Pucciniales on Annona (Annonaceae) with special focus on the genus Phakopsora

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ORIGINAL ARTICLE

Pucciniales on *Annona* (Annonaceae) with special focus on the genus *Phakopsora*

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Abstract The known species of Pucciniales on the tree genus *Annona* (Annonaceae), *Phakopsora cherimoliae, Batistopsora crucis-filii*, *B. pistila*, as well as the anamorphic species of *Aecidium annonae* and *Uredo rolliniae*, were investigated by light microscopy and DNA sequencing. For DNA extraction, N-Phenacylthiazolium bromide (PTB) was used to achieve a higher yield of DNA from herbarium specimens. The phylogenetic analyses were based on the ITS1–5.8S–ITS2 region, partial LSU and SSU of the nuclear rDNA, and the mitochondrial cytochrome oxidase subunit 3. The molecular as well as the morphologic investigations indicated that the genus *Batistopsora* is synonymous with *Phakopsora*. The two *Batistopsora* species appeared in all phylogenies within *Phakopsora*. They form a monophyletic clade together with *P. cherimoliae* as well as with the anamorphic *Uredo rolliniae* and the herein newly described species *Phakopsora annonae-sylvaticae*. Therefore, the following new combinations have been made: *Phakopsora crucis-filii*, *P. pistila* and *P. rolliniae*. *Phakopsora crucis-filii* and *P. pistila* could not be distinguished by the used sequences but are morphologically and ecologically well separated. This contradiction is discussed. *Phakopsora crucis-filii* is firstly reported as a pathogen on the fruit tree *Annona squamosa*. The species show host preferences to species groups of *Annona* at the subgeneric level and distribution patterns similar to those of their hosts. In comparison with the rust fungal genus *Dasyspora*, which occurs on *Xylopia* (Annonaceae) also in the Neotropics, the *Phakopsora* spp. on *Annona* show similar phylogeographical patterns. The redetermination of the host plants has shown that *A. annonae* does not occur on Annonaceae but on *Diospyros hispida* (Ebenaceae). Therefore, the new species, *Aecidium verannonae*, has to be described for the *Aecidium* species, which occurs really on *Annona*. It did not appear to be closely related to the Phakopsoraceae in the phylogenetic analysis. An identification key for all known rust fungi on *Annona* is given.

Keywords *Aecidium* · *Batistopsora* · N-Phenacylthiazolium bromide (PTB) · Phakopsoraceae · Phylogeography · *Uredo*

Taxonomical novelties:

Aecidium verannonae Beenken sp. nov.

Phakopsora annonae-sylvaticae Beenken sp. nov.

Phakopsora crucis-filii (Dianese, R.B. Medeiros & L.T.P. Santos) Beenken comb. nov.

Phakopsora pistila (Buriticá & J. F. Hennen) Beenken comb. nov.

Phakopsora rolliniae (W. T. Dale) Beenken comb. nov.

Introduction

The genus *Annona* comprises several economically important fruit trees that are cultivated worldwide in tropical and subtropical areas (e.g. Morton 1987) such as *A. cherimola* Mill. (cherimoya), *A. reticulata* L. (custard apple), *A. squamosa* L. (sugar apple), *A. muricata* L. (soursop), and hybrids like the so-called atemoya (*A. squamosa* x *A. cherimola*). Additionally, local people use and cultivate several other species as sources of fruits, timber and medicine (Ploetz 2003; Chatrou et al. 2004; Gottsberger and Silberbauer-Gottsberger 2006; Morton 1987). With nearly 200 species, including the recently synonymized genera *Rollinia* and *Raimondia* (Rainer 2007; Richardson et al. 2004), the genus *Annona* is one of the largest tree genera in tropical ecosystems. The genus is located in the Neotropics with the exception of four species located in Africa

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(Chatrou et al. 2004). Plant diseases that harm these fruit trees are of agricultural importance and ecological interest. Ploetz (2003) listed a number of diseases on cultivated Annonaceae but only one of them belongs to the rust fungi: *Phakopsora cherimoliae* (Lagerh.) Cummins on *Annona*. Further rusts belonging to Phakopsoraceae were described from wild *Annona* species (Buriticá 1999). Besides *P. cherimoliae*, two *Batistopsora* species, *B. crucis-filii* Dianese, R.B. Medeiros & L.T.P. Santos and *B. pistila* Buriticá & J. F. Hennen, are reported occurring on several *Annona* species (Buriticá 1999). The Phakopsoraceae is a large family with 12–18 genera and includes more than 200 species (Buriticá and Hennen 1994; Cummins and Hiratsuka 2003; Kirk et al. 2008). The genus *Phakopsora* itself comprises approximately 110 species occurring on more than 30 dicotyledonous plant families worldwide mainly in the Tropics (Kirk et al. 2008). With *Phakopsora pachyrhizi* Syd. & P. Syd. on soybeans, *P. euvitis* Y. Ono on grape vine and *P. gossypii* (Arthur) Hirats. f. on cotton plants, the genus includes some of the most dreaded plant pests on cultivated crops worldwide.

Thus, the aim of the third part of monographic studies of rust fungi on Annonaceae (Beenken and Berndt 2010; Beenken et al. 2012) was to investigate the systematic and phylogenetic relationship of the rust fungi on *Annona* with a special focus on the Phakopsoraceae. Collections of *Aecidium annonae* Henn. and *Uredo rolliniae* W.T. Dale, anamorphic species of Pucciniales described also from *Annona* species, were examined to determine if they have affinities with the Phakopsoraceae. Samples recently collected in French Guiana and herbarium specimens loan from several herbaria were studied using microscopy and DNA sequencing. All host plants were newly determined to species level to record the host preferences of all species. It was found that any hosts from herbarium specimens were wrongly identified. The specimens that did not belong to *Annona* were excluded from this study and will be described subsequently (Beenken in preparation). To give an understanding of distribution patterns and evolutionary traits in rust fungi on Annonaceae in the Neotropics, the results from the present study on Phakopsoraceae were compared with the phylogeographic patterns of the genus *Dasyspora*, which also occurs on *Xylopia* in the Neotropics (Beenken et al. 2012).

Materials and methods

Fungal collection and morphology

Rust infected leaves of several *Annona* species were collected during a field trip in French Guiana in 2009. Small pieces of leaves bearing fungal infections were cut out and dried for DNA extraction using silica gel. Additionally, host plants with and without fungal material were dried between papers in a plant press for morphological investigations and host identification. Small pieces of infected leaves were taken from herbarium specimens borrowed from B, BPI, BRUX, M, NY, PC, PUR, S, W and Z + ZT (acronyms according to Index Herbariorum, Thiers 2011) for molecular and morphological investigations, too. Herbarium numbers of samples chosen for DNA extractions are given in bold type together with numbers of isolation with in the specimen lists (see also Table 1). All host plants were identified or determination was revised, respectively, using the works of Fries (1931, 1939), Maas and Westra (1992), Funk et al. (2007), Cavalcanti and Ramos (2003) and Castro et al. (1999). Finally, specimens were compared with scans of specimens (http://plants.jstor.org) and directly with specimens in the Herbaria in Berlin (B), Cayenne (CAY), Munich (M), Vienna (W, WU) and Zurich (Z + ZT). Additionally anatomic characteristics were used to identify host specimens whose determination was dubious (Metcalfe and Chalk 1957; Busch 1913; Maas and Westra 1992; Contreras and Lersten 1984; Wallnöfer 2012). Finally, B. Wallnöfer, a specialist of Ebenaceae, and H. Rainer, specialist of Annonaceae, (both members of the Naturhistorisches Museum Wien, Vienna, Austria) identified some of the host plants.

Micromorphology of fungi was examined by light microscopy from spore scrapes and hand sections as described in Beenken et al. (2012). Measurements of 25–50 spores are given as minimum–arithmetic mean–maximum or minimum–maximum, respectively. All specimens contain only uredinia unless otherwise stated by Roman numerals for spore states. Terminology follows Cummins and Hiratsuka (2003). Modified Flora Neotropica base map no. 1 (prepared by Hendrik R. Rypkema, Department of Systematic Botany, State University of Utrecht, the Netherlands 1989) was used to illustrate the distributions of the species (cf. Beenken et al. 2012).

Molecular investigations

DNA was extracted using NucleoSpin Plant II extraction kit (Macherey-Nagel, Düren, Germany) following the manufacturer's standard protocol for plant tissue (cf. Beenken et al. 2012). Lysis was carried out with buffer PL1, and DNA was eluted in 100 µl elution buffer of the kit. To achieve a higher yield of DNA from herbarium or other difficult specimens, the lysis step was modified according Telle and Thines (2008): N-Phenacylthiazolium bromide (PTB) was added to the lysis buffer PL1 of the extraction kit to a final concentration of 2.5 mM (≈ 0.7 mg/ml); the incubation was executed on a shaker (Eppendorf Thermomixer) for 1–2 h at 65 °C; the remaining steps followed the manufacturer's standard protocol. A second method was developed to increase the success of PCR amplification from DNA, which was already extracted by the standard protocol, as follows. The DNA extract was

Table 1 Specimens, DNA extraction methods and GenBank accession numbers used in the phylogenetic analyses

Species	Host	Location	Collection-date	Isolate	PTB[a]	Herbarium voucher	ITS-LSU	SSU	CO3
Aecidium verannonae	*Annona spraguei*	Panama, Juan Diaz	21 Aug 1923	87	N	PUR 43011	KF528007	KF528037	-
Phakopsora annonae-sylvaticae	*Annona sylvatica*	Brazil, Minas Gerais	16 Jun 1983	86	N	PUR 87311	KF528008	KF528038	KF528046
Phakopsora argentinensis	*Croton* cf. *anisodontus*	Brazil, Ceará	26 Jun 2003	82	N	RB 8248 in ZT	KF528009	KF528039	KF528047
Phakopsora cherimoliae	*Annona cherimola*	Costa Rica, San José	3 Mar 1991	34	N	M-0142451	KF528010	-	-
Phakopsora cherimoliae	*Annona cherimola*	Costa Rica, San José	26 Mar 1992	85	N	RB 3096 in ZT	KF528011	KF528040	KF528048
Phakopsora cherimoliae	*Annona cherimola x A. squamosa*	USA, Florida	2 Feb 1989	30	N	PUR 89695	KF528012	-	-
Phakopsora crucis-filii	*Annona paludosa*	French Guiana, Iracubo	19 Jul 2009	80	N	ZT Myc 48987	KF528013	-	-
Phakopsora crucis-filii	*Annona paludosa*	French Guiana, Kourou	9 Aug 2009	41	N	ZT Myc 48988	KF528014	-	-
Phakopsora crucis-filii	*Annona paludosa*	French Guiana, Mana	23 Jul 2009	43	N	ZT Myc 48989	KF528015	-	-
Phakopsora crucis-filii	*Annona paludosa*	French Guiana, Sinnamary	24 Jul 2009	12	N	ZT Myc 48990	KF528016	KF528041	KF528049
Phakopsora crucis-filii	*Annona squamosa*	Brazil, Ceará	19 Nov 2009	61	N	ZT Myc 48991	KF528017	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Goias	9 May1979	152	A	PUR 66576	KF528018	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Goias	18 Jul 1988	153	N	PUR N3599	KF528019	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Mato Grosso	27 Jul 1988	31	N	PUR N3129	KF528020	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Minas Gerais	28 Apr 1986	98	N	PUR 90234	KF528021	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Minas Gerais	11 Jun 1988	99	N	PUR N3600	KF528022	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Minas Gerais	14 Nov 1983	154	N	PUR 87629	KF528023	-	-
Phakopsora crucis-filii	*Annona tomentosa*	Brazil, Minas Gerais	14 Apr 1986	155	N	PUR 90140	KF528024	-	-
Phakopsora phyllanthi	*Phyllanthus acidus*	Brazil, Ceará	10 Jan 2006	83	N	RB 8581 in ZT	KF528025	KF528042	KF528050
Phakopsora pistila	*Annona sericea*	French Guiana, Iracubo	19 Jul 2009	26	N	ZT Myc 48992	KF528026	KF528043	KF528051
Phakopsora pistila	*Annona sericea*	French Guiana, Saint Laurent du Maroni	22 Jul 2009	42	N	ZT Myc 48994	KF528027	-	-
Phakopsora pistila	*Annona sericea*	Guyana, Pakaraima Mountains	28 Jan 2003	156	N	BPI 863563	KF528028	-	-
Phakopsora pistila	*Annona spraguei*	Panama, Juan Diaz	21 Aug 1923	32	N	PUR 66577	KF528029	-	-
Phakopsora rolliniae	*Annona exsucca*	French Guiana, Iracubo	19 Jul 2009	188	B	ZT Myc 49004	KF528030	-	KF528052
Phakopsora rolliniae	*Annona exsucca*	French Guiana, Mana	23 Jul 2009	190	B	ZT Myc 48995	KF528031	-	KF528053
Phakopsora rolliniae	*Annona exsucca*	French Guiana, Roura	8 Aug 2009	23	A	ZT Myc 48999	KF528032	KF528044	-
Phakopsora rolliniae	*Annona exsucca*	French Guiana, Roura	28 Jul 2009	191	B	ZT Myc 48996	KF528033	-	-
Phakopsora rolliniae	*Annona exsucca*	French Guiana, Saint Laurent du Maroni	22 Jul 2009	189	B	ZT Myc 49000	KF528034	KF528045	KF528054
Phakopsora rolliniae	*Annona exsucca*	Trinidad and Tobago, Lopinot valley	Feb 1949	157	A	PUR F11845	KF528035	-	-
Phakopsora rolliniae	*Annona exsucca*	Trinidad and Tobago, La Seiva Valley	13 May 1913	159	A	NY 3237	KF528036	-	-

