

## Hymenochaetaceae from the Guineo-Congolian rainforest: *Phylloporia flabelliforma* sp. nov. and *Phylloporia gabonensis* sp. nov., two undescribed species from Gabon

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**Abstract** – *Phylloporia flabelliforma* sp. nov. and *Phylloporia gabonensis* sp. nov. are described on the basis of specimens originating from the western edge of the lower Guineo-Congolian rainforest, in Gabon. Both species form seasonal, gregarious and sessile basidiomata, with spatulate to flabelliform pilei, emerging in dense clusters at the lower part of living trunks of *Dichostemma glaucescens* and *Anthostema aubryanum*, two small-stemmed Euphorbiaceae from the understorey compartment. Both taxa have a pileus surface in a grayish orange tone when fresh, 5-6 irregular pores/mm and a homogeneous, pale cork-colored context, briefly discoloring to reddish or reddish brown in 3% alkali. The hyphal system is monomitic and the basidiospores are ellipsoid to broadly ellipsoid in both species. *Phylloporia flabelliforma* is specifically characterized by thin pilei,  $\leq 1.5$  mm at the thickest, with a shiny surface and a thin, and regular white margin. *Phylloporia gabonensis* has thicker pilei with a dull surface and a lobed, incised margin. Phylogenetic inferences using DNA sequence data from partial nuc 28S (region including the D1/D2/D3 domains) resolved these two species as two distinct but closely related sister clades within the *Phylloporia* lineage. Phylogenetic inferences using DNA sequence data from the ITS regions confirmed the results obtained from the nuc 28S. These two species are compared to *P. fulva* and *P. inonotoides*, both occurring in the same phytochorion in Gabon. They are also compared to *Inonotus pusillus* and *I. dentatus*, two species known only from the Neotropics.

**Africa / Basidiomycota / Hymenochaetales / lower Guineo-Congolian rainforest / polypores**

### INTRODUCTION

Hymenochaetaceae (Basidiomycota, Hymenochaetales) remains critically underexplored in Central Africa or, in biogeographical terms, the Guineo-Congolian phytogeographic region (Ryvarden 1998, 2000). A comprehensive, modern treatment

of this family combining morphological and molecular, DNA-based studies is badly needed for this large phytochorion.

Yet, using combined morphological and molecular, DNA-based, approaches, a handful of taxonomic novelties emerged from this phytogeographic region, mostly from its western edge, in the lower Guinean subregion in Gabon (White 1979, 1983). It includes *Fomitiporia gabonensis* Amalfi & Decock, *F. ivindoensis* Decock *et al.* and *F. nobilissima* Decock & Yombiyeni (Amalfi *et al.*, 2010), *Phellinus gabonensis* Decock & Yombiyeni (Yombiyeni *et al.* 2011), *Phylloporia afrospathulata*<sup>1</sup>, *P. inonotoides* and *P. fulva* (Yombiyeni *et al.* 2015). Previously, Douanla-Meli *et al.* (2007) had described *Phylloporia resupinata* from Cameroon based on morphological data only; later on, however, molecular data have shown that this species belongs to the *Fomitiporella* and not to the *Phylloporia* lineage (Decock *et al.* 2013, Valenzuela *et al.* 2011).

As far as *Phylloporia* is concerned, in addition to the three species listed above, currently, 5 other species are reported from this phytochorion *viz.* *P. chrysites*, *P. fruticum*, *P. minutispora*, *P. spathulata* and *P. weberiana* (Douanla-Meli *et al.* 2007, Hjortstam *et al.* 1993, Ipulet and Ryvarden 2005, Núñez and Daniëls 1999, Roberts and Ryvarden 2006, Ryvarden 2000, Ryvarden and Johansen 1980, Wagner and Ryvarden 2002, Yombiyeni *et al.* 2015). *Phylloporia minutispora* is originating and so far only known from the eastern edge of this phytochorion, in western Uganda (type locality, Ipulet and Ryvarden 2005) and eastern Congo RDC (Ipulet and Ryvarden 2005, Yombiyeni *et al.* 2015). The remaining four species were originally described from very distant geographic areas and phytochoria. Their reports from Central Africa (*e.g.* Hjortstam *et al.* 1993, Núñez and Daniëls 1999, Roberts and Ryvarden 2006) should be taken with caution (Yombiyeni *et al.* 2015).

