Two new species of *Roccellaceae* (Ascomycota: *Arthoniales*) from Brazil, with the description of the new genus *Sergipea*

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Abstract: The new lichen genus Sergipea M. Cáceres, Ertz & Aptroot is described in the Roccellaceae, based on the new species Sergipea aurata M. Cáceres, Ertz & Aptroot from NE Brazil. The species was found in a remnant of Atlantic transition forest in Sergipe. It is similar in many respects to species of the genus Enterographa, but it is characterized by bright orange stromata, due to the presence of an anthraquinone, and a thallus with a somewhat byssoid hypothallus. Phylogenetically it is close to the genera Dichosporidium and Erythrodecton. The phylogenetic position of the generic type of Dichosporidium confirms the close relationship of the genus to Erythrodecton in the basal branch of the Roccellaceae. A new species of Enterographa is also described from NE Brazil. Enterographa rotundata E. L. Lima, M. Cáceres & Aptroot has solitary, round apothecia, which is unusual in this genus with mainly elongated apothecia or punctiform apothecia arranged in lines. It was found in Caatinga forest in Pernambuco.

Key words: Atlantic rainforest, Catinga, corticolous, Dichosporidium, Enterographa, Erythrodecton, lichens, Pernambuco, Sergipe

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Introduction

The Roccellaceae are a predominantly tropical group of lichens. The basal branch in the phylogeny contains the core group of Enterographa [including its type, E. crassa (DC.) Fée], as well as Erythrodecton (Ertz & Tehler 2011). The genus Enterographa Fée is a predominantly tropical group of crustose Roccellaceae comprising c. 50 species (Sparrius 2004; Ertz et al. 2005; Sparrius et al. 2006; Sparrius & Aptroot 2007; Jagadeesh Ram et al. 2008; Lücking 2008; Sparrius & Björk 2008; Ertz 2009a, b; Seaward & Aptroot

2009; Yeshitela et al. 2009; Sipman 2011). Phylogenetic studies have shown the genus to be polyphyletic (Ertz et al. 2009), and two species that were accepted in the genus by Sparrius (2004) were shown to fall into a different clade than the type species of Enterographa, E. crassa (DC.) Fée. These were transferred to the genera Opegrapha Ach. (Ertz et al. 2009) and Fulvophyton Ertz & Tehler (Ertz & Tehler 2011). It is likely that the many species remaining in the genus still do not form a monophyletic group.

The Atlantic rainforests and the Caatinga forests in NE Brazil are rich in lichen species but still incompletely explored (Cáceres 2007; Menezes et al. 2011; Lima et al. 2013). The Arthoniales, including the Roccellaceae, is one of the three dominant crustose lichen groups on bark in NE Brazil, and crustose Roccellaceae are common and sometimes abundant (Cáceres et al. 2007, 2008).

During ecological studies focusing on epiphytic crustose lichens in NE Brazil, an *Enterographa*-like lichen was found with

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Table 1. Specimens and DNA sequences used in this study, with their respective voucher information. GenBank accession numbers **in bold** refer to sequences (3) generated by this project. All other sequences (17 GenBank accession numbers) were obtained directly from GenBank.

Name	Voucher	Substratum	mtSSU GenBank number
Chiodecton natalense	Zambia, D. Ertz 6576 (BR)	bark	EU704051
Dendrographa leucophaea	California, L. Sparrius 7999 (DUKE)	bark	AY548811
Dichosporidium boschianum	Fiji, T. Lumbsch 19815a (F))	GU327692
D. nigrocinctum 1	Guadeloupe, D. Ertz 15377 (BR)	bark	KC820664
D. nigrocinctum 2	Guadeloupe, D. Ertz 15923 (BR)	bark	KC820665
Enterographa crassa 1	France, D. Ertz 5041 (BR)	bark	EU704056
E. crassa 2	France, D. Ertz 7554 (BR)	bark	EU747080
E. crassa 3	Belgium, D. Ertz 7561 (BR)	bark	EU747081
E. crassa 4	Luxembourg; D. Ertz 7621 (BR)	bark	EU747082
E. hutchinsiae 1	Belgium, D. Ertz 10064 (BR)	rock	EU747083
E. hutchinsiae 2	Belgium, D. Ertz 10066 (BR)	bark	EU704057
E. zonata 1	Belgium, N. Vigneron 104 (BR)	bark	EU704081
E. zonata 2	Belgium, D. Ertz 9230 (BR)	rock	EU747084
Erythrodecton granulatum	Gabon, D. Ertz 9908 (BR)	bark	EU704058
Lecanactis abietina	Belgium, D. Ertz 5068 (DUKE)	bark	AY548813
Opegrapha rufescens	Belgium, N. Vigneron 75 (BR)	bark	EU704074
O. vulgata	Belgium, D. Ertz 7564 (BR)	bark	EU704080
Roccella fuciformis	Tenerife, P. Diederich 15572 (DUKE)	rock	EU704082
Schismatomma pericleum	A. Tehler 7701 (S)	bark	AY571390
Sergipea aurata	Brazil, M. E. S. Cáceres & K. A. Jesus 12539 (ISE)	bark	KC820666