[a] DNA extractions: *N* no treatment with PTB, *A* treatment with PTB after extration, *B* treatment with lysis buffer containing PTB

mixed at a ratio of 1:1 with an aqueous solution of PTB (1.4 mg/ml) to a final concentration of ca. 2.5 mM and incubated for 0.5 h at 65 °C; this DNA solution was cleaned afterwards and reconstituted using a purification kit (Genomic DNA Clean & Concentrator, Zymo Research, Irvine, CA, USA) to remove the PTB. Success of each DNA extraction was controlled by PCR amplification of the ITS region with rust fungal specific primer. The internal transcribed spacer region (ITS1–5.8S–ITS2), partial LSU (28S) and SSU (18S) of the nuclear rDNA, as well as the mitochondrial cytochrome oxidase subunit 3 (CO3) were amplified and sequenced. PCR amplification and sequencing follow exactly the protocol in Beenken et al. (2012) using the following primer combinations: *ITS1–5.8S–ITS2*: ITS5-u/ITS4rust (Pfunder et al. 2001; Beenken et al. 2012). *LSU*: LRust1R/LR6; LRust1R/LRust3 and LRust3R/LR6 (Beenken et al. 2012; Vilgalys and Hester 1990). *SSU*: NS1/Rust18SR; NS1/NSrust3R and NSrust2/Rust18SR; NS1/NSrust1R, NSrust1/NSrust2R, NS3/NSrust3R, NSrust2/NSrust5R, NS5/NSrust7R and NSrust6/Rust18SR (White et al. 1990; Aime 2006; Beenken et al. 2012). *CO3*: CO3_F1/CO3_R1 (Vialle et al. 2009). Sequences were deposited in GenBank (accession numbers in Table 1).

Alignment

The sequences were assembled with Sequencher version 4.10.1 (Gene Codes, Ann Arbor, MI, USA) and aligned with MacClade 4.06 (Maddison and Maddison 2003). Ambiguously aligned regions were delimited and excluded from phylogenetic analyses with Gblocks version 0.91b (Castresana 2000). Three datasets with a total of six sub-matrices were created: (i) a combined SSU-LSU dataset consisting of 48 taxa and two sub-matrices (SSU with 1,636 sites, LSU with 831 sites); (ii) an ITS-LSU dataset consisting of 23 taxa and 1,669 nucleotide sites; (iii) a CO3 dataset consisting of 12 taxa and with 627 sites; accession numbers of sequences taken from GenBank are given in Figs. 1 and 3. Sequences in the combined SSU-LSU dataset (Fig. 1) were derived mainly from Aime (2006) and Beenken et al. (2012), respectively.

Phylogenetic analysis

The aligned datasets and sub-matrices were analyzed with maximum likelihood methods as implemented in RAxML version 7.2.8 (Stamatakis 2006). For the SSU-LSU dataset, *Caeoma torreyae* was selected as outgroup (cf. Aime 2006). For the ITS-LSU dataset, *Phakopsora phyllanthi* was used as the outgroup because it appears as a sister species to the *Phakopsora* related species on *Annona* in the SSU-LSU phylogeny (Fig. 1). The CO3 dataset consists of all *Phakopsora* related species of our study with *P. meibomiae* and *P. pachyrhiza* as outgroup (cf. the SSU-LSU phylogeny, Fig. 1). All analyses were performed assuming a general time reversible model of nucleotide substitution (GTR), estimating a discrete gamma distribution (GTRGAMMA option in RAxML). One thousand rapid hill-climbing runs with distinct starting trees were completed for each dataset. Maximum likelihood bootstrap analyses with 1,000 replicates were performed on the individual sub-matrices to test for potential conflict among the genes. Because no conflict was found (i.e. no well supported differences in the topology) additional analyses on the combined datasets were run. Again, a GTR with gamma model was used, but with partitions according to the sub-matrices, allowing for multiple models of substitution. Three partitions were used for the CO3 dataset, according to the codon position.

Bayesian analysis was performed with MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2005) on the same datasets as the maximum likelihood analysis. Independent general time-reversible models (GTR) with gamma distribution approximated by four categories were implemented for all data partitions. Three independent Bayesian runs were conducted for every dataset, each with four chains and 10 million generations, sampling every 100th tree. Post-burn-in trees were collected and the summarizations calculated only when the standard deviation of split frequencies had reached levels below 0.01. To ensure further that the runs reached stationarity and converged on the same ln-likelihood scores, the resulting likelihoods, tree topologies and model estimates were examined and compared by eye. Posterior probability values equal to or greater than 0.95 were considered significant. Lower posterior probability values will be given, if the corresponding ML bootstrap values are higher than 50 %. Phylogenetic trees were visualized using the program Dendroscope (Huson et al. 2007).

Fig. 1 Position of *Phakopsora* and other species on Annonaceae (*in bold text*) within the Pucciniales. Maximum-likelihood analysis with RAxML version 7.2.6 recovered from combined LSU and SSU sequence data. *Numbers at nodes* indicate RAxML bootstrap support >50 %/Bayesian posterior probabilities >0.90. GenBank accession numbers of LSU/SSU in *brackets*, of new sequences in Table 1; *numbers in italic* asign the isolates in Table 1

Result

Five species of *Phakopsora* on *Annona* species (Table 2) are recognized including one new species and three requiring new combinations. The molecular data did not allow a separation of the genus *Batistopsora* with the two species *B. crucis-filii* and *B. pistila* from *Phakopsora*. *Uredo rolliniae* belongs to *Phakopsora*, too. One sample assigned as *B. crucis-filii* turn out as new *Phakopsora* species that is also only known from its uredinial stage. Additionally one *Aecidium* species on *Annona* was described as new to science and one undescribed


100/1.00
Dasyspora segregaria (JF263488/JF263507)
Dasyspora gregaria (JF263477/JF263502)
100/1.00
Dasyspora emarginatae (JF263465/JF263498)
98/
1.00
Dasyspora guianensis (JF263479/JF263503)
Dasyspora mesoamericana (JF263480/JF263504)
62/0.93
Dasyspora winteri (JF263492/JF263508)
97/-
Dasyspora amazonica (JF263460/JF263496)
100/1.00
Dasyspora frutescentis (JF263468/JF263500)
Dasyspora ferrugineae (JF263467/JF263499)
100/1.00
Dasyspora nitidae (JF263484/JF263505)
-/0,73
98/1.00
Puccinia popowiae (JF263495/JF263511)
Diorchidium polyalthiae (JF263493/JF263509)
Aecidium sp. on Annona sparguei 87
100/1.00
Cronartium ribicola (DQ354560/M94338)
100/1.00
Endocronartium harknesii (AY700193/AY665785)
Pucciniastrum epilobii (AF522178/AF522179)
Pileolaria toxiodendri (AY745698/DQ092921)
Allodus podophylli (DQ354543/DQ354544)
Gymnosporangium clavipes (DQ354545/DQ354546)
Puccinia caricis (DQ354514/DQ354515)
-/0.99
Puccinia violae (DQ354509/DQ354508)
87/1.00
Puccinia convolvuli (DQ354512/DQ354511)
Puccinia hemerocallidis (DQ354519/DQ354518)
69/
0.98
Puccinia smilacis (DQ354533/DQ354532)
Puccinia coronata (DQ354526/DQ354525)
Uromyces ari-triphylli (DQ354529/DQ354528)
75/
1.00
72/
1.00
Puccinia poarum (DQ831028/DQ831029)
Cumminsiella mirabilissima (DQ354531/DQ354530)
Sphenospora kevorkianii (DQ354521/DQ354520)
79/1.00
Puccinia physalidis (DQ354522/DQ354523)
77/1.00
Frommeella mexicana (DQ354553/DQ354552)
Trachyspora intrusa (DQ354550/DQ354549)
Uromycladium fusisporum (AF522182/DQ354510)
-/0.95
Batistopsora pistila 26
100/0.97
Batistopsora crucis-filii 12
Batistopsora pistila (DQ354568/DQ354567)
97/1.00
Uredo rolliniae 189
100/1.00
Uredo rolliniae 23
99/1.00
Phakopsora cherimoliae 85
60/-
Uredo sp. on Annona sylvatica 86
52/0.91
68/0.99
Phakopsora phyllanthi 83
Phakopsora argentiniensis 82
100/1.00
Phakopsora tecta (DQ354535/AY125397)
99/1.00
Phakopsora pachyrhizi (DQ354537/DQ354536)
Phakopsora
58/0.96
Ravenelia havanensis (DQ354557/DQ354556)
Endoraecium acaciae (DQ323916/DQ323917)
Porotenus biporus (JF263490/JF263510)
Caeoma torreyae (AF522183/AY123284)

Uredo sp. was found. The hosts of *Aecidium annonae* were determined as belonging to the genus *Diospyros* (Ebenaceae) by morphological and anatomical characters.

Morphology

Six taxa could be distinguished by the morphology of their uredinia on *Annona* (see key). The presence or absence of periphyses and their arrangement in combination with their length are key characteristics of species groups. Further features to separate single species are size and especially the length-wide ratio of urediniospores, as well as their ornamentation with short or long spines.

Telia are only found in *P. cherimoliae*, *B. crucis-filii* and *B. pistila*. They are all very similar: crust-like, subepidermal, and formed by several layers of teliospores. Spermogonia and aecia were never observed in combination with uredinia or telia but a new *Aecidium* sp. occurs independently on *A. holosericea* Saff. and *A. spraguei* Saff., the same host plant species as *B. pistila* (Table 2).

Hosts

All species, which could be separated morphologically, showed preferences to special taxonomical groups, mainly sections, within the genus *Annona* (Table 2). Thus, the host range of each species supports the present morphological classification. One single collection of *P. crucis-filii,* which occurs mainly on species of sect. *Helogenia*, was observed on *A. squamosa* (sect. *Atta*).

Table 2 Hosts and distribution of rust fungi on *Annona*

1. Phakopsora cherimoliae	
On *Annona* sect. *Atta*:	
Annona cherimola	Tropical and subtropical Americas, Philippines
Annona reticulata	Costa Rica
Annona squamosa	USA, Cuba
2. Phakopsora crucis-filii	
On *Annona* sect. *Helogenia*:	
Annona paludosa	French Guiana
Annona tomentosa	Brazil
On *Annona* sect. *Atta*:	
Annona squamosa	Brazil
3. Phakopsora pistila	
On *Annona* sect. *Pilannona*:	
Annona holosericea	Honduras
Annona sericea	Guyana, French Guiana
Annona spraguei	Panama
4. Phakopsora rolliniae	
On *Annona exsucca*	
(= *Rollinia exsucca*)	Trinidad, French Guiana
5. Phakopsora annonae-sylvaticae sp. *nov.*	
On *Annona sylvatica*	
(= *Rollinia sylvatica*)	Brazil
6. Uredo sp.	
On *Annona* sp.	Venezuela
7. Aecidium verannonae sp. nov.	
On *Annona* sect. *Pilannona*:	
Annona holosericea	Honduras, El Salvador
Annona spraguei	Panama

Molecular investigations

Most of the DNA extractions from herbarium material using only NucleoSpin Plant II extraction kit (Macherey-Nagel, Düren, Germany) succeeded, with some exceptions (Table 1). The DNA extracts from one sample of *B. crucis-filii* and from two older samples of *U. rolliniae*, which could firstly not be amplified by PCR with ITS primers, were treated subsequently with PTB (Table 1). Afterwards, PCR amplifications of up to 800 bp long pieces of ITS and LSU, respectively, were possible. Additionally, four samples of *U. rolliniae* collected 2009 (Table 1) were extracted using the modified DNA extraction with PTB added to the lysis buffer because preliminary tests with several samples of *Puccinales* has shown that a treatment with PTB could enlarge success of PCR amplifications in general (Beenken et al. in preparation).

Phylogeny

The resulting RaxML maximum likelihood and Bayesian analyses of each dataset produced trees with more or less congruent topologies. Figure 1 shows a ML analysis from combined LSU and SSU datasets; Fig. 2 shows a ML analysis from combined ITS1-5.8S-ITS2 and LSU dataset; and Fig. 3 shows a ML analysis from mitochondrial CO3 sequence data.

