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New records and data on rust fungi (Pucciniales, Basidiomycota) in Benin

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Abstract

The documentation of rust fungi in tropical Africa is very incomplete. For West Africa, 332 species of rust fungi (Pucciniales, Basidiomycota) are recorded, including only six species from Benin. By a recent survey of rust fungi in Benin, 22 specimens representing 11 species of Pucciniales were found. These species were identified based on morphological characteristics observed by light microscopy. Nine species represent new records of rust fungi for Benin. Among them, *Neophysopella tropicalis* on *Vitis* sp. is recorded for Africa for the first time. Nucleotide sequences of the LSU region of ribosomal DNA confirmed identifications of five species, for which sequences were available in GenBank for comparison. For three species, annotated LSU and ITS barcode sequences are provided for the first time. *Puccinia canaliculata* var. *tenuis* on *Cyperus* spp., described from South Africa and recorded for Guinea as well as for Benin, is treated as *Puccinia doidgeae nom. nov*., based on morphological and sequence data.

Keywords: Africa, *Neoolivea tectonae*, *Neophysopella tropicalis*, *Puccinia arachidis*, *Puccinia canaliculata*, *Sphaerophragmium acaciae*, Uredinales, *Uromyces decoratus*

Introduction

With more than 7,000 species known worldwide, the Pucciniales (Pucciniomycotina) is the most species rich order of phytopathogenic fungi in the Basidiomycota (e.g. Aime & McTaggart 2020). Rust fungi are biotrophic parasites responsible for economic losses when they attack cultivated plants., e.g. wheat, soybeans, or coffee, as well as trees planted for wood production, such as eucalyptus, pines, or poplar, and ornamental plants (e.g. Fisher *et al*. 2012, Lorrain *et al*. 2018).

While rust fungi on economically important crops have been studied extensively in extratropical countries, knowledge about their distribution in tropical latitudes is incomplete, especially for most countries in Africa (Piepenbring *et al*. 2020). Difficulties in identification are due to numerous species which characteristics are poorly known and often lacking specimens. Morphological descriptions of tropical species are often short and based on one or few specimens only, photos and drawings of cellular structures often are missing, data on spore stages are incomplete, the geographical distribution is incompletely documented, exact ranges of host plants are unknown, and DNA barcode sequences of many tropical species are not yet included in databases. Only correctly applied names allow the compilation of information on geographical distribution ranges, to develop efficient control measures, and to establish meaningful quarantine regulations.

Despite the economic importance of rust fungi, very few surveys with a focus on this group have been conducted in Africa. Representative lists of rust species in Africa are available for South Africa (Doidge 1948, 1950, McTaggart *et al*. 2017), Ghana (Dade 1940), Guinea (Viennot-Bourgin 1959, Kranz 1966), Ivory Coast (e.g. Viennot-Bourgin 1958, Yen 1976a, b), Nigeria (Eboh 1978 and further studies cited by Piepenbring *et al*. 2020), and Sierra Leone (Deighton 1936). For West Africa, 332 species have been reported (Piepenbring *et al*. 2020; electronic supplement 1). Among

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them, 213 species (64%) are cited only once (138) or twice (75) for only one or two of 14 West African countries. For Benin, only six species have been documented in the literature (Roger 1951, Subrahmanyam *et al*. 1985, Marley *et al*. 2002, Beed *et al*. 2011, Piepenbring *et al*. 2020; comp. electronic supplement 1).

Salazar Yepes & de Carvalho Junior (2013) proposed a ratio of 1: 9 for species of rust fungi: species of vascular plants in a given area in the tropics. Using 7072 as the number of species of vascular plants known for West Africa (Hepper 1972), there should theoretically be 786 species of rust fungi in this area. If this estimation is correct, the known 332 species in West Africa correspond to 42% of the potential rust species richness.

The present publication is the result of identifications of specimens collected recently in Benin based on morphology using available literature. 28S and ITS regions of rDNA of specimens were sequenced and used for confirmation of identifications if possible.

Methods

Fieldwork and morphological analyses

Fresh and dried rust specimens collected by A. Tabé, M. Piepenbring, and collaborators in diverse regions of Benin were used for the present study. Host plants were identified based on descriptions in literature (Akoègninou *et al*. 2006) and comparison with specimens available in the National Herbarium of Benin (BENIN). Taxonomic identifications of host plants were confirmed by botanists of the National Herbarium. Rust sori were observed by light microscopy with material scraped from sori or from hand sections of sori. Drawings were made with the help of a camera lucida. Specimens were deposited at the Mycological Herbarium of the University of Parakou, Benin (UNIPAR).

Sequencing and phylogenetic analysis

DNA was extracted from specimens as reported by Aime (2006) and Aime *et al*. (2018) using the UltraClean Plant DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). The large subunit (28S, LSU) of the rDNA repeat was amplified with Rust2inv (Aime 2006) /LR6 (Vilgalys & Hester 1990) and nested with Rust 28SF (Aime *et al*. 2018) /LR5 (Vilgalys & Hester 1990) following the protocols of Aime *et al*. (2018). The nested 28S reaction had an initial denaturation step of 3 min at 94°C; 42 cycles of 30s at 94°C, 1 min at 58°C, and 1.5 min at 72°C; with a final extension for 7 min at 72°C. The internal transcribed spacer (ITS) region of the rDNA repeat was amplified and sequenced with primers Rust ITS1-F/Rust ITS2-R (Toome & Aime 2015), following protocols reported by Toome & Aime (2015). PCR products were purified and sequenced at Genewiz (Plainfield, New Jersey). Sequence reads were assembled, trimmed, and edited in Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan). Generated sequences were deposited to NCBI GenBank.

To determine phylogenetic relationships of specimens on *Cyperus* sp. from Benin with related species, phylogenetic analyses based on 28S rDNA were conducted. To construct the dataset, rust species on Cyperaceae were borrowed from Arthur Fungarium, Purdue University, USA (PUR) and their sequences were generated using the same methods as described above. Sequence data of related species was also retrieved from GenBank (table 1). Sequences were aligned with MUSCLE 3.8 within Geneious Prime ver. 2020.2.3 (Biomatters Ltd). The RAxML analyses were run with a rapid Bootstrap analysis using a random starting tree and 1200 maximum likelihood bootstrap replicates, under a GTR GAMMA model.

Results

Coleosporium plumeriae Pat., Bull. Soc. Mycol. France 18(2): 178 (1902)
Figure 1

Uredinia hypophyllous, scattered, yellow-orange, with a chlorotic spot on the upper side of the leaf, tissue around older sori necrotic. **Urediniospores** globose, ellipsoidal to broadly ellipsoidal, occasionally cylindrical, (20–)26–33(–38) × (13–)14–21(–30) µm (n = 90), cellular content bright yellow, wall 2–3 µm thick, hyaline, verrucose, germ pores not seen. **Telia** not observed.

28S rDNA sequences from three specimens of *C. plumeriae* from Benin share 100% (899/902 bp) sequence identity with previously published sequences of *C. plumeriae* (e.g. MF769679, McTaggart & Aime 2018).

TABLE 1. Information on sequence data of rust fungi generated and/or used in the context of the present study.

Species	Collection No.	Herb. Voucher No.	Host species	Host family	Country	28S rDNA	ITS rDNA	Source
Coleosporium plumeriae	TA423		*Plumeria alba*	Apocynaceae	Benin	OL437020	--	this paper
C. plumeriae	TA435		*Plumeria alba*	Apocynaceae	Benin	OL437021	--	this paper
C. plumeriae	TA433		*Plumeria rubra*	Apocynaceae	Benin	OL437022	--	this paper
Kweilingia divina	TA403		*Bambusa vulgaris*	Poaceae	Benin	OL437023	--	this paper
Neophysopella tropicalis	MP5348		*Vitis* sp. cult.	Vitaceae	Benin	OL437024	--	this paper
Puccinia arachidis	TA408		*Arachis hypogaea*	Fabaceae	Benin	OL437025	--	this paper
P. arachidis	TA413		*Arachis hypogaea*	Fabaceae	Benin	OL437026	--	this paper
Puccinia canaliculata	TA427		*Cyperus rotundus*	Cyperaceae	Benin	OL437027	--	this paper
P. canaliculata	TA436		*Cyperus rotundus*	Cyperaceae	Benin	OL437028	OL437017	this paper
P. canaliculata	U677	BPI 881120a	*Cyperus rotundus*	Cyperaceae	Oman	HQ412647	--	Deadman *et al.* (2011)
P. canaliculata		BRIP 57789	*Cyperus* sp.	Cyperaceae	Australia	MW147046	--	Aime & McTaggart (2020)
P. canaliculata		BRIP 40326	*Cyperus rotundus*	Cyperaceae	Australia	OL437029	--	this paper
Puccinia caricina		BRIP 57951	*Carex appressa*	Cyperaceae	Australia	KX999870	--	Marin-Felix *et al.* (2017)
Puccinia caricis s.l.	U1676	PUR N22692	*Carex debilis* var. *rodgei*	Cyperaceae	USA	OL437030	--	this paper
P. caricis s.l.	MCA3087	BPI 878008	*Carex* sp.	Cyperaceae	Canada	OL437031	--	this paper
Puccinia cladii		PUR 50994	*Cladium jamaicense*	Cyperaceae	USA	OL437032	--	this paper
Puccinia doidgeae	MP5444=TA430		*Cyperus esculentus*	Cyperaceae	Benin	OL437033	OL437018	this paper
Puccinia junci		PDD 99243	*Juncus tenuis*	Juncaceae	New Zealand	KX985745	--	Padamsee & McKenzie (2017)
Puccinia liberta		BRIP 59686	*Eleocharis ochrostachys*	Cyperaceae	Australia	KX999881	--	Marin-Felix *et al.* (2017)
Puccinia obscura		KR 14322	*Luzula sylvatica*	Juncaceae	Germany	FJ669234	--	Scholler *et al.* (2011)
Puccinia polysora	TA402		*Zea mays*	Poaceae	Benin	OL437034	--	this paper
P. polysora	TA410		*Zea mays*	Poaceae	Benin	OL437035	--	this paper
Puccinia silvatica		TUB 011528	*Taraxacum* sp.	Asteraceae	Germany	AY222048	--	Lutz *et al.* (2004)
Puccinia thaliae	TA419		*Canna indica*	Cannaceae	Benin	OL437036	--	this paper
Puccinia tirolensis	URB969	PUR N5681	*Carex digitata*	Cyperaceae	Germany	OL437037	--	this paper
Puccinia urticata	U1002	BPI 872251	*Carex acutiformis*	Cyperaceae	Germany	OL437038	--	this paper
Sphaerophragmium acaciae	TA432		*Albizia lebbeck*	Fabaceae	Benin	OL437039	--	this paper
Uromyces decoratus	TA431		*Crotalaria retusa*	Fabaceae	Benin	OL437040	OL437019	this paper