Pursuing our ongoing survey of Hymenochaetaceae in the Guineo-Congolian rainforest (Amalfi *et al.* 2010, Yombiyeni *et al.* 2011, 2015), additional materials of *Phylloporia* were gathered from lower Guinean rainforest (White 1979, 1983) in southwestern Gabon, more specifically in the Rabi forest monitoring plot, Center for Tropical Forest Science – Global Earth Observatories (CTFS-ForestGEO).

On the basis of morphological and DNA sequence data, as well as considering their ecological specificities, two additional undescribed species are added for the region: *Phylloporia flabelliforma* sp. nov. and *Phylloporia gabonensis* sp. nov. Both species are commented.

## MATERIALS AND METHODS

*Collection localities.* – The specimens of the new species were collected in southwestern Gabon, in the CTFS-ForestGEO Rabi forest monitoring plot (about 0.597988 S, 9.786291 E, elevation approx. 30-60 m, <http://www.ctfs.si.edu/site/Rabi>). The CTFS-ForestGEO Rabi forest belongs to lower Guinean sub-region of the Guineo-Congolian phytochorion (Vande weghe 2005, White 1979, 1983).

*Specimen's description.* – Type specimens of the new species are deposited at NY (holotype), MUCL and Libreville (isotypes) (Herbarium acronyms are

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1. As a rule authorship of scientific names included in the Table 1 are not repeated in the text.

according to Thiers [<http://sweetgum.nybg.org/ih/>]. Colors are described according to Kornerup and Wanscher (1981). Sections of the basidiomata were incubated for one hour at 40°C in 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 basidiomata 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, Q = the ratio of length/width of basidiospores, and ave<sub>q</sub> = arithmetic mean of the ratio R. As a rule, whenever possible, 30 microscopic elements of the basidiomata (pores/basidiospores/hyphae) were measured from each specimen.

*Molecular study and phylogenetic analysis, nuc 28S data set.* – DNA extraction, amplification and sequencing of the 5' end of the nuc 28S gene were as described in Decock *et al.* (2007), Decock *et al.* (2013) and Yombiyeni *et al.* (2015). Primers LR0R and LR6 (Vilgalys and Hester 1990) were used to amplify the portion of the nuc 28S that includes the D1/D2/D3 domains.

One hundred and two specimens or cultures representing 62 taxa or potential species clades were included in the phylogenetic analysis. Materials and sequences used in this study are listed in Table 1. The dataset used in the present study to infer phylogenetic inferences is the same as previously used by Yombiyeni *et al.* (2015) and available at TreeBASE under study accession S17120, to which 5 newly generated sequences were added.

The methodologies and parameters for running phylogenetic analyses [maximum parsimony as implemented in PAUP\* version 4.0b10 (Swofford 2003), Bayesian inference as implemented in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) and Maximum likelihood as implemented in RAxML 7.0.4 (Stamatakis 2006)] are described in details in Decock *et al.* (2013) and Yombiyeni *et al.* (2015) and not repeated here. *Inonotus micantissimus*, MUCL52413, a species of the *Inonotus sensu* Wagner and Fischer (2002) clade, was designated as outgroup (Larsson *et al.* 2006).

*Molecular study and phylogenetic analysis, ITS data set* – Primers ITS5 and ITS4 (White *et al.* 1990) were used for the amplification and sequencing of the ITS region (ITS1, ITS2, 5.8S). ITS sequences of 12 specimens representing 8 taxa, or potential species clades, were included in the analysis; they are *Phylloporia* sp. MUCL 55568 (KU198357), MUCL 55569 (KU198356), MUCL 55570 (KU198358), *Phylloporia* sp. MUCL 55571 (KU198355), MUCL 55572 (KU198354), *Phylloporia parasitica* LR19843 (KU198361), *Phylloporia fruticum* MUCL 52762 (KU198363), *Phylloporia* sp. FG/13-726 (KU198360), FG/13-749 (KU198359), *Phylloporia ribis* MUCL (KU198362), *Phylloporia ribis f. ulicis* (AY558644, AF200237).

