

Hymenochaetaceae (Basidiomycota, Hymenochaetales) from the Guineo-Congolian phytochorion: *Phylloporia rinoreae* sp. nov., an additional undescribed species from the Forest Global Earth Observatory Plot in Gabon

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Background and aims – The aim of this study is the continuation of an ongoing survey of Hymenochaetaceae (Basidiomycota, Hymenochaetales) from the lower Guinean sub-region in Central Africa. In this frame, a new species of *Phylloporia* is described from Gabon, based on morphological, ecological and phylogenetic data.

Methods – The species is described using morphological methods, and ecological data. DNA-based phylogenetic analysis are also used to search for affinities.

Key results – A new species of *Phylloporia*, *P. rinoreae*, is described based on specimens collected on living twigs of a shrubby *Rinorea* species (Violaceae), occurring in the Guineo-Congolian rain forest. Phylogenetic inferences using DNA sequence data from partial nuc 28S (region including the D1/D2/D3 domains) resolved this species as a distinct clade within the *Phylloporia* lineage. An identification key to the species reported from the Guineo-Congolian phytochorion is provided.

Conclusion – *Phylloporia rinoreae* is the seventh species of the genus described from and so far only known from Gabon in the Lower Guinean phytogeographical sub-region.

Keywords - Africa; Basidiomycota; Hymenochaetales; Phylloporia; polypores.

INTRODUCTION

Phylloporia (Basidiomycota, Hymenochaetaceae), originally described with a single species, *P. parasitica* Murrill (Murrill 1904), has received much attention in the last 20 years. Numerous species have been described since then and the genus nowadays contains about sixty described species (e.g., Ipulet & Ryvarden 2005; Cui et al. 2010; Valenzuela et al. 2011; Zhou & Dai 2012; Decock et al. 2013, 2015; Zhou 2013, 2015, 2016; Gafforov et al. 2014; Liu et al. 2015; Yombiyeni & Decock 2017; Yombiyeni et al. 2015; Ferreira Lopes et al. 2016; Chen et al. 2017; Ren & Wu 2017; Bittencourt et al.

2018; Quin et al. 2018; Wu et al. 2019; Rajchenberg et al. 2019).

Yombiyeni & Decock (2017) summarized the current knowledge of the genus in tropical Africa: thirteen named and five still unnamed species are listed for this area (Ryvarden & Johansen 1980; Hjortstam et al. 1993; Núñez & Daniëls 1999; Ryvarden 2000; Wagner & Ryvarden 2002; Ipulet & Ryvarden 2005; Roberts & Ryvarden 2006; Decock et al. 2015; Yombiyeni et al. 2015).

Pursuing an ongoing survey of Hymenochaetaceae in the Guineo-Congolian phytochorion (Amalfi et al. 2010; Yombi-

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yeni et al. 2010, 2015; Decock et al. 2015; Yombiyeni & Decock 2017), additional material from a *Phylloporia* species, found growing on twigs of an understorey species of *Rinorea* Aubl. (Violaceae) in several spots of rain forest in Gabon, could not be identified to any of the described species. They also form a single, distant terminal clade in phylogenetic inferences based on partial 28S DNA sequence data (region including domains D1, D2, and D3).

On this basis, as well as considering its ecological specificities, *Phylloporia rinoreae* is described as a new species, and illustrated. A key to the species currently reported from the Guineo-Congolian phytochorion is presented.

MATERIALS AND METHODS

Collection localities

The specimens of the new species were collected in several spots of lower Guinean rain forest in Gabon: the CTFS-ForestGEO (Centre for Tropical Forest Science - Forest Global Earth Observatory, Anderson-Teixeira et al. 2014), Rabi forest monitoring Plot (~ S 01°55′28,5″ – E 009°52′48″, elev. ~ 30–60 m); the Moka Island, Akanda National Park (~ N 00°36′54″ – E 009°29′59″, elev. ~ 49 m); the CEB forest concession in Lastourville (~ S 0°15′11″ – E 013°17′34″, elev. ~ 720 m).