Following species forming uredinia on *Annona spp.* appeared as a single clade within *Phakopsora* in the analyses of combined LSU-SSU datasets including species of several genera of the Pucciniales: *P. cherimoliae*, *B. crucis-filii*, *B. pistila*, *U. rolliniae* and the *Uredo* sp. on *Annona sylvatica* A. St.-Hil. (Fig. 1). This monophyletic clade shows 99 % bootstrap support and a Bayesian posterior probability of 1.00. Herein, the indistinguishable *Batistopsora* spp. and *U. rolliniae* form a well-supported sub-clade as sister taxa. *P. cherimoliae* and the *Uredo* sp. on *Annona sylvatica* appear also as sister taxa but with a low bootstrap support. The *Phakopsora* clade itself represented here additionally by two species from Euphorbiaceae, *P. phyllanthi* Dietel and *P. argentiniensis* (Speg.) Arthur, and two species from Fabaceae, *P. pachyrhizi* Syd. & P. Syd., and *P. tecta* H.S. Jacks. & Holw. is also highly supported. Within the *Phakopsora* clade, the species on *Annona* appeared next to *P. phyllanthi,*

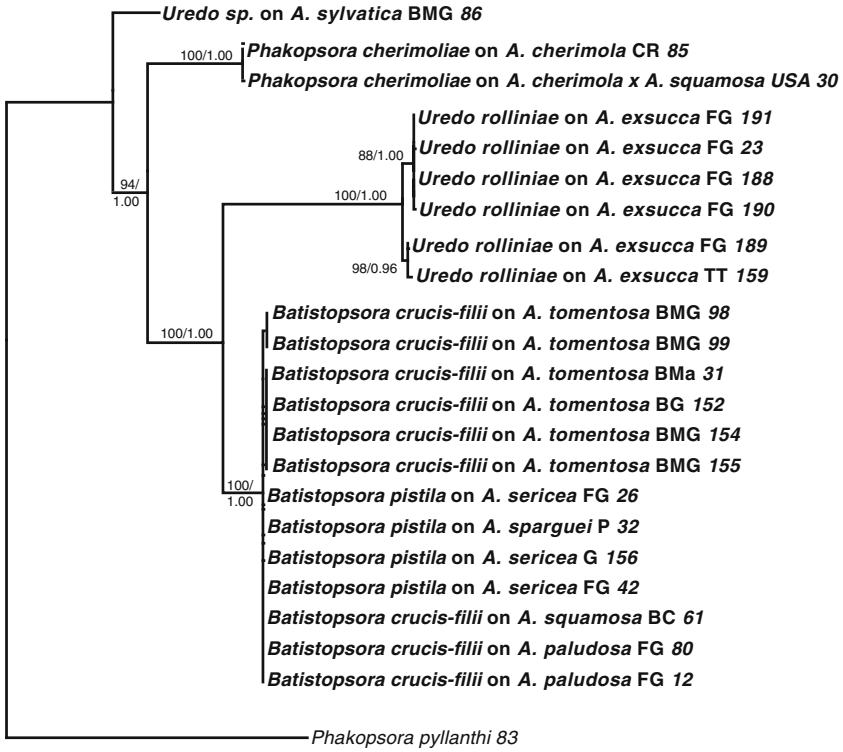

Fig. 2 Phylogeny of *Phakopsora* and related species on *Annona* species from several localities. Maximum likelihood analysis with RAxML version 7.2.6 recovered from combined ITS-LSU sequence data. *Numbers at nodes* indicate RAxML bootstrap support >50 %/Bayesian posterior probabilities >0.90. GenBank accession numbers in Table 1. The countries of origin are abbreviated as follows: *BC* Brazil, Ceará, *BG* Brazil, Goias, *BMa* Brazil, Mato Grosso, *BMG* Brazil, Minas Gerais, *CR* Costa Rica, *FG* French Guiana, *G* Guyana, *P* Panama, *TT* Trinidad and Tobago, *USA* USA, Florida. *Numbers in italic* asign the isolates in Table 1

a species occurring on Euphorbiaceae. The *Aecidium* on *A. spraguei* did not belong to the *Phakopsora* clade but appeared weakly supported in a sister position to the second clade concerning rust fungi on Annonaceae with *Dasyspora* spp. on *Xylopia* spp., *Puccinia popowiae* Cooke on *Monanthotaxis caffra* Verdc. and *Diorchidium polyalthiae* Syd. & P. Syd. on *Polyalthia longifolia* (Sonn.) Thwaites. The material of *Uredo* sp. assigned as *P. cherimoliae* from Venezuela (Sydow, Fungi venezuelani No. 307) and of *A. annonae* were not accessible for DNA-investigations.

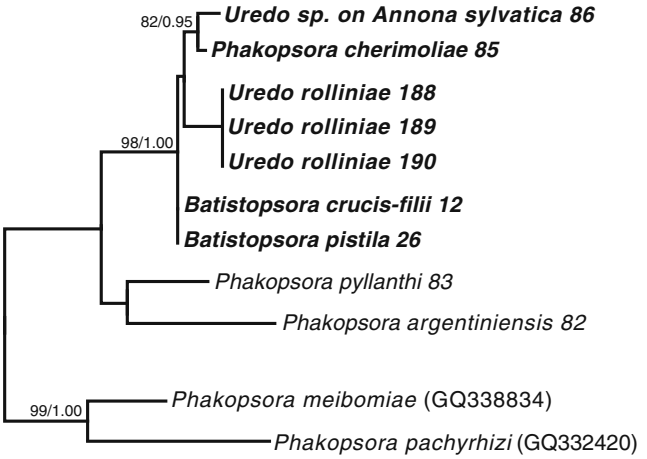

Fig. 3 Phylogeny of *Phakopsora* and related species on *Annona.* Maximum likelihood analysis with RAxML version 7.2.6 recovered from combined CO3 sequence data. *Numbers at nodes* indicate RAxML bootstrap support >50 %/Bayesian posterior probabilities >0.90. GenBank accession numbers *in brackets*, of new sequences in Table 1; *numbers in italic* asign the isolates in Table 1

The ITS-LSU tree (Fig. 2) shows a similar topology as the corresponding sub-tree in the LSU-SSU analyses. *B. crucis-filii* and *B. pistila* were not distinguishable by the ITS-LSU sequences, too. There were single base changes at nine positions of the ITS alignment between several samples of *B. crucis-filii* from Brazil and *B. pistila* but they did not allow separating one distinct sub-clades for each species. *U. rolliniae* is highly supported in sister position to the *Batistopsora* spp. Within *U. rolliniae*, two subgroups appeared that differed in a few base pairs of the ITS (1.3 % differences) and LSU (0.6 % differences) sequences. One subgroup originated from Trinidad (type locality) and northwestern French Guiana, the second group was formed by samples collected in the Southeast of it in French Guiana. The *Uredo* sp. on *A. sylvatica* is sister species to all the other species on *Annona*.

In phylogenetic analyses of the mitochondrial CO3 (Fig. 3) the tree topology differs slightly from them of the nuclear rDNAs. *P. cherimoliae* and *Uredo* sp. on *A. sylvatica* are sister taxa with a good support. The *Batistopsora* spp. have identical CO3 sequences, too. Their positions and that of *U. rolliniae* are not resolved with any support.

Taxonomy

1. ***Phakopsora cherimoliae*** **(Lagerh.) Cummins**, *Bull. Torrey bot. Club* **68**: 467 (1941)

Figs. 4a and 5a

Basionym: *Uredo cherimoliae* Lagerh., in Patouillard and Lagerheim *Bull. Soc. mycol. Fr.* 11(4): 215 (1895)

= *Physopella cherimoliae* (Lagerh.) Arthur, *Résult. Sci. Congr. Bot. Wien 1905*: 338 (1906)

= *Phakopsora cherimoliae* Cummins, *Mycologia* 48(4): 604 (1956)

= *Phakopsora neocherimoliae* Buriticá & J.F. Hennen, in Buriticá, *Revta ICNE*, Instit. Cienc. Nat. Ecol. 5: 176 (1994)

= *Uredo cupulata* Ellis & Everh., *Publications of the Field Museum of Natural History*, Botany Series 2: 16 (1900)

Spermogonia and aecia unknown. Uredinia in small groups or scattered on abaxial leaf sides, 0.1–0.5 mm in diameter, of *Calidion*-type (Cummins and Hiratsuka 2003), subepidermal, erumpent, surrounded by free, clavate periphyses forming a dense ring; periphyses up to 60 μm long, basal 5–10 μm wide at the apex up to 15 μm slightly enlarged, walls basally 1 μm at the apex up to 8 μm thick; paraphyses similar to periphyses but smaller and thinner walled; urediniospores ovate to subglobose, 20–23.5–30×16–19.2–21 μm, wall pale brown, 1–1.5 μm thick, ornamentation densely fine echinulate with thin, conical, 0.5–1.0 μm high spines, germpores obscure. Telia close to the uredinia, subepidermal, crust-like, dark brown, 0.1–1.0 mm in diameter, teliospores in 3–6 layers, cubical to oblong-ellipsoid, 7–13(17)×12–26 μm, walls 1–2 μm thick or up to 3 μm in the outermost spore layer, yellow to light brown. D-haustoria, intracellular haustorial bodies cylindrical, arched (sausage shaped), 10–15×3–4 μm, thinly, laterally or nearly terminally stalked.

Host and distribution: On leaves of the following species of *Annona* sect. *Atta*: *A. cherimola* Mill., *A. reticulata* L., *A. squamosa* L. and hybrids between them. *Phakopsora cherimolia* is known from Argentina, Brazil (Ferrari et al. 2004), Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Honduras, Mexico, Venezuela, USA (Florida, Texas) and the Philippines.

Notes: *Phakopsora cherimoliae* is morphologically distinguished by fine echinulate urediniospores and free, clavate periphyses with apically thickened walls. While the hosts of *P. cherimoliae* are cultivated in all subtropical and tropical regions of the world, records of *P. cherimoliae* are almost exclusively from the Americas. It occurs from the southern USA in the north to northern Argentina in the south. Only one old sample comes from outside of the Americas. It was

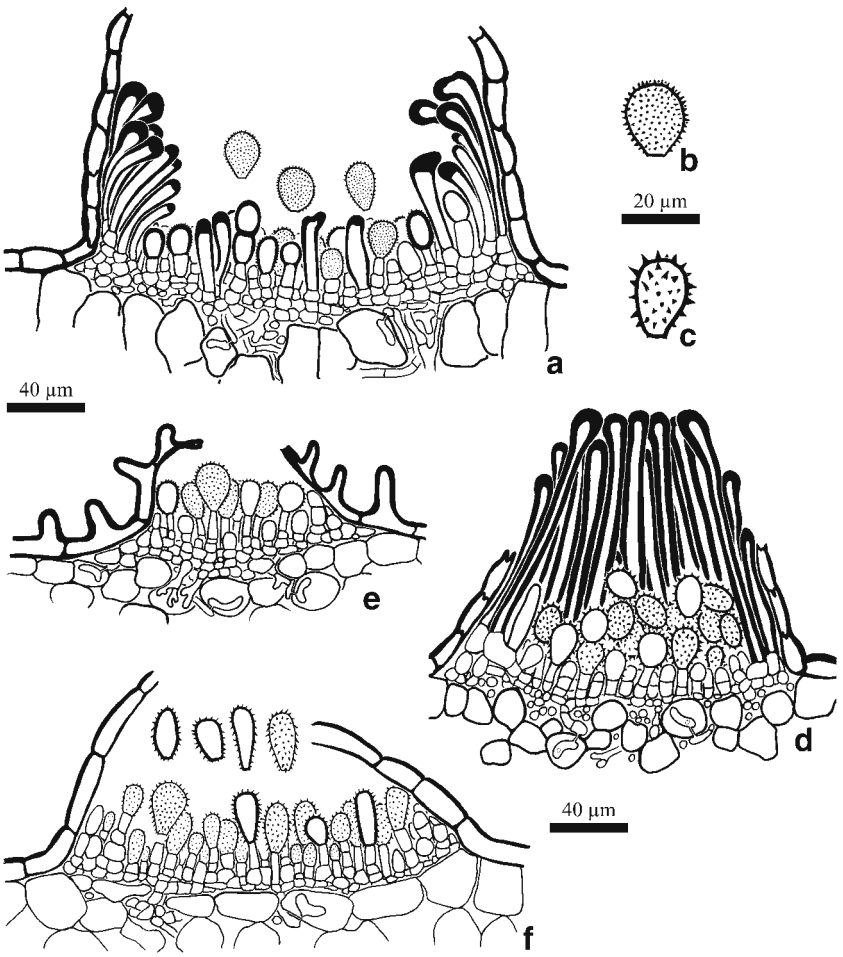

Fig. 4 Uredinia of *Phakopsora* species on *Annona* spp. **a.** *P. cherimolia* on *A. cherimola*, uredinium surrounded by periphyses and with paraphyses between the sporogenous cells. **b.** *P. crucis-filii* on *A. tomentosa*, single urediniospore. **c - d.** *P. pistila* on *A. holosericea*: **c.** single urediniospore; **d.** uredinium with fused periphyses forming a tube. **e.** *P. rolliniae*, uredinium without periphyses breaking through the papillate leaf epidermis of *A. exsucca*. **f.** *P. annonae-sylvaticae* on *A. sylvatica*, uredinium without periphyses. All samples show D-haustoria in the host cells of mesophyll. Sections are shown upside down. (**a** from PUR 66521; **b** from PUR N3599; **c**.-**f**. from type specimens)

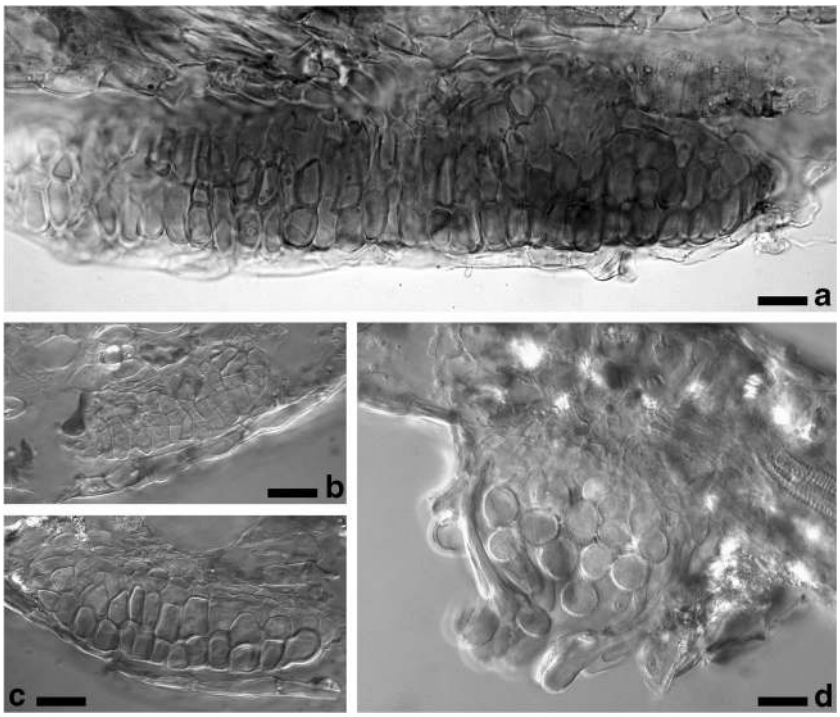

Fig. 5 **a.** *Phakopsora cherimolia* on *Annona cherimola*, telium (from PUR 66521). **b – d.** *P. crucis-filii* on *A. squamosa* (from ZT Myc 48991, prepared and photographed by R. Berndt): **b.** young telium; **c.** ripe telium; **d.** uredinium. *Bars*=20 μm

collected in 1923 in the Philippines and misidentified as *Physopella artocarpi* (Berk. & Broome) Arthur on *Artocarpus communis* J.R. Forst. & G. Forst.

