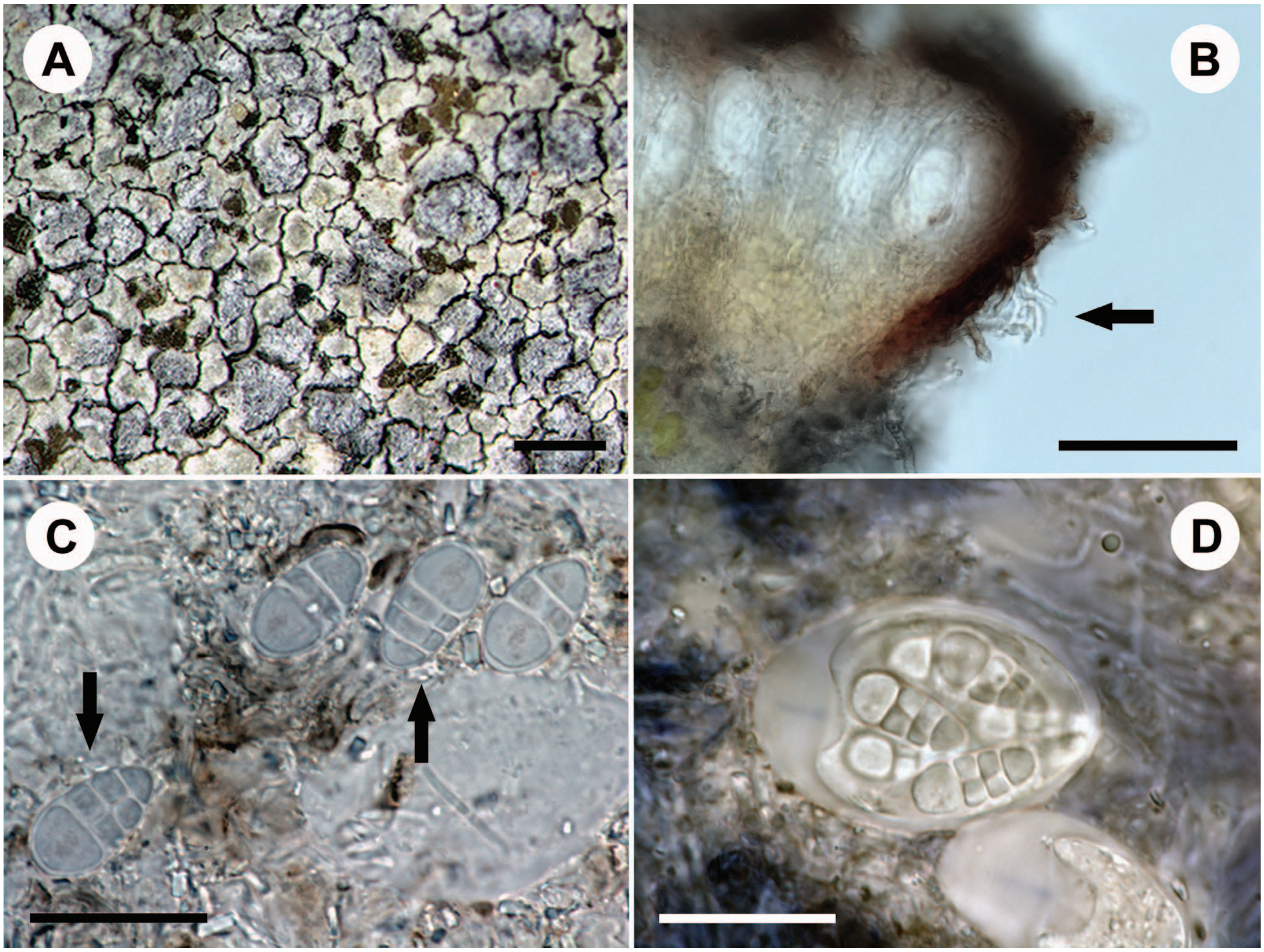

Figure 1. *Reichlingia americana*. **A.** Habitus. **B.** Section of exciple showing microtomentum (arrow). **C.** Mature ascospores (arrows, showing longitudinal cells walls). **D.** Mature ascospores in ascus, post treatment with KI. (A, C from *Morse 18874b*; B, D from *Morse 26007*). Scales: A = 0.5 mm; B = 50 μm; C–D = 20 μm.

was performed at KANU using solvents A, B′ and C following Orange et al. (2001). Associated taxa are provided below without taxonomic authorities, following the concepts and authorities in Esslinger (2019).

