Cora timucua (Hygrophoraceae), a new and potentially extinct, previously misidentified basidiolichen of Florida inland scrub documented from historical collections

Robert Lücking[1,7], Laurel Kaminsky[2], Gary B. Perlmutter[3], James D. Lawrey[4] and Manuela Dal Forno[5,6]

[1] *Botanischer Garten und Botanisches Museum, Freie Universität Berlin, Königin-Luise-Straße 6–8, 14195 Berlin, Germany;* [2] *Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, U.S.A.;* [3] *University of North Carolina Herbarium, North Carolina Botanical Garden, CB# 3280, Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280, U.S.A;* [4] *Department of Biology, George Mason University, Fairfax, VA 22030-4444, U.S.A.;* [5] *Botanical Research Institute of Texas, Fort Worth, TX, 76107, U.S.A.* [6] *Department of Botany, Smithsonian Institution, National Museum of Natural History, 10th St & Constitution Ave NW, Washington, DC, 20560, U.S.A.*

Abstract. The known collections of the genus *Cora* in continental North America north of Mexico, all restricted to Florida, are shown to belong to a single species, representing a previously unrecognized taxon formally described herein as *C. timucua*. Based on data of the fungal ITS barcoding marker, obtained through Sanger and Illumina sequencing from two historical collections, the new species is phylogenetically most closely related to *C. casanarensis* from Colombia and *C. itabaiana* from Brazil, although it is morphologically most similar to the only distantly related *C. hymenocarpa* from Costa Rica. Based on data from the *Consortium of North American Lichen Herbaria* (CNALH) and from the *Global Biodiversity Information Facility* (GBIF), most of the collections of *C. timucua* originate from around the turn of the 19th century, while a few were made in the second half of the 20th century, all between 1968 and 1985. Almost all collections originate from Florida sand pine scrub, apparently the preferred habitat of this taxon. Neither modern collections nor extant localities are known. Based on these findings and the substantial degree of land use change in Florida in the past decades, we assessed the conservation status of *C. timucua* using the IUCN Red List criteria and found that it should be classified as critically endangered (CR), in line with the status of another Florida endemic, *Cladonia perforata*, which was the first federally red-listed lichen in the United States. The most likely location where *C. timucua* may still be extant is Ocala National Forest in the north-central portion of the Florida peninsula, although recent macrolichen surveys in that area did not encounter this species.

Keywords. Basidiolichens, Consortium of North American Lichen Herbaria, endemism, high throughput sequencing, metabarcoding, phenotype-based phylogenetic binning, species delimitation.

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Massive and continuous land use change through human activities in the past decades has led to what is being considered the sixth (or perhaps more precisely, the seventh) mass extinction (Barnosky et al. 2011; Kolbert 2014; Leakey & Lewin 1995; Rampino & Shen 2019; Wake & Vredenburg 2008). While this phenomenon is largely perceived through well-documented extinctions of charismatic vertebrates, particularly mammals (Ceballos et al. 2010, 2017; Plotnick et al. 2016), it also applies to other groups of organisms, such as invertebrates, bryophytes, and fungi, including lichens. However, it is challenging to reliably assess the conservation status or factual extinction of small organisms.

In lichens, population decline or extinction can only reliably be documented for conspicuous, frequently collected macrolichens, such as the

[7] Corresponding author's e-mail: r.luecking@bgbm.org

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example of *Usnea angulata* in the eastern United States highlighted by Allen et al. (2019), or for well-known populations of particular species with restricted distribution (Sparrius et al. 2017). In general, for any detected individual, there might be dozens, hundreds, and sometimes thousands that escape the attention of the observer, especially when visually inconspicuous or when growing in hard to reach microhabitats such as the forest canopy. Quantitative long-term monitoring which would have to be performed on numerous species for this purpose is only feasible for selected taxa that have already been classified as threatened, such as *Erioderma pedicellatum* (Hue) P.M.Jørg. (Allen et al. 2019; Cameron & Neily 2008; Cameron & Toms 2016; Goudie et al. 2011; Wiersma & Skinner 2011). However, even such species may suddenly turn up in places where they had not been documented before (Nelson et al. 2009; Stehn et al. 2013). Threat to entire habitats is therefore often used as a proxy to assess lichen conservation, including estimating future extinctions due to climate change (Allen & Lendemer 2016; Lendemer & Allen 2014). In addition to the challenge of properly documenting species abundances in microbiota formed by lichens, bryophytes, invertebrates and other small organisms, threatened biota may contain large numbers of undescribed species at risk of extinction before they have even been discovered (Dirzo & Raven 2003; Pereira et al. 2018).

The lichen biota of North America north of Mexico is well-documented, with over 4,800 species recorded (Esslinger 2019). This number corresponds to an increase of 111% since the first published checklist more than six decades ago (Hale & Culberson 1956) and 36% since two and a half decades ago (Esslinger & Egan 1995). The regularly published checklists document the continued growth of our knowledge of the North American lichen biota, with ongoing discoveries of new and previously unrecognized species (e.g., Brodo & Lendemer 2015; Dal Forno et al. 2019; Esslinger et al. 2017; Fryday & McCarthy 2018; Gasparyan et al. 2017; Knudsen et al. 2016; Lendemer & Goffinet 2015; Lendemer et al. 2016; Lücking et al. 2017a; McCune et al. 2019; Sanders & Lücking 2015; Tønsberg & Goward 2016). A major resource to unlock access to this diversity and to detect potentially novel lichen taxa are online occurrence data repositories, such as the *Global Biodiversity Information* Facility (GBIF; https://www.gbif.org). The *Consortium of North American Lichen Herbaria* (CNALH; https://lichenportal.org/cnalh) has assembled over 1.7 million occurrence records for North America, largely based on natural history collections hosted in over 121 regional and international herbaria. These records do not replace physical examination of critical specimens, but it is the digitally available names that trigger such research in the first place (e.g., Perlmutter et al. 2018). One example is the genus *Cora*, currently listed in the North American lichen checklist with a single species, *Cora glabrata* (Spreng.) Fr. (Esslinger 2019). In his revision of the taxon, under the name *Dictyonema pavonia* [sic], Parmasto (1978: 106) gave its presence in North America with the statement "U.S.A. (Florida, 3)", with no indication of actual voucher specimens or corresponding herbaria. Thus, without the digital occurrence data now available, it would have been a difficult task to figure out where exactly specimens of this lichen have been previously collected and deposited.

Although representing conspicuous, presumably well-known macrolichens, the basidiolichen genus *Cora* Fr. is a prime example of previously unrecognized species richness in lichen-forming fungi. Until about a decade ago, a single species was accepted in this taxon, recognized variously under the names *C. glabrata*, *C. pavonia* (Sw.) Fr., *Dictyonema glabratum* (Spreng.) D.Hawksw., and *D. pavonium* (Fr.) Parmasto (Chaves et al. 2004; Fritz-Sheridan & Portecop 1987; Hawksworth 1988; Larcher & Vareschi 1988; Lawrey et al. 2009; Oberwinkler 1984; Parmasto 1978; Yánez et al. 2012). Analysis of the fungal ITS barcoding marker (Schoch et al. 2012) of numerous collections, together with reassessment of morphological, anatomical and chemical features, revealed a staggering number of species, with currently 189 taxa recognized and 92 of them formally named (Dal Forno et al. 2017; Lücking et al. 2013, 2014a, 2017b; Vargas et al. 2014). Surprisingly, these species are not cryptic but can be readily distinguished by a combination of phenotype features, substrate ecology, and geographic distribution, with numerous regional and local endemics (Lücking et al 2014a, 2017b).