Specimen's identification and description

The holotype specimen of the new species is deposited at NY. Isotypes are deposited at MUCL and LBV (herbarium acronyms are according to Thiers, continuously updated). Colours are described according to Kornerup & Wanscher (1981). Sections of the basidiomes were incubated for one hour at 40°C in a NaOH 3% solution, then carefully dissected under a stereomicroscope and examined in NaOH 3% solution at room temperature (Decock et al. 2010, 2013). To study the staining reaction of the hyphae and basidiospores, sections of the basidiomes were examined in Melzer's reagent, lactic acid Cotton blue, and KOH 4%. All microscopic measurements were done in Melzer's reagent. In presenting the size range of microscopic elements, 5% of the measurements at each end of the range are given in parentheses when relevant. The following abbreviations are used: ave = arithmetic mean, R = the ratio of length/width of basidiospores, and ave_{R} = arithmetic mean of the ratio R. As a rule, whenever possible, 30 microscopic elements of the basidiomes (pores / hyphae / basidiospores) were measured from each specimen.

Molecular study and phylogenetic analysis

DNA extraction, amplification and sequencing of the 5' end of the nuc DNA 28S gene (region including the D1/D2/D3 domains) were as described in Decock et al. (2007). Primers LR0R and LR6 (Vilgalys & Hester 1990) were used to amplify and to sequence the portion of the 28S gene.

One hundred and forty-nine entries representing 89 taxa or potential species clades were included in the phylogenetic analysis. Materials and sequences used in this study are listed in supplementary file 1. The dataset is deposited at Tree-BASE under the accession number S24857.

The methodologies and parameters for running phylogenetic analyses [Bayesian inference as implemented in Mr-Bayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001), Maximum likelihood as implemented in RAxML ver. 7.0.4 (Stamatakis 2006) and Maximum Parsimony as implemented in PAUP* ver. 4.0b10 (Swofford 2003)] are described in Decock et al. (2015) and Yombiyeni et al. (2015) and not repeated in details here. For the Maximum Parsimony, the heuristic search was restricted to keep 20 trees at each search with a predefined tree length \geq 2550, in order to limit the final number of trees. *Inonotus micantissimus*, MUCL 52413, a species of the *Inonotus* clade *sensu* Wagner & Fischer (2002), was designated as outgroup (Larsson et al. 2006).

RESULTS

Phylogenetic analysis

Within *Phylloporia*, the length of the 28S fragment ranges from 866 to 884 bps. The final alignment of 149 sequences resulted in 966 positions (514 were constant and 387 were parsimony informative). Using the Akaike Information Criterium (AIC) of MrModeltest ver. 2.3 (Posada & Crandall 1998), the best-fit model for the 28S data set was determined as GTR+I+G with unequal base frequencies (A = 0.2436, C = 0.2831, G = 0.3235, T = 0.2499), a gamma distribution shape parameter of 0.5760 and a proportion of invariable sites of 0.3650. The nucleotide substitution rates estimated according to this model were A/C= 1.17, A/G=10.97, A/T=1.52, C/ G=1.0, C/T=25.52 and G/T = 1.0.

The MP analysis produced 30 most parsimonious trees (2601 steps, consistency index = 0.245, retention index = 0.652). The two Bayesian runs converged to stable likelihood values after 10,990,000 generations (out of a total of 20×10^6 generations). The remaining stationary trees from each analysis were used to compute a 50% majority rule consensus tree (BC) and to calculate posterior probabilities. In the ML searches, the 28S alignment had 520 distinct patterns with a proportion of gaps and undetermined characters of 9.97%.

The consensus of the most parsimonious trees, the consensus tree of the BI and the most likelihood tree of ML were congruent as far as the terminal clades are concerned. One of the equally most parsimonious trees, representing the dominant topology, is presented in fig. 1.