Types: on *Annona cherimola*: **Ecuador**, Prov. Pichincha, San Nicolas, OCT 1891, leg. G. Lagerheim (Lectotype determined here: S F102360; Isotypes: S F29646, S F102361, PUR F8884, NY 00618226, NY 00618228, NY 00618229).

Paratypes: on *Annona cherimola*: **Ecuador,** Balao, Dec1890, G. Lagerheim (NY 00618227, NY 00618230, NY 00618231, BPI US0195599, BPI US0025702, BPI US0025694, S F32075); —, Dec 1890G. Lagerheim No. 2976 (PUR F8883, S F102362)

Additional specimens:

On *Annona cherimola:*

Argentina: Prov. Tucuman, Dept. Capital, 15 Sep 1995, leg. J.R. Hernández (230A BPI 841111); —, Dept. Famaillá, Sauce Huascho, 23 Apr 1993, leg. N.E.V. de Ramallo (BPI 843845). **Colombia**: Dept. Antioquia, Medellin, alt. 1,540 m, Sep 1941, Herbario Del Laboratorio De Fitopatologia No. 00952, (PUR F10664); —, Medellin Park, 16 Apr 1926, leg C.E. Chardon No. 2 (BPI 856045); —, near Iitiribi Cafetal "La Suiza", 28 May1926, leg C.E. Chardon No. 126 (BPI 856043); —, Medellin, Sep 1941, leg. C. Garces 59 (BPI 856049); —, Medellin, Sep 1941, leg. C. Garces, ex Herb. Arthur Herb. Lab. Fitopat. Dept. Agric. Colombia 952 (BPI 856050); —, Medellin, alt 1540, Nov 1942, Fungi of Colombia 1837, leg. C. Garces 582 (BPI 856039, BPI 856047); Dept. Caldas, Villa aria, 2 Jan1942, leg. M. Becerra-Agudelo (BPI US0025697); —, along Quindio River near Armenia, 14 Dec 1929, leg. C.E. Chardon 711 (BPI 856044); Dept. Valle del Cauca, Finca Piedra Grande, S of Cali, 14 May1929, Leg. C.E. Chardon, J.A.B.. Nolla no. 243 (BPI 856045). **Costa Rica:** Asseri, F. ex Herb. Petrak, Jan 1925, leg H. Sydow (M-0142452); —, Sydow, Fungi exotici exsiccate 601, 1 Jan 1925, leg. H. Sydow (M-0142455, PUR 47440, NY sn., BPI US0025708, BRUX 77494.88, S F102368, ZT Myc 48984, ZT Myc 48985); Prov. Heredia, Cordillera Central, E of San José de la Montaña, alt. 1600–1,640 m, 13 Oct 1991, leg. P. Döbbeler 6547 (M-0142450); —, 15 Sep 1991, leg. P. Döbbeler 6549 (M-0142447); Prov. San José, San José, campus of the university, 26 Mar 1992, leg. R. Berndt (**RB 3096 = isolate 85**, in ZT); —, S of San José, Cerros de Esazú, close to San Juan de Dios, alt. 1550–1650, 17 Aug 1991, leg. P. Döbbeler 6579 (M-0142448); —, Cerros de Esazú, SW of Esazú, alt 1,470 m, 3 Mar 1991, leg. P. Döbbeler 6639 (**M-0142451 = isolate 34**); —, SW of San José, E of Palmichal, alt. 1,180–1,350 m, 22 Sep 1990 leg. P. Döbbeler 6436 (M-0142449); —, Vicinity of San José, alt. 1,130 m, 4 Dec 1925–10 Feb 1926, leg. P.C. Standley No. 47391 (BPI US0025699), No 41227 (BPI US0025703); —, Vicinity of Santa Maria de Dota, alt 1,500–1,800 m, 14–26 Dec 1925, leg. P.C. Standley No. 42089 (BPI US0025704). **Ecuador**, Prov. Pichincha, 66 km from Quito, on the Quito-Otavalo road via Minas, alt. 6500 ft., 8 Aug 1975, leg. K.P. Dumont 2435 & P. Buriticá 75-E284 (PUR 66523). **Guatemala**, Aguacatán, 27 Dec 1940, J.R. Johnston 1966 (PUR 49858); Moran, 11 Feb 1905, W. A. Kellerman 5463 (PUR 49391 Type of *P. cherimoliae* Cummins, 1956 and of

P. neocherimoliae Buriticá & J.F. Hennen, 1994, BPI US0025707) (II, III). **Honduras**, Escuela Agricola Panamericana, 12 Dec 1951, A.S. Muller 654 (PUR 53061, BPI US0025696). **Mexico**, Chiapas, Comitan, 5.2 miles S of Comitan on Mexico highway 190, 27 Nov 1974, J.F. Hennen & P. Buriticá 74–450 (PUR 66522); —, 49.8 miles SE of San Chritóbal near Comitan, Mexico highway 190, 26 Nov 1974, Hennen & P. Buriticá 74–448 (PUR 66521) (II, III). **USA**, Florida, Miami, Experiment Station, 10 May1919, leg E. Simmonds 21058 (PUR 42787), leg E. Simmonds 42899 (PUR 42788); —, Miami, 5 Feb 1921, leg. J. A. Stevenson No 5660 (PUR 42793); —, Miami, Plant Intr. garden, Feb 1922, leg. J.A. Stevenson 6135F.H.B. (PUR 42789, BPI US0025701), sn. (BPI US0025698, BPI US0025706); —, Miami, Dade College, Woodlawn Cemetery, 26 Feb 1944, leg. S.R. Arthur (BPI US0025695, BPI 856046). **Venezuela:** Est. Aragua, Gardens at Las Delicias, near Maracay, alt 450 m, 16 Jun 1932, Mycological Explorations of Venezuela, University of Puerto Rico, Cornell University, Ministerio de Salubridad. Agricultura y Cria No. 165, leg. Chardon, Toro & Alamo (BPI 856040, BPI US0025705); Est. Miranda, El Cedral road to Los Teques, alt 1,200–1,300 m, 26 Jun 1932, Mycological Explorations of Venezuela, University of Puerto Rico, Cornell University, Ministerio de Salubridad. Agricultura y Cria No. 313, leg. Chardon, Toro & Alamo (BPI 856041).

Philippines: Prov. Pampanga, Luzon, Mt Arayat, Apr 1923, F. Petrak, Mycotheca generalis 1673 (as *Physopella artocarpi* (Berk. & Broome) Arthur on *Artocarpus communis* J.R. Forst. & G. Forst.) leg. M. S. Clemens, (S F102376, M-0206138).

On *Annona squamosa*

Cuba, Santiago de las Vegas, Estacion Expeimental Agronomica, 25 Jun 1916, leg. J.R. Johnston No 848 (PUR 42792); —, 3 Nov 1917, leg. J.R. Johnston 951 (PUR 42794); Havana. Los Pinos, 7Jan1919, leg. J.R. Johnston (BPI US0025690); Arroyo Arenas, 16 Dec 1919, leg. J.R. Johnston (BPI US0025691). **USA**, Florida, Miami, Experiment Station, 17 Mar 1919, leg J.C. Arthur (PUR 42795); —, Miami, Intercepted at Miami 45950, 21 Nov 1967, leg. J.C. Buff (BPI US0025720); —, Miami, Intercepted at Miami 45866, 06 Nov 1967, leg. J.C. Buff (BPI US0025722); —, Miami, Intercepted at Miami 48008, 14 Nov 1967, leg. H.L. Rubin (BPI US0025720); —, Miami, Dade College, U.S. Plant Introduction Garden at Chapman Field, 1 Mar 1944, leg. S.R. Arthur (BPI US0025121); —, Miami, Feb 1924, leg. J.A. Stevenson 7047 (BPI US0025712, BPI US0025714, BPI US0025716); —, Miami, 3 Feb 1917, leg. G.R. Lyman (BPI US0025715); —, Miami, 10 Feb 1924, leg. J.A. Stevenson (BPI US0025717); —, Miami, 08 Feb 1924, leg. J.A. Stevenson 7049 (BPI US0025719, BPI US0025710); —, Miami, Feb 1921, leg. J.A. Stevenson 1416 (BPI US0025709, BPI US0025711, BPI US0025718).

On *Annona cherimola x A. squamosa*

USA: Florida, Homestead, 336 St & 205 Av., Brooks tropical orchard, 2 Feb 1989, M. Jackson (**PUR 89695 = isolate 30**); —, Miami, Exper. Station, 17 Mar 1919, leg J.C. Arthur (PUR 42796); —, Miami, Dade College, U.S. Plant Introduction Garden at Chapman Field, 1 Mar 1944, leg. S.R. Arthur (BPI 856038).

On *Annona reticulata*:

Costa Rica: San José, R. W. Davidson 0 (BPI US0025708). **Cuba**, Santiago de las Vegas, Estacion Expeimental Agronomica, 2 Mar 1916, J.A. Johnston 492 (PUR 42791).

On *Annona* sp.:

Colombia: La Vega (El Encanto), 27 Nov 1937, Leg R. Barrios R. (BPI US0025680). **Costa Rica:** near San José, 20 Oct 1928, leg. H. Schmidt (BPI US0025684); near San José, 1930, leg. H. Schmidt (BPI US0025679). **Mexico**: Yucatan, Feb 1899, leg. F. Millspough VIII, ex Herb. Ellis, (PUR 42797, Isotype of *Uredo cupulata*). **USA:** Florida, Miami, Pl. Intr. garden, Feb 1922, leg. J.A. Stevenson 6136 (NY sn., S F102364), sn. (BPI US0025682), —, Miami, 10 Feb 1924, leg. J.A. Stevenson 7054 (BPI US0025685), sn. (BPI US0025688); —, Miami, Feb 1924, leg. J.A. Stevenson 7048 (BPI US0025686), sn. (BPI US0025687, BPI US0025681); —, ex Jamaica intercepted at Miami, 16 Aug1971, leg. F. Matthews 003071 (BPI US0025989).

Excluded collections: The following collection does not belong to *P. cherimoliae* because its host is not a species of Annonaceae and its uredinia have neither periphyses nor other sterile elements: Costa Rica: La Caja, close to San José, F. Petrak, Mycotheca generalis 303. (as *Physopella cherimoliae* on *Annona cherimola*), Dec 1924, leg. H. Sydow (M-0142453, ZT Myc 48986, NY sn.).

2. *Phakopsora crucis-filii* (Dianese, R.B. Medeiros & L.T.P. Santos) Beenken comb. nov.