Cora is predominantly found in the Neotropics, but its range extends far south into southern Chile and Argentina, with additional species known from the South Atlantic islands, the Mascarenes, South

Africa and Sri Lanka, and potentially Hawaii (Lücking et al. 2014a, 2015a, 2017b; Parmasto 1978). The northernmost extension of the genus is Florida, the only reported occurrence of *Cora* in continental North America north of Mexico (Esslinger 2019; Fink 1935; Moore 1970; Parmasto 1978). Although this material had previously been identified as *D. pavonium* (Parmasto 1978) and is currently listed under the name *C. glabrata* (Esslinger 2019), it is highly unlikely that either name provides a correct identification for it (Lücking et al. 2013, 2014a,b, 2015a, 2017b).

While Parmasto (1978) mentioned three collections assigned to *D. pavonium* from Florida without details, a search in CNALH, GBIF and other online repositories, under all possible synonyms, returned 34 records from 12 herbaria, unlocking access to a broad sample of this lichen, all from Florida. Hence, it can be assumed that *Cora* in the continental United States was never recorded outside Florida. The fact that almost all species of *Cora* now recognized are regional or local endemics and the notion that Florida represents the northernmost extension of the genus, with subtropical habitats well outside its usual ecological range, led us to believe that the Florida material represented an unrecognized species. Notably, most collections were made around the turn of the 19th century, and the most recent collections originate from between 1968 and 1985. After that, there appears to be no record of this lichen in any herbarium. Search in the citizen science portals *iNaturalist* (https://www.inaturalist.org) and *The Mushroom Observer* (https://mushroomobserver.org) did not turn up any observations either.

All herbarium specimens originate from the northern Florida peninsula, from Columbia and Clay south to Citrus, Marion, Lake, Volusia and Seminole counties in north-central peninsular Florida. Florida has been under heavy development in the past decades (Bueno et al. 1995; Chimney & Goforth 2001; Milesi et al. 2003; Volk et al. 2017; Xian et al. 2007). Extensive land use change has caused substantial destruction of natural habitats, leading to the decline of species populations such as anurans, gopher tortoises and lichens (e.g., Delis et al. 1996; McCoy et al. 2006; Yahr 2000, 2003). This would suggest that *Cora* might now be extinct in Florida, and therefore in North America north of Mexico as a whole, providing an instance of a potentially novel taxon known only from natural history collections (Barrabé 2014; Rivas Plata et al. 2014; Sparrius 2004; van den Boom et al. 2018; White et al. 2019), and the second example in the genus *Cora* (Lücking et al. 2015a).

In order to assess whether the Florida material indeed constitutes an undescribed entity, we obtained molecular data of the ITS from two historical herbarium specimens. We further examined almost all available collections to circumscribe this taxon phylogenetically and morphologically, and based on our results, we are recognizing it here as a new species under the name *Cora timucua*. We also present an assessment of the conservation status of this novel lichen.

Materials and Methods

Phenotype assessment. A total of 32 specimens labeled under the names *Cora glabrata*, *C. pavonia*, *Dictyonema glabratum*, and *D. pavonia*, and originating from Florida were studied in various herbaria or obtained on loan (DUKE, F, FH, FLAS, MICH, MIN, NY, SFSU, UC, US, YU). Specimens were studied either at the corresponding herbarium (DUKE, FH, NY) or at the Field Museum (F), the BGBM (B), and the US National Herbarium (US), employing a standardized protocol (Lücking et al. 2017b). For morpho-anatomical details, we used a ZEISS Axioskop compound microscope and a LEICA Zoom 2000 dissecting microscope. To assess the potential fresh color of the taxon, individual lobes of selected specimens were carefully rewetted (Lücking et al. 2017b).

Phenotype-based phylogenetic binning. After confirming that all material represented a phenotypically homogeneous unit, agreeing in all diagnostic characters, the scores for 11 selected phenotype characters, for substrate ecology, and for geographic distribution were analyzed against 87 known, sequenced and formally named species of *Cora* using the *Cora* PhyloKey 1.0 (Lücking et al. 2016). In this approach, a phenotypically defined unit (query taxon) is binned into a reference tree consisting of taxa for which both molecular and phenotype data are available, by first reconstructing the molecular phylogeny, then computing phenotype maximum likelihood character weights based on their distribution on the molecular phylogeny, and then binning the query taxon into the tree based on its phenotype affinity; the best match is thereby subjected to non-parametric bootstrapping (Berger et al. 2011; Lücking & Kalb 2018; Lücking et al. 2015b; Rivas Plata et al. 2012). For the molecular reference tree, we used an ITS alignment of representative sequences for each of the 87 reference taxa in Phylip format (**Supplementary File S1**), and a matrix of 11 phenotype characters plus substrate plus distribution (see Lücking et al. 2017b) for the 87 reference taxa and the query taxon, also in Phylip format (**Supplementary File S2**). The columns in the character matrix were thereby as follows: (1) substrate (1 = saxicolous, 2 = terricolous directly on soil, 3 = terrestrial between grasses or mosses, 4 = epiphytic); (2) thallus size (1 = small, 2 = intermediate, 3 = large), (3) sutures (0 = absent, 1 = short, 2 = distinct); (4) main surface color when fresh (1 = grey, 2 = brown, 3 = olive, 4 = green, 5 = bluish); (5) surface structure (0 = even, 1 = rugose, 2 = pitted, 3 = broadly undulate, 4 = narrowly undulate); (6) surface trichomes (0 = absent, 1 = felty, 2 = setose, 3 = strigose); (7) lobe margins (0 = glabrous, 1 = pilose, 2 = granulose, 3 = pilose-granular); (8) soredia (0 = absent, 1 = present); (9) cortex type (1 = viaduct, 2 = collapsed viaduct; 3 = compacted, 4 = prosoplectenchymatous, 5 = paraplectenchymatous); (10) lower medullary papillae (0 = absent, 1 = present); (11) hymenophore type (1 = adnate, 2 = concentric, 3 = concentric-cyphelloid, 4 = cyphelloid); (12) bleeding pigment in herbarium after rewetting (0 = absent, 1 = present); (13) Central America (0 = absent, 1 = present); (14) Caribbean (0 = absent, 1 = present); (15) Galapagos (0 = absent, 1 = present); (16) Northern Andes (0 = absent, 1 = present); (17) Central Andres (0 = absent, 1 = present); (18) Southern Andes to Patagonia (0 = absent, 1 = present); (19) Brazil (0 = absent, 1 = present); (20) Paleotropics (0 = absent, 1 = present). For the binning approach, we employed maximum likelihood weights as described in Berger et al. (2011). Binning was performed locally in RAxML 7.2.6 (Stamatakis 2006).

Molecular phylogenetic analysis. Based on an objective, quantitative assessment, the binning approach reveals taxa that are phenotypically congruent with the query taxon, but it cannot reliably reconstruct its true phylogenetic relationships. We therefore also sequenced the fungal ITS barcoding marker for two herbarium collections housed at US (Florida, *Rapp 581*, US-1034276, DNA extract US-244; Florida, *Thaxter s.n.*, US-1034207, DNA extract US-266). For both samples, we first employed Sanger sequencing, using the methods of DNA extraction, PCR, and sequence assembly described in Dal Forno et al. (2017), except that a different DNA extraction kit was used (PowerSoil-HTP 96 Well Soil DNA Isolation Kit, MO BIO, U.S.A.). Subsequently, the two samples were prepared for an NGS metabarcoding approach in a pooled library according to Caporaso et al. (2012) and the Earth Microbiome Project (http://www.earthmicrobiome.org), sequencing the ITS1 region. The dataset was processed in Qiime2 (Bolyen et al. 2018) utilizing the plug-ins import, demux, tools extract and DADA2 (Callahan et al. 2016). Analyses were run on the Smithsonian Institution High Performance Computing Cluster.