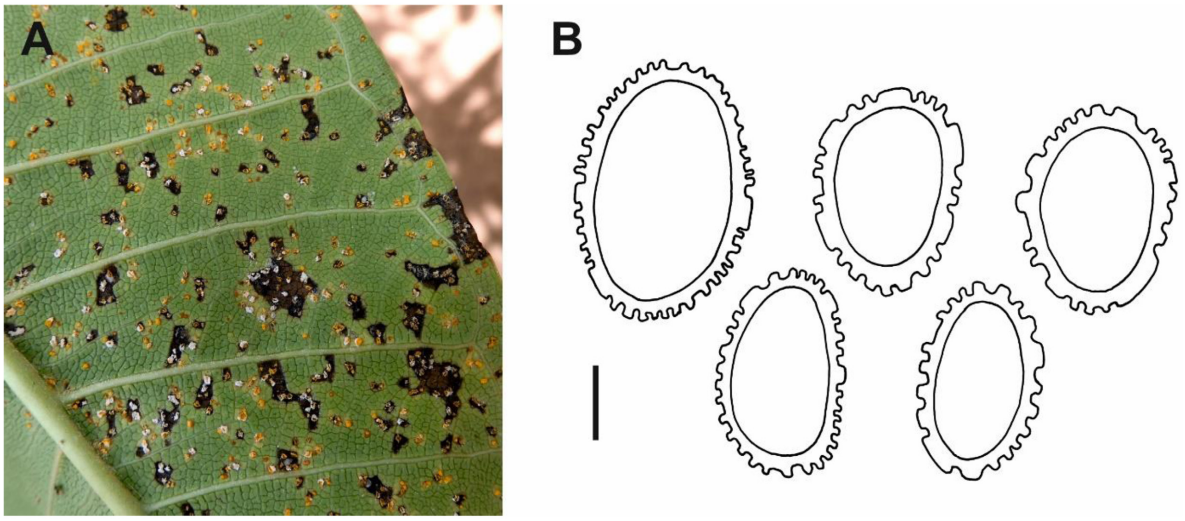

FIGURE 1. *Coleosporium plumeriae* on *Plumeria alba* (TA423). **A** Hypophyllous uredinia. Many sori are colonized by a white hyperparasite. **B** Urediniospores drawn in optical sections. Scale bar: 10 µm.

Specimens examined:—Uredinia on *Plumeria alba*: BENIN. Borgou: N'Dali, elev. 384 m, 9°51' N, 2°42' E, 1 September 2019, A. Tabé, Y. Meswaet, and S. Boni, *TA423* (UNIPAR), GenBank Acc. no. LSU: *OL437020*; Atlantique: Abomey-Calavi, elev. 7 m, 6°27' N, 2°20' E, 25 September 2019, A. Tabé and Y. Meswaet, *TA435* (UNIPAR), GenBank Acc. no. LSU: *OL437021*. Uredinia on *Plumeria rubra*: BENIN. Atlantique: Abomey-Calavi, elev. 7 m, 6°27' N, 2°20' E, 24 September 2019, A. Tabé and Y. Meswaet, *TA433* (UNIPAR), GenBank Acc. no. LSU: *OL437022*.

Hosts and distribution in Benin:—Uredinia on *Plumeria alba* L. and *P. rubra* L. (Apocynaceae), Northern and Southern Benin.

Host species:—Primary host species: *Plumeria acuminata* W.T. Aiton, *P. acutifolia* Poir., *P. alba*, *P. clusioides* Griseb., *P. obtusa* L., *P. pudica* Jacq., *P. rubra*; Apocynaceae (Farr & Rossman 2019). **Secondary host species:** *Pinus* sp.; Pinaceae (Traquair & Kokko 1980, Weeraratne & Adikaram 2006).

Distribution:—Africa [Nigeria (Hernández *et al.* 2005), Benin (this study)], Asia, Central America, North America, Oceania, South America, Caribbean (Farr & Rossman 2020).

Comments:—*Coleosporium plumeriae* is reported here for the first time for Benin. As the secondary host (*Pinus* sp.) is not present in Benin, it most likely cycles continuously as urediniospores. The morphological characteristics of the uredinial stage observed for specimens from Benin are identical with those reported for *C. plumeriae* from other countries (e.g. Chung *et al*. 2006, Baiswar *et al*. 2008, Wang *et al*. 2011).

Kweilingia divina (Syd.) Buriticá, Revista Soc. Colomb. Ci. Nat. 22 (no. 84): 330 (1998)
Figure 2

Uredinia amphigenous, individual or in groups, associated with brown lesions surrounded by an orange-yellow halo. **Urediniospores** broadly ellipsoidal to ellipsoidal, sometimes subglobose, or oblong, (22–)23–30 × (15–)17–22(–25) µm (n = 30), light brown to hyaline, wall 1–2 µm thick, echinulate, germ pores indistinct. **Paraphyses** curved, (22–)28–48(–57) × (6–)8–10(–12) µm (n = 15), hyaline or brownish yellow, wall smooth, 2 µm thick. **Telia** not observed.

A 28S rDNA sequence from the specimen of *Kweilingia divina* from Benin shares 100% (893/894 bp) sequence identity with previously published sequences of *K. divina* (e.g. MG907215, Aime *et al*. 2018).

Specimen examined:—Uredinia on *Bambusa vulgaris*: BENIN. Zou: Zogbodomey, Lokoli river, elev. 50 m, 7°02' N, 2°11' E, 11 August 2017, A. Tabé, M. Piepenbring, participants of Summer School 2017, *TA403* (UNIPAR), GenBank Acc. no. LSU: *OL437023*.

Host and distribution in Benin:—Uredinia on *Bambusa vulgaris* Schrad. ex J.C. Wendl. (Poaceae), Southern Benin.

Host species:—Primary host species: *Bambusa* spp., *Dendrocalamus* spp., *Gigantochloa apus* (Schult. & Schult.f.) Kurz, *Guadua latifolia* (Bonpl.) Kunth, *Ochlandra* spp., *Oxytenanthera* sp., *Thyrsostachys* spp.; Poaceae. **Secondary host species:** *Randia brandisii* (Wight & Arn.) Gamble, *R. candolleana* Wight & Arn., *R. dumetorum* (Retz.) Poir., *R. uliginosa* (Retz.) Poir., *Randia* sp.; Rubiaceae (Gautam & Avasthi 2018).

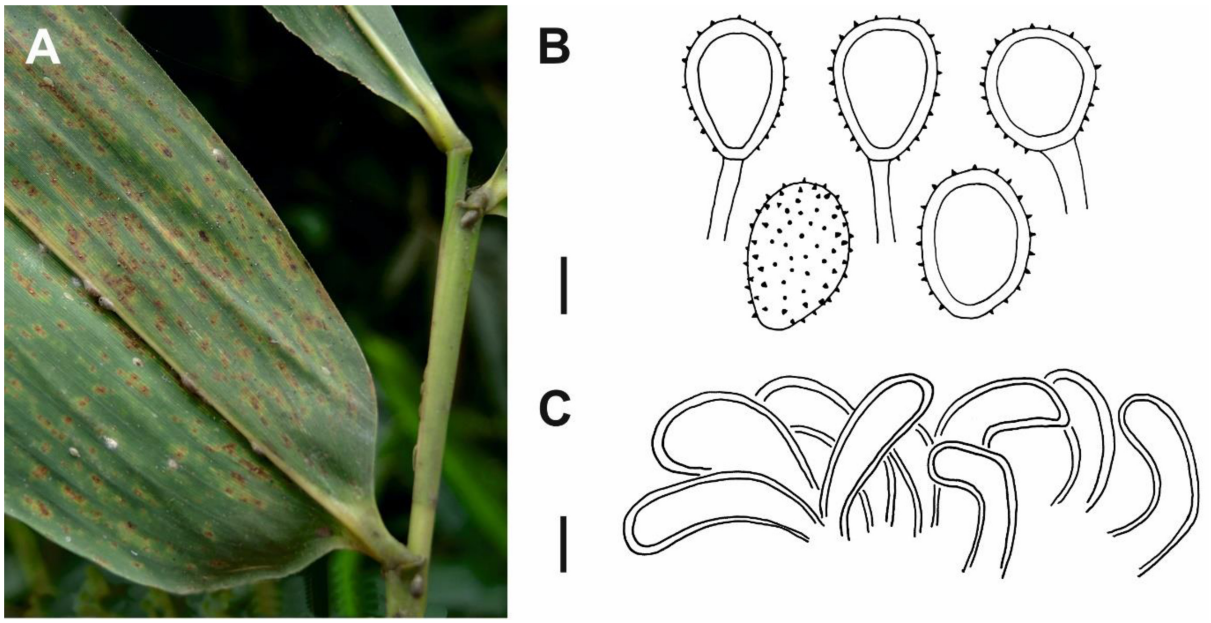

FIGURE 2. *Kweilingia divina* on *Bambusa vulgaris* (TA403). **A** Hypophyllous uredinia **B** urediniospores drawn in optical sections except one spore **C** paraphyses. Scale bars: 10 µm.

Distribution:—Africa [Ivory Coast (Yen 1976a), Nigeria (Eboh 1985), Benin (this study)], Asia, Central America, North America, Oceania, South America (Farr & Rossman 2020).

Comments:—*Kweilingia divina* on *Bambusa vulgaris* is reported here for the first time for Benin. The morphological characteristics observed for specimens from Benin are identical with those reported for *K. divina* from other countries (e.g. Eboh 1985, Blomquist *et al*. 2009, Gautam & Avasthi 2018).