The topologies of the trees regarding the recovery and the relative positions of the different genera of Hymenochaetaceae included were identical whatever the phylogenetic methodology, in accordance with previous results (Decock et al. 2013, 2015; Yombiyeni & Decock 2017). The *Phylloporia* clade is strongly supported (fig. 1). The terminal clades received significant support whereas most of the internal clades were barely supported (fig. 1), making it difficult to infer the interspecific phylogenetic relationships within the genus.

The phylogenetic analyses recovered two specimens of a *Phylloporia* species from two areas of rain forest in Gabon, as a distinct terminal species clade (fig. 1, clade "*P*.

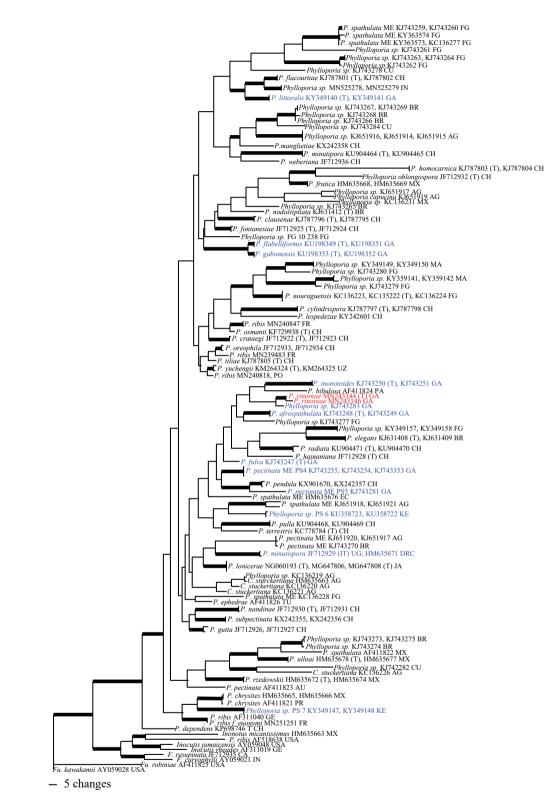


Figure 1 – Phylogenetic relationships of *Phylloporia* species inferred from *nuc 28S* rDNA sequences. The tree was rooted with *Inonotus micantissimus* MUCL52413. Thickened branches represent bootstrap value (MP, ML) and PP value greater than 90%/0.90. Names highlighted in blue represent species known from tropical Africa, in red = the new species *P. rinoreae*. Abbreviations used. AG = Argentina; AU = Australia; BR = Brazil; CH = China; CA = Cameroon; CU = Cuba; DRC = Democratic Republic of Congo; EC = Ecuador; FG = French Guiana; FR = France; GA = Gabon; GE = Germany; IN = India; JA = Japan; KE = Kenya; MA = Martinique; MX = Mexico; PA = Pakistan; PR = Puerto Rica; TU = Turkmenistan; UG = Uganda; USA = United States of America; UZ = Uzbekistan; (T) = Type specimen; ME = Morpho-ecological type.

rinoreae"). This species clade is distant from all other species clades shown to date (fig. 1, e.g., Decock et al. 2013, 2015; Yombiyeni et al. 2015; Ferreira Lopes et al. 2016; Zhou 2015, 2016; Chen et al. 2017).

Morphological and ecological characteristics

The voucher specimens of the clade "*P. rinoreae*" are characterized by seasonal basidiomes, solitary, embracing to pending (thickly button-shaped) at maturity (fig. 2A–H), applano-convex in section, with a cinnamon to cocoa brown, spongy tomentum covering the pileus. The context is homogeneous, covered by a thin, dense layer of melanized hyphae (so-called black line) subtending the tomentum. The hyphal system is dimitic in the contextual and hymenophoral trama, and the basidiospores are mostly oblong to ellipsoid in face view, the abaxial side straight to faintly incurved, averaging $4.3 \times 2.7 \mu m$.

The species was found emerging from small twigs / branches (3–5 mm diam.) of a bushy species of *Rinorea* (Violaceae), inhabiting the understorey of Guineo-Congolian rain forest.