Phylogenetic analyses were performed using maximum parsimony (MP) as implemented in PAUP\* 4.0b10 (Swofford 2003). The most parsimonious trees (MPT) were identified using the exhaustive search option, further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority-rule in the bootstrap consensus tree. Clades with bootstrap support value (BS) above 90% were considered strongly supported by the data.

Table 1. List of species/specimens (country of origin, collection reference, substrate/host, and accession numbers of sequences (nuc 28S) used in the phylogenetic analyses

<i>Genera/Species</i>	<i>Country of origin</i>	<i>Collection reference</i>	<i>Substrate/host</i>	<i>LSU GenBank Accession</i>
<i>Aurificaria</i>				
<i>A. luteoumbrina</i> (Romell) D.A. Reid	Puerto Rico	LF 39116	<i>Pinus sylvestris</i>	AY059033
<i>Coltricia</i>				
<i>C. cf. stueckertiana</i> (Speg.) Rajchenb. & J.E. Wright	Argentina	MUCL 47643, Robledo 728	Root, unidentified angiosperm	HM635663
	Argentina	CORD, Robledo 219	Root, unidentified angiosperm	KC136219
	Argentina	CORD, Robledo 218	Root, unidentified angiosperm	KC136220
	Argentina	CORD, Robledo 281	Root, unidentified angiosperm	KC136221
	Argentina	CORD, Robledo 351	Root, unidentified angiosperm	KC136226
<i>Fomitiporella</i>				
<i>F. caryophylli</i> (Racib.) T. Wagner & M. Fisch.	India	BBS 448.76	<i>Shorea robusta</i>	AY059021
<i>F. cavicola</i> (Kotl. & Pouzar) T. Wagner & M. Fisch.	UK	N 153	<i>Fagus sylvatica</i>	AY059052
<i>Fulviformes</i>				
<i>F. karwakamii</i> (M.J. Larsen <i>et al.</i> ) T. Wagner & M. Fisch.	USA	CBS 428.86	<i>Casuarina equisetifolia</i>	AY059028
<i>F. robiniae</i> (Murrill) Murrill	USA	CBS 211.36	<i>Robinia pseudoacacia</i>	AY411825
<i>Inocutis</i>				
<i>I. jamaicensis</i> (Murrill) A.M. Gottlieb <i>et al.</i>	USA	Gilb. 14740	<i>Quercus virginia</i>	AY059048
<i>I. rheades</i> (Pers.) Fiasson & Niemelä	Germany	TW 385	<i>Populus tremula</i>	AF311019
<i>Inonotus</i>				
<i>I. micantissimus</i> Rick) Rajchenb.	Mexico	MUCL 52413	wood, Unidentified angiosperm	HM635663
<i>Phylloporia</i> Murrill				
<i>P. afrospathulata</i> Yombiyeni <i>et al.</i>	Gabon	MUCL 54511/NY (T)	Root, unidentified angiosperm	KJ743248
	Gabon	MUCL 53983 (PT)	Root, unidentified angiosperm	KJ743249
<i>P. bibulosa</i> (Lloyd) Ryvarden	Pakistan	Ahmad 27088	<i>Peristrophe bicalyculata</i>	AF411824
<i>P. cf. capucina</i> (Mont.) Ryvarden	Argentina	CORD, Robledo 1610	Stem, unidentified angiosperm	KJ651919