Neoolivea tectonae (Racib.) Aime & McTaggart, Fungal Syst. Evol. 7: 40 (2020)

≡ ***Olivea tectonae*** (Racib.) Thirum., Curr. Sci. 18: 176. 1949.

= ***Olivea tectonae*** (T.S. Ramakr. & K. Ramakr.) R.L. Mulder, in Mulder and Gibson, C.M.I. Descr. Pathog. Fungi Bact. 365: 1. 1973. *Chaconia tectonae* T.S. Ramakr. & K. Ramakr., Indian Phytopathol. 2: 19. 1949, *nom. cons. prop.*

(For further synonyms see Aime & McTaggert 2020.)

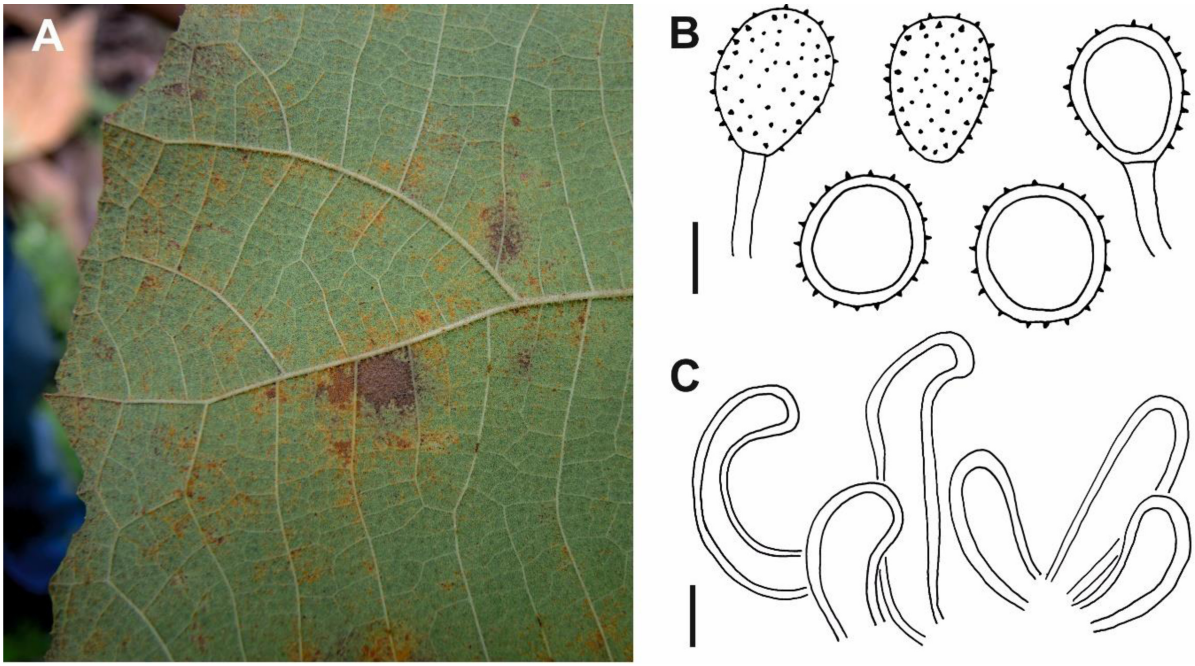

FIGURE 3. *Neoolivea tectonae* on *Tectona grandis* (TA404). **A** Hypophyllous uredinia **B** urediniospores drawn in surface view or in optical section **C** apical parts of paraphyses. Scale bars: 10 µm.

Uredinia hypophyllous, subepidermal, erumpent, powdery, paraphysate, associated with orange to dark brown chlorotic spots on the upper side of the leaves. **Urediniospores** subglobose to broadly ellipsoidal, occasionally globose, (19–)20–24(–30) × 17–19(–21) µm (n = 90), mostly hyaline, yellow-orange, walls echinulate or covered by granular ornamentation, 1.5–2 µm thick, germ pores not seen. **Paraphyses** cylindrical and more or less irregularly curved, 20–42 × 8–12 µm, wall up to 4 µm thick, up to 5 µm thick at the apex, hyaline, smooth. **Telia** not observed.

Several attempts to isolate 28S and ITS rDNA sequences from the specimens of *N. tectonae* from Benin were unsuccessful.

Specimens examined:—Uredinia on *Tectona grandis:* BENIN. Zou: Zogbodomey, Lama forest, elev. 47 m, 7°03' N, 2°10' E, 11 August 2017, A. Tabé, M. Piepenbring, and participants of Summer School 2017, *TA404* (UNIPAR) ; Borgou: Parakou, Nima, elev. 363 m, 9°21' N, 2°38' E, 22 November 2017, A. Tabé, *TA412* (UNIPAR).

Host and distribution in Benin:—Uredinia on *Tectona grandis* (Lamiacae), Central and Southern Benin.

Host species:—Primary host species: *Tectona grandis*, *Tectona* spp.; Lamiaceae (Yun 2020). **Secondary host species:** not known.

Distribution:—Africa [Ivory Coast (Koffi *et al.* 2018), Benin (this study)], Asia, Central America, Oceania, Southern America, Caribbean (Farr & Rossman 2020).

Comments:—*Neoolivea tectonae* is reported for the first time for Benin and for the second time for Africa. The morphological characteristics observed for specimens from Benin are identical with those reported for *N. tectonae* from other countries (e.g. Daly *et al*. 2006, Cabral *et al*. 2010).

Neophysopella tropicalis Y. Ono, S. Chatasiri, Pota & Okane, in Ono *et al.*, Mycol. Progr. 19: 914 (2020)
Figure 4.

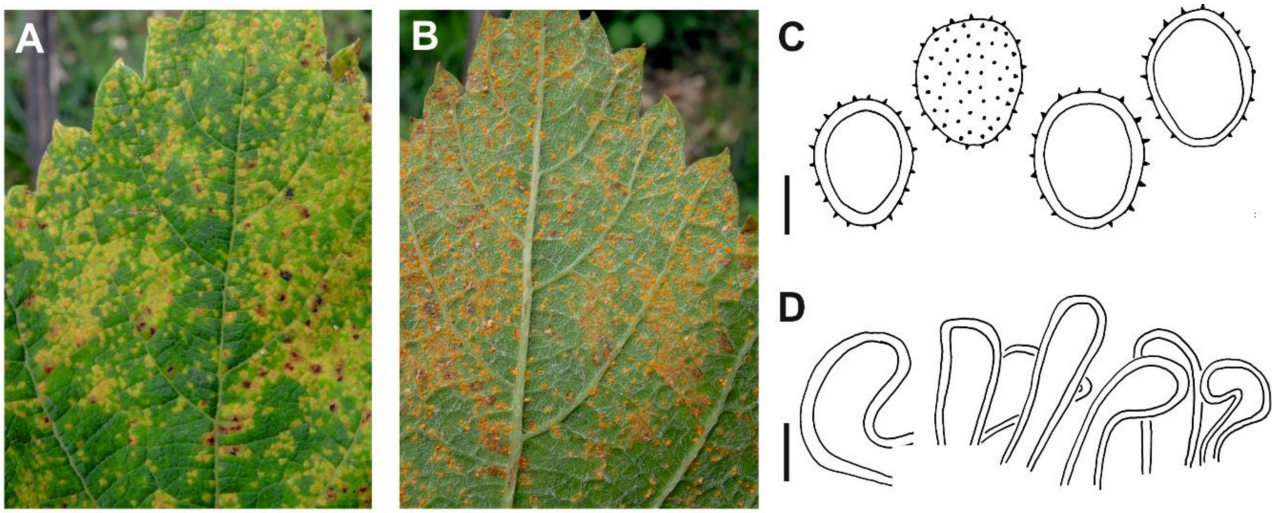

FIGURE 4. *Neophysopella tropicalis* on *Vitis* sp. cult. (MP5348). **A** Infected leaf seen from above **B** the same leaf seen from below, with hypophyllous uredinia **C** urediniospores drawn in optical sections except one spore **D** apical parts of paraphyses. Scale bars: 10 µm.

Uredinia hypophyllous, erumpent, individual or in groups, yellow-orange, associated with angular, necrotic spots on the upper side of the leaves, urediniospores surrounded by paraphyses. **Urediniospores** ellipsoidal to broadly ellipsoidal, sometimes globular, (18–)19–23(–26) × 14–17(–20) µm (n = 30), densely and finely echinulate, spines approx. 1 µm high, pale yellow to hyaline, wall 1–2 µm thick, germ pores not observed. **Paraphyses** cylindrical, straight or sometimes curved, 24–60 × 8–16 µm (n = 15), wall light brown, 2–4 µm thick, sometimes thicker at the apex, smooth. **Telia** not observed.

A 28S rDNA sequence obtained from the specimen from Benin presents 99% (896/901) sequence identity with previously published sequences of *N. tropicalis* (e.g. LC534274, Ono *et al*. 2020).

Specimen examined:—Uredinia on *Vitis* sp. cult.: BENIN. Borgou: Parakou, Atagara, Songhaï center, elev. 372 m, 9°41' N, 2°69' E, 2 August 2017, M. Piepenbring and participants of Summer School 2017, *MP5348* (UNIPAR), GenBank Acc. no. LSU: *OL437024.*

Host and distribution in Benin:—Uredinia on *Vitis* sp. cult. (Vitaceae), Central Benin.

Host species:—Primary host species: cultivars derived from *Vitis vinifera* L. and *V. labrusca* L.; Vitaceae. **Secondary host species:** not known (Ono *et al*. 2020).

Distribution:—Africa [Benin (this study)], Asia, Oceania, Central America, North America, Southern America (Ono *et al*. 2020).

Comments:—*N. tropicalis* is reported here for the first time for Benin and for the first time for Africa. Characteristics of the specimens from Benin were compared to those described for *N. meliosmae-myrianthae* (Henn. & Shirai) Jing X. Ji & Kakish. (Hennen *et al*. 2005; Ono *et al*. 2012), *N. montana* (Y. Ono & Chatasiri) Jing X. Ji & Kakish. (Ono *et al*. 2012), and *N. tropicalis* (Ono *et al*. 2020) from other countries. According to Ono *et al*. (2020), the longest paraphyses of *N. tropicalis* are shorter (23–67 µm) than those of *N. meliosmae-myrianthae* (19–79 µm) and *N. montana* (22–80 µm), and the walls of the paraphyses are only up to 4 µm thick in *N. tropicalis* in contrast to values of 5 µm and higher in the other two species. These characteristics indicate that the specimen found in Benin represents *N. tropicalis*. They are, however, rather difficult to assess.