On the basis of the results of both the phylogenetic inferences and the morphological studies, and considering the autecological parameters, we conclude that these specimens represent a distinct species, which is described below as *Phylloporia rinoreae*.

TAXONOMY

Phylloporia rinoreae Decock, Jerusalem & Yombiyeni, **sp. nov.**

MycoBank no: MB832131

Fig. 2

Diagnosis – *Phylloporia rinoreae* is characterized by basidiomes emerging from narrow twigs of a unidentified Rinorea species (Violaceae), small, amplectans to pending (thickly button-shaped) basidiomes, roughly to, gradually, narrowly sulcate pilei toward the margin, in light brown to brown shade, presenting in section a thin, dense layer of deeply melanized hyphae (black line) separating an upper tomentum from a comparatively much thinner, homogeneous context, a dimitic hyphal system, and pale yellowish, oblong to ellipsoid (to broadly ellipsoid) in face view, mostly 4.0–4.5 × 2.5– $3.0 \mu m$ (ave = $4.3 \times 2.7 \mu m$).

Type – Gabon, Ogooué Maritime, Gamba complex, CTFS-ForestGEO Rabi forest monitoring Plot, ~ S $01^{\circ}55'28.5''$ – E $009^{\circ}52'48''$, elevation ~ 30-60 m, on small stem of living *Rinorea* sp. (Violaceae), Oct. 2016, *P. Yombiyeni s.n.* (holotype: NY, barcode NY03684213; isotypes: MUCL, barcode MUCL 56283, LBV).

Description – <u>Basidiomes</u> solitary, seasonal, pileate, sessile, semi-circular or dimidiate first, then amplectens, or pending then thickly button-shaped, attached by the vertex, applano-convex in section, bent downward toward the margin, projecting 7–15 mm long, 10–20 mm wide, from 0.5–1 mm thick at the very margin up to 5–7 mm at the base or the attachment point (vertex); <u>pileus surface</u> tomentose, spongy,

velvety to the touch, broadly sulcate with a few (1-4) deep furrows near the base, more narrowly and densely sulcate near the margin, dull, mostly uniformly light brown to brown when dry (cinnamon to cocoa brown, 6[D-E]6, 6E[6-7]) (no data from fresh basidiomes but likely slightly darker), progressively darker near the margin (dark brown, 6F6), the furrows darker, dark brown to almost black; margin wellmarked, forming a well-defined narrow rim, entire, regular in outline, greyish orange on drying; pore surface plane to mostly concave, the pore field starting immediately behind the very margin, mostly light brown when dry (5D[3-4] up to 5[D-E]6, honey yellow, oak brown, mustard brown); pores regular, mostly round, occasionally slightly radially ellipsoid, mostly 9-10 / mm, (80-)85-100(-110) µm wide (ave = 93.5 μ m wide) when round, 105–115 × 80–90 μ m when ellipsoid; dissepiments thin, entire, agglutinated, 20-50 µm thick (ave = $30.5 \mu m$); in section, tomentum spongy, loose, brown, from 1 mm thick at the margin to 3 mm thick at the thickest part; context 0.2-1.0 mm thick toward the base or the attachment point, gradually thinning to the margin where it is very thin to absent, hard corky, shiny, light brown to brown, topped with a thin, dense black line, up to 100 µm thick, subtending the tomentum; tube layer up to 0.5-1.0 mm deep, light brown to brown; all parts darkening in alkali 3%.