<i>Genera/Species</i>	<i>Country of origin</i>	<i>Collection reference</i>	<i>Substrate/host</i>	<i>LSU GenBank Accession</i>
<i>P. chrysis</i> (Berk.) Ryvarden	Puerto Rico Mexico Mexico Mexico	N.W. Legon MUCL 52763 MUCL 52764 MUCL 52862	Unidentified angiosperm Unidentified angiosperm Unidentified angiosperm <i>Neopringle sp.</i>	AF411821 HM635665 HM635666 HM635667
<i>P. crataegi</i> L.W. Zhou & Y.C. Dai	China China	IFP, Dai 11014 (T) IFP, Dai 11016 (PT)	Root, <i>Crataegus sp.</i> Root, <i>Crataegus sp.</i>	JF712922 JF712923
<i>P. ephedrae</i> (Woron.) Parmasto	Turkmenistan	TAA 72-2	<i>Ephedra sp.</i>	AF411826
<i>P. flabelliforma</i> Decock & Yombiyeni	Gabon Gabon Gabon	MUCL 55568 MUCL 55569 (T) MUCL 55570	Living trunk, <i>Dichostemma</i> Living trunk, <i>Dichostemma</i> Living trunk, <i>Dichostemma</i>	KU198350 KU198349 KU198351
<i>P. fontanesiae</i> L.W. Zhou & Y.C. Dai	China China	IFP, Li 199 (T) IFP, Li 194 (PT)	Living <i>Fontanesia sp.</i> Living <i>Fontanesia sp.</i>	JF712925 JF712924
<i>P. cf. fruticum</i> (Berk. & M.A. Curtis) Ryvarden	Mexico Mexico Mexico	MUCL 52762 ENCB TR&RV858 MUCL 52863	Unidentified angiosperm Unidentified angiosperm Unidentified angiosperm	HM635668 HM635669 HM635670
<i>P. fulva</i> Yombiyeni & Decock	Gabon	MUCL 54472/NY (T)	Trunk, unidentified angiosperm	KJ743247
<i>P. gabonensis</i> Decock & Yombiyeni	Gabon Gabon	MUCL 55571 MUCL 55572 (T)	Living trunk, <i>Dichostemma</i> Living trunk, <i>Dichostemma</i>	KU198352 KU198353
<i>P. gutta</i> L.W. Zhou & Y.C. Dai	China China	IFP, Dai 4103 (PT) IFP, Dai 4197 (T)	Unidentified angiosperm <i>Abelta sp.</i>	JF712926 JF712927
<i>P. hainaniana</i> Y.C. Dai & B.K. Cui	China	IFP, Dai 9640 (T)	Twig, unidentified angiosperm	JF712928
<i>P. inonotooides</i> Yombiyeni & Decock	Gabon Gabon Gabon	MUCL 54468/NY (T) MUCL 54469 (PT) MUCL 54470 (PT)	Trunk, <i>Crotonogyne manniana</i> Trunk, <i>Crotonogyne manniana</i> Trunk, <i>Crotonogyne manniana</i>	KJ743250 KJ743251 KJ743252
<i>P. minutispora</i> Ipulet & Ryvarden	RDC Uganda	MUCL 52865 O, Ipulet 706 (IT)	Root, unidentified angiosperm Root, unidentified angiosperm	HM635671 JF712929
<i>P. nandinae</i> L.W. Zhou & Y.C. Dai	China China	IFP, Dai 10625 (PT) IFP, Dai 10588 (T)	Living <i>Nandina domestica</i> Living <i>Nandina domestica</i>	JF712931 JF712930
<i>P. nouraguensis</i> Decock & Castillo	French Guiana French Guiana	MUCL 53816 (T) MUCL 53817 (PT)	Living twig, <i>Myrcia sp.</i> Living twig, <i>Myrcia sp.</i>	KC136222 KC136223

Table 1. List of species/specimens (country of origin, collection reference, substrate/host, and accession numbers of sequences (nuc 28S) used in the phylogenetic analyses (*continued*)