Puccinia arachidis Speg. var. ***arachidis***, Anales Soc. Ci. Argent. 17: 90 (1884)
Figure 5

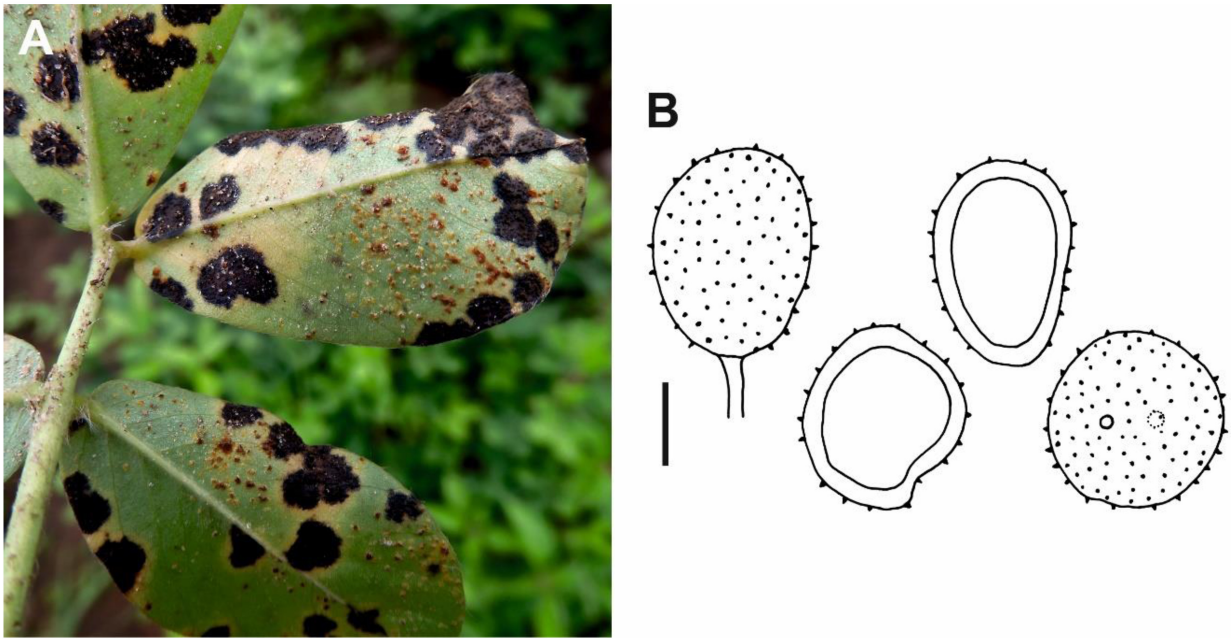

FIGURE 5. *Puccinia arachidis* on *Arachis hypogaea*. **A** Hypophyllous uredinia and dark brown lesions caused by *Nothopassalora personata* (Benin, Tontarou, without specimen) **B** urediniospores drawn in surface view or in optical sections (TA420). Scale bar: 10 µm.

Uredinia amphigenous, more abundant on the lower side of leaves, subepidermal, covered by the host epidermis when young, 0.5–1 mm diam., surrounded by chlorotic spot, green to yellowish spots on the upper side, first orange-brown, later brown. **Urediniospores** broadly ellipsoidal to ellipsoidal, (20–)24–28(–34) × (17–)19–23(–26) µm (n = 120), brown, wall 1–3 µm thick, echinulate, spines 1–2 µm long, 1–4 equatorial germ pores, pedicels up to 27 µm long. **Telia** not observed.

Two 28S rDNA sequences from two specimens of *P. arachidis* from Benin were obtained which together cover 633 base positions and differ at two base positions (similarity 631/633, i.e., 100%). They differ from previously published sequences of other rust fungi by 4% or 6% respectively. Barcode sequence data of *P. arachidis* are not available for comparison; they are published here for the first time.

Specimens examined:—Uredinia on *Arachis hypogaea*: BENIN. Atlantique: Allada, Sékou, elev. 19 m, 6°37' N, 2°14' E, 15 August 2017, A. Tabé and Y. Meswaet, *TA405* (UNIPAR); Donga: Djougou, Pabégou, elev. 445 m, 9°42' N, 1°39' E, 11 September 2017, A. Tabé and Y. Meswaet, *TA408* (UNIPAR), Acc. no. LSU: *OL437025*; Borgou: Parakou, Nima, elev. 376 m, 9°20' N, 2°36' E, 22 November 2018, A. Tabé, *TA413* (UNIPAR), GenBank Acc. no. LSU: *OL437026*; Borgou: Parakou, Barrière, elev. 376 m, 9°20' N, 2°36' E, 27 August 2019, A. Tabé and Y. Meswaet, *TA420* (UNIPAR).

Host and distribution in Benin:—Uredinia on *Arachis hypogaea* L. (Fabaceae), Northern and Southern Benin.

Host species:—Primary host species: *Arachis glabrata* Benth., *A. hypogaea*, *A. nambyquarae* Hoehne, *A. prostrata* Benth., *Arachis* sp.; Fabaceae (Farr & Rossman 2019). **Secondary host species:** not known.

Distribution:—Africa [Ivory Coast (Savary *et al.* 1988), Mauritius (Orieux & Felix 1968), Nigeria (Eboh 1981), Southern Africa (Crous *et al.* 2000), Tanzania (Ebbels & Allen 1979) Benin, Botswana, Burkina Faso, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Senegal, Somalia, Sudan, Uganda, Zambia, Zimbabwe (Subrahmanyam *et al*. 1985)], Asia, Central America, North America, Oceania, Southern America, Caribbean (Farr & Rossman 2020).

Comments:—This is the second report of this rust for Benin. The morphological characteristics observed for specimens from Benin are identical with *Puccinia arachidis* as described from the other countries (e.g. Subrahmanyam *et al*. 1985, Hennen *et al*. 2005, Anco & Wang 2017).

Puccinia doidgeae **Tabé, Aime & M. Piepenbr., *nom. nov., stat. nov.***
MycoBank: 841915
Figures 6–7

Replaced synonym: *Puccinia canaliculata* var. *tenuis* Doidge, Bothalia 4: 900 (1948). **Type:** On *Cyperus esculentus* L., SOUTH AFRICA. Brits, Hartbeespoort, (no date), F. M. du Toit 33120 (type probably in PREM, n.v., not available upon request). [non *Puccinia tenuis* (Schwein.) Burrill 1884]

Etymology:—Honouring Ethel Mary Doidge, who noticed that this taxon is distinct from typical *Puccinia canaliculata*.

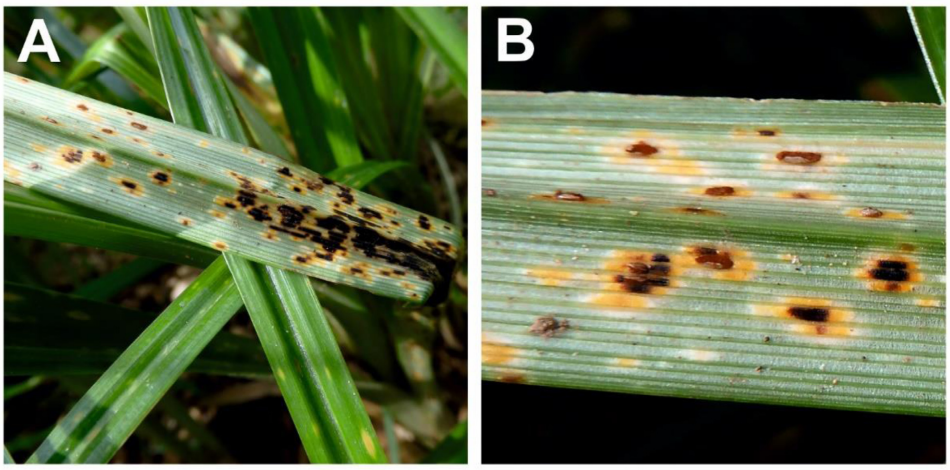

FIGURE 6. *Puccinia doidgeae* on *Cyperus esculentus* (MP5444). **A** Abaxial uredinia and telia **B** orange-brown, erumpent uredinia, black embedded telia, and whitish to orange young sori.

Uredinia amphigenous, more abundant on the abaxial side of the leaves, elongated, parallel to the veins of the leaves, first subepidermal, later erumpent, orange-brown, containing only urediniospores or sometimes mixed with teliospores. **Urediniospores** broadly ellipsoidal, globose, or pyriform, (22–)24–28(–30) × (16–)18–22(–24) µm (n = 33), yellow to pale yellow, finely echinulate, golden brown, wall 2 µm thick, with 2 equatorial germ pores, pedicels hyaline, mostly 60–70 µm long. Urediniospores easily break off the pedicels, so urediniospores with pedicels attached to them are rarely observed. After the liberation of numerous urediniospores, a uredinium presents a 'cushion' formed by disused pedicels. **Telia** abaxial, elongated between veins of the leaves, usually 0.5–1 mm long, rarely up to 2 mm, approximately 200 µm broad, subepidermal, black, with teliospores in locules, delimited by pseudoparaphyses evident in transverse sections observed by light microscopy. **Pseudoparaphyses** densely packed, more or less curved, 50–80 µm long and 1–3 µm broad, slightly enlarged (up to 5 µm) at the tips, light brown. **Teliospores** clavate, not or slightly constricted at the septa, apex acuminate or rounded, two-celled, (40–)43–55(–70) × (12–)13–16(–17) µm (n = 20), yellowish brown, more strongly pigmented towards the tips, wall 1–1.5 µm thick at the sides, 6–7(–9) µm thick at the apex, pedicels reddish brown, pigmentation darker than at the tips of the teliospores, up to 45 µm long (n = 10).