Hyphal system dimitic in the context and hymenophoral trama; generative hyphae simple septate, thin- to slightly thick-walled, hyaline to yellowish, darker in KOH, scarcely ramified, the branches constricted at their emergence point, 1.5–3.0 µm diam.; in the tomentum, next to the black line, hyphae horizontal, with a near parallel orientation, soon erected (vertical) and loosely packed, near the margin slightly incurving backward; individual hyphae straight to variably sinuous, rarely geniculated, mostly unbranched, ending rounded, slightly thick-walled, the lumen > wall thickness, with both primary and secondary septa, pale golden brown, from 3.0 µm diam. near the base gradually enlarging up to $(3.5-)4.0-5.5 \ \mu m$ (ave = 4.6 μm diam.); in the context, skeletal hyphae horizontal, with a near parallel orientation, golden brown, darker in KOH, moderately thick-walled with the lumen widely open, $(2.5-)3.5-4.0 \mu m$ diam. (ave = 3.6 µm); in the hymenophoral trama, skeletal hyphae golden brown, darker in KOH, with a interwoven disposition, straight, thick-walled, the wall internally straight to slightly sinuous, the lumen \leq wall thickness, aseptate or with occasional secondary septa, from 2.5–3.0 µm at the basal septum to $3.0-3.8(-4.0) \ \mu m$ diam. (ave = $3.5 \ \mu m$) in the main part, occasionally, locally inflated (up to 5 µm).

<u>Hymenium</u>: <u>basidioles</u> slightly pyriform to clavate; mature <u>basidia</u> mostly clavate, $12.5 \times 4.5 \mu m$, with four sterigmata; <u>cystidioles</u> absent; <u>basidiospores</u> oblong to elliptical (to broadly elliptical) in face view, the abaxial side straight to faintly incurved in side view, appearing slightly angular on drying, thick-walled, smooth, pale yellowish in KOH, without reaction in Melzer's reagent, $(3.8-)4.0-4.5(-5.0) \times 2.5-3.0(-3.2) \mu m$ (ave = $4.3 \times 2.7 \mu m$), R = (1.3-)1.4-1.8(-1.9) (ave_R = 1.6). Fig. 2.

Phylogenetic affinities – The species, hitherto, is related to an unnamed *Phylloporia* species known from a single specimen from Gabon (fig. 1).

Ecology (substrate, host, habitat) – Living branches or twigs (3–5 mm diam) of unidentified species of *Rinorea* Aubl. (Violaceae), understorey compartment, rain forest.

Geographic distribution – Currently known from three spots of the Guineo-Congolian phytochorion, rain forest, in Gabon (cf. list of specimens).

Phenology – Actively growing basidiomes were observed from December to February.

Etymology – The specific epithet "*rinoreae*" (Latin) refers to its host plant, *Rinorea* sp.

Additional specimens examined – Gabon: Prov. Ogooué Maritime, CTFS-ForestGEO Rabi forest monitoring Plot, ~S 01°55′28,5″ – E 009°52′48″, elev. ~30–60 m, small living twigs (~4 mm diam), *Rinorea* sp. (Violaceae), Dec. 2016, *P. Yombiyeni s.n.* (MUCL, barcodes MUCL 56282, MUCL 56284, MUCL 56285); Prov. Estuaire, Akanda National Park, Moka Island (*île* aux oiseaux), ~N 0.614985 – E 9.499697, elev. ~50 m, old forest, on living twigs of *Rinorea* sp., 1 Dec. 2016, *Nguema Ekomo Diosdado s.n.* (MUCL, barcode MUCL 57327); Prov. Ogooué-Lolo, Lastourville, CEB forest concession, ~S 0.2531389 – E 13.29269, elev. ~700 m, old forest on crest, living twigs, *Rinorea* sp., 28 Feb. 2017, *Nguema Ekomo Diosdado s.n.* (MUCL, barcode MUCL 57328).

Remarks on conservation – The IUCN red list category of *P. rinoreae* is difficult to assess because it is intimately linked to the IUCN status of its host plant, an understorey species of *Rinorea* (Violaceae), which remains to be determined at the species level. *Phylloporia rinoreae* should be, for the time being, classified as Data Deficient.

DISCUSSION

Phylloporia rinoreae is the seventh *Phylloporia* described and hitherto only known from the western edge of the Guineo-Congolian phytogeographic region in Gabon (Yombiyeni et al. 2015, Decock et al. 2015, Yombiyeni & Decock 2017).