Mycobank no. MB805024 Figs. 4b, 5b–d and 6

Basionym: *Batistopsora crucis-filii* Dianese, R.B. Medeiros & L.T.P. Santos, *Fitopatol. Brasil.* 18(3): 437 (1993)

=*Uredostilbe crucis-filii* Buriticá, *Rev. Acad. Colomb. Cienc.* 23(87): 272 (1999)

Spermogonia and aecia not found. Uredinia in small groups or scattered on abaxial leaf sides, 0.1–0.4 in diameter, of *Uredostilbe*-type (Cummins and Hiratsuka 2003), subepidermal, erumpent, palisade-like surrounded by periphyses fused to each other forming a tube; periphyses 40–110 μm long, basal 5–8 μm wide at the apex up to 15 μm slightly enlarged to capitate, walls colorless to pale brown, basally 1–3 μm and at the apex up to 5(8) μm thick; paraphyses not found; urediniospores ovate to subgobose, 20–21.5–26×15–17–20 μm, length-wide ratio 1.06–1.31–1.73, wall pale yellow, ca. 1 μm thick, ornamentation densely fine echinulate with

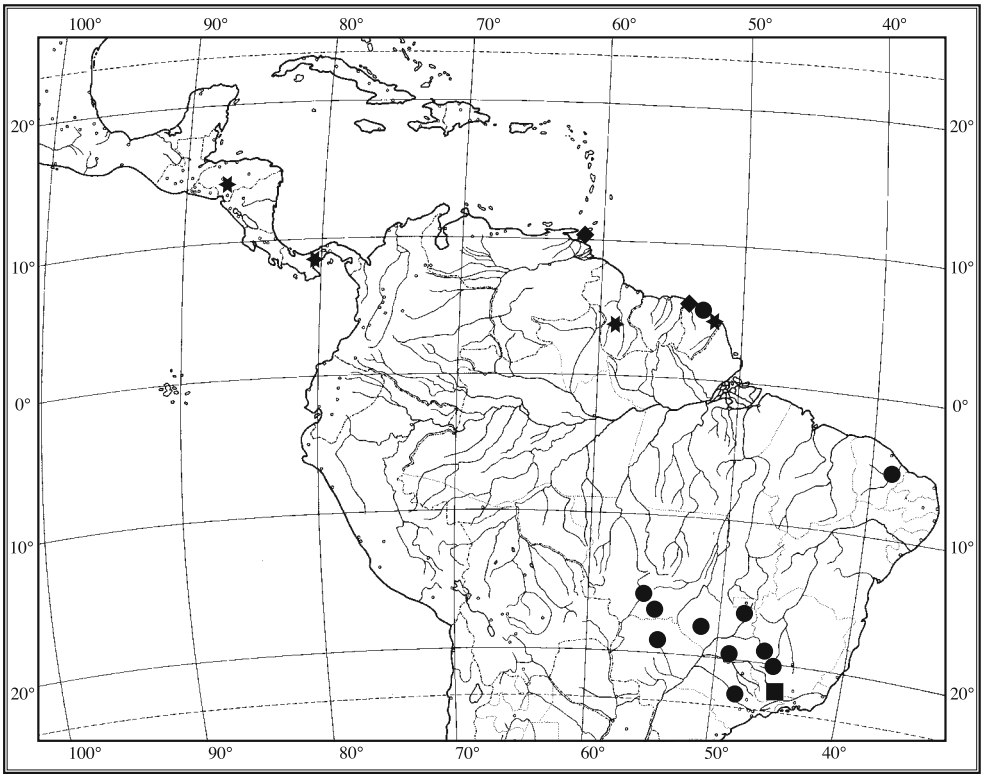

Fig. 6 Collection sites of four *Phakopsora* species on *Annona* illustrating their known natural distributions: *circles = P. crucis-filii*; *stars = P. pistila; diamonds = P. rolliniae*; *square = P. annonae-sylvaticae*. Collecting points of each species in French Guiana are pooled. Distribution of *P. cherimoliae* is not shown because it has been spread all over the tropical and subtropical Americas by the cultivation of its host plants

thin, conical, 0.5–1.0 μm high spines, germpores obscure. Telia close to the uredinia, subepidermal, crust-like, dark brown, 0.1–0.2 mm in diameter, teliospores in columns, in 3–4(5) layers, cubical to oblong-ellipsoid, 6–10×13–18 μm, walls 1–2 μm thick, yellow to light brown. D-haustoria, intracellular haustorial bodies cylindrical, arched (sausage shaped), 9–16×3–5 μm, thinly, laterally or nearly terminally stalked.

Host and distribution: On leaves of the following species of *Annona* sect. *Helogenia*: *A. tomentosa* R.E. Fr. and *A. paludosa* Aubl., a single collection on *A. squamosa* L. (sect. *Atta*). Found in the cerrados and savanna-like habitats of Brazil and French Guiana.

Notes: *Phakopsora crucis-filii* is similar to *P. cherimolia* in that it has densely fine echinulate urediniospores but differs morphologically by the longer periphyses of its uredinia that are fused to a tube. In contrast to Dianese et al. (1993), who described spermogonia of group VI, type 5 (Cummins and Hiratsuka 2003) and young undeveloped aecia, it could be found neither spermogonia nor aecia in the present material. The findings of *P. crucis-filii* on *A. paludosa* in French Guiana are the first reports for this host species and first records out of Brazil (Berndt 2013, as *B. crucis-filii*). *A. squamosa* is a new host plant, too.

Type: on *Annona tomentosa*, **Brazil:** Minas Gerais, Município Paracatú, in cerrado at Fazenda Puniel, 6 Jun 1993, leg. J. C. Dianese (Holotype: UB col. mycol. 3917, not seen).

Investigated specimens:

On *Annona tomentosa*

Brazil: Goias, Jataí, 50 km SSW of Jataí, highway 364, 18 Jul 1988, leg. J.F. Hennen, M.M. Hennen R.M. López 88–571 (**PUR N3599 = isolate 153**); —, Luziânia (host misidentified as *A. glaucophylla*), 9 May1979, leg. E.P. Heringer 1981 (**PUR 66576 = isolate 152**); Mato Grosso, Chapada dos Guimarães, ca. 14 km W (SW) of Chapada dos Guimarães, cerrado highway to Cuiabá, 21 Jul 1988, leg. J.F. Hennen, R.M. López 88–594 (PUR N3601); —, Rondonópolis, 11 km S of Rondonópolis, Balneário Lago Azul road, 27 Jul 1988, leg. J.F. Hennen & López B 88–674 (**PUR N3129 = isolate 31**) (II, III); Mato Grosso do Sul, Rio Verde, W of Iate Club Rio Verde, about 05 km on the river, 16 Apr 1983, leg. J.F, Hennen, M.M. Hennen, R. Antunes 83–179 (PUR N3598); Minas Gerais, 14 km S of Ibiá, cerrado, leg. J.F. Hennen 86–178 (PUR 90188); —, Ibiá, 11 Jun 1988, leg. J.F. Hennen, Y. Ono, M.M. Hennen 88–264 (**PUR N3600 = isolate 99**); —, 34 km W of Formiga, cerrado, 14 Apr 1986 leg. JF. Hennen 86–134 (**PUR 90140 = isolate 155**); —, 6 km NO of Lagoa da Prata, cerrado 28 Apr 1986, leg. J.F. Hennen 86–220 (**PUR 90234 = isolate 98**); —, N of Frutal, higway Br. 153, 14 Nov 1983, leg. J.F. Hennen, M.M. Hennen, C. Adell 83–735 (**PUR 87629 = isolate 154**); São Paulo, Bauru, km 337 da Estrada Marechal Rondon, 22 Mar 1989, leg. A.A. de Carvalho Jr. 89–29 (PUR N3597);

On *Annona paludosa*

French Guiana: Iracubo canton, branch from road N1 west of Iracubo, under transmission line, 5°29′24″ N, 53°18′ 25″ W, alt 25 m, 19 Jul 2009, leg. L. Beenken & R. Berndt LB 19.07.09/7 (PC, **ZT Myc 48987 = isolate 80**); Kourou canton, road N1, Savanne de Pères, between turnoff to Guatemala and Kourou River bridge, 5°07′18″ N, 53°39′04″ W, alt 10 m, 9 Aug 2009, leg. L. Beenken & R. Berndt LB 09.08.09/1 (PC, **ZT Myc 48988 = isolate 41**); Mana canton, Piste Montagne de Fer, side road of road N1 at km 200, alt 70 m, L. Beenken & R. Berndt, 23 Jul 2009, 5°23′58″ N, 53°33′36″ W, LB 23.07.09/13 (PC, **ZT Myc 48989 = isolate 43**); Sinnamary canton, road N1, small savanna close to "Maison de la nature Sinnamary, Les Pripris de Yiyi", 5°19′25.4″ N, 53°02′21.6″ W, 24 Jul 2009, leg. L. Beenken & R. Berndt LB 24.07.09/2 (PC, **ZT Myc 48990 = isolate 12**).

On *Annona squamosa*

Brazil: Ceará, Limoeiro do norte county, Chapada do Apodi, 19 Nov 2009, leg. F. Freire (**ZT Myc 48991 = isolate 61**) (II, III)

3. *Phakopsora pistila* (Buriticá & J. F. Hennen) Beenken comb. nov.

Mycobank no. MB805025 Figs. 4c,d and 6

Basionym: *Uredostilbe pistila* Buriticá & J. F. Hennen, *Revta Acad. Colomb. Cienc.* 19(72): 49 (1994)

= *Batistopsora pistila* Buriticá & J. F. Hennen in Buriticá, *Rev. Acad. Colomb. Cienc.* 23(87): 272 (1999)

Spermogonia and aecia unknown. Uredinia in small groups or scattered on abaxial leaf sides, 0.1–0.4 mm in diameter, of *Uredostilbe*-type (Cummins and Hiratsuka 2003), subepidermal, erumpent, palisade-like surrounded by periphyses fused to each other forming a tube; periphyses 40–120 μm long, basal 5–10 μm wide at the apex up to 14 μm slightly enlarged to capitate, walls colorless to pale brown, basally 1–3 μm and at the apex up to 5 μm thick; paraphyses not found; urediniospores ovate to subgobose, (17) 20–22.4–25×14–16.5–19 μm, length-wide ratio 1.11–1.37–1.64, wall pale yellow, ca. 1 μm thick, ornamentation echinulate with broad, conical, 1–2 μm high spines, germpores obscure. Telia close to the uredinia, subepidermal, crust-like, dark brown, 0.1–0.2 mm in diameter, teliospores in columns, in 3–4 layers, cubical to oblong-ellipsoid, (5)8–10×14–18 μm, walls ca. 1 μm thick, apically up to 4 μm thick in the outermost spore row, yellow to light brown. D-haustoria, intracellular haustorial bodies cylindrical, arched (sausage shaped), 12–17×3–5 μm, thinly, laterally or nearly terminally stalked.

Host and distribution: On leaves of species following of *Annona* sect. *Pilanona*: *A. holosericea* Saff., *A. sericea* Dunal and *A. spraguei* Saff. Records in Central America from Honduras and Panama; records in northern South America from French Guiana and Guyana.

Notes: *Phakopsora pistila* shows the same type of uredinia with tube forming periphyses as *P. crucis-filii* but it is easily distinguishable by the widely-spaced, long spines on its urediniospores. The presented collections from French Guiana reported *Annona sericea* as a host of *P. pistila* for the first time (Berndt 2013, as *B. pistila*). The collection J.R. Hernández 2003–085 (BPI 863563) from Guyana was determined as *B. crucis-filii* (Hernández et al. 2005, cited in Aime 2006) but its morphology and its host, *A. sericea*, fit better to *P. pistila*.

Type: on *Annona holosericea* (misspelled as *A. nolosericea* in Buriticá 1999; Buriticá and Hennen 1994; Hennen et al. 2005), **Honduras**: Santa Clara, 22 Jul 1950, G.S. Muller 97 (Holotype: PUR 53060, also holotype of *Batistopsora pistila*) (II, III)

Additional specimens:

On *Annona sericea*

French Guiana: Iracubo canton, branch from road N1 west of Iracubo, under transmission line, 5°29′24″ N, 53°18′ 25″ W, alt 25 m, 19 Jul 2009, leg. L. Beenken & R. Berndt LB 19.07.09/6 (PC, **ZT Myc 48992 = isolate 26**); Kourou canton, Dégrad Saramaka, near waterworks, forest at river Kourou, 5°00′54″ N, 52°41′56″ W, alt 10 m, 18 Jul 2009, leg. L. Beenken & R. Berndt LB 18.07.09/1 (PC, ZT Myc 48993); Saint Laurent du Maroni canton, Saint Jean, road to Plateau des Mines, 5°24′ N, 54°03″ W, alt 50 m, 22 Jul 2009, leg. L. Beenken & R. Berndt LB 22.07.09/5 (PC, **ZT Myc 48994 = isolate 42**). **Guyana**: Pakaraima Mountains, upper Potaro River, Ayanganna old airstrip (as *B. crucis-filii*), 28 Jan 2003, J.R. Hernández 2003–085 (**BPI 863563 = isolate 156**).

On *Annona spraguei*

Panama: Juan Diaz, 21 Aug 1923, F.L. Stevens 1240a (**PUR 66577 = isolate 32**)

4. *Phakopsora rolliniae* (W. T. Dale) Beenken comb. nov.

Mycobank no. MB805026 Figs. 4e and 6

Basionym: *Uredo rolliniae* W.T. Dale, *Mycol. Pap.* 59: 8 (1955)

Spermogonia and aecia unknown. Uredinia scattered on abaxial leaf sides, 0.1–0.2 mm in diameter, of *Uredo*-type (Cummins and Hiratsuka 2003), subepidermal, erumpent; peri- and paraphyses lacking; urediniospores subglobose to ovate, 17–19.5–26×12–14.9–17 μm, length-wide ratio 1.00–1.32–1.71, wall pale yellow, ca. 1 μm thick, ornamentation densely fine echinulate with thin, conical, 0.5–1.0 μm high spines, germpores obscure. Telia not found. D-haustoria, intracellular haustorial bodies cylindrical, arched (sausage shaped), 12–22×3–4 μm, thinly, laterally or nearly terminally stalked.