bright orange stromata, due to the presence of an anthraquinone. It was found in a remnant of Atlantic transition forest in Sergipe. It was clear that it was an undescribed species, but as this would be the first Enterographa with anthraguinones, it was sequenced to ascertain that it really belongs to the genus Enterographa. However, it clustered with strong support in the sister group of the well-supported core group of Enterographa, together with species of Dichosporidium and Erythrodecton. Although it shares some characters with Erythrodecton in particular (the presence of an anthraquinone), it differs in several respects from both genera, markedly by the firm, not byssoid thallus, the absence of ascoma carbonization, and the ascospore type. Therefore, this new species is described here in the new genus Sergipea.

Also, a new species of the genus *Enterographa* was found as part of the ecological studies. *Enterographa rotundata* has solitary round apothecia, which is unusual in this genus with mainly elongated apothecia or puncti-

form apothecia arranged in lines. It was found in the Vale do Catimbau National Park, a Caatinga forest reserve, in Pernambuco State.

Material and Methods

Identification and descriptive work was carried out in Itabaiana, Universidade Federal de Sergipe, using a Leica EZ4 stereomicroscope and a Leica DM500 compound microscope, and also in Soest using an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contrast, connected to a Nikon Coolpix digital camera. Sections were mounted in tap water, on which all measurements were also taken. The specimens from this study are preserved in ISE. The chemistry of the type specimens was investigated by thin-layer chromatography (TLC) using solvent A (Orange et al. 2001).

The mtSSU of the holotype of Sergipea aurata and of two specimens of Dichosporidium nigrocinctum (Ehrenb.) G. Thor were sequenced, using primers mrSSU1 and mrSSU3R (Zoller et al. 1999) and following the procedures cited by Ertz et al. (2009). For the phylogenetic analyses, 17 sequences were retrieved from GenBank in addition to our own sequences (Table 1). The outgroup species, Opegrapha vulgata Ach., was chosen based on Ertz & Tehler (2011).

The mtSSU sequences were aligned manually to the existing alignment of Ertz *et al.* (2009) using MacClade 4.05 (Maddison & Maddison 2002). Ambiguously aligned regions and introns representing a total of 727 bp (mainly due to an intron in the sequence of *Roccella fuciformis*) were delimited manually and excluded from the analyses.

Maximum Parsimony (MP) analysis was performed in PAUP*4.0b10 (Swofford 2002) on the mtSSU matrix of 735 unambiguously aligned characters, including 251 variable characters of which 195 are parsimony-informative. Heuristic searches were used with 1000 random addition sequence replicates, MaxTrees set to autoincrease, tree-bisection-reconnection (TBR) branch swapping, MulTrees option in effect and gaps treated as missing data. The MP analysis yielded four most parsimonious trees (448 steps) with topological differences present only in the clade *Lecanactis-Chiodecton*. Bootstrap values (MP-bs) were obtained from 1000 replicates with three random addition sequences (all other parameters identical to the original MP search).