It could be compared to P. weberiana, considered sensu Ryvarden & Johansen (1980; cf. also Yombiyeni & Decock 2017), also reported in Central Africa from Cameroon and the Democratic Republic of Congo (Ryvarden & Johansen 1980; Hjortstam et al. 1993). Both species share the roughly sulcate pileus and a similar anatomy, with a tomentum lying on a thin black line and, comparatively, a thinner underlying context. Ryvarden & Johansen (1980) described P. weberiana with basidiomes 30-100 mm wide and 10-30 mm thick, therefore much larger than in *P. rinoreae*, for which the available basidiomes did not exceed 20 mm wide and 7 mm thick. Phylloporia rinoreae and P. weberiana also differ by their number of pores / mm, respectively 9-10(-11)/ mm (or 85-100 µm diam.) and mostly 5-6/mm (Ryvarden & Johansen 1980). Furthermore, P. weberiana has likely a different autecology, its basidiomes emerging preferentially from trunks of small-stemmed trees whereas the basidiomes of P. rinoreae emerge from small (2-5 mm diam.) terminal or subterminal branches or twigs of a bushy plant.

To a lesser degree, locally, *P. rinoreae* could be compared to *P. littoralis*. Both species share the basidiome anatomy (a tomentum over a comparatively much thinner context) and autecological features (basidiome emerging from small branches of small bushy plants). *Phylloporia rinoreae* and

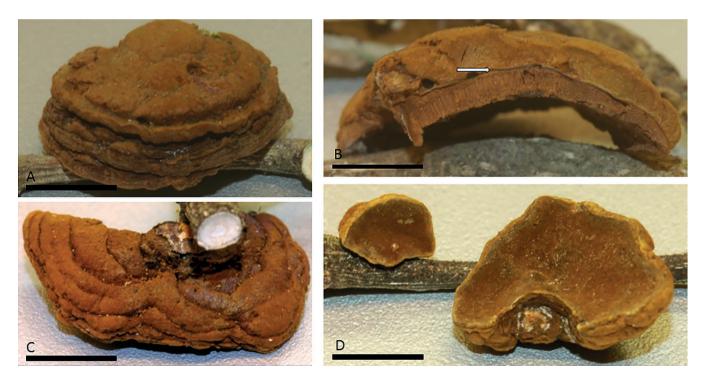


Figure 2 – Basidiomata of *Phylloporia rinoreae*. **A**. MUCL 56282, basidiome, upper view. **B**. MUCL 57328, section of basidiome, showing the black line above the context (arrow). **C**. MUCL 56284, basidiome, upper view. **D**. MUCL 57327, basidiome, view of the pore surface. Scale bars: A = 10 mm; B, C = 7 mm; D = 5 mm.

1.	Basidiomes stipitate; emerging from underground organs (roots) of trees or bushes
1'.	Basidiomes sessile, emerging from aboveground organs (trunk, branches, twigs, petioles) of small trees, bushes, or liana
2.	Pileus surface homogeneous; context without thin black line; pores 7–9/mm; basidiospores $2.0-3.0 \times 2.5 \mu\text{m}$
2'.	Pileus surface with silvery concentric lines; context with a thin black line; pores $10-11 / \text{mm}$ asidiospores ellipsoid $3.4-4.0 \times 2.2-2.7 \ \mu\text{m}$
3. 3'.	Pileus with a tomentum thicker than the underlying context
4.	Black line absent between context and tomentum; pileus not sulcated
4'.	Description in Yombiyeni & Decock (2017). Known from Gabon. Black line present between the context and the tomentum; pileus concentrically sulcate
5.	Basidiospores broadly ovoid to subglobose, 2.5–3.5 µm in the longest dimension
5'.	Basidiospores ellipsoid, $3-4.5 \times 2-3.5 \ \mu m$
5.	Basidiomes from 30–100 mm wide; pores 5–6/mm
	Description in Ryvarden & Johansen (1980); also in Corner (1991) for south-east Asian specimens. In Central Africa, reported from Cameroon and DRC.
5'.	Basidiomes up to 20 mm wide; pores 9–10(–11)/mm <i>P. rinoreae</i> Description in this paper. Known from Gabon.
7. 7'.	Pileus with a thin trichoderm over a thin black line; hyphal system dimitic
8.	Basidiomes flabelliform to conical, pendant; pileus greyish orange to pale light brown
8'.	Basidiomes semi-circular, triquetrous in section; pileus dark brown
9.	Basidiospores oblong ellipsoid, 4.5–5.5 \times 2.0–2.5 µm; pores 2–3/mm <i>P. inonotoides</i>
9'.	Description in Yombiyeni et al. (2015). Known from Gabon. Basidiospores ellipsoid to ovoid, shorter, on average < 4 µm long; pores 5–6/mm
10.	Basidiomes ≤ 1.5 mm thick; margin regular, entire; pileus shining
	Description in Decock et al. (2015). Known from Gabon.
ιυ.	Basidiomes > 1.5 thick; margin irregular, incised; pileus dull <i>P. gabonensi</i> . Description in Decock et al. (2015). Known from Gabon.