Host and distribution: On leaves of *Annona exsucca* DC [= *Rollinia exsucca* (DC.) A. DC], known from the island Trinidad and from French Guiana.

Notes: In contrast to the previous *Phakopsora* species, the uredinia of *P. rolliniae* lack peri- or paraphyses. Its subglobous urediniospores are fine echinulate. The collections from French Guiana were the first records from the South American mainland (Berndt 2013, as *U. rolliniae* on *Rollinia exsucca*).

Types: on *Annona exsucca*, **Trinidad and Tobago:** Fungi of Trinidad No. 2105, Lopinot valley, Feb 1949, leg R.E.D. Baker (Lectotype designated here: BPI 843305 ex PACMA 595; Isotype: **PUR F11845 = isolate 157**); Fungi of Trinidad No. 2031, Cumaca road, 27 Dec 1948, leg W.T. Dale (Paratypes: BPI 847264, PUR F14460); Trinidad, B. WJan, La Seiva Valley, 13 May 1913, leg. R. Thaxter No.14 (Paratypes: BPI US0025124, PUR F8885, NY s.n., NY s.n. ex Farlow Herbarium Acc.no. **3237 = isolate 159**);

Additional specimens:

French Guiana: Iracubo canton, branch from road N1 west of Iracubo, under transmission line, 5°29′24″ N, 53°18′25″ W, alt 25 m, 19 Jul 2009, leg. L. Beenken & R. Berndt LB 19.07.09/8 (PC, ZT Myc 49003); —, 19 Jul 2009, leg. L. Beenken & R. Berndt LB 19.07.09/10 (PC, **ZT Myc 49004 = isolate 188**); Mana canton, Piste Montagne de Fer, side road of road N1 at km 200, 5°23′58″ N, 53°33′36″ W, alt 70 m, 23 Jul 2009, leg. L. Beenken & R. Berndt LB 23.07.09/14 (PC, **ZT Myc 48995 = isolate 190**); Roura canton, road to Kaw (D6), side road to Fougasier, Montagne de Kaw, 4°30′ N, 52°10′ W, alt 100 m, 28 Jul 2009, leg. L. Beenken & R. Berndt LB 28.07.09/1 (PC, **ZT Myc 48996 = isolate 191**); —, road to Kaw (D6), side road to Fougasier, Montagne de Kaw, 4°30′ N, 52°10′ W, alt 100 m, 28 Jul 2009, L. Beenken & R. Berndt LB 28.07.09/4 (PC, ZT Myc 48997); —, road N2 to Regina, branching forest road, ca. 19 km SE from the Cacao crossing, secondary forest, Foret de Tiponni, 4°27′25.6″ N, 52°21′01.7″ W, 8 Aug 2009, leg. L. Beenken & R. Berndt LB 08.08.09/2 (PC, ZT Myc 48998); —, road N2 to Regina, Coralie, side road to Cacao, 4°52′ N, 52°21″ W, alt. 100 m, 8 Aug 2009, L. Beenken & R. Berndt LB 08.08.09/4 (PC, **ZT Myc 48999 = isolate 23**); Saint Laurent du Maroni canton, Saint Jean, road to Plateau des Mines, 5°24′ N, 54°03′ W, alt 50 m, 22 Jul 2009, leg. L. Beenken & R. Berndt LB 22.07.09/3 (PC, **ZT Myc 49000 = isolate 189**);

5. *Phakopsora annonae-sylvaticae* Beenken sp. nov.

Mycobank no. MB805027 Figs. 4f and 6

Etymology: from the host *Annona sylvatica*

Spermogonia and aecia unknown. Uredinia scattered on abaxial leaf sides, 0.1–0.2 mm in diameter, of *Uredo*-type (Cummins and Hiratsuka 2003), subepidermal, erumpent; peri- and paraphyses lacking; urediniospores elongate ovate to clavate, 17–25.7–30×12–14.2–17 μm, length-wide ratio 1.5–1.83–2.38, wall pale yellow, ca. 1 μm thick, ornamentation echinulate with thin, conical, 1–1.5 μm high spines, germpores obscure. Telia not found. D-haustoria, intracellular haustorial bodies cylindrical, arched (sausage shaped), 10–20×4–5 μm, thinly, laterally or nearly terminally stalked.

Host and distribution: On leaves of *Annona sylvatica* A. St.-Hil [= *Rollinia sylvatica* (A. St.-Hil.)]. Only known from the type locality in southwest Brazil.

Notes: *Phakopsora annonae-sylvaticae* has uredinia without sterile elements like *P. rolliniae* but differs from it by elongate urediniospores with longer and thinner spines.

Types: on *Annona sylvatica*, **Brazil**: Minas Gerais, Monte Verde, 16 Jun 1983, leg. J.F. Hennen, M.B. Figueiredo, C. Adell, S. Laudonia 83–415, labeled as *B. crucis-filii* on *Annona* sp. (Holotype: **PUR 87311 = isolate 86**; Isotype: IBI 14566, not seen).

6. *Uredo* sp.

Spermogonia and aecia unknown. Uredinia scattered on abaxial leaf sides, 0.1–0.2 mm in diameter, subepidermal, irregularly erumpent, remains of cellular peridia present, peri- or paraphyses lacking. Uredospores globose to ovoid 18–21–23×14–17–19 μm, length-wide ratio 1.00–1.25–1.64, wall pale yellow, 0.5(−1) μm thick, ornamentation densely fine echinulate with 0.5–1 μm high spines. germpores obscure. Telia not found.

On cf. *Annona* sp.: Venezuela: El Limón, valle de Puerto la Cruz, D. F. 20. Jan.1928, leg H. Sydow, Fungi venezuelani No. 307, labeled as *Physopella cherimoliae* Arth. (M-0142454, BPI US0025683, S F102366).

Note: The host plant belongs to Annonaceae but could not certainly be determined to genus level.

7. *Aecidium verannonae* Beenken sp. nov.

Mycobank no. MB805028 Fig. 7

Etymology: from Latin *verus*, *-a*, *-um* (= true, real, right) and the host genus *Annona* to indicate that this *Aecidium* occurs really on a true *Annona* in contrast to *A. annonae* that does not occur on an *Annona* species (see below).

Spermogonia and aecia in large groups on discolored (brown in herbarium specimens) leaf spots of 5–15 mm diameter, slightly hypertrophied to 1 mm thick with age. Spermogonia adaxial, light brown to black, of group VI, type 5 (Hiratsuka and Hiratsuka 1980), subepidermal, lens shaped, 100–130×120–140 μm, hymenia slightly concave. Aecia of Aecidium-type (Cummins and Hiratsuka 2003), abaxial, 0.17–0.25 mm in diameter, surrounded by white cup to tube shaped peridia split in several lobes; aeciospores nearly cubic to polyhedral with rounded corners, 15–16.28–18(20) x 12–14.91–18 μm, length-wide ratio 1–1.09–1.4, wall 1–1.5(2) μm thick, colorless to pale yellow, ornamentation irregularly, coarsely verrucose to areolate, with angular and cubical, 1–2 μm high and 0.5–1.5(2) μm wide warts, refracting cylindrical bodies, 3 μm high and 3–5 μm in diameter. Peridial cells in

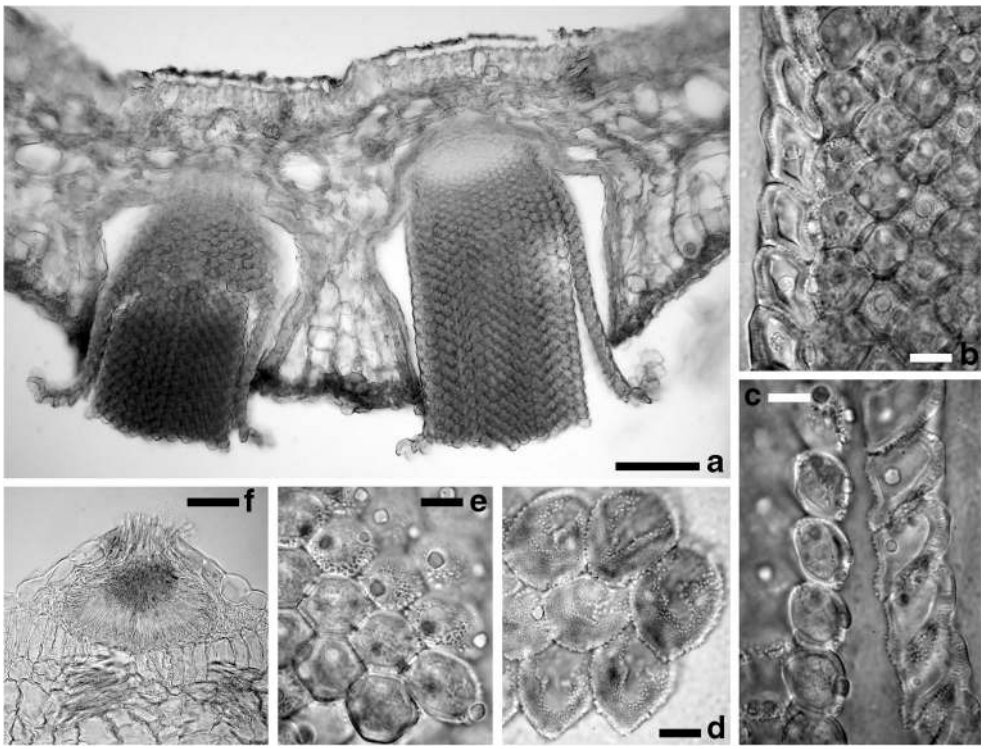

Fig. 7 *Aecidium verannonae* sp. nov. **a – e.** Aecia on *Annona spraguei* (from Holotype): **a**. two aecia in section; **b**.-**c**. peridial cells in longitudinal section and aeciospores; **d**. inner side of peridial cells in top view; **e**. aeciospores with refracting bodies in top view and optical section; **f**. spermogonium in section on *A. holosericea* (from PUR 53051). *Bars* in a=100 μm, in b–e=10 μm, in f=50 μm

longitudinal Section 15–25 μm long, 10–15 μm thick, wall at the inner side 1–2 μm thick with a low verrucose ornamentation, walls at the outer side 1 μm thick with 2–4 μm high rod-like thin warts densely tuberculate, peridial cells in plan view scale-like arranged, ovoid, 16–28 (31) μm long, 15–24 μm wide, ornamentations of inner sides densely verruculose, of outer sides appear densely fine-dotted to labyrinthine in parts.

M-haustoria, intracellular haustorial bodies cylindrical to oblong ovoid, 5–10×2–3 μm, with a terminal, ca. 1 μm thick, thin walled, filiform appendix.

Host and distribution: On leaves of *Annona spraguei* Saff. and *A. holosericea* Saff. in Central America with records from El Salvador, Honduras and Panama.

Notes: *Aecidium verannonae* is the only known *Aecidium* species from *Annona* spp. up to now. *Aecidium annonae*, the epithet of which indicates the host genus *Annona*, occur neither on *Annona* nor on other Annonaceae but on *Diospyrus* (Ebenaceae). Both species differs also distinctly in their morphology. *Aecidium verannonae* has aeciospores with a rough ornamentation and refracting bodies that lack on the fine verrucous aeciospores of *A. annonae*. In contrast to *A. verannonae*, the inner sides of peridial cells of *A. annonae* are smooth.

Types: on leaves of *Annona spraguei*, **Panama:** Juan Diaz, 21 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1240 (Holotype: BPI US0151224; Isotypes: BPI US0151226, **PUR 43011 = isolate 87**) (0, I).

Additional specimens:

On *Annona spraguei*

Panama: Alehjuela, 18 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1123 (BPI US0151227, PUR 43006) (0, I); —, 18 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1139 (PUR 43010); Baillemona, 20 Sep 1924, leg. F. L. Stevens, Fungi of Panama no. 679 (BPI US0151217, BPI US0151218, NY s.n.) (0, I); Barro Colorado, 10 Sep 1924, leg. F. L. Stevens, Fungi of Panama no. 426 (BPI US0151215, BPI US0151216, NY s.n.) (0, I); Frijoles, 14 Oct 1924, leg. F. L. Stevens, Fungi of Panama no. 1278 (PUR 43005, NY s.n.) (0, I); Gatuncillo, 18 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1147 (BPI US0151225, PUR 43009) (0, I); Juan Mina, 18 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1162 (BPI US0151228, PUR 43007) (0, I); Tompia, 15 Aug 1923, leg. F. L. Stevens, Fungi of Panama no. 1038 (BPI US0151223, PUR 43008) (0, I).

on *Annona holosericea*

El Salvador: 10 km N of San Miguel, 18 Jul 1946, leg. F. L. Wellman no. 1432 (PUR 52842) (0, I). **Honduras**: Jamastian, 12 Aug 1950, leg. A.S. Mueller 176 (BPI US015122, PUR 53051) (0).

Species excluded from rust fungi on Annonaceae

***Aecidium annonae* Henn.** *Hedwigia* 34: 100 (1895)

= *Aecidium annonaceicola* Henn. *Hedwigia* 34: 101 (1895) (Sydow and Sydow 1924)

Types: **Brazil**: Goiás, Meia Ponte, Oct 1892, on *Diospyros hispida* A. DC. (det. B. Wallnöfer), host mislabeled as *Annona*

sp., leg. E. Ule no. 1919 (Holotype: B 700014119; Isotypes: BPI US0151214) (0, I).