Taxonomy

Reichlingia americana C.A.Morse & Ladd, *sp. nov.* **Fig. 1**

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A saxicolous member of Reichlingia distinguished by its epilithic, rimose thallus; submuriform ascospores (12.6–)13.8–16.1(–17.9) × (5.6–)6.5–8.2 (–8.8) μm; and the presence of 2′-O-methylperlatolic acid.

Type: U.S.A. Oklahoma: Osage Co., ca. 13.3–13.8 mi N, 0.25–1 mi E of Pawhuska, Osage Wildlife Management Area: Western Wall Unit: NW part, 36.86°–36.87°N, 96.33°–96.32°W, elev. 850–970 ft, Crosstimbers canyon system with boulders and low cliffs of Pennsylvanian sandstone along South Fork Pond Creek and unnamed tributaries, 14 Mar 2018, *C.A. Morse 26007 & D. Ladd* (KANU 405788!, holotype; HB. LADD!, isotype; other isotypes to be distributed with *Lichenes Exsiccati Magnicamporum*).

Description. Thallus epilithic, thin, rimose, creamy white to greyish or greenish grey, epruinose to finely pruinose; areoles contiguous (less commonly dispersed among protruding grains of substrate), irregularly polygonal, 0.2–0.4 mm in diam., 0.1–0.3 mm thick, ecorticate, compact felty, smooth to distinctly roughened; pruina rectangular, 2.0–12.0 × 1.1–4.4 μm; white fibrous prothallus occasionally present, or thallus sometimes blackening along contact zone with other lichens; medulla I+ blue (or I+ reddish along outer edges of areoles), KI+ blue, of thick-walled, irregularly branching hyphae, (1.1–)1.3–2.0 μm wide (measured in K), densely inspersed with and largely obscured by fine, POL+ granules (these persisting in 10% H_2SO_4, clearing in K); photobiont trentepohlioid, the cells 10.8–19.7 μm in diameter, irregularly dispersed through medulla. Ascomata initiating marginally, immersed in areoles, at first discrete, oblong, becoming submoniliform or continuous, linear or crescent shaped, 0.1–0.5 × 0.07 mm, eventually expanding to occupy the areole, plane and level with upper surface of adjacent areoles, 0.1–0.4 mm in diam., angular to rounded, dark brown, usually densely white pruinose and contrasting with creamy white color of areoles or rarely epruinose; epihymenium a dense layer of pruina; epithecium reddish brown to brown, K– (grey); hymenium ca. 65–80 μm high, colorless to pale yellowish, I+ red, KI+ blue; paraphysoids branching, slender, 0.7–1.7 μm wide at midpoint, not expanded distally; exciple narrow, reddish brown, K– (grey) above to colorless below, basally constricted, ca. 14 μm wide, of tangled, thick-walled hyphae 1.9–2.6 μm wide, the free tips of some of these forming a sparse tomentum visible microscopically; hypothecium ca. 60 μm high, colorless or pale yellowish. Asci *Arthonia*-type, with or without KI+ blue ring or sometimes with a very narrow KI+ blue cone, broadly clavate to ovate, 33–44 × 18–24 μm, 8-spored; ascospores colorless, not becoming brown or warted at maturity, narrowly obovoid, ellipsoid, or oblong, (12.6–)13.8–16.1(–17.9) × (5.6–)6.5–8.2(–8.8) μm, length:breadth 1.8–2.3(–2.6) [n=39], usually distinctly macrocephalic, submuriform, with (2–)3(–4) transverse septa and one or both medial cells with a longitudinal septum, with walls ca. 0.1 μm thick. Conidiomata not observed. Sporodochia not observed.

Chemistry. Spot tests of thallus: K–, KC–, C–, Pd–, UV–. TLC: 2′-*O*-methylperlatolic acid.

Etymology. The specific epithet refers to the known distribution of the new species.

Distribution and ecology. Known only from three collections, on sheltered faces of non-calcareous sandstone cliffs, in areas protected from direct wetting but with relatively high light intensities. Associated lichens include *Arthonia* cf. *madreana*, *Caloplaca yuchiorum*, *Chrysothrix xanthina*, *Cresponea premnea* var. *saxicola*, *Dirina* sp., *Dirinaria frostii*, *Fuscidea recensa*, *Lecanora* cf. *sulphurescens*, *Lepraria normandinoides*, *Pertusaria pseudocorallina* and *Phlyctis petraea*.