We obtained one full-length Sanger consensus sequence for the sample *Rapp 581* (collected in 1923), a total of 6,604 ITS1 Illumina reads (eight haplotypes; **Table 1**) for the same sample *Rapp 581* and 16,895 reads (four haplotypes; **Table 1**) for the sample *Thaxter s.n.* (likely collected before or around 1900). Using a previous global ITS alignment (Lücking et al. 2017b), the newly generated sequences (one per haplotype for the Illumina reads) were aligned with 651 ingroup sequences of *Cora* and two outgroup sequences of *Corella* (**Supplementary File S3**), to infer the global phylogenetic relationships of the query taxon. The best-scoring maximum likelihood tree was computed in RAxML 8.2.0 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010), using the RAxML-HPC BlackBox, with 402 bootstrap replicates (automated stop criterion), under the default GTR-Gamma model.

Based on the placement of the query sequence in the global phylogeny, we subsequently computed a partial phylogeny of the strongly supported (100%) containing clade, including 16 ingroup sequences representing five species, plus 13 query sequences. The best-scoring maximum likelihood tree for this clade was computed locally in RAxML 8.2.0 (Stamatakis 2014), with 1000 bootstrap replicates, under the default GTR-Gamma model.

Conservation assessment. We inferred approximate geocoordinates based on the label information. Subsequently, we mapped all known collections on a *Google Maps* satellite map of the northern Florida peninsula and added a layer

Table 1. Voucher information for newly generated ITS sequences used in this study. Accession numbers of other sequences used are indicated in the tree figures (Fig. 3, Supplementary File S5).

Isolate	Voucher	Technique/Haplotype	ITS accession
US-244	Florida, *Rapp 581*, US-1034276	Illumina/ASV01	MT908260
US-244	Florida, *Rapp 581*, US-1034276	llumina/ASV02	MT908261
US-244	Florida, *Rapp 581*, US-1034276	Illumina/ASV03	MT908262
US-244	Florida, *Rapp 581*, US-1034276	Illumina/ASV04	MT908263
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV01	MT908264
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV02	MT908265
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV03	MT908266
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV04	MT908267
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV05	MT908268
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV06	MT908269
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV07	MT908270
US-266	Florida, *Thaxter s.n.*, US-1034207	Illumina/ASV08	MT908271
US-266	Florida, *Thaxter s.n.*, US-1034207	Sanger	MT908272

showing the natural distribution of Florida sand pine scrub, based on Davis (1967), according to label and mapping data the preferred natural habitat of this species. According to Allen et al. (2019), the most important information to gather for a lichen IUCN assessment is the present and past distribution of the species. We therefore calculated the historical and current Area of Occupancy (AOO) and Extent of Occurrence (EOO) for the species using *GeoCat* (http://geocat.kew.org/editor). Potential current distribution of the species was based on documented land use changes over the past decades (Kautz 1998; Volk et al. 2017) and information regarding the protection status of geographical locations of herbarium specimens (e.g., National Parks vs. cities). We subsequently assessed the corresponding IUCN category for this species following the listed criteria.

Results and Discussion

Phenotype-based phylogenetic binning. The binning approach placed the query taxon with full support next to *Cora hymenocarpa* Lücking, Chaves & Lawrey (**Fig. 1**). Both agreed in substrate and in phenotype character states except the much smaller thallus (<5 cm *vs*. up to 20 cm across) and smaller lobe size (<4 cm *vs*. 5–15 cm broad) in the query taxon and the presence of a bleeding reddish brown pigment in herbarium specimens after rewetting (not observed in *C. hymenocarpa*). Given these differences and the different distribution (*C. hymenocarpa* is known from Costa Rica; Lücking et al. 2017), this result supported the notion that the Florida material represented a previously unrecognized taxon, not conspecific with any of the 92 formally described species.

Molecular phylogenetic analysis. The full-length Sanger consensus sequence obtained from *Rapp 581* (DNA extract US-244) contained two ambiguous sites marked W (IUPAC: A or T) in position 210 and Y (IUPAC: C or T) in position 237 (**Supplementary File S4**), the numbering starting from the ITS1F primer (CTTGGTCATTTAGAGGAAGTAA) utilized for both sequencing techniques. The eight ITS1 haplotypes obtained through Illumina sequencing for the same sample exhibited corresponding polymorphism (A vs. T, C vs. T) at the same sites (**Supplementary File S4**). In addition, autapomorphic substitutions were detected at five further sites in *Rapp 581* and at four sites in *Thaxter s.n.* (**Supplementary File S4**). Whether these single-base substitutions represent real intragenomic variation or sequencing errors is difficult to assess. The absence of any variation in the conserved 5.8S portion (58 constant sites) suggests that the remaining variation may be genuine: when dividing the ITS1 region into blocks of ten sites, the mean value of variable sites per block was found to be 0.7, i.e. 3.5 per five consecutive blocks. In contrast, the conserved 5.8S region had no variable sites over five consecutive blocks (**Supplementary File S4**). Given the observed variation, we computed pairwise sequence identity for the ITS1 region between all 13 variants (one Sanger consensus, 12 Illumina haplotypes). Mean pairwise identity was 98.8%, slightly above the UNITE species hypothesis thresh-