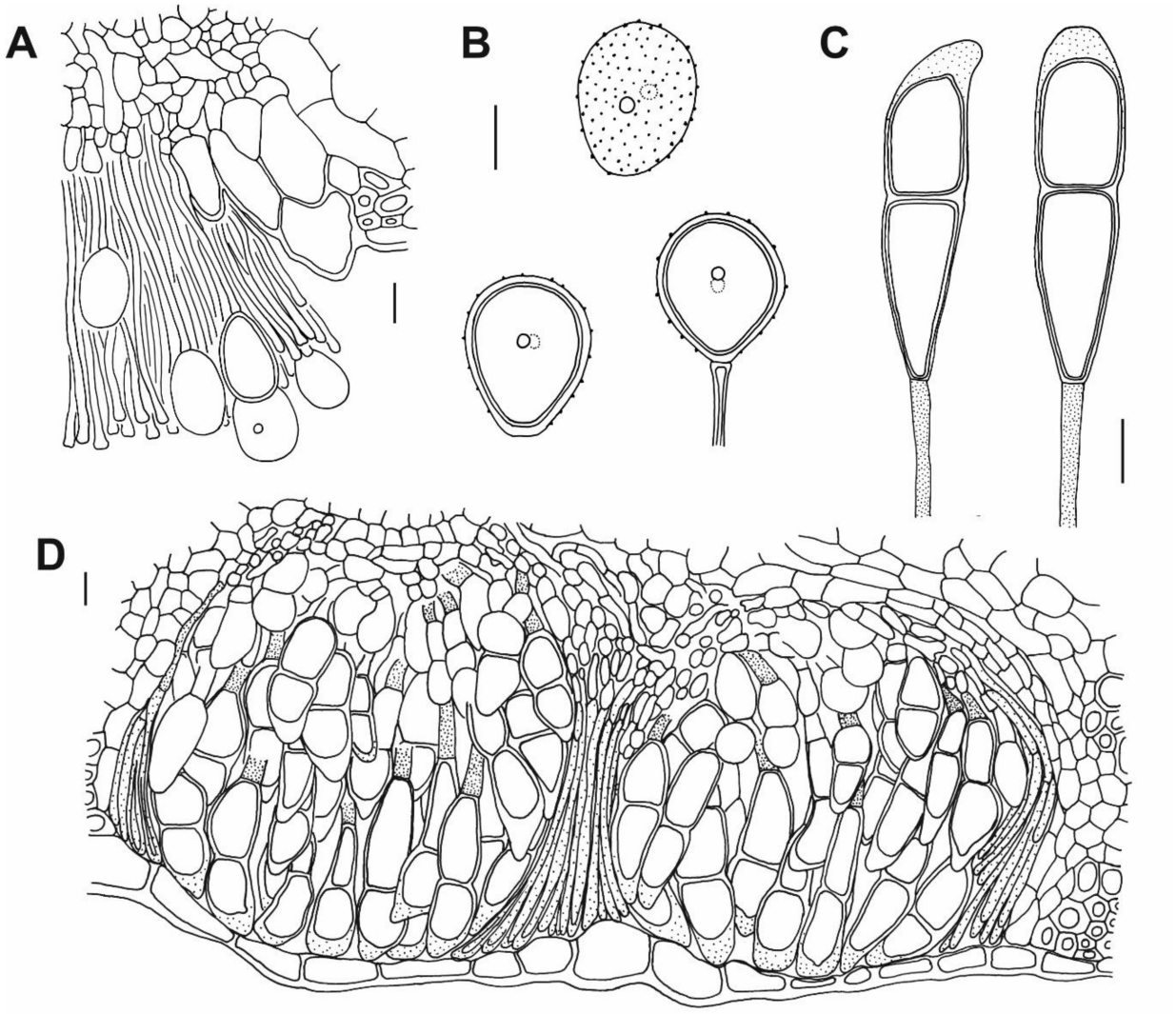

FIGURE 7. *Puccinia doidgeae* on *Cyperus esculentus* (MP5444). **A** Part of a uredinium with ruptured host tissue, numerous pedicels, and some urediniospores in various developmental stages **B** mature urediniospores with germ pores drawn in surface view or in optical sections **C** teliospores with pigmented pedicels and slightly pigmented tips **D** a telium with two locules delimited by pseudoparaphyses, filled with teliospores, and surrounded by host tissue. Dots of different density indicate more or less strong pigmentation. Scale bars: 10 µm (**A**, **D**); 5 µm (**B**, **C**).

Sequence data of the 28S ribosomal DNA indicate that *P. canaliculata* var. *tenuis* is not conspecific with other specimens of *P. canaliculata* (Fig. 8). Therefore and because of morphological differences (see below), we propose to raise the variety to species rank. As the name at the new rank, *Puccinia tenuis*, is occupied by the name *P. tenuis* (Schwein.) Burrill, we propose to replace it by the new name *P. doidgeae*.

Host and distribution in Benin:—Uredinia and telia on *Cyperus esculentus* (Cyperaceae), Northwestern Benin.

Specimen examined:—Uredinia and telia on *Cyperus esculentus*: BENIN. Atacora: Boukoumbé, Koutatiégou village, Koussogou hamlet, elev. 234 m, 10°11’ N, 1°05’ E, 15 September 2019, M. Piepenbring, M.U. Schmidt, and participants of the symposium 2019, *MP5444* (= *TA430*) (UNIPAR), GenBank Acc. no. LSU: *OL437033*, ITS: *OL437018.*

Specimens examined for comparison:—*Puccinia canaliculata* (Schwein.) Lagerh. on *Cyperus rotundus* L.: BENIN. Borgou: Parakou, around Campus of the University of Parakou, elev. 356 m, 9°20’ N, 2°38’ E, 15 September 2019, A. Tabé, Y. Meswaet, R. Dramani, TA427 (UNIPAR), GenBank Acc. no. LSU: *OL437027*. On the same host plant with spikelets of different colour, at the same locality, elev. 356 m, 9°20’ N, 2°38’ E, 15 September 2019, A. Tabé, Y. Meswaet, R. Dramani, TA436 (UNIPAR), GenBank Acc. no. LSU: *OL437028*, ITS: *OL437017*. These two specimens only present uredinia and were identified as *P. canaliculata* based on molecular sequence data (Fig. 8).

Host species of *P. doidgeae:*—Primary host species: *Cyperus esculentus*, *Cyperus* sp. (Cyperaceae) (Doidge 1948, Viennot-Bourgin 1959). **Secondary host species:** not known.

Distribution:—Africa [Guinea (Viennot-Bourgin 1959), South Africa (Doidge 1948)]. This species has not been reported outside Africa and is reported here for the first time for Benin. Specimens of *P. doidgeae* probably have been identified as *P. canaliculata* in the past.

Comments:—According to the key for the identification of species of *Puccinia* infecting *Cyperus* spp. (Kern 1919), the specimen from Benin represents *P. canaliculata*, due to urediniospores with two equatorial germ pores and walls uniformly 1–2 µm thick. In addition, *P. canaliculata* differs from *P. cyperi* Arthur by the presence of loculate telia, that have also been referred to as telia with stroma. The specimen from Benin, however, differs from *P. canaliculata* by slenderer teliospores (13–16 µm wide) versus 15–21 µm (Kern 1919) and 14–22 µm (Barreto & Evans 1995) for typical teliospores of *P. canaliculata*. Based on this difference, Doidge (1948) established the new variety, *P. canaliculata* var. *tenuis*, that was later confirmed by Viennot-Bourgin (1959).

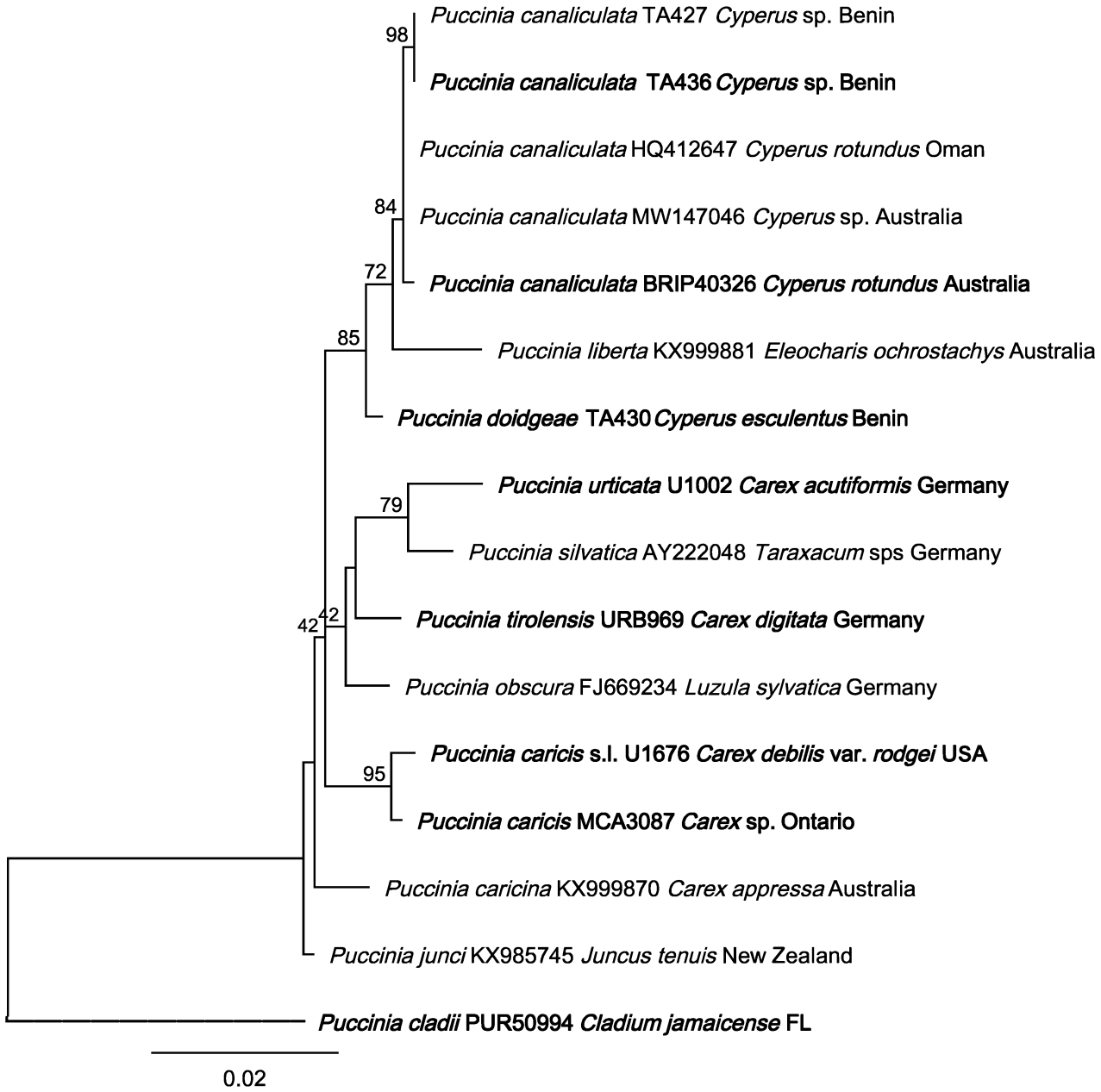

FIGURE 8. RAxML analysis of 28S rDNA sequences of *Puccinia* spp. mostly on species of Cyperaceae. Sequences newly generated for this study are indicated in bold. RAxML bootstrap support >70 is indicated next to supported node.