Discussion. 2′-*O*-methylperlatolic acid has been documented from several other genera of Arthoniomycetes, including *Cryptothecia* Stirt., *Glomerulophoron* Frisch, Ertz & G.Thor, *Inoderma* (Ach.) Gray, *Myriostigma* Kremp., and *Sporodophoron* Frisch, Y.Ohmura, Ertz & G.Thor, as well as several species currently assigned to *Arthothelium* A.Massal. (Frisch et al. 2014a, 2015). *Reichlingia americana* differs from members of these genera in its thallus or spore morphology. However, it shares with fertile species of *Reichlingia* pruinose ascomata with a basally constricted exciple and a thin microtomentum formed by the free tips of the excipular hyphae, a well-developed pale hypothecium, and oblong-ovoid, hyaline, septate ascospores (Frisch et al. 2014b). A microtomentum of free tips of paraphysoids, discussed by Frisch et al. (2014b), was not evident in the new species, but this feature may be obscured by the dense layer of pruina in the epihymenium. While the new species appears to differ from most other members of *Reichlingia* in its rimose thallus and immersed ascomata, in other respects, particularly in its microtomentose exciple and chemistry, the species is easily accommodated within the genus.

Within *Reichlingia*, the new species is distinguished by a combination of immersed ascomata, comparatively short, submuriform ascospores, chemistry, substrate, habitat and geographic distribution. The only other *Reichlingia* with submuriform ascospores, *R. virginea* (Müll.Arg.) Frisch (≡*Arthothelium virgineum* Müll.Arg.), a corticolous species known only from Tanzania, produces 5 × 0–1-septate ascospores and perlatolic acid plus an unidentified xanthone (Frisch 2014b; Müller 1895). A key to species currently included in *Reichlingia* is presented below.

If *Reichlingia americana* had been described previously, most likely it would have been as an *Arthothelium*. Eleven species of that genus are exclusively or primarily saxicolous. Of these, four species—*A. evanescens* Øvstedal, *A. diffluens* (Nyl.) Imshaug & Fryday, *A. feuereri* Aptroot & Seaward, and *A. halophilum* Follm.—produce larger or more richly muriform ascospores and different secondary metabolites (Follmann 1968; Fryday 2002; Øvstedal & Gremmen 2001; Seaward & Aptroot 2004). *Allarthothelium elliottii* (Vainio) Zahlbr., known only from the type collection from the Dominican Republic, has larger muriform ascospores and a trebouxioid photobiont (Wainio 1896). The remaining saxicolous species—*Arthothelium desertorum* Aptroot & Wirth, *A. galapagoense* Huneck & Follman, *A. miesii* Van den Broeck, *A. pacificum* Follmann, *A. saxicolum* Makhija & Patw., and *A. spilomatoides* (Nyl.) Zahlbr.—produce submuriform ascospores of similar size, which differ in having 5 or more transverse septa and in lacking enlarged distal cells; these all also differ from *R. americana* in their chemistry (Aptroot & Wirth 2006; Follmann 1968; Makhiha & Patwardhan 1995, 1997; Van den Broeck et al. 2017; F. Bungartz, J. Elix & D. Ertz in litt.).

A larger number of corticolous species of *Arthothelium* produce submuriform, macrocephalic ascospores of similar size to those of *Reichlingia americana*. Most of these are poorly known, bearing scant descriptions and lacking chemistry data, but several species with ascospores bearing fewer than 5 transverse septa warrant comparison. Among them, *A. impolitellum* (Nyl.) Makhija & Patw., known only from the Indian type collection, produces slightly smaller ascospores (12–14 × 4–6 µm) with a thinner hymenium, brown hypothecium, and proximal cells of the ascospores bear a longitudinal division (Makhiha & Patwardhan 1995). Secondary metabolites have not been reported for *A. impolitellum*, but two other species—*A. ramosum* Makhijan & Patw. and *A. fuscoroseum* Makhija & Patw.—differ in their chemistry and thalline pigments, respectively (Makhiha & Patwardhan 1995). The North American species *Arthonia leucastraea* Tuck. also produces submuriform, macrocephalic ascospores, which bear 4 transverse septa and are 12–16 × 5–7 µm fide Tuckerman (1872; width 5.5–7.5 µm fide Fink 1935). *Arthonia leucastraea* has not been the subject of a modern study and was not available for in-person examination by the authors, but reportedly differs in producing a dark brown hypothecium (Tuckerman 1872; Fink 1935). Images of type material (U.S.A. Texas: 1848, *Wright s.n.* [fh, hb tuck. 3618; mich 74911]) also indicate that the ascomata of this species differ from those of *R. americana*, although *A. leucastraea* may also belong in *Reichlingia*.