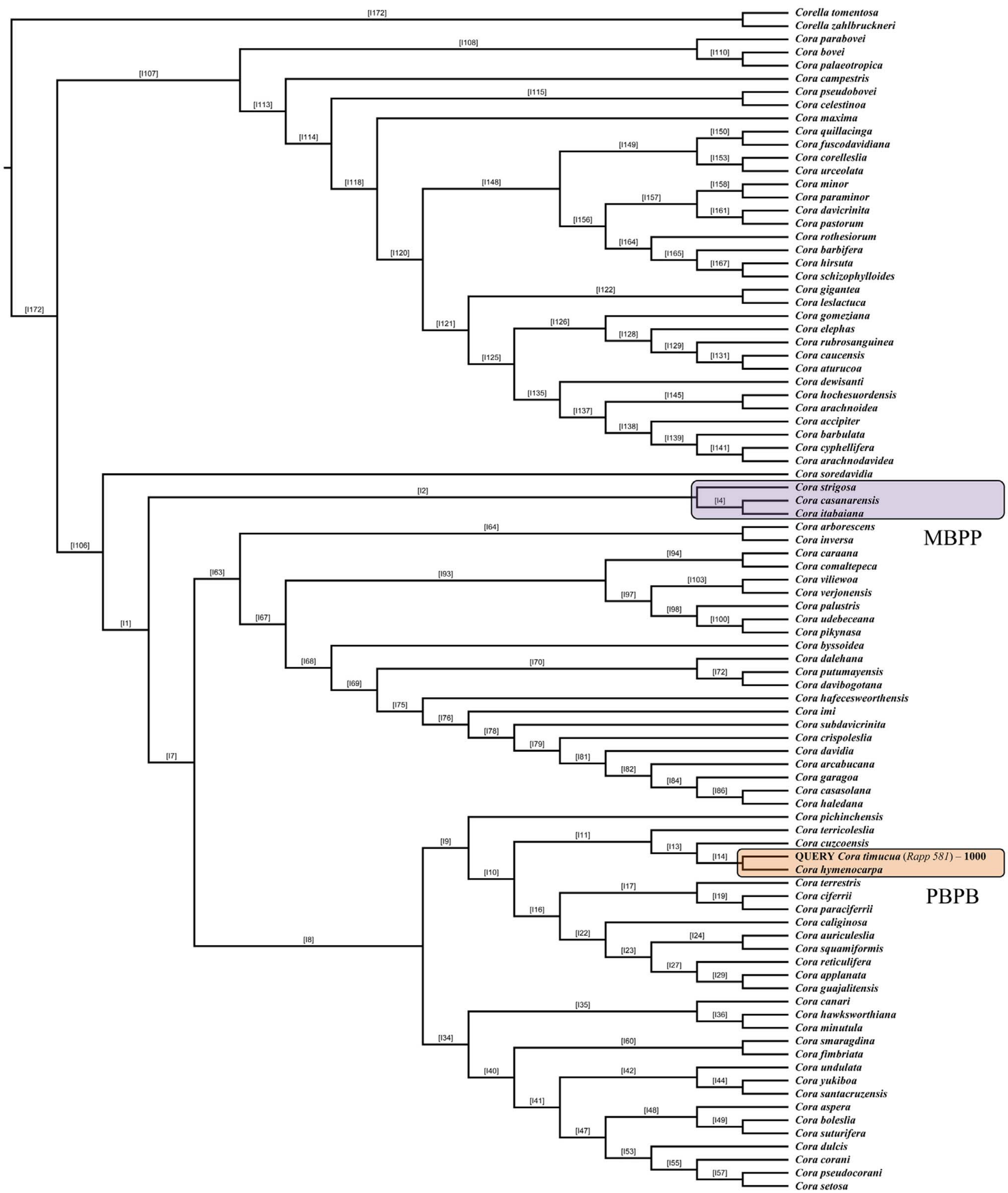

Figure 1. Classification tree for the query sample using phenotype-based phylogenetic binning (PBPB). The query sample clustered with 100% support (all 1,000 bootstrap replicates) with *Cora hymenocarpa* (highlighted in beige). Using molecular-based phylogenetic placement (MBPP), the species would fall in a clade including *C. strigosa*, *C. casanarensis*, and *C. itabaiana* (see Fig. 3, Supplementary File S4; highlighted in purple).

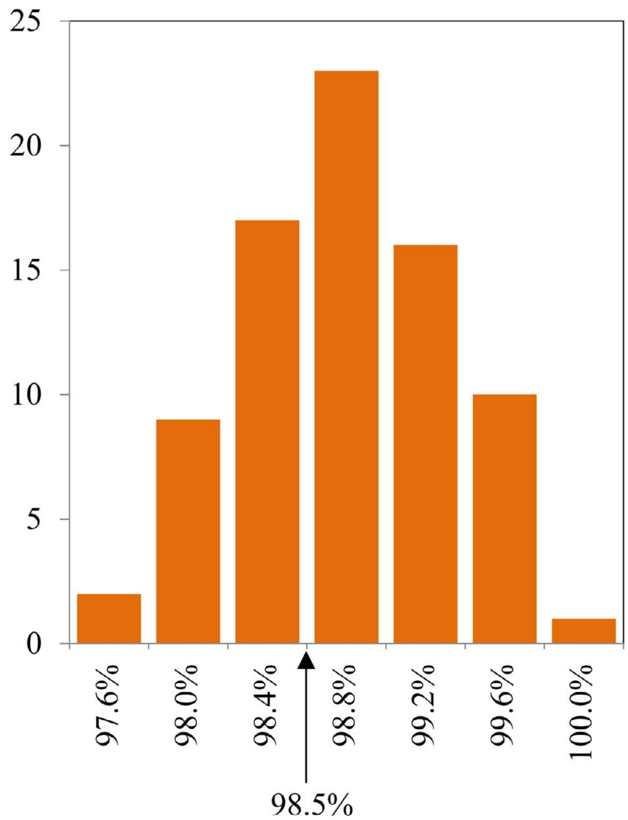

Figure 2. Proportion of sequence identities through pairwise comparison of the 13 ITS1 variants in the single Sanger consensus and the 12 Illumina haplotypes of two samples of *Cora timucua*.

old of 98.5% (Abarenkov et al. 2010), with a range between 97.6% and 100% (**Fig. 2**).

The global molecular phylogeny of *Cora* based on the ITS barcoding marker (**Supplementary File S5**) placed the query sequences with full support in a clade containing the species *C. hirsuta* (Moncada & Lücking) Moncada & Lücking (Colombia), *C. casanarensis* L.Y.Vargas (Colombia), Moncada & Lücking, *C. itabaiana* Dal-Forno, Aptroot & M.Cáceres (Brazil), and two as yet undescribed species (also from Brazil). Analysis of the supported containing clade resulted in the same ingroup topology (**Fig. 3**), with the query sequences recovered as strongly supported sister (99%) to a clade containing the two species *C. casanarensis* and *C. itabaiana*. Compared to *C. casanarensis*, *C. timucua* exhibits 22 substitutions and 16 indels out of 723 positions in the ITS, resulting in a BLAST identity of 94.7%, strongly supporting the phylogenetic distinction of these two sister taxa. The phenotypically best matching species, *C. hymenocarpa*, was not closely related and appeared in a different portion of the global phylogeny (**Supplementary File S5**), illustrating a surprising degree of parallel phenotypic evolution.

The 12 Illumina haplotypes clustered with strong support (94%) together with the Sanger consensus sequence in a single clade (**Fig. 3**). The internal topology of this clade, caused by stochastic point variation and two polymorphic sites (see above), was not supported. Thus, in contrast to clustering algorithms potentially suggesting the presence of four OTUs (see above), alignment-based phylogenetic analysis recovered a single species. This finding is in line with analysis of intragenomic and intrathalline variation in another species of the genus *Cora*, *C. inversa* Lücking & Moncada (Lücking et al. 2014b), representing the second report for *Cora* where very low intragenomic and/or intrathalline ITS variation has been demonstrated. The rather homogeneous ITS in species of this genus, together with the high level of resolution and support in both the terminal clades and the backbone, and the strong correlation between lineages delimited by ITS and their phenotype features (Lücking et al. 2017b), underlines the utility of the fungal ITS barcoding marker to document species diversity in the genus *Cora*.

Successful sequencing of the ITS of two historical specimens, one likely collected before 1900 and the second in 1923, using two different methods yielding congruent results, was surprising. Sanger sequencing has been previously employed to obtain molecular data from herbarium collections of lichens as old as 151 years, but for more or less crustose species growing on rock and/or in (semi-)arid climates (Redchenko et al. 2012; Sohrabi et al. 2010), which are more likely to retain intact DNA over prolonged times in the herbarium. Next-gen sequencing has been applied to herbarium samples of various taxa, including macrolichens, but of more recent ages (Gueidan et al. 2019; Leavitt et al. 2019). To our knowledge, the two specimens of *Cora* that we sequenced, one 96 years old and the other likely over 120 years old, are the oldest epiphytic macrolichen herbarium samples successfully sequenced using either Sanger or NGS techniques.