P. littoralis differ by their pileus surface, sulcate and brown vs. even and pale corky, presence vs. absence of a black line separating the context from the tomentum, 9-10(-11)/mm vs. (3-)4(-5)/mm, and, as far as it is known, by their habitat and hosts; *Phylloporia littoralis* is known from *Nichallea* (Rubiaceae) only, in an open, coastal, and seasonally dry forest (Yombiyeni & Decock 2017) whereas *P. rinoreae* inhabits buffered, closed rain forest.

In a phylogenetic perspective (fig. 1), until now, *P. rinoreae* has no known relative but a putative species repre-

sented by one unnamed specimen, which also was collected in the Guineo-Congolian rain forest in Gabon (Ivindo National Park, ~ N 00°06′50″ – E 12°37′43″, GenBank Acc. KJ743283). Additional material is necessary to characterize in more detail this latter species.

Phylloporia rinoreae is the second species known from Violaceae worldwide. *Phylloporia rzedowskii* (Valenzuela et al. 2011) was described growing from *Hybanthus mexicanus* Ging. ex DC., a bushy Violaceae growing in the understorey, in seasonally dry forest of eastern Mexico. *Phylloporia*

rinoreae and *Phylloporia rzedowskii* are, however, unrelated phylogenetically. They also differ morphologically; *Phylloporia rzedowskii* has large pores (2–3/mm), a monomitic hyphal system, and cylindrical basidiospores (Valenzuela et al. 2011).

With the addition of *P. rinoreae*, eleven named *Phylloporia* species are currently reported from the Guineo-Congolian phytogeographic region (Ryvarden & Johansen 1980; Hjortstam et al. 1993; Núñez & Daniëls 1999; Ryvarden 2000; Wagner & Ryvarden 2002; Ipulet & Ryvarden 2005; Roberts & Ryvarden 2006; Decock et al. 2015; Yombiyeni et al. 2015; Yombiyeni & Decock 2017). Seven species are described and, for the time being, only known from this phytochorion (Ipulet & Ryvarden 2005; Decock et al. 2015; Yombiyeni & Decock 2017; Yombiyeni et al. 2015). In addition, still, three unnamed species from the Guineo-Congolian region and two from mountainous area in Eastern Africa (fig. 1, PS 4, PS 5, PS 6, PS7) remain to be described (fig. 1, Yombiyeni & Decock 2017).

It should be noted also that the reports in Central Africa of species, originally described from other biogeographic areas, South America or the Southern Pacific, such as *P. chrysites*, *P. fruticum* and *P. weberiana*, must be taken with great care, as already emphasized by Yombiyeni & Decock (2017).

SUPPLEMENTARY FILE

One supplementary file is associated to this paper: List of species / specimens used in the phylogenetic analyses (pdf)

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