The best-fit model of DNA evolution GTR+G was chosen using the Akaike Information Criterion (AIC), as implemented in Modeltest v. 3.06 (Posada & Crandall 1998). Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES portal (Miller et al. 2010). Analyses were run under the GTR model of nucleotide substitution, including a discrete gamma distribution with six rate categories. Two parallel MCMCMC runs were performed, each using four independent chains and 10 000 000 generations, sampling trees every 1000th generation. TRACER v.1.5 (Rambaut & Drummond 2007) was used to ensure that stationarity was reached by plotting the log-likelihood values of the sample points against generation time. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the last 15 002 of the 20 002 trees sampled.

The MP tree did not contradict the Bayesian tree topology for the strongly supported branches, and hence only the majority-rule consensus tree of the Bayesian analysis is shown here with the branch supports of the MP analysis. PP $\geq 95\%$ and MP-bs $\geq 70\%$ were considered to be significant.

The Species

Sergipea aurata M. Cáceres, Ertz & Aptroot gen. et sp. nov.

MycoBank No.: MB 803755 (genus) & 803756 (species)

Roccellaceae with non-carbonized ascomata immersed in stromata covered with orange anthraquinone, ascospores 7–9-septate, $35-40(-50)\times 5-6~\mu m$, narrowly clavate, rather thick-walled with elongated lumina, thallus with lichexanthone, stromata with anthraquinone.

Type: Brazil, Sergipe, Areia Branca, Fonte da Bica, on tree bark, c. 400 m alt., 19 May 2012, M. E. S. Cáceres & K. A. Jesus 12539 (ISE—holotype).

(Fig. 1)

Thallus crustose, cracked, not corticate, dull, pale greenish grey, very thin and closely following the bark surface, patchily interspersed with slightly byssoid hypothallus (visible only at high magnification), surrounded by a brown prothallus line. Algae trentepohlioid.

Ascomata not carbonized, c. 0·1 mm diam., globose, immersed and visible from above only by the ostiole, 5–25 clustered together in 1–2 mm wide, bright orange stromata. Excipulum hyaline, continuous below the hymenium. Epihymenium internally with pale brown pigmentation and externally with orange crystals. Hamathecium not inspersed, IKI+ blue (amyloid), paraphysoids 1·0–1·5 μm wide, anastomosing and often somewhat curled. Asci cylindrico-clavate, 73–89 × 21–27 μm. Ascospores 8 per ascus, hyaline, 7–9-septate, narrowly clavate, 35–40(–50) × 5–6 μm, without gelatinous sheath, rather thickwalled with elongated lumina.

Pycnidia not observed.

Chemistry. Thallus partly UV+ yellow, partly UV-, C-, P-, K-, stromata K+ purple. TLC: thallus with lichexanthone, stromata with an unidentified anthraquinone that resembles parietin in Rf-value and KOH-reaction.

Ecology and distribution. On smooth bark of trees in Caatinga forest. Known only from Brazil. It grows together with the equally endemic Enterographa subquassiaecola M. Cáceres & Lücking (Cáceres 2007), which is quite common in NE Brazil.

Discussion. As only one species is known in this genus, the description above refers to both the genus and the species (a so-called descriptio generico-specifica, ICBN Art. 38), as it would be impossible to discern generic and specific characters at this stage. The thallus and the internal structures, including the internal pigmentation, fit some species in the genus Enterographa well. It seems in habitus close to E. pertusarioides Sparrius (Sparrius 2004), which however lacks anthraquinones, contains psoromic acid and has 3-septate ascospores of a much smaller size. Sergipea

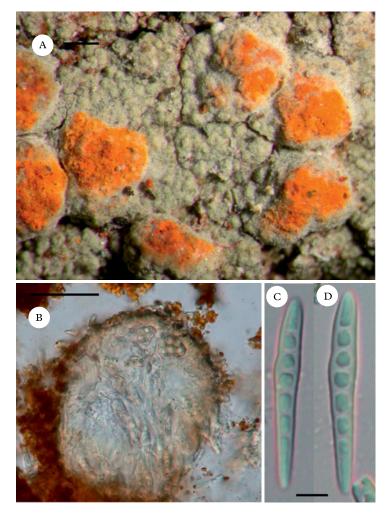


Fig. 1. Sergipea aurata, holotype. A, habitus; B, section through ascoma; C & D, ascospores. Scales: A = 0.5 mm; $B = 50 \mu m$; C & $D = 5 \mu m$. In colour online

aurata is a truly remarkable and unexpected species.