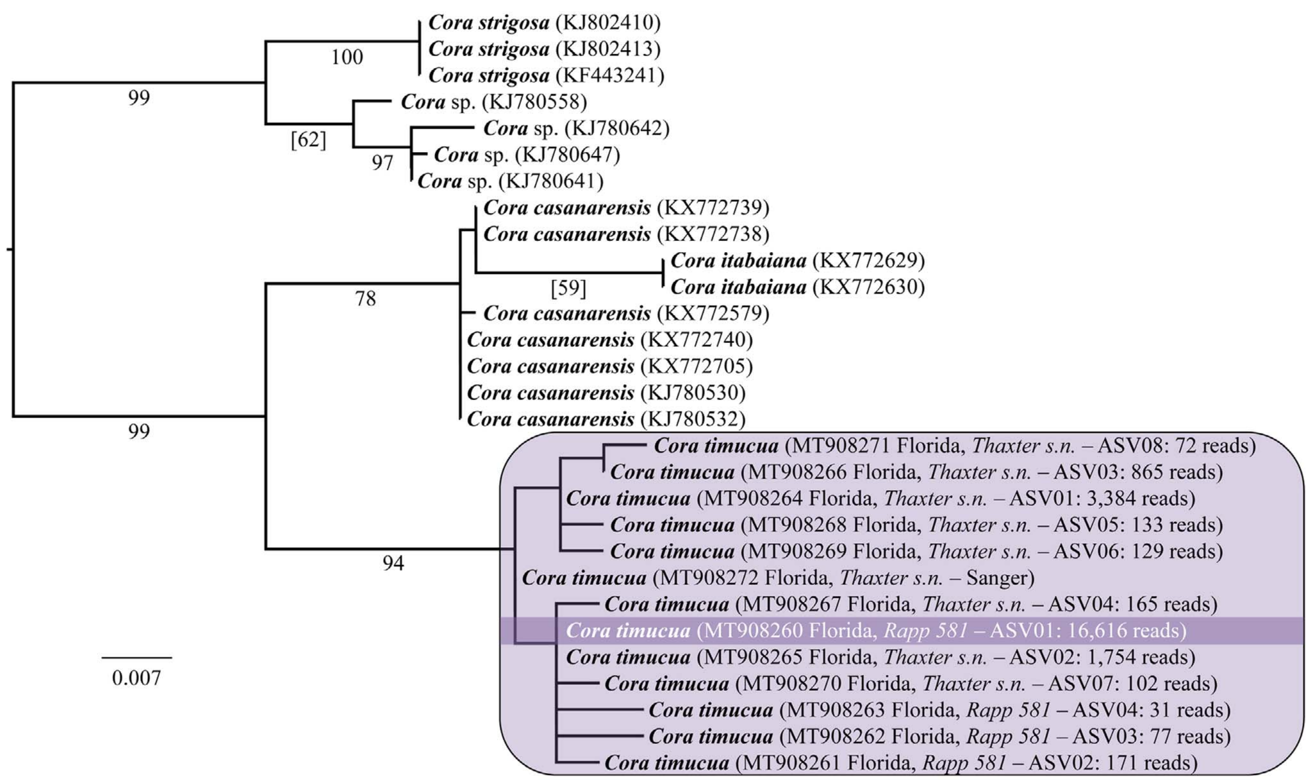

Figure 3. Best-scoring maximum likelihood tree of the clade including the query taxon, based on the fungal ITS barcoding marker (for the underlying global phylogeny, see Supplementary File S5). The most frequent haplotype corresponding to the holotype is highlighted.

Taxonomy

Cora timucua Dal Forno, Kaminsky & Lücking, *sp. nov.* **Fig. 4A–O**

MycoBank MB 836688

Similar to Cora hymenocarpa, with distinct sutures, a completely adnate, initially spot-like but soon confluent hymenophore and lower medullary papillae; differing from the latter in the smaller thalli and lobes and in the reddish brown pigment bleeding from rewetted herbarium specimens; differing from the closely related C. casanarensis in substrate (epiphytic vs. rock-dwelling) and color of hydrated thalli (aeruginous vs. olive-grey to grey-brown), and from the closely related C. itabaiana in the larger thallus and lobe size, the color of hydrated thalli (aeruginous vs. green-grey) and the presence of lower medullary papillae, and from both also in the bleeding pigment.

Type: U.S.A. Florida: [Seminole County], Oviedo; [28°40′N, 81°12′W], [15 m]; without data on habitat and substrate; May 1923, *S. Rapp 581* (us-1034276!, holotype; fh-00394661!, isotype).

ITS barcoding sequence: MT908260 (Florida, *Rapp 581*, us-1034276; holotype; Illumina); MT908272 (Florida, *Thaxter s.n.*, us-1034207; paratype; Sanger).

Description. Thallus epiphytic on stems, branches and twigs of shrubs, foliose, 2.5–7 cm across, composed of (1–)3–5 semicircular, adjacent to subimbricate lobes; individual lobes 1–3(–4) cm wide and 1–3 cm long, moderately to frequently branched, with long, radial branching sutures, surface aeruginous green when rewetted, with slight concentric color zonation, olive-green to olive-brown towards or along the margins, with thin, involute, grey to grey-green or grey-brown margins, becoming grey in the herbarium; margins upon rewetting often yellowish to reddish brown and bleeding a reddish brown pigment. Upper surface shallowly and broadly undulate when rewetted, undulate-rugose when dry, glabrous; involute margins glabrous but becoming pilose near the sutures; lower surface ecorticate, felty-arachnoid (representing the exposed medulla), green-grey when rewetted, becoming light grey in the herbarium. Thallus

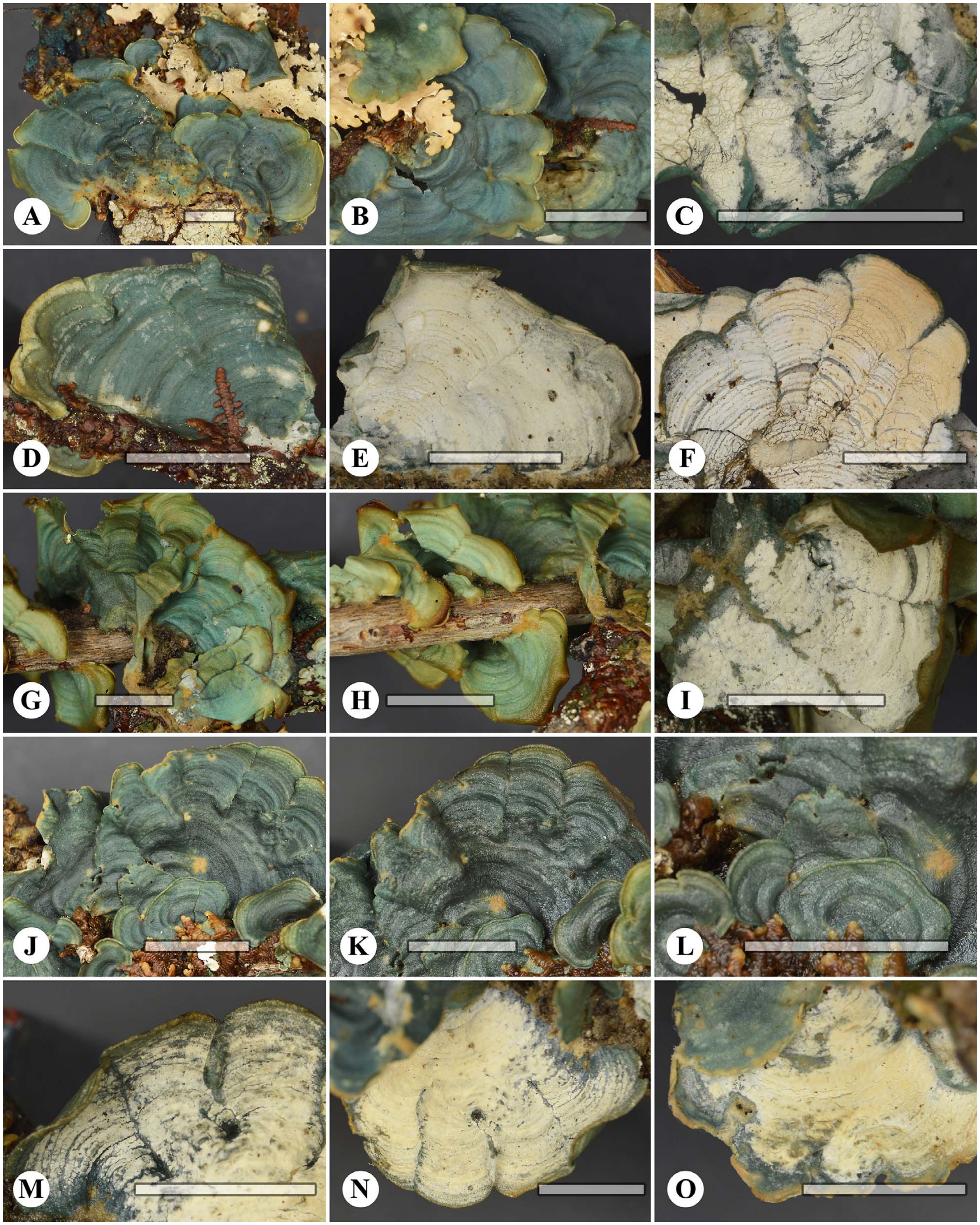

Figure 4. Habit of *Cora timucua*, showing upper and lower surface of rewetted thalli from herbarium collections. **A–C.** Isotype, *S. Rapp 581* (FH-00394661). **D–E.** *S. Rapp 581* (FH-00394662). **F.** *True s.n.* (FH-00394658). **G–I.** *R. Thaxter s.n.* (FH-00394660). **J–K.** *D. G. Griffin III 100* (UC-1406931)]. Scale = 10 mm.