<i>Genera/Species</i>	<i>Country of origin</i>	<i>Collection reference</i>	<i>Substrate/host</i>	<i>LSU GenBank Accession</i>
<i>P. oblongospora</i> Y.C. Dai & H.S. Yuan	China	IFP, Zhou 179 (T)	Branch, unidentified angiosperm	JF712932
<i>P. oreophila</i> L.W. Zhou & Y.C. Dai	China	IFP, Cui 2219 (PT)	Bush, unidentified angiosperm	JF712933
	China	IFP, Cui 9503 (T)	Fallen, unidentified angiosperm	JF712934
<i>P. osmanthi</i> L.W. Zhou	China	Yuan 5655 (T)	<i>Osmanthus</i> sp.	KF729938
<i>P. pectinata</i> (Klotzsch) Ryvarden	Australia	R. Coveny 113	Trunk, <i>Rhodania rubescens</i>	AF411823
<i>P. ME pectinata</i>	Gabon	MUCL/GA-12-813	Living trunk, Melastomataceae	KJ743253
	Gabon	MUCL/GA-12-846	Living trunk, Melastomataceae	KJ743254
	Gabon	MUCL/GA-12-812	Living trunk, Melastomataceae	KJ743281
	Argentina	CORD, Robledo 429	Dead stem, <i>Magfadyena unguis-cati</i>	KJ651913
	Brazil	ICN/ISA xxx		KJ743270
<i>Phylloporia pseudofruticum</i> (Lloyd) Campos-Santana & Decock	Brazil	ICN/ISA 333	Living liana	KJ743272
	Brazil	ICN/ISA 610	Living liana	KJ743273
	Brazil	ICN/ISA 117	Living liana	KJ743271
	Cameroon	O, DMC 476 (IT)	Trunk, <i>Entandrophragma</i> sp.	JF712935
<i>P. resupinata</i> Douanla-Meli & Ryvarden	Germany	MF 82-828	<i>Ribes uva-crispa</i>	AF311040
	France	MUCL	Base of living trunk, <i>Euomyzus europaeus</i>	KU358724
<i>P. rzedowskii</i> R. Valenz. & Decock	Mexico	MUCL 52868 (T)	Branch, <i>Hybanthus mexicanus</i>	HM635672
	Mexico	MUCL 52859 (PT)	Branch, <i>Hybanthus mexicanus</i>	HM635673
	Mexico	MUCL 52860 (PT)	Branch, <i>Hybanthus mexicanus</i>	HM635674
	Mexico	MUCL 52861 (PT)	Branch, <i>Hybanthus mexicanus</i>	HM635675
<i>Phylloporia</i> sp.	Argentina	CORD, Robledo 527	Living twig, <i>Allophylus edulis</i>	KJ651915
	Argentina	CORD, Robledo 968	Living twig, <i>Allophylus edulis</i>	KJ651916
	Brazil	ICN/ISA 007	No data	KJ743265
	Brazil	ICN/ISA 553	No data	KJ743266
	Brazil	MUCL 54295/ICN	No data	KJ743269
	French Guiana	MUCL, FG-13-721	Trunk, unidentified angiosperm	KJ743263
	French Guiana	MUCL, FG-13-722	Trunk, unidentified angiosperm	KJ743264
French Guiana	MUCL, FG-13-670	Trunk, unidentified angiosperm	KJ743262	

General/Species	Country of origin	Collection reference	Substrate/host	LSU GenBank Accession	
<i>Phylloporia</i> sp.	French Guiana	MUCL, FG-13-754	Root, unidentified angiosperm	KJ743261	
	French Guiana	MUCL, FG-10-321	Trunk, unidentified angiosperm	KJ743277	
	French Guiana	MUCL, FG-13-726	Root, unidentified angiosperm	KJ743279	
	French Guiana	MUCL, FG-13-749	Root, unidentified angiosperm	KJ743280	
	Kenya	KE-15-02	Trunk, <i>Rawsonia lucida</i> ,	KU358722	
	Kenya	KE-15-19	Trunk, <i>Rawsonia lucida</i> ,	KU358723	
	Cuba	MUCL 43733	No data	KJ743278	
	Mexico	MUCL 53433	Unidentified angiosperm	KC136231	
	Cuba	MUCL, CU-05-249	Branch, unidentified angiosperm	KJ743282	
	Cuba	MUCL 45062	Trunk, unidentified angiosperm	KJ743284	
	Gabon	MUCL, YOM 5	Unidentified living liana	KJ743283	
	Mexico	Chay 456	Root, <i>Apocynaceae</i>	AF411822	
	<i>P. spathulata</i> (Hook.) Ryvarden	Ecuador	MUCL 52864	Root, unidentified angiosperm	HM635676
	<i>P. ME spathulata</i>	Argentina	CORD, Robledo 1467	Root, unidentified angiosperm	KJ651918
		Argentina	CORD, Robledo 1790	Root, unidentified angiosperm	KJ651921
French Guiana		MUCL, FG-12-522	Root, unidentified angiosperm	KJ743259	
French Guiana		MUCL, FG-12-523	Root, unidentified angiosperm	KJ743260	
French Guiana		MUCL, FG-11-506	Root, unidentified angiosperm	KC136227	
French Guiana		MUCL, FG-11-462	Root, unidentified angiosperm	KC136228	
<i>P. terrestris</i>	China	T	Unidentified angiosperm	KC778784	
<i>P. ulloai</i> R. Valenz. et al.	Mexico	MUCL 52866 (PT)	Unidentified living liana	HM635677	
	Mexico	MUCL 52867 (T)	Unidentified living liana	HM635678	
	Mexico	MUCL 52870 (PT)	Unidentified living liana	HM635679	
<i>P. weberiana</i> (Bres. & Henn.: Sacc.) Ryvarden	China	IFP, Dai 9242	Unidentified angiosperm	JF712936	
	Uzbekistan	YG 033, TASM (T)	Dead unidentified angiosperm	KM264324	
<i>P. yuchengii</i>	Uzbekistan	YG 051, TASM	Dead unidentified angiosperm	KM264325	