Specimens on Euphorbiaceae used for molecular investigations

***Phakopsora argentiniensis* (Speg.) Arthur** on *Croton* cf. *anisodontus* Müll. Arg. (det. I. Cordeiro and D. Carneiro-Torres), **Brazil:** Ceará, Cascavel county, Preaoca District, 26 Jun 2003, leg. F. Freire, det. R. Berndt (**RB 8248 = isolate 82,** in ZT).

Note: The type of *C. anisodontus* has leaves with long pedicellate glands as in the host plant of *P. argentiniensis* but its leaves differ slightly in format and the margins are more crenate. However, these small differences maybe can be accepted within the variability of *C. anisodontus* (Cordeiro pers. com.).

***Phakopsora phyllanthi* Dietel** on *Phyllanthus acidus* (L.) Skeels, **Brazil:** Ceará, Fortaleza, 10 Jan 2006, leg. F. Freire, det. R. Berndt (**RB 8581 = isolate 83**, in ZT).

Key of rust fungi on *Annona*

1 Only spermogonia and aecia present, uredinia absent ..... 7. *Aecidium verannonae*
1* Uredinia present
2 Uredinia with periphyses
3 Periphyses free, up to 60 μm long..... 1. *Phakopsora cherimoliae*
3* Periphyses fused to a tube, more than 80 μm long
4 Urediniospores with up to 1 μm long spines..... 2. *Phakopsora crucis-filii*
4* Urediniospores with up to 2 μm long spines ..... 3. *Phakopsora pistila*
2* Uredinia without periphyses
5 Uredinia with peridia..... 6. *Uredo* sp.
5* Uredinia without peridia
6 Urediniospores globose to ovoid, length-wide ratio 1.00–1.7, up to 26 μm long, ornamentation with up to 1 μm long spines..... 4. *Phakopsora rolliniae*
6* Urediniospores elongated, length-wide ratio 1.5–2.4, up to 30 μm long, ornamentation with up to 1.5 μm long spines..... 5. *Phakopsora annonae-sylvaticae*

Discussion

Taxonomy

Dianese et al. (1993) established the new genus *Batistopsora* Dianese, R.B. Medeiros & L.T.P. Santos with the type species *B. crucis-filii* in the family of Phakopsoraceae since it has spermogonia of group VI, type 5 and uredinia of *Uredostilbe*-type. *Phakopsora* has group VI, type 7 spermogonia insofar as they are known (Cummins and Hiratsuka 2003). In connection with the spermogonia, Dianese et al. (1993) described additionally young undeveloped aecia. In contrast to Dianese's observation, neither spermogonia nor aecia could be found in the present abundant material of *B. crucis-filii* and *B. pistila*. On the other hand, an *Aecidium* sp. occurs on the same *Annona* spp. on which *B. pistila* occurs (Table 2). They were never observed both together on one single leaf but F.L. Stevens collected both at the same place and date from *A. spraguei* in Panama (his collection numbers 1240 and 1240a). It turned out, after sequencing, that this *Aecidium* neither belongs to *B. pistila* nor is related to another *Phakopsoraceae* but represents a new species, *A. verannonae* (Fig. 1). *Aecidium verannonae* has spermogonia (Fig. 5f) of type 5 (Hiratsuka and Hiratsuka 1980) that are similar to those described and illustrated by Dianese et al. (1993) in its dimensions and position in the leaf tissue. Unfortunately, the original material of Dianese et al. (1993) was not available from Brazil to verify with molecular techniques whether the described spermogonia and aecia belong to *Batistopsora* or possibly to another *Aecidium* species co-occurring with telia and uredinia of *B. crucis-filii* on the same leaf. However, the differences between spermogonia of types 5 and 7 within group VI are minor (Hiratsuka and Hiratsuka 1980) and any further morphological characters were not observed that could justify a separation of *Batistopsora* from *Phakopsora* (cf. Berndt et al. 2008; Cummins and Hiratsuka 2003). The arrangement of teliospores into columns or irregular patterns seems to be very variable, even within single species (Ono et al. 1992) and is not suitable for genus characterization (Cummins and Hiratsuka 2003). The differences between the uredinia surrounding periphyses of *Batistopsora* and *Phakopsora* are more of quantitative than qualitative character. The periphyses of *Batistopsora* are of the similar shape as in *P. cherimoliae* but much longer and laterally fused. However, both species of the genus, *Batistopsora crucis-filii* and *B. pistila*, appeared together with *P. cherimoliae* within *Phakopsora* in all molecular phylogenies with high supports (Figs. 1, 2 and 3). Therefore, *Batistopsora* is congeneric to *Phakopsora* and its species have to be transferred to *Phakopsora*, as Cummins and Hiratsuka (2003) already assumed.

Uredo rolliniae and the new *Uredo* sp. on *A. sylvatica* belong also to the clade of *Phakopsora* species on *Annona*. Thus, *Uredo rolliniae* was placed into *Phakopsora* and the new species was described as *Phakopsora annonae-sylvaticae*.

In conclusion, all rusts of Phakopsoraceae occurring on *Annona* belong to a highly supported monophyletic clade that appears within the genus *Phakopsora* in the present phylogenetic analyses (Figs. 1, 2 and 3). It is also noteworthy that all these *Phakopsora* species are parasitic exclusively on species

of the genus *Annona* from the Neotropics including the synonymized genus *Rollinia*.

Aime (2006) concluded that the "family and the genus *Phakopsora* itself are polyphyletic, divided into two monophyletic but unrelated lineages". One lineage consists of species occurring on dicotyledonous plants (the "true" *Phakopsora* according to Aime 2006). This group of *Phakopsora* is represented here by species on *Annona*, Euphorbiaceae and Fabaceae, including the economically important soybean rusts, *P. pachyrhizi*. The species of the second lineage occurs on monocotyledonous Poaceae (Aime 2006), which is not included in our study. However, the type species of the genus *Phakopsora*, *P. punctiformis* (Barclay & Dietel) Dietel on *Galium aparine* L. (Rubiaceae) from India, is still not sequenced but this would be necessary to clear finally the taxonomy of the genus (Aime 2006).

Nomenclature

According to the new International Code of Nomenclature for algae, fungi and plants from 2011 (Melbourne Code) Article 59 (McNeill et al. 2012), names of anamorphs and teliomorphs are "treated equally for the purposes of establishing priority, regardless of the life history stage of the type". Consequently *Phakopsora cherimoliae* (Lagerh.) Cummins is the current name for this species (cf. Hawksworth et al. 2013), because *Uredo cherimoliae* Lagerh., its basionym, is the oldest name. Thus, the often-used name, *Phakopsora neocherimoliae* Buriticá & J.F. Hennen, is a later heterotypic synonym. *Uredostilbe pistila* Buriticá & J. F. Hennen is older then *Batistopsora pistila* Buriticá & J. F. Hennen, thus the former has to be used as basionym of the new combination *Phakopsora pistila* (Buriticá & J. F. Hennen) Beenken. Following these new rules of the Code (McNeill et al. 2012; Braun 2012), the recombination of *Uredo rolliniae* into *Phakopsora rolliniae* and the new description as *Phakopsora annonae-sylvaticae* is allowed, even through only the anamorphic uredinia stages of them are known, because these could clearly be assigned to the teleomorphic genus *Phakopsora* by DNA analyses.

Species delimitation within *Phakopsora* on *Annona*

Whereas telia in *Phakopsora* show few characteristics to distinguish species, the present study shows that the morphology of the uredinia is very diverse, even in a group of closely related species (cf. Ono et al. 1992; Buriticá and Hennen 1994). Thus, uredina could be well used to delimit the five *Phakopsora* species and one *Uredo* sp. on *Annona*, and were most suitable for creating a key, particularly since telia were very rarely found. Host preferences to single species or sections of *Annona* (Table 2) could be also used as additional ecological features, albeit with restrictions. *Phakopsora crucis-filii* occurs mainly on species of sect. *Helogenia* but it was also detected once on *A. squamosa* belonging to *Annona* sect. *Atta*, which is mainly the host section of *P. cherimoliae*. The host jump of *P. crucis-filii* on it is reported here for the first time. It is noteworthy to monitor because *A. squamosa* is an often cultivated fruit tree and *P. crucis-filii* may develop as a newly problematic pathogen on it.

The sequencing data fit very well with the present morphological species delimitation but with one exception. The two species *P. crucis-filii* and *P. pistila* are not distinguishable by the investigated DNA sequences, not even by the ITS-sequences (Fig. 2). Both species have similarly formed uredinia but are well-separated by the ornamentation of their urediniospores (Buriticá 1999). The urediniospores of *P. pistila* bear long spines in contrast to the fine-echinulate spore ornamentations of *P. crucis-filii* (Fig. 4b, c). They differ strictly in their host range, too (Table 2). This becomes particularly obvious in French Guiana, where their distribution areas overlap and both species occur sympatrically at edges of forests at savannas. A strong correlation between the morphologically defined species and their host preferences was found there. *Phakopsora crucis-filii* was only observed on *A. paludosa* and never on *A. sericea* and versa vice *P. pistila* only on *A. sericea* and never on *A. paludosa*; even if they were found on adjacent trees at the same location together (collections LB 19.07.09/6 and LB 19.07.09/7). From these observations of host specificity in the field it can be assumed that *P. crucis-filii* and *P. pistila* are not only morphologically but also ecologically well-separated species. Thus, there is no reason for synonymizing these well-established species (Buriticá 1999) in spite of their sequence similarities of the ITS region. It is known that ITS sequences cannot always separate closely related but morphologically and ecologically well-separated species and "lack of ITS variation does not provide evidence of conspecificity" (Bruns 2001). Harrington and Rizzo (1999) also criticize that "there may be an over reliance on rDNA and their spacer regions for phylogenetic analyses of the fungi, especially for species-level comparisons". Even Schoch et al. (2012), who proposed the ITS sequence for barcoding of fungi, conceded the "limitation of ITS sequences for identifying species in some groups".

Beenken et al. (2012) gave further examples from the rust fungal genus *Dasyspora*, which is comparable to present *Phakopsoa* species. The genus *Dasyspora* occurs also on Neotropical Annonaceae, on *Xylopia*, but in contrast to the presented *Phakopsora* species, which show a more or less strict host preference on sub generic level (Table 2), the *Dasyspora* species show host preferences on species level (Beenken et al. 2012). It is distributed in about the same regions of Central America and South America as the *Phakopsora* species on *Annona* (Beenken et al. 2012). Within Dasyspora, the species of the *D. gregaria* complex are also quite indistinguishable in their ITS sequences. *Dasyspora segregaria* Beenken and *D. gregaria* (Kunze) Henn. occur in

Central America and the Guianas, respectively, the morphologically distinct *D. echinata* Beenken & Berndt were only found in the cerrados of Brazil (Beenken et al. 2012). *Phakopsora pistila* and *P. crucis-filii* show more or less the same distinction in their distribution pattern and habitat preferences in correlation to their hosts. *Phakopsora pistila* occurs in the more humid climates of Central America and adjacent northern South America the hotspot of *Annona*, sect. *Pilanona*, (Fries 1931) and *P. crucis-filii* prefer the dryer cerrados of Brazil where the most species of sect. *Helogenia* grow (Fries 1931). In contrast to both *Phakopsora* species, an overlapping zone in the *D. gregaria* complex is unknown.

The found differences in spore ornamentation between the species in each complex could be interpreted as adaption to the different environments and hosts (Beenken et al. 2012). Furthermore, it could be speculated that these differentiations in morphology and in host preferences have arisen faster than changes in the nuclear rDNA could be taken place. Raffaele et al. (2010) compared the genomes of four extremely closely related species of the fungus-like plant pathogenic *Phytophthora infestans* complex (Oomycetes) that have ca. 99.9 % identity between their ITS sequences. They showed that host jumps and following specializations to different hosts have effected a rapid evolution of genes that are involved in the pathogen-host interaction. Aside from host jumps, hybridization, which is also known from rust fungi, is a further mechanism inducing fast speciation (reviewed in Park and Wellings 2012). The offspring of hybridization could become a new species that have the same ITS sequence of one of its parental species or a combination of both parental sequences, as Newcombe et al. (2000) could show for the rust fungal hybrid *Melampsora* × *columbiana* G. Newc. and its parental species *M. medusae* Thüm. and *M. occidentalis* H.S. Jacks. Stukenbrock et al. (2012a, b) identified a hybridisation in the ascomycete grass pathogen genus *Zymoseptoria*, in which the hybrid, *Zymoseptoria pseudotritici* B.A. McDonald, Stukenbr. & Crous, share the identical ITS sequence with its parental species *Z. tritici* (Desm.) Quaedvl. & Crous. However, there are clear evidence neighter of host jump nor of hybridisation from the present data. Incongruences between the nuclear (Figs. 1 and 2) and mitochondrial (Fig. 3) phylogenies that would indicate hybridisation were not found.