in section 200–250 µm thick, with upper cortex, photobiont layer, and medulla; upper cortex distinctly viaduct-shaped, formed by a 20–30 µm thick layer of densely packed, periclinal, 4–6 µm thick hyphae supported by a 30–50 µm high 'medullary' layer of spaced groups of more or less anticlinal, 4–6 µm thick hyphae; photobiont layer 60–100 µm thick, aeruginous; medulla 30–50 µm thick, hydrophobic; clamp connections absent, but lower medullary hyphae with numerous short, unbranched to shortly coralloid, straight to hooked, papilliform hyphae. Hymenophore developed as initially rounded to irregular, resupinate, completely adnate, emarginate patches soon becoming confluent and covering the entire lobe underside, then usually with irregular to concentric cracks, patches at first 0.5–1 mm diam. before becoming confluent, with whitish to cream-colored, smooth surface and indistinct margins; hymenophore in section 70–80 µm thick, resting on a dense layer of medullary hyphae, strongly hydrophobic; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles 20–30 $\times$ 4–6 µm; basidia 25–35 $\times$ 4–6 µm, 4-sterigmate; basidiospores not observed.

Chemistry. No substances detected by TLC, but lobe margins upon rewetting bleeding with a reddish brown pigment.

Etymology. The epithet is a noun in apposition, referring to the Native American Timucua people, which occupied a large territory covering the northern Florida peninsula and southeasternmost Georgia. With an estimated population of approximately 200,000 at the onset of European colonization, the Timucua became extinct towards the second half of the 18th century due to infectious diseases brought from Eurasia, warfare and the slave trade (Ehrmann 1940; Hann 1996; Milanich 1996; Stojanowski 2005). We selected this epithet to symbolize the problem of extinction of natural habitats, species, and indigenous ethnicities as result of the modern colonization of the Americas.

Distribution and ecology. The new species is presumably endemic to the southeastern United States (Florida). According to label information, which frequently mentions "white sand scrub" as habitat, the reported phorophytes, and the reconstructed distribution of known specimens (see below), the species was chiefly collected as epiphyte on shrubs in the Florida (sand pine) scrub vegetation, specifically Florida peninsula inland scrub and oak-dominated hardwood forest, at an elevation range of 90–120 ft (about 25–35 m). Identified phorophytes were mostly *Lyonia ferruginea* (Walter) Nutt. (rusty staggerbush) and *Quercus virginiana* Mill. (southern live oak), and thalli of this lichen were found associated with bryophytes and other lichens, including *Coccocarpia erythroxyli* (Spreng.) Swinscow & Krog, *Dictyonema* spp., *Leptogium* spp., *Normandina pulchella* (Borrer) Nyl., *Sticta beauvoisii* Delise, and various parmelioid taxa including *Parmotrema reticulatum* (Taylor) M.Choisy. Given the number of historical collections, it is difficult to ascertain whether this species was (or is) genuinely rare and was relatively frequently collected due to its unique morphology, or once was more common (see below).

Remarks. The material here recognized as a new species, *Cora timucua*, had previously been identified with the names *C. glabrata*, *C. pavonia*, *Dictyonema glabratum*, or *D. pavonium* (Esslinger 2019; Hale & Culberson 1956, 1970; Parmasto 1978). The earliest collections date to 1885 and the species was accepted, as *C. pavonia*, in Fink's (1935) *The Lichen Flora of the United States* (listed for Florida) and in the first checklist of North American lichens (Hale & Culberson 1956). However, in the two subsequent editions of the checklists it was excluded, given as "... *apparently not in our area* ..." (Hale & Culberson 1960: 167; Hale & Culberson 1966: 173), without any explanation. Moore (1970) confirmed the presence of the species, as *C. pavonia*, in Florida and it was then again listed for North America in the fourth checklist (Hale & Culberson 1970) and up to the most recent version (Esslinger 2019).

Given the findings of recent phylogenetic revisions of the genus *Cora* and the substantial level of previously unrecognized phenotypic and ecological divergence of species-level clades in this genus, together with the inferred high level of regional endemism (Lücking et al. 2014a, 2017), the finding that the North American material from Florida does not represent *C. pavonia* or *C. glabrata* is not surprising. Lücking et al. (2014b) argued that *C. pavonia* is likely a Caribbean endemic, thus far only known from Jamaica. Likewise, *C. glabrata* is considered a Caribbean endemic thus far only known from Guadeloupe (Lücking et al. 2013).

Morphologically and ecologically, *C. glabrata* differs in being terrestrial, the brownish color when hydrated, the thicker, revolute lobe margins and the absence of distinct branching sutures, and the more or less concentric, corticiform hymenophore composed of small, sessile, irregular to elongate patches with slightly upturned margins (Lücking et al. 2013). *Cora pavonia* is similar to *C. timucua* in size, but it grows over bryophytes mostly on rock and soil, its branching sutures are less conspicuous, the fresh color is more greyish green without a strong aeruginous tinge, and the hymenophore is similar to that of *C. glabrata*, forming concentric circles of small, irregular to elongate patches with slightly upturned margins (also based on topotype material recently collected by MDF; unpublished).

Compared to many other recently described species in the genus (Lücking et al. 2017b), a rather large number of collections is available for this taxon. The morphology of these specimens is remarkably uniform, characterized by epiphytic growth, mid-sized thalli and lobes with distinct branching sutures, a strong aeruginous tinge of rehydrated thalli with a reddish brown, bleeding pigment, lower medullary papillae, and the adnate hymenophore, at first forming rounded, whitish patches similar to ascomata of *Cryptothecia* but then becoming confluent and covering the entire lower surface (**Fig. 4**). Therefore, even if only two collections have sequence data available, we conclude that a single species is present. Based on the above features, and adding the undulate lobes, the absence of soredia and trichomes, and the presence of a viaduct-like cortex, the binning analysis identified *Cora hymenocarpa* as closest morphological match to *C. timucua*. However, *C. hymenocarpa* forms much larger thalli (up to 20 cm across) with broader lobes (5–15 cm broad) and a bleeding reddish brown pigment upon rewetting was not observed in that species (Lücking et al. 2017). *Cora hymenocarpa* is only distantly related to *C. timucua*.

Another phenotypically similar, also distantly related species is *Cora galapagoensis* Dal-Forno, Bungartz & Lücking, which agrees in the branching sutures and anatomical details. However, like *C. hymenocarpa*, *C. galapagoensis* also forms much larger thalli with a much branched, partly imbricate lobes that take a grey-brown to olive-grey color when fresh, and its hymenophore is formed by well-delimited, concentrically arranged patches (Dal Forno et al. 2017).

Based on our ITS-derived phylogeny, *Cora timucua* is most closely related to *C. casanarensis* from Colombia and *C. itabaiana* from Brazil. The first is a rock-dwelling species that agrees with *C. timucua* in size and in the distinct branching sutures, but its fresh color is olive-grey to grey-brown, without any aeruginous tinge (Vargas et al. 2014). The epiphytic *C. itabaiana* is overall smaller than *C. timucua* and lacks an aeruginous tinge (green-grey when fresh), and it also lacks lower medullary papillae (Lücking et al. 2017). Overall, the distinctiveness of *C. timucua* is thus demonstrated both phylogenetically and phenotypically, and our data suggest that this is the only species present in Florida (and all of North America north of Mexico) and that is likely endemic to that region.