Additional specimen seen. **Brazil:** same as the type, M. E. S. Cáceres & K. A. Jesus 12540 (ISE).

Enterographa rotundata E. L. Lima, M. Cáceres & Aptroot sp. nov.

MycoBank No.: MB 802592

Corticolous *Enterographa* species with round apothecia, ascospores (3–)7-septate, $50-60\times3\cdot5-5\cdot0$ µm, without substances.

Type: Brazil, Pernambuco, Buíque, Vale do Catimbau National Park, on bark of tree, *c.* 900 m alt., 3 February 2012, *E. L. Lima* 668 (ISE—holotype).

(Fig. 2)

Thallus crustose, continuous but often seemingly absent, not corticate, dull, pale pinkish to pale brownish, very thin and closely following the bark surface, without prothallus. Algae trentepohlioid.

Ascomata round or slightly ellipsoidal, dark brown to black, 0.1-0.2 mm wide, margin raised above the disc, ochraceous

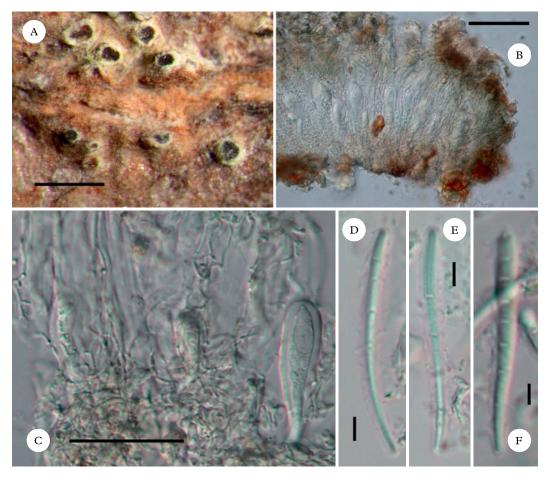


Fig. 2. Enterographa rotundata, holotype. A, habitus; B, section through ascoma; C, hamathecium and young ascus; D-F, ascospores. Scales: A = 0.5 mm, $B \& C = 50 \mu m$; $D-F = 5 \mu m$. In colour online

white, c. 0.05 mm wide. Excipulum hyaline to pale brownish. Epihymenium internally with pale brown pigmentation. Hamathecium not inspersed, IKI+ pale blue (weakly amyloid), paraphysoids 1.0-1.5 µm wide, anastomosing and often somewhat curled. Asci cylindrico-clavate, $65-79\times15-23$ µm, tholus amyloid. Ascospores 8 per ascus, hyaline, (3–)7-septate, narrowly clavate, $50-60\times3.5-5.0$ µm, surrounded by a 1.5-2.5 µm wide gelatinous sheath, lumina somewhat rounded.

Pvcnidia not observed.

Chemistry. Thallus and apothecia UV-, C-, P-, K-. TLC: no substances detected.

Ecology and distribution. On smooth bark of trees in Caatinga forest. Known only from Brazil. It grows together with, for example, Dirinaria leopoldii (Stein) D. D. Awasthi, Lecanora leprosa Fée, L. leproplaca Zahlbr., Pertusaria ventosa Malme, Phaeographis ventosa Redinger, and Ramboldia haematites (Fée) Kalb et al.

Discussion. One of very few Enterographa species with round apothecia and the third corticolous one with that character. Enterographa lecanoracea Sipman (Sipman 2011) differs by having larger ascomata (0·5–1·5 mm) that are sessile to nearly stalked, and a C+red thallus. Enterographa mesomela Sparrius