Puccinia polysora Underwood, Bull. Torrey Bot. Club 24: 86 (1897)
Figure 9

Uredinia amphigenous, sparse, mostly elongated or oval, 2–3 mm long, subepidermal, erumpent. **Urediniospores** mostly ellipsoidal to oblong or globose, sometimes broadly ellipsoidal, rarely cylindrical, (23–)28–35(–38) × (18–)21–24(–29) µm (n = 120), brown to golden brown, echinulate, spines 1.5–2 µm long, wall 1–2 µm thick, with 2 or 3 equatorial germ pores. **Paraphyses** cylindrical, spatula-shaped, or slightly curved, rounded and sometimes thicker at the tip, (33–)40–52(–55) × (5–)6–9(–10) µm (n = 15, width measured in the middle of the paraphyses), hyaline, wall 1–2 µm thick. **Telia** not observed.

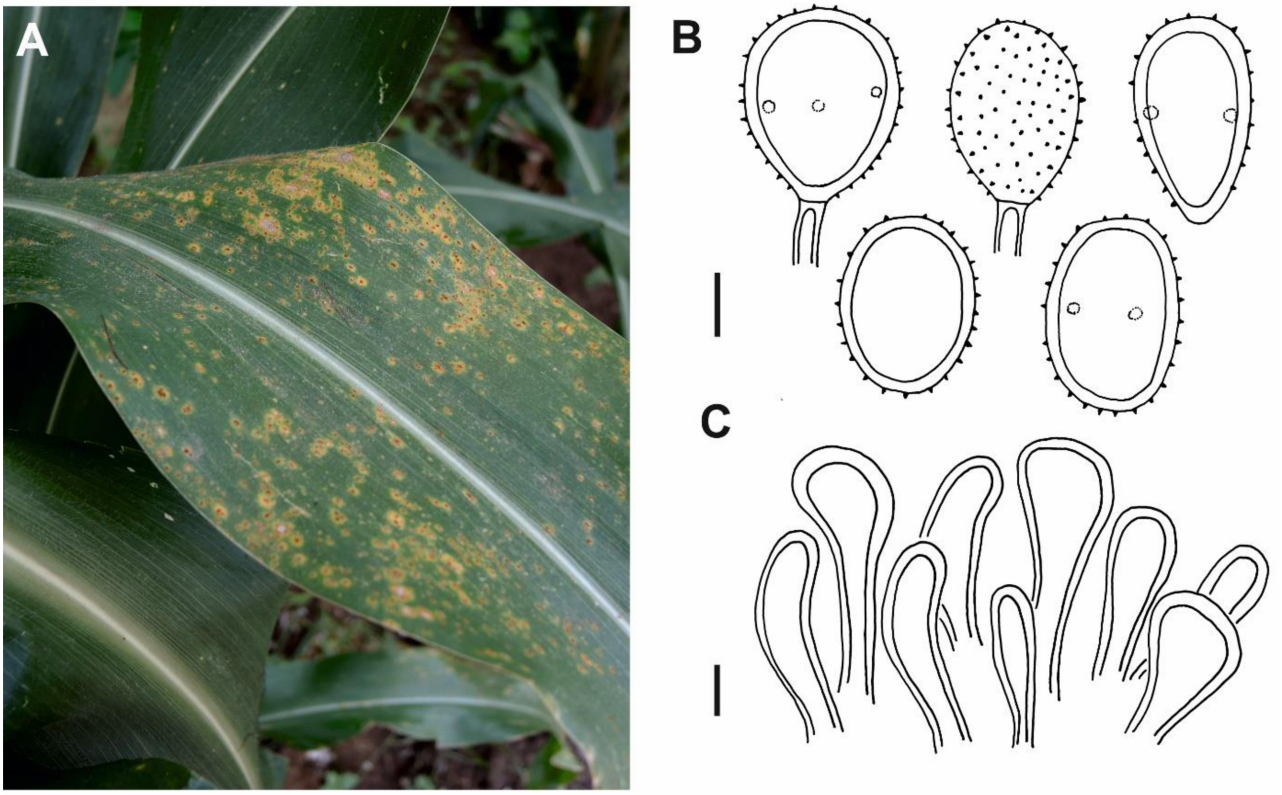

FIGURE 9. *Puccinia polysora* on *Zea mays* (TA401). **A** Epiphyllous uredinia **B** urediniospores drawn in optical sections except one spore **C** apical parts of paraphyses. Scale bars: 10 µm.

28S rDNA sequences from two specimens of *P. polysora* collected in Benin share 100% (897/901) sequence identity with a previously published sequence of *P. polysora* (GU058024, Dixon *et al*. 2010).

Specimens examined:—Uredinia on *Zea mays*: BENIN. Borgou: Parakou, Atagara, Songhaï center, elev. 372 m, 9°41’ N, 2°69’ E, 2 August 2017, A. Tabé, N.S. Yorou, M. Piepenbring, participants of Summer School 2017, *TA401* (UNIPAR); Borgou: Tchaourou, Wari-Maro, elev. 302 m, 9°07’ N, 2°07’ E, 4 August 2017, A. Tabé, N.S. Yorou, M. Piepenbring, Y. Meswaet, participants of Summer School 2017, *TA402* (UNIPAR), GenBank Acc. no. LSU: *OL437034*; Borgou: N’Dali, elev. 179 m, 9°51’ N, 2°44’ E, 25 August 2017, A. Tabé, Y. Meswaet, *TA406* (UNIPAR); Borgou: Parakou, Nima, elev. 376 m, 9°20’ N, 2°36’ E, 10 October 2017, A. Tabé, *TA410* (UNIPAR), GenBank acc. no. LSU: *OL437035*.

Host and distribution in Benin:—Uredinia on *Zea mays* L. (Poaceae), Central and Northern Benin.

Host species:—Primary host species: *Tripsacum australe* H.C. Cutler & E.S. Anderson, *T. dactyloides* L., *T. laxum* Nash, *Tripsacum* sp., *Zea mays*; Poaceae (Crouch & Szabo 2011, Hennen *et al*. 2005). **Secondary host species:** not known (Hennen *et al*. 2005).

Distribution:—Africa [Ivory Coast, Guinea, Madagascar, Malawi, Mauritius, Senegal, Sierra Leone, Southern Africa, Tanzania, Uganda, Zimbabwe (Riley 1956, Kranz 1964a, b, Peregrine & Siddiqi 1972, Yen 1976a, Crouch & Szabo 2011), Benin (this study)], Asia, Central America, Europe, North America, Oceania, Southern America, Caribbean (Farr & Rossman 2021).

Comments:—*Zea mays* can be infected by three species of Pucciniales, common rust (*Puccinia sorghi* Schwein.), southern rust (*P. polysora*), and *P. purpurea* Cooke. The morphological characteristics of the uredinia and uredinial paraphyses of Benin material are identical with those reported for *P. polysora* from other countries (Huguenin 1959, Pavgi 1972, Hennen *et al*. 2005, Crouch & Szabo 2011, Ramirez-Cabral *et al*. 2017, Tsatsia & Jackson 2017). *Puccinia polysora* is reported for the first time for Benin.

Puccinia thaliae Dietel, Hedwigia 38: 250 (1899)
Figure 10

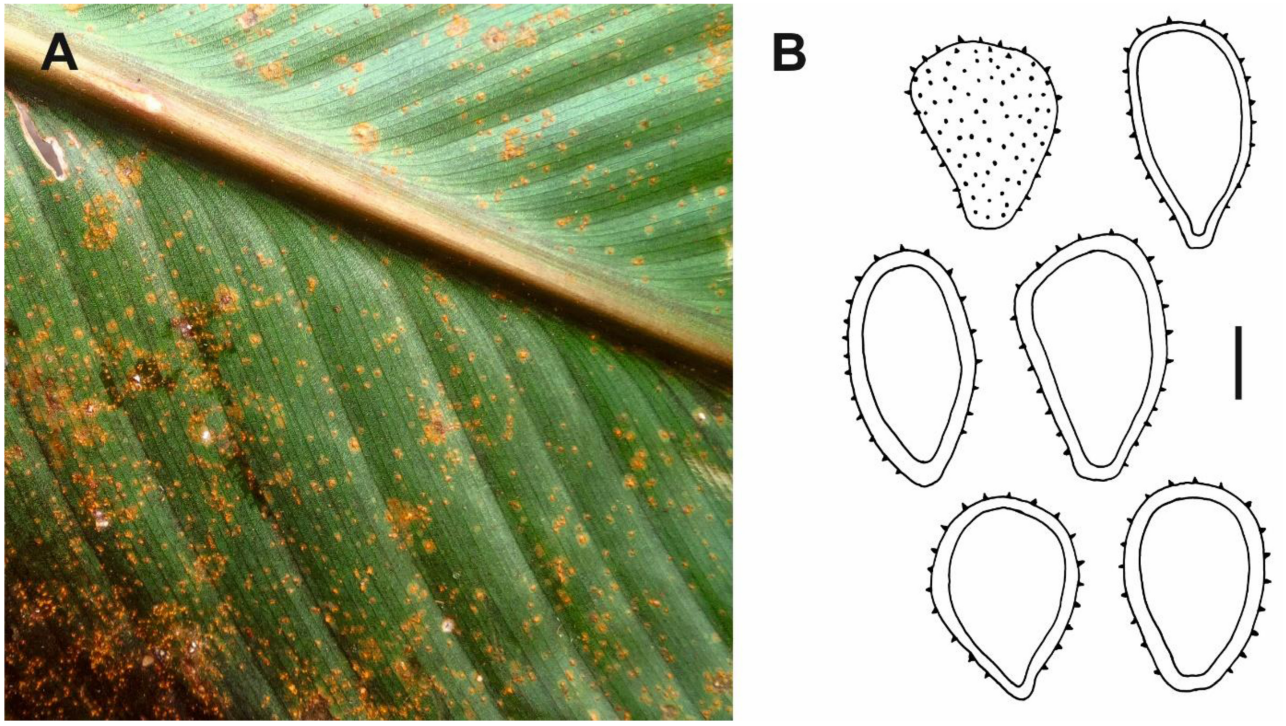

FIGURE 10. *Puccinia thaliae* Dietel on *Canna indica* (TA419). **A** Hypophyllous uredinia **B** urediniospores drawn in optical sections except one spore. Scale bar: 10 µm.