T, PT = type, paratype. ME = Morpho-ecological group.

## RESULTS

Within *Phylloporia*, the length of the 28S fragment ranged from 866 to 884 bps. The alignment of the 102 sequences resulted in 948 positions of which 23 were excluded as ambiguous, 486 were constant, and 366 were parsimony informative. The MP analysis produced 2 most parsimonious trees (2057 steps, consistency index = 0.304, retention index = 0.641). One of the equally most parsimonious trees is presented in Fig. 1.

Using the AIC of MrModeltest 2.3 (Posada and Crandall 1998), the best-fit model for the nuc 28S data set was GTR+I+G with unequal base frequencies (A = 0.2387, C = 0.1908, G = 0.3288, T = 0.2416), a gamma distribution shape parameter of 0.5539 and a proportion of invariable sites of 0.3482.

The two Bayesian runs converged to stable likelihood values after 5,000,000 generations. The remaining stationary trees from each analysis were used to compute a 50% majority rule consensus tree and to calculate posterior probabilities. In the ML searches with RAxML, the LSU alignment had 448 distinct patterns with a proportion of gaps and undetermined characters of 7.75%.

The consensus of the most parsimonious trees was mostly identical to the BC tree and to the optimal ML tree.

The topologies of the trees regarding the recovery and the relative positions of the different genera of Hymenochaetaceae considered were identical in all the phylogenetic inferences, in accordance with previous results (Decock *et al.* 2013, Valenzuela *et al.* 2011, Yombiyeni *et al.* 2015). The *Phylloporia* clade is very well supported (Fig. 1).