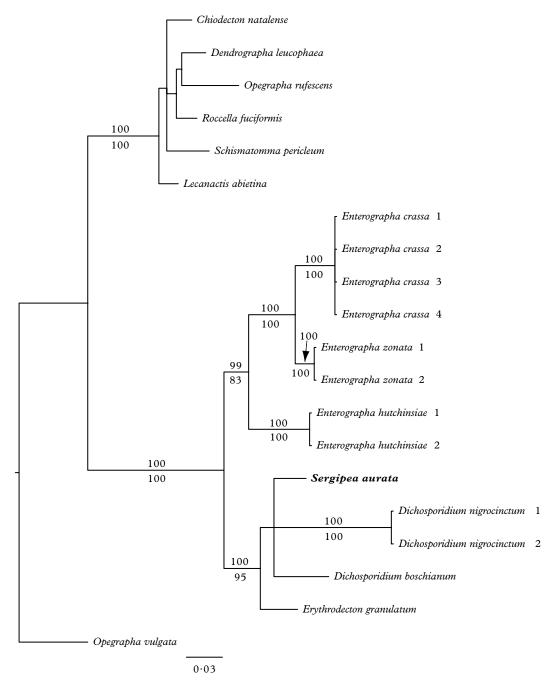


Fig. 3. One locus (mtSSU) 50% majority-rule consensus tree produced by the Bayesian analysis and representing the proposed phylogenetic relationships among 20 specimens of *Arthoniales*. $PP \ge 95\%$ and MP-bs values $\ge 70\%$ are considered strongly supported and shown respectively above and below internal branches. The new genus and species *Sergipea aurata* is noted in bold.

et al. is superficially similar to the new species but differs by a grey-green, slightly cracked to areolate thallus, slightly shorter ascospores (32-40 µm) and a different chemistry (confluentic acid in the thallus, and norstictic acid in the hypothecium; Sparrius et al. 2006). The morphs of E. anguinella (Nyl.) Redinger [now Opegrapha anguinella (Nyl.) Ertz & Diederich] that were described as E. lecanoroides R. C. Harris also have nearly round apothecia (Sparrius 2004), but they differ by nearly flush apothecia without a prominent margin and by a different chemistry (presence of psoromic acid). Three foliicolous species of Enterographa, viz. E. batistae Lücking & Sérus. (Lücking et al. 1998), E. byssoidea Lücking (Lücking 1991) and E. perez-higaredae Herrera-Campos & Lücking (Herrera-Campos & Lücking 2002), also have usually rounded apothecia (Lücking 2008). All differ by various characters from the new species, besides their foliicolous habitat, in each case also by very tiny ascomata which are smallest in E. batistae (c. 0.1mm diam.); E. byssoidea differs also by the byssoid apothecium margin and E. perezhigaredae by the presence of psoromic acid. A superficially similar species is *Lecano*grapha atropunctata Sparrius et al.; it differs from the new species by a thick dark brown to black excipulum and hypothecium, more septate ascospores (7-13-septate) and a different chemistry (presence of schizopeltic acid; Sparrius et al. 2006).

Additional specimens examined. **Brazil:** same as the type, E. L. Lima 662, 665, 670 & 673 (ISE).

Discussion of the phylogenetic position of Sergipea and Dichosporidium

In our phylogenetic tree (Fig. 3), Sergipea aurata clusters close to the genera Dichosporidium and Erythrodecton in the basal branch of the Roccellaceae s.str. as defined by Ertz & Tehler (2011). It is included in a strongly supported clade sister to the genus Enterographa and thus cannot be described in this latter genus. The generic type of Dichosporidium, D. nigrocinctum, was here newly sequenced and our phylogenetic tree confirms

the position of the genus close to *Erythrodecton granulatum* (Mont.) G. Thor, as first shown by Nelsen *et al.* (2009) for *D. boschianum* (Mont.) G. Thor. The relationships within the clade *Erythrodecton-Sergipea* are not supported. Therefore the generic position of the new taxon is uncertain within this clade.

Erythrodecton mainly differs from Sergipea by biclavate ascospores, a red pigment in the thallus medulla and internal carbonization of the ascomata. All species of Dichosporidium have internal carbonization of the ascomata, a different chemistry and a distinctly byssoid thallus, contrasting with Sergipea. Moreover, D. nigrocinctum differs, for example, by biclavate ascospores and D. boschianum by hooked ascospores. As a consequence, we prefer to describe a new genus to accommodate our new species.

It should be noted here that both species of *Dichosporidium* do not cluster together in our phylogenetic tree, suggesting that the genus might be paraphyletic. More data will have to be included in future phylogenetic studies to resolve the generic delimitation of these taxa.

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