Uredinia amphigenous, more abundant on the adaxial side, scattered, yellow orange, erumpent, subepidermal, associated with chlorotic yellow-orange spots, spots dark brown in severe infections. **Urediniospores** ellipsoidal to broadly ellipsoidal or pyriform, rarely globose, (27–)30–39(–45) × (15–)19–25(–28) µm (n = 30), pale yellow to hyaline, spore wall 2–3 µm thick, echinulate, germ pores not visible. **Telia** not observed.

A 28S rDNA sequence of *P. thaliae* from a specimen from Benin shares 100% (890/892) sequence identity with previously published sequences of *P. thaliae* (e.g. JX206994, Padamsee & McKenzie 2012).

Host and distribution in Benin:—Uredinia on *Canna indica* L. (Cannaceae), Southern Benin.

Specimen examined:—Uredinia on *Canna indica*: BENIN. Atlantique: Allada, Atogon, Niaouli CRA-Sud, elev. 24 m, 6°39’ N, 2°09’ E, 8 May 2019, A. Tabé, *TA419* (UNIPAR), GenBank Acc. no. LSU: *OL437036*.

Host species:—Primary host species: *Canna coccinea* Mill., *C. compacta* Roscoe, *C. edulis* Ker Gawl., *C. generalis* L.H. Bailey, *C. glauca* L., *C. indica*, *C. patens* (Aiton) Roscoe, *Canna* sp.; Cannaceae. *Calathea* sp., *Ctenanthe* sp., *Ischnosiphon arouma* (Aublet) Körn., *I. leucophaeus* (Poepp. & Endl.) Körn., *I. simplex* Huber, *Ischnosiphon* sp., *Maranta arundinacea* L., *Maranta* sp., *Thalia dealbata* Fraser (Hennen *et al*. 2005), *Stromanthe tonckat* (Aubl.) Eichler (Nelson 2013); Marantaceae. **Secondary host species:** not known (Hennen *et al*. 2005).

Distribution:—Africa [Uganda (Gjaerum *et al.* 2003), Nigeria (Eboh 1986), Southern Africa (Van Jaarsveld *et al.* 2007), Benin (this study)], Asia, Central America, Europe, North America, Oceania, Southern America, Caribbean (Farr & Rossman 2020).

Comments:—The size of the urediniospores is rather variable according to data in diverse publications (Eboh 1986, Hennen *et al*. 2005, Cedas de Jesus *et al*. 2018). The size of the urediniospores of the present specimen is similar to sizes reported by Padamsee & McKenzie [2012; (23–)26–36(–43) × 17–23(–27) µm] and Nelson [2013; 28–40 × 20–25 µm]. *Puccinia thaliae* is reported here for the first time for Benin.

Sphaerophragmium acaciae (Cooke) Magnus, Ber. Deutsch. Bot. Ges. 9: 121 (1891)
Figure 11

Uredinia hypophyllous, scattered, individual or grouped, circular, subepidermal, erumpent, brown. **Urediniospores** ovoid, ellipsoidal to broadly ellipsoidal, or reniform, (18–)22–26(–27) × (15–)16–18(–20) µm (n = 30), pale yellow, wall 1–2 µm thick, echinulate, with 4–5 inconspicuous, more or less equatorial germ pores. **Telia** not observed.

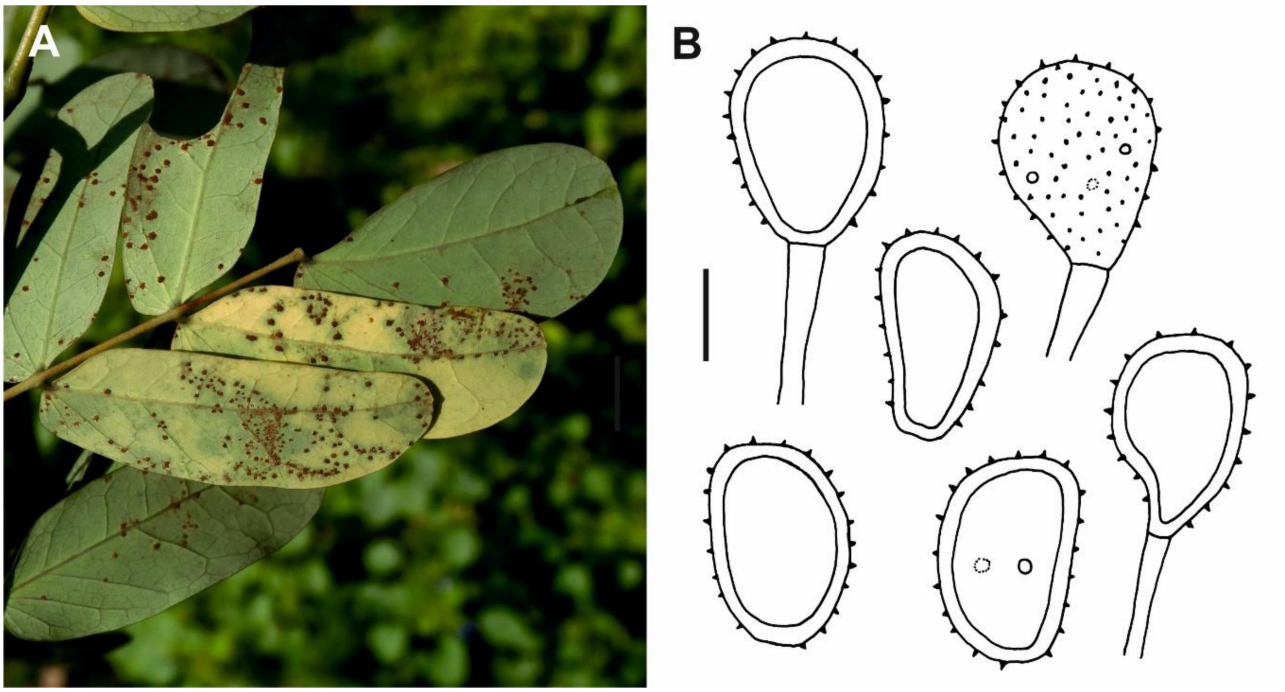

FIGURE 11. *Sphaerophragmium acaciae* on *Albizia lebbeck* (TA432). **A** Hypophyllous uredinia **B** urediniospores drawn in optical sections except one spore. Scale bar: 10 µm.

The 28S rDNA sequence of the specimen of *S. acaciae* from Benin shares 99.9% (875/876 bp) sequence identity with a sequence deposited as *Sphaerophragmium* sp. on *Albizia* sp. (KJ862350, specimen BRIP 56910 from Australia; McTaggart *et al*. 2015) as well as 100% with two specimens deposited in BPI and identified as *S. acaciae* (BPI863973 from Puerto Rico; BPI878100 from Nigeria; C. Aime, unpublished). Here, we publish the first completely annotated LSU rDNA sequence for *S. acaciae*.

Host and distribution within the studied area:—Uredinia on *Albizia lebbeck* (L.) Benth. (Fabaceae), Central Benin.

Specimen examined:—Uredinia on *Albizia lebbeck*: BENIN. Collines: Glazoué, elev. 192 m, 7°58’ N, 2°14’ E, 22 September 2019, A. Tabé, Y. Meswaet, M. Piepenbring, N.S. Yorou, M.U. Schmidt, *TA432* (UNIPAR), GenBank Acc. no. LSU: *OL437039*.

Host species:—Primary host species: *Albizia acle* (Blanco) Merr., *A. coriaria* Welw. ex Oliv., *A. corniculata* (Lour.) Druce, *A. falcataria* (L.) Fosberg, *A. julibrissin* Durazz., *A. kalkora* (Roxb.) Prain, *A. lebbeck*, *A. mollis* (Wall.) Boivin, *A. myriantha* Merr., *A. odoratissima* (L.f.) Benth., *A. procera* (Roxb.) Benth., *A. saponaria* (Lour.) Blume, *Albizia* sp.; Fabaceae (Farr & Rossman 2021). **Secondary host species:** not known (Beenken & Berndt 2010).

Distribution:—Africa [Ghana (Wakefield & Hansford 1949), Madagascar (Spaulding 1961), Malawi (Corbett 1964), Mauritius (Spaulding 1961), Nigeria (Eboh 1978), Reunion (Boa & Lenné 1994), Sierra Leone, Somalia (Castellani & Ciferri 1937), Sudan (Tarr 1963), Uganda (Boa & Lenné 1994), Benin (this study)], Asia, North America, Oceania, Southern America, Caribbean (Farr & Rossman 2020).