Additional specimens examined. U.S.A. FLORIDA: Citrus County, about 6 miles WNW of Inverness; [28°51′44″N, 82°27′45″W], [33 m]; March 1914, *R.M. Harper 203* (NY-1302987!; NY-1302988!). Clay County, Green Cove Springs; [29°59′34″N, 81°40′43″W], [7 m]; February 1885, *G. Martin 10* (F-C0370560F!). Columbia County, O'Leno State Park; [29°55′09″N, 82°35′47″W], 120 ft [37 m]; white sand scrub, on branches of hardwoods; March 1969, *D.G. Griffin III s.n.* (FLAS-L162!); ibid.; [29°55′09″N, 82°35′47″W], 100 ft [30 m]; white sand scrub community, on branches of *Quercus virginiana*; 7 June 1970, *D.G. Griffin III 100* (FLAS-L161!, UC-1406931!); ibid., 20 mi S of Lake City on Hwy 41; [29°55′09″N, 82°35′47″W], [17 m]; deciduous forest, on deciduous tree; 1972, *J.P. Dey 6852* (NY-1750064!); ibid., NW of High Springs; [29°50′48″N, 82°37′36″W], 100 ft [30 m]; white sand scrub, on branches of *Lyonia ferruginea* and *Quercus virginiana*; July 1973, *N. Faria s.n.* (FLAS-L847!, FLAS-L849!). [Lake County], Eustis; [28°51′13″N, 81°41′07″W], [20 m]; on wood; *R. Thaxter s.n.* (US-1034207!); ibid.; on scrub oak; s.dat., *R. Thaxter s.n.* [hb. Fink 11851] (MICH-102758!); ibid.; October 1899, *R. Thaxter s.n.* (FH-00394663!); ibid.; s.dat., *R. Thaxter 532* (NYSFSU-L-004236), *R. Thaxter s.n.* (MICH-102761!). Marion County, Ocala National Forest, near Juniper Springs; [29°12′26″N, 81°44′23″W], [20 m]; white sand scrub, on oak twigs; December 1968, *B. Moore 6235* (DUKE-0222621!); ibid., north of 40-19 intersection, southeast of river bridge; [29°11′26″N, 81°38′27″W], 90 ft [27 m]; white sand scrub; July 1973, *K. Kendall s.n.* (FLAS-L846!); ibid.; on branches of *Lyonia ferruginea*; July 1973, *N.H. Can s.n.* (FLAS-L848!); ibid.; on branches; July 1973, *C.C. Tu s.n.* (FLAS-L850!); ibid., between Eureka and Salt Springs, just E of Ocklawaha River; [29°21′52″N, 81°51′38″W], [33 m]; August 1985, *Egan 13017* (MIN-941405!). [Seminole County], Oviedo; [28°40′07″N, 81°12′28″W], [14 m]; on *Andromeda*; April 1921, *S. Rapp s.n.* (FH-00394664!); ibid.; 19 November 1922, *S. Rapp 581 p.p.* (FH-00394662!); ibid.; May 1923, *S. Rapp 581 p.p.* (FH-00394661!, US-1034276!); ibid.; on branches; March 1927, *S. Rapp s.n.* (FLAS-L8151!). Sanford; [28°48′17″N, 81°16′07″W], [9 m]; on shrubs; November 1918, *S. Rapp s.n.* (FLAS-L8150!); ibid.; September 1922, *S. Rapp s.n.* (YU-240846!); ibid.; in bush, on shrubs; April 1929, *S. Rapp 581* (YU-240845!). Vicinity of Sanford; s.dat., *S. Rapp s.n.* (BALT-L-0004660, not seen; BALT-L-0004662, not seen). [Volusia County], Orange City, Blue Spring [since 1972 Blue Spring State Park]; [28°57′N, 81°20′W]; October 1909, *True s.n.* (FH-00394658!). Near Orange City junction; s.dat., *True s.n.* [hb. Fink 12815] (MICH-102759!). Unknown locality; 1897–1898, *R. Thaxter s.n.* [hb. Fink 7271] (FH-00394660!). Unknown locality; unknown date; *R. Thaxter s.n.* (FH-00394659!). Unknown locality; unknown date; *Underwood & Griggs s.n.* [hb. Fink 11722] (MICH-102760!).

Conservation assessment

The rather rich available material of *Cora timucua* was collected over a period of exactly 100 years between 1885 (*G. Martin 10*, F) and 1985 (*Egan 13017*, MIN). Eight collections originate from the 19th century, thirteen from the early 20th century (1909–1929), and nine from the second half of the 20th century (1968–1985) with a total of 32 specimens studied (including some duplicates). Apparently, the species has not been re-collected after 1985 and there are no records in *iNaturalist* and *The Mushroom Observer* (see above). All collections from 1968 onwards originate from two sites, O'Leno State Park in Columbia County and Ocala National Forest in Marion County (**Fig. 5**). This provided an excellent base to assess the conservation status of this species based on herbarium specimens only (Nualart et al. 2017).

Florida is the state with the highest proportion of land conversion through urban development within the continental United States (Bueno et al. 1995; Chimney & Goforth 2001; Milesi et al. 2003; Volk et al. 2017; Xian et al. 2007). About 75% of urban development occurred since the 1950s after World War II, and currently urban areas make up 16% of the total state area (Kautz 1998; Volk et al. 2017). Together with other forms of land conversion, such as tree plantations, pasture and agriculture, over 50% of the state area has been converted, whereas only about 5% remain as mixed hardwood-coniferous and upland hardwood forest and hammock (Volk et al. 2017). As outlined above, the principal habitat of *Cora timucua* is Florida (sand pine) scrub, in particular Florida peninsula inland scrub and *Quercus*-dominated hardwood forest, also known as mesic hammock, which largely fits the map overlay of this habitat with the known collection sites (**Fig. 5**). However, without more precise quantitative data, this ecological classification is somewhat tentative. The lack of more precise locality and plant community data particularly for the historical specimens collected around the turn of the 19th century make relocating this species difficult, because the identified phorophytes can be found in multiple scrub communities ranging from sand pine-dominated to *Quercus*/*Lyonia* forest with little or no pine.

The fact that this species has not been re-collected in the past 35 years indicates that it may be extinct or at the very least strongly reduced in abundance and range, given the substantial level of land use change in the area and the observation that other epiphytic species of *Cora* are usually confined to undisturbed habitats (Lücking et al. 2017). "The Villages", located inside the range of *C. timucua*, is the fastest growing residential area in the United States (Bartling 2008; Simpson 2016; Stebbins 2019). Sumter, Osceola and St. Johns Counties, which are also in or adjacent to the range of *C. timucua*, represent three of the ten counties with the fastest growing populations in the United States (Crossett et al. 2004). As a result, most of the historical localities in the periphery of the inferred original range (**Fig. 5**; outer polygon) have been converted, with the exception of O'Leno State Park, which was established in the 1930s, making it one of the oldest state parks in Florida. Ocala National Forest (**Fig. 5**; inner polygon) appears to retain the only substantial