Reichlingia americana represents the first member of the genus to be reported from the Americas, although the phylogenetic data in Van den Broeck et al. (2018) indicate that the corticolous species *Arthonia anglica* Coppins and an undescribed taxon from Florida (*Arthonia* sp. 9090 of Van den Broeck et al. 2018) may also belong to the genus. *Arthonia anglica* is locally common in more mesic woodlands in regions east of the range of *R. americana*, and differs from other members of *Reichlingia* by its chemistry, producing gyrophoric acid. The biogeographic implications of a distribution encompassing western Europe, Africa and central North America are unclear and may be an artifact of low numbers of collections. The lichen biota of the Crosstimbers region includes significant influences from both a better-studied southeast Coastal Plain element, and a comparatively poorly known southwestern element, as well as a smaller, apparently endemic element (e.g., Brodo et al. 2001; Morse & Sheard 2020). Several trentepohlioid saxicolous lichens with similar ecological requirements occurring in western Europe are widely distributed in the southeast Great Plains, southern Ozarks, and Appalachians. Included among these are *Cresponea premnea* var. *saxicola* and *Enterographa hutchinsiae*.

Other specimens examined. U.S.A. Oklahoma: Osage Co., 4.5–5 mi S, 1.5–2 mi E of New Prue, City of Sand Springs Keystone Ancient Forest Preserve: N part, 36.18°N, 96.23°–96.24°W, elev. 850–1050 ft, brushy Crosstimbers forest on steep, rocky, W, E and N-facing slopes, on deeply sheltered, N-facing crevice of W-facing cliff above ravine, 16 Mar 2018, *C.A. Morse 26202 & D. Ladd* (hb. ladd, kanu); on sheltered face of E-facing cliff, 01 May 2009, *C.A. Morse 18874b & D. Ladd* (kanu).

Key to *Reichlingia* as presently circumscribed

Note that unidentified substance 'A' of Coppins & James (1978), reported for *R. dendritica* (Leight.) Ertz & Sandersson (as *A. altantica* P.James var. *atlantica*) and *R. zwackhii* (Sandst.) Frisch & G.Thor (as *Arthonia zwackhii* Sandst.) and 'anombrophila unknown' of Coppins (1989) reported for *R. anombrophila* (Coppins & P.James) Frisch (as *Arthonia anombrophila* Coppins & P.James) have subsequently been determined as 2′-*O*-methylperlatolic acid (Frisch et al. 2014b, 2020). Recent studies by Ertz et al. (2019) and Thiyagarja et al. (2020) suggest that the lichenicolous taxa *Arthonia invadens* Coppins and *A. graphidicola* Coppins belong to a clade containing *Coniocarpon*, *Reichlingia* and *Synarthonia*, although their position within the clade remains uncertain. *Arthonia invadens* inhabits thalli of the sterile sorediate crust *Schizotrema quericola* (Coppins & P.James) Ertz, Frisch & Sanderson, while *A. graphidicola* inhabits the thalli of *Graphis* Adans. species (Cannon et al. 2020). Both species produce epruinose ascomata and 2–3 transversely septate ascopores.

1a. Thallus bearing dark brown sporodochia-like conidiomata; ascomata unknown.......... **Reichlingia leopoldii**; central Europe (Cannon et al. 2020)
1b. Thallus lacking sporodochia; ascomata present 2
2a. Ascospores submuriform 3
2b. Ascospores transversely septate 4
3a. Ascospores with 4–5 transverse septa, the terminal cell not enlarged; perlatolic acid plus an unidentified xanthone present; corticolous .... **Reichlingia virginea**; Tanzania (Frisch 2014b; Müller 1895)
3b. Ascospores with 3(4) transverse septa, the terminal cell enlarged; 2′-O-methylperlatolic acid present; saxicolous .... **Reichlingia americana**; U.S.A.
4a. Thallus saxicolous; atranorin, confluentic acid, stictic acid, and unidentified substance ‘B’ present in addition to 2′-O-methylperlatolic acid...... **Reichlingia dendritica**; western Europe (Cannon et al. 2020)
4b. Thallus corticolous; only 2′-O-methylperlatolic acid present 5
5a. Ascospores 12–14 (16) × 4–5 (6) μm with (1)3 transverse septa ... . **Reichlingia anombrophila**; western Europe and Madeira (Cannon et al. 2020)
5b. Ascospores ≥16 μm long, with ≥3 transverse septa 6
6a. Paraphysoids unpigmented; ascospores 19–23 × 7–8 μm with 4–5 transverse septa .... **Reichlingia syncesioides** Frisch & G.Thor; Uganda (Frisch et al. 2014b)
6b. Paraphysoids brown-pigmented apically; ascospores 16–22 (24) × 5–7 μm with 3–4 transverse septa .... **Reichlingia zwackhii**; Europe (Cannon et al. 2020)

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