Comments:—The 28S rDNA sequence of the specimen on *A. lebbeck* from Benin indicates that it represents a species of *Sphaerophragmium*. Three species of *Sphaerophragmium* are known on *Albizia* spp., namely *S. acaciae* (Cooke) Magnus, *S. albiziae* Lohsomb., Kakish. & Y. Ono, and *S. clemensiae* Syd. (Lohsomboon *et al*. 1994). *S. acaciae* is reported from many countries with warm to hot climate, while *S. albiziae* and *S. clemensiae* are only known from East Asia. *S. albiziae* forms amphigenous uredinia and urediniospores that are longer than those of the specimen from Benin, i.e., 24–39 µm (Lohsomboon *et al*. 1994) vs. 22–26 µm (specimen from Benin). The urediniospores

of *S. clemensiae* are as long as those of the present specimen, but they are slenderer than those from Benin (16–18 µm) by being 12–17 µm wide (Lohsomboon *et al*. 1994). With a size of (18–)22–26(–27) × (15–)16–18(–20) µm, the urediniospores of the present specimen are within the size range of those of *S. acaciae* (20–33 × 14–25 µm; Lohsomboon *et al*. 1994). Further similarities are the exclusively hypophyllous position of the uredinia and 4 to 5 germ pores on each urediniospore which are however difficult to see. Based on these similarities, the specimen from Benin is identified as *S. acaciae* and represents the first report for Benin.

Uromyces decoratus Syd. & P.Syd., in Sydow *et al.*, Ann. Mycol. 5(6): 491 (1907)
Figure 12

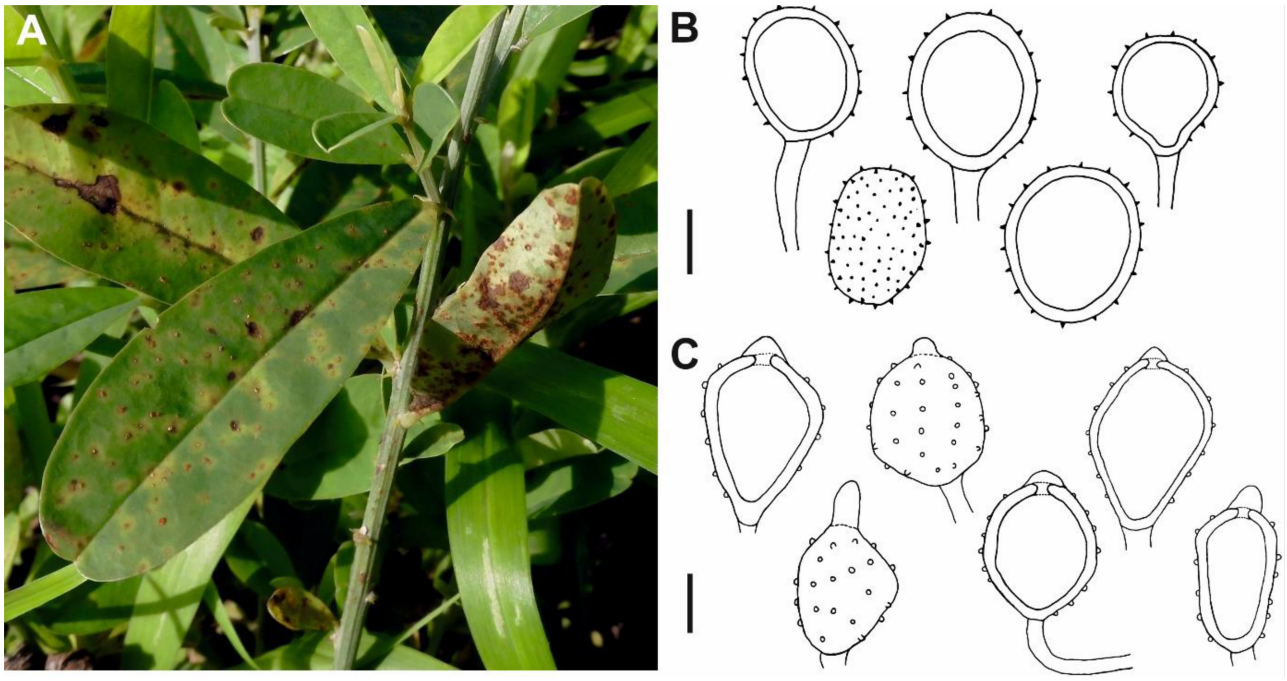

FIGURE 12. *Uromyces decoratus* on *Crotalaria retusa* (TA431). **A** Uredinia and telia on leaves **B–C** Spores drawn in optical sections or in surface view **B** urediniospores **C** teliospores. Scale bars: 10 µm.

Uredinia amphigenous, mostly hypophyllous, circular or elongated, brownish, erumpent, subepidermal or exposed when the epidermis ruptures. **Urediniospores** globose or ellipsoidal, sometimes cylindrical or pyriform, (20–)16–23(–30) × (15–)18–21(–23) µm (n = 60), pale yellow to brown, wall 1.5–2 µm thick, echinulate to warty, germ pores not seen. **Telia** hypophyllous, circular, dark brown to black, subepidermal, erumpent. **Teliospores** uni-cellular, globose, ovoid, ellipsoidal to broadly ellipsoidal, rarely cylindrical or angular, (25–)26–32(–35) × (15–)18–20 µm (n = 20), yellowish brown to dark brown, wall 2 µm thick, with relatively broad, shallow warts which are scattered or in irregular lines, each teliospore with one germ pore at the apex covered by a hyaline cap that is mostly 2–5(–7.5) µm long, pedicels hyaline, up to 75 µm long.

For specimen TA431 from Benin two sequences were obtained. The 28S rDNA sequence (904 bp) shows a similarity of 98% with *U. galegae* on *Galega officinalis* L. (Fabaceae) (DQ250133, unpublished sequence deposited by C. Aime in 2006). The ITS rDNA sequence (542 bp) shows a similarity of 93% with *U. striolatus* which is a microcyclic rust on *Euphorbia cyparissias* L. (Euphorbiaceae) closely related to species heterocyclic on *E. cyparissias* and diverse species of Fabaceae (AF180201, Pfunder *et al*. 2001). According to these results, *U. decoratus* is closely related to rust fungi infecting beans, but there are no sequences available for this species until now.

Host and distribution in Benin:—Uredinia and telia on *Crotalaria retusa* L. (Fabaceae), Central and Northeastern Benin.

Specimens examined:—Uredinia and telia on *Crotalaria retusa:* BENIN. Borgou: Nikki, Sèrékali, elev. 423 m, 9°55’ N, 3°02’ E, 18 September 2019, A. Tabé, M. Piepenbring, Y. Meswaet, and M.U. Schmidt, *TA431* (UNIPAR), GenBank Acc. no. LSU: *OL437040*, ITS: *OL437019*; Borgou: Parakou, Nima, elev. 364 m, 9°24’ N, 2°41’ E, 30 December 2020, A. Tabé, *TA437* (UNIPAR).

Known host species:—Primary hosts species: *Crotalaria retusa* and numerous other species of *Crotalaria* (Farr & Rossman 2020). **Secondary host species:** not known.

Distribution:—Africa [Ghana (Leather 1959), Guinea (Kranz 1964a), Ivory Coast (Yen 1976b), Nigeria (Lenné 1990), Senegal (Bouhot 1966), Benin (this study)], Asia, Central America, South America (Farr & Rossman 2020).

Comments:—In Africa, *Crotalaria* spp. have been reported as hosts of *Uromyces crotalariae* (Arthur) J.W.Baxter, *U. decoratus*, and *U. occidentalis* Dietel. *U. crotalariae* is characterized by teliospores that can be 20–27 µm long and 27–32 µm broad or 24–30 × 20–27 µm (versus 26–32 × 18–20 µm in the specimen from Benin) and have verrucose to echinulate ornamentation (Hennen *et al*. 2005) in contrast to flat warts partly in striae in the present specimen. *U. occidentalis* was described from *Lupinus* spp. from the USA (Dietel 1903) and was mentioned once on a species of *Crotalaria* in Nigeria (Eboh 1978). According to Dietel (1903), *U. occidentalis* is characterized by densely (not striate) warty teliospores measuring 17–26 × 16–21 µm (versus 26–32 × 18–20 µm in the Benin specimen). We consider that the identification proposed by Eboh (1978) is erroneous and that the specimen collected in Nigeria corresponds to *U. decoratus*, because he described urediniospores similar to those presented here for Benin as well as teliospores with warts partly in striae.

The present specimen is identified as *U. decoratus* based on the presence of teliospores with flat warts partly in lines and sizes similar to those reported for the type (20–28 × 14–20 µm; Sydow *et al*. 1907) or approximately 19–31 × 14–20 µm as measured from diverse specimens by Bouhot (1966). This species is reported here for the first time for Benin.

Discussion

For the present investigation, we focused on rust fungi infecting cultivated plants and common useful plants in wild vegetation in Benin, West Africa. Therefore, we are not surprised that we did not discover species new to science. The challenge of the present study was to attribute new specimens to existing species concepts. For most specimens, this was not trivial because monographs with keys are not available for rust fungi in tropical Africa, old descriptions are often incomplete, and drawings are often missing. Completely annotated molecular sequence data for comparison were lacking for four of nine species for which we obtained barcode sequence data. In addition to this, the intraspecific variability of characteristics, especially sizes of spores, is not well known, and there are erroneous identifications in literature as well as in databases. The checklist of fungi known for West Africa (Piepenbring *et al*. 2020) was helpful for the identification process because it shows which species of rust fungi have been reported in West African countries, provides information on host plant species, and references to morphological descriptions.

The present publication increases the knowledge on species diversity of rust fungi in Benin from six to 16 species, including the report of *Neophysopella tropicalis* on *Vitis* sp. cult. that is new for the African continent (Electronic supplement 1). We publish original photos and drawings of several rust fungi for the first time for West Africa. Four species of rust fungi are annotated with molecular sequence data for the first time. As a result of this taxonomic study, *Puccinia canaliculata* var. *tenuis* on *Cyperus esculentus* is raised to species level as *P. doidgeae*.

Only two of ten species were observed in both the uredinial and telial stage. Spermatogonia or aecidia were not found at all. We thereby confirm the general dominance of uredinia as spore generation of rust fungi in the tropics (Piepenbring *et al*. 2011).

Acknowledgement

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Supplementary material

Electronic supplement 1. Checklist for rust fungi (Pucciniales) in West Africa, based on Piepenbring *et al*. (2020) and including new records presented here. For references to literature cited in the checklist and not cited here, see Additional File 3 in Piepenbring *et al*. (2020).

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