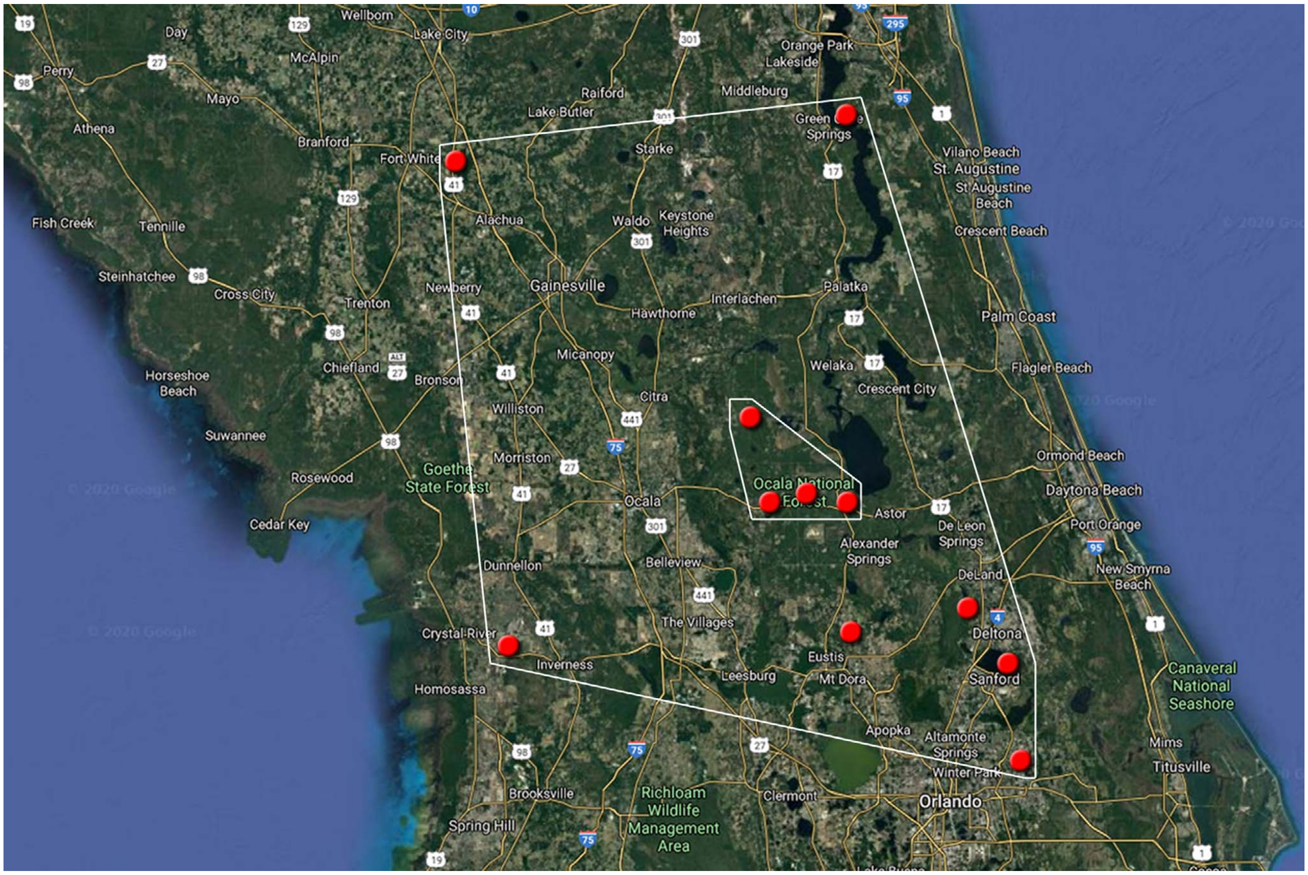

Figure 5. Origin of historical collections of *Cora timucua* gathered between 1885 and 1985 with inferred geographical coordinates based on label data, overlaid on a satellite map image (*Google Maps*) of the northern portion of the Florida peninsula. The outer polygon indicates the inferred historical extent of occurrence based on all known collections; the inner polygon estimates the potential extent of occurrence of extant populations.

area of the presumed preferred habitat of this species. O'Leno and Ocala are the only localities from which the species has been reported in the second half of the 20th century. Both sites harbor rare macrolichens not collected elsewhere in the Florida Peninsula since the year 2000, including *Tuckermanella fendleri* (Nyl.) Essl. (Ocala) and *Crocodia aurata* (Ach.) Link (O'Leno & Ocala) (DeBolt et al. 2007; Kaminsky 2011).

The Florida scrub ecosystem as a whole is listed as Critical/Endangered, with 85–90% of its original extension converted into *Citrus* groves and urban areas (https://www.worldwildlife.org/ecoregions/na0513; Myers 1990). Logging practices and silviculture with tree species not native to the ecosystem represent another threat, particularly if implemented as surrogate of natural fire succession (Greenberg et al. 1994, 1995; Means et al. 1996; Menges 2007; Weekley et al. 2008, 2013). Even land under conservation may be threatened by controlled burns with varying intensity and frequency of fire, affecting lichen recovery in terms of diversity and composition (Pharo et al. 2001), including in critical habitats such as grasslands (Johansson & Reich 2005) and in Florida rosemary scrub (Yahr 2000). Degradation of natural ecosystems can also shift plant community composition. *Quercus* will replace pine under disturbance and convert sand pine scrub into mesic hardwood forest (Abrams 1992). While *Cora timucua* has also been collected on *Quercus*, the quality of its natural habitat may be in decline due to such disturbance effects.

The historically documented Extent of Occupancy (EOO) of *Cora timucua* was 14,059.639 km^2, while the potential current EOO was 73.277 km^2 and the Area of Occupancy (AOO) 44 km^2 and 12 km^2, respectively. The potential current areas were based on the assumption that the species has indeed survived in Ocala National Forest, which largely corresponded to the extent of Florida peninsula inland scrub preserved in that area (Myers 1990). These data would place the species under IUCN Criteria B1, category CR (critically endangered), based on EOO, and IUCN Criteria B2, EN (endangered), based on AOO. However, none of the additional criteria required for assessments under Criteria B (a) (severely fragmented) and (b) (continuing decline) of this category [criterion (c) did not apply] could be assessed, since not all localities have been revisited. However, it is noteworthy that this species has not been reported in more recent surveys of Florida (DeBolt et al. 2007; Kaminsky 2011), and targeted attempts by LK and colleagues to relocate the taxon at O'Leno State Park along Santa Fe River (*Quercus*/*Lyonia* forest) and off the Dogwood Trail (old growth mixed pine/ *Quercus* forest) (Rosentreter et al. 2020) were unsuccessful. We therefore propose *Cora timucua* to be listed under IUCN Criteria D, critically endangered (CR), based on very small or restricted inferred population size. This genus of basidiolichens, *Cora*, is generally easily observed and frequently collected given its peculiar thalli, so even if this species still existed in nature, the available data and our recent attempts to locate it indicate that it is now extremely rare.

Our current study highlights the critical conservation value of the Florida sand pine scrub and other similar habitats that are rapidly disappearing through human activities. The Florida perforate reindeer lichen, *Cladonia perforata* A.Evans, is another Florida endemic species restricted to Florida scrub, although it occurs in a different, coastal subtype, rosemary bald scrub. Since 1993, it is listed as a federally endangered (EN) species (Teague & Riplay 2000; White 1994; Yahr 2000, 2003). Based on our present knowledge, *Cora timucua* is the only species in the genus to have inhabited the United States and is, at best, endangered, and at worst, extinct. It compares to the recently described *Dirina calcicola* Sparrius, now placed in *Fulvophyton* as *F. calcicola* (Sparrius) Tehler & Ertz, which was based on two Florida collections made by Roland Thaxter from 1898 and has apparently not been recollected since (Sparrius 2004; Tehler et al. 2013).

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Supplementary documents online:

Supplementary File S1. ITS alignment of the reference taxa for the binning analysis.

Supplementary File S2. Character matrix for the reference taxa and the query taxon for the binning analysis.

Supplementary File S3. Global ITS alignment of the genus *Cora* including one target sequence for *C. timucua* (US-266, Thaxter s.n.).

Supplementary File S4. Local ITS alignment of the *Cora casanarensis* clade including the target species *C. timucua*.

Supplementary File S5. Best-scoring maximum likelihood global ITS tree of the genus *Cora* including one target sequence for *C. timucua* (US-266, Thaxter s.n.).