Contrary to this, the analyses of ITS and LSU sequences split *P. rolliniae* into two subgroups (Fig. 2). One includes collections from Trinidad, the type location, and northern French Guiana, and the second group originates from southeast French Guiana. The analyzed members of both groups were identical in their sequences of the mitochondrial CO3 (Fig. 3). No differences in their morphology or hosts preferences were found, thus they were assigned to one single species. *Dasyspora nitidae* Beenken, which was also collected in French Guiana, shows a bifurcation into two subgroups in the ITS-LSU analyses (Beenken et al. 2012).

Phakopsora annonae-sylvaticae is morphologically very similar to *P. rolliniae*. Both have uredinia without peri- or paraphyses and differ only in ornamentation, size and shape of uredinospores. Both occur on *Annona* species, which formerly belonged to the genus *Rollinia* (Rainer 2007). Unexpectedly, they did not appear as sister species in any of our phylogenies but were in sister positions to species with periphyses. *Phakopsora phyllanthi*, which appear in sister position to all species on *Annona* (Fig. 1), has uredinia with well-developed peri- and paraphyses. Such sterile elements are quite common in the uredinia of *Phakopsora* (Ono et al. 1992; Buriticá and Hennen 1994). Thus, the loss of periphyses seems to be happened twice in *Phakopsora* on *Annona*. *Phakopsora annonae-sylvaticae* is only known from the type collection from southeastern Brazil far away from the distribution area of *P. rolliniae* in the north of South America. The same differences in distribution areas show their hosts *A. sylvatica* and *A. exsucca* (Fries 1939; Maas and Westra 1992). Thus, *P. rolliniae* and *P. annonae-sylvaticae* could be regarded as cryptic or nearly cryptic species. In the genus *Dasyspora*, *D. mesoamericana* Beenken and *D. frutescentis* Beenken are cryptic species that occur both one *X. frutescens* but the former in Central America and the latter in South America (Beenken et al. 2012). It may be that the morphological conformities reflect in each case analogous adaptation to similar hosts.

In conclusion, the found incongruences between the sequence data and morphology, respectively, host preferences of species illustrate our limited knowledge and understanding of the intra- and intergeneric variability of the ITS region of rust fungi in general, but especially of those from the tropics, at this time. Thus, the morphology and host preferences were assessed as important characters for species delimitation in the present species concept but the sequence data could help to interpret them, as it was already discussed extensively for the rust fungal genus *Dasyspora* in Beenken et al. (2012).

The found conspicuous analogies between evolutionary and phylogeographic patterns of *Phakopsora* and *Dasyspora* are obviously not coincidental. They may indicate parallel evolution of both genera, each together with its host genus, driven by common influences of historical geomorphological processes and climatic changes in the Neotropics as it is reported from several South American organisms (reviewed in Turchetto-Zolet et al. 2013). However, because the evolution of plant pathogens cannot explain independently from their hosts, it would be firstly necessary to study the phylogeographies of *Annona* and *Xylopia*, respectively.

Additional rust fungi on *Annona*

The collection of H. Sydow, Fungi venezuelani No. 307, labeled as *Physopella cherimoliae* was detected as an unknown *Uredo* species. Because of its lack of paraphyses and

having a peridium, these uredinia belong neither to *P. cherimoliae* as they were labeled, nor to one of the other *Phakopsora* species on *Annona.* The remnants of cellular peridia bring to mind Milesia-type uredinia, as they are known from Pucciniastraceae (Cummins and Hiratsuka 2003), but their typical ostiolar cells were not observed. Milesia-type uredinia are also reported from *Phakopsora* species (Ono et al. 1992; Buriticá and Hennen 1994). Molecular data were not available from the old specimen to clear its taxonomy and the determination of its host plant to an *Annona* species was not certain. For these reasons, describing a new species was not done.

The *Aecidium* sp. found on *Annona spraguei* and *A. holosericea* did not belong to *A. annonae* because it differed in their hosts, which is not an Annonaceae in *A. annonae* but *Diospyros hispida* (Ebenaceae). They differed in their ornamentation of aeciospores and peridial cells as well. Therefore, the new species, *A. verannonae*, is described in the anamorphic "form" genus *Aecidium*. *Aecidium* should be used here as a provisionally taxonomical but not as a phylogenetic classification (cf. Braun 2012) because it could be assigned to any existing teleomorphic genus neither morphological features nor by molecular data up to now (Fig. 1). *Aecidium verannonae* is the only one further rust fungus that could be certainly reported from *Annona* but it is less closely related to the Phakopsoraceae (Fig. 1).

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References

Aime MC (2006) Toward resolving family-level relationships in rust fungi (Uredinales). Mycoscience 47:112–122

Beenken L, Berndt R (2010) Rust fungi on Annonaceae: the genus *Sphaerophragmium*. Mycologia 102(3):650–663

Beenken L, Zoller S, Berndt R (2012) Rust fungi on Annonaceae II: the genus *Dasyspora* Berk. & M. A. Curtis. Mycologia 104:659–681

Berndt R (2013) First catalogue of the rust fungi of French Guiana, northern South America. Mycol Prog 12:193–211

Berndt R, Freire F, Piatek M, Wood AR (2008) New species of *Phakopsora* (Basidiomycota, Uredinales) on *Dissotis*, *Melhania* and *Merremia* from Cameroon, South Africa and Brazil. Sydowia 60:15–24

Braun U (2012) The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. IMA Fungus 3(1):81–86

Bruns TD (2001) ITS reality. Inoculum 52(6):2–3

Buriticá CP (1999) La familia Phakopsoraceae en el Neotrópico III, generos: *Batistopsora* y *Phakopsora*. Rev Acad Colomb Cienc 23: 271–305

Buriticá P, Hennen JF (1994) Familia Phakopsoraceae (Uredinales). 1. Géneros anamórphicos y teleomórphicos. Rev Acad Colomb Cienc 19:47–62

Busch P (1913) Anatomisch-systematische Untersuchung der Gattung *Diospyros*. Thesis, Erlangen

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Castro AAJF, Martins FR, Tamashiro JY, Shepherd GJ (1999) How rich is the flora of Brazilian cerrados? Ann Mo Bot Gard 86:192–224

Cavalcanti TB, Ramos AE (eds) (2003) Flora do Distrito Federal Brasil. Vol. 3. Brasilia.

Chatrou LW, Rainer H, Maas PJM (2004) Annonaceae (Soursop Family). In: Smith N, Mori SA, Hendreson A, Stevenson DW, Heald SV (eds) Flowering plants of the neotropics. The New York botanical garden. Princeton University Press, Princeton, pp 18–20

Contreras LS, Lersten NR (1984) Extrafloral nectaries in Ebenaceae: anatomy, morphology, and distribution. Am J Bot 71(6):865–872

Cummins GB, Hiratsuka Y (2003) Illustrated genera of rust fungi, 3rd edn. APS Press, St. Paul

Dianese JC, Medeiros RB, Santos LTP, Furlanetto C, Sanchez M, Dianese AC (1993) *Batistopsora* gen. nov. and new *Phakopsora*, *Ravenelia*, *Cerotelium*, and *Skierka* species from the Brazilian cerrado. Fitopatol Bras 18(3):436–450

Ferrari JT, de C Nogeira EM, Hennen JF, Figueiredo MB, Coutinho LN, Louzeiro IM (2004) Ocorrência e controle da ferrugem em Cherimólia no estado de São Paulo. Arq Inst Biol São Paulo 71: 95–98

Fries RE (1931) Revision der Arten einiger Anonaceen-Gattungen II. Acta Horti Bergani 10:129–341, 27 plates

Fries RE (1939) Revision der Arten einiger Anonaceen-Gattungen III. Acta Horti Bergani 12:1–220, 20 plates

Funk V, Hollowell T, Berry P, Kelloff C, Alexander SN (2007) Checklist of the plants of the Guiana shield (Venezuela: Amazonas, Bolivar, Delta Amacuro; Guyana, Surinam, French Guiana). Contr US Natl Herb 55:1–584

Gottsberger G, Silberbauer-Gottsberger I (2006) Life in the Cerrado: a South American tropical seasonal vegetation. Vol. I. Origin, structure, dynamics and plant use. Reta Verlag, Ulm, pp 1–279

Harrington TC, Rizzo DM (1999) Defining species in the fungi. In: Worrall JJ (ed) Structure and dynamics of fungal populations. Kluwer, Dordrecht, pp 43–71

Hawksworth DL, McNeill J, de Beer ZW, Wingfield MJ (2013) Names of fungal species with the same epithet applied to different morphs: how to treat them. IMA Fungus 4(1):53–56

Hennen JF, Figueiredo MB. de Carvalho AA, Hennen PG (2005) Catalogue of the species of plant rust fungi (Uredinales) of Brazil, Jardim Botânico do Rio de Janeiro, Rio de Janeiro http://www.jbrj.gov.br/publica/uredinales/Brazil_Catalogue1drevisado.pdf

Hernández JR, Aime MC, Henkel TW (2005) The rust fungi (Uredinales) of Guyana. Sydowia 57(2):189–222

Hiratsuka Y, Hiratsuka N (1980) Morphology of spermogonia and taxonomy of rust fungi. Rept Toottori Mycol Inst 18:257–268

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Huson DH, Richter DC, Rausch C, Dezulian T, Franz M, Rupp R (2007) Dendroscope - an interactive viewer for large phylogenetic trees. BMC Bioinforma 8:460

Kirk PM, Cannon PF, David JC, Stalpers J (eds) (2008) Dictionary of fungi, 10th edn. CABI Publishing, Oxon

Maas PJM, Westra LYT (1992) *Rollinia*. Flora Neotropica 57:1–188

Maddison DR, Maddison WP (2003) MacClade 4.0. Sinauer Associates, Sunderland

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (2012) International code of nomenclature for algae, fungi and plants (Melbourne code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Koeltz Scientific Books, Regnum Vegetabile 154: i–xxx, 1–208. http://www.iapt-taxon.org/nomen/main.php

Metcalfe CR, Chalk L (1957) Anatomy of the Dicotyledons, vol I +II. Clarendon, Oxford

Morton JF (1987) Fruits of warm climates. Creative Resource Systems, Winterville

Newcombe G, Stirling B, McDonald S, Bradshaw HDJ (2000) *Melampsora* × *columbiana*, a natural hybridof *M. medusae* and *M. occidentalis*. Mycol Res 104:261–274

Ono Y, Buriticá P, Hennen JF (1992) Delimitation of *Phakopsora, Physopella,* and *Cerotelium* and their species on Leguminosae. Mycol Res 96:825–850

Park RF, Wellings CR (2012) Somatic hybridization in the uredinales. Annu Rev Phytopathol 50:219–239

Pfunder M, Schürch S, Roy BA (2001) Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol Res 105: 57–66

Ploetz RC (2003) Diseases of atemoya, cherimoya, soursop, sugar apple and related fruit crops. In: Ploetz RC (ed) Diseases of tropical fruit crops. CABI Publishing, Cambridge, pp 21–34

Raffaele S, Farrer RA, Cano LM, Studholme DJ, MacLean D, Thines M, Jiang RHY, Zody MC, Kunjeti SG, Donofrio NM, Meyers BC, Nusbaum C, Kamoun S (2010) Genome evolution following host jumps in the Irish potato famine pathogen lineage. Science 330: 1540–1543

Rainer H (2007) Monographic studies in the genus *Annona* L. (Annonaceae): inclusion of the genus *Rollinia* A.St.-Hil. Ann Naturhist Mus Wien B 108:191–205

Richardson JE, Chatrou LW, Mols JB, Erkens RHJ, Pirie MD (2004) Historical biogeography of two cosmopolitan families of flowering plants: annonaceae and rhamnaceae. Phil Trans R Soc London B 359:1495–1508

Ronquist F, Huelsenbeck JP (2005) MrBayes. Version 3.1.2. Bayesian analysis of phylogeny. GNU General Public License

Schoch CL, Seifert KA, Huhndorf S, Spouge RV, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. PNAS 109:6241–6246. doi:10.1073/pnas.1117018109

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21):2688–2690

Stukenbrock EH, Quaedvlieg W, Javan-Nikhah M, Zala M, Crous PW, McDonald BA (2012a) *Zymoseptoria ardabilia* and *Z. pseudotritici*, two progenitor species of the septoria tritici leaf blotch fungus *Z. tritici* (synonym: *Mycosphaerella graminicola*). Mycologia 104: 1397–1407

Stukenbrock EH, Christiansen FB, Hansen TT, Dutheil JY, Schierup MH (2012b) Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. PNAS 109: 10954–10959

Sydow P, Sydow H (1924) Monographia Uredinarum, Vol IV Uredineae imperfectae. Gebrüder Bornträger, Leipzig, pp 1–671

Telle S, Thines M (2008) Amplification of cox2 (620 bp) from 2 mg of up to 129 years old Herbarium specimens, comparing 19 extraction methods and 15 polymerases. PLoS ONE 3(10):e3584

Thiers B (2011) [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C (2013) Phylogeographic patterns shed light on evolutionary process in South America. Mol Ecol 22:1193–1213

Vialle A, Feau N, Allaire M, Didukh M, Martin F, Moncalvo JM, Hamelin RC (2009) Evaluation of mitochondrial genes as DNA barcode for Basidiomycota. Mol Ecol Resour 9(1):99–113

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172(8):4238–4246

Wallnöfer B (2012) A revision of neotropical *Diospyros* (Ebenaceae): part 5. Ann Naturhist Mus Wien B 113:223–251

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322