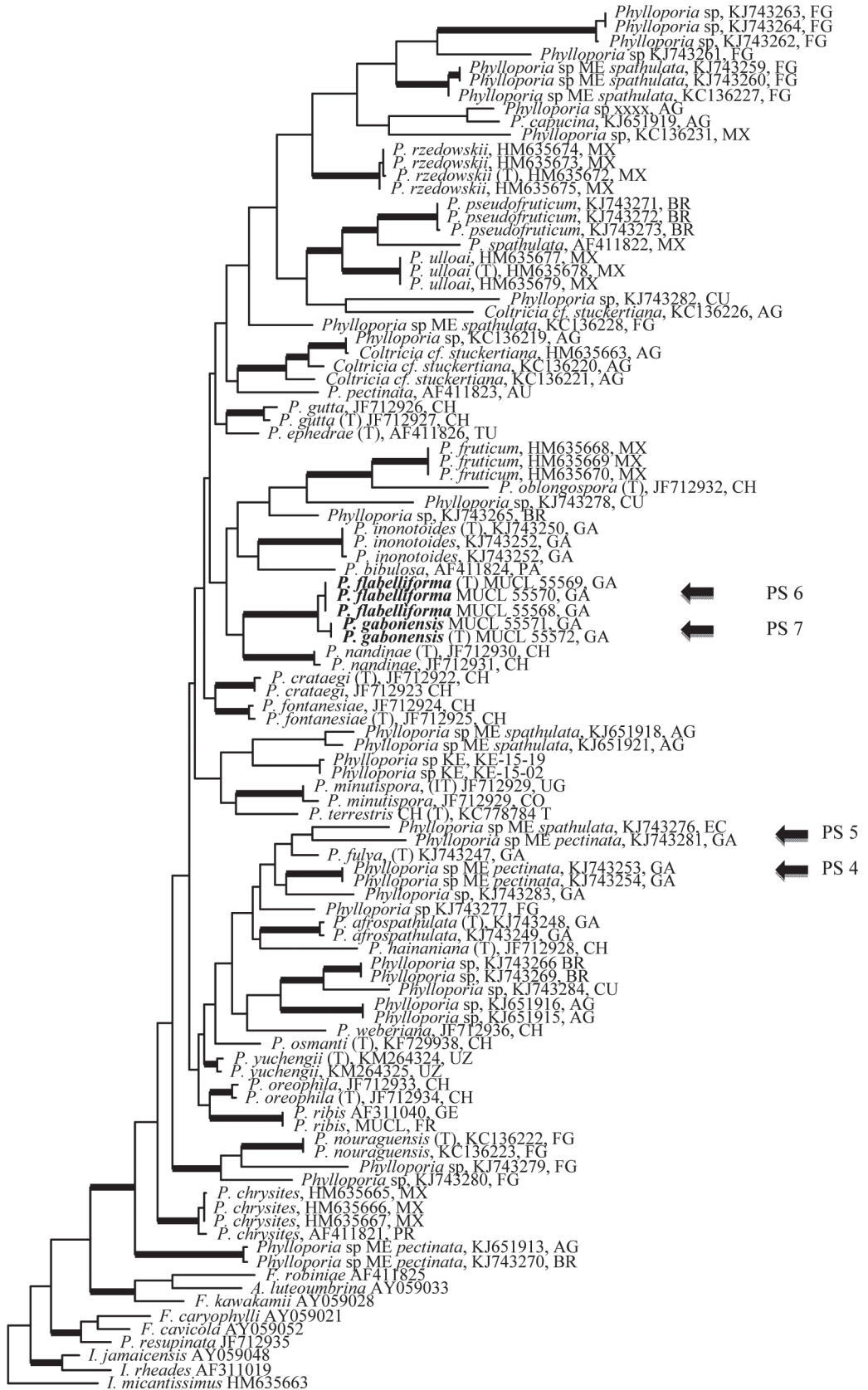
The ITS alignment resulted in 812 positions, of which 98 were excluded, 405 were constant and 163 were parsimony informative. An 85 bp long indel is present in the ITS1 region of the specimens MUCL 55568, MUCL 55569, MUCL 55570, MUCL 55571 and MUCL 55572 but absent in all the other collections sequenced for this study or downloaded from GenBank. It was removed from the analysis. A second shorter, 13 bp long indel present in the ITS 2 was also removed from the analysis. The MP analysis produced 2 most parsimonious trees (466 steps, CI = 0.845, RI = 0.820). One of the equally most parsimonious trees is presented in Fig. 2.

In the 28S phylogenetic analysis, *Phylloporia* specimens from the CTFS-ForestGEO Rabi plot formed 4 distinct species clades (Fig. 1, PSs 4-7, cf. Yombiyeni *et al.* 2015 for numbering). These four species clades are distant from all other species clades shown to date (Decock *et al.* 2013, Yombiyeni *et al.* 2015). Two of these species clades *viz.* PS 6 and PS 7 are very closely related, sister clades (Fig. 1). Their partial 28S DNA sequence differs in only 8 base pair positions. Phylogenetic analysis based on a data set of ITS sequences confirmed they represent two divergent clades (Fig. 2). Their ITS sequences differ in 18 positions.

Their voucher specimens are characterized by small, seasonal basidiomata emerging in large clusters, irregular pores, monomitic hyphal system and ellipsoid

Fig. 1. Phylogenetic relationships of *Phylloporia* species inferred from nuc 28S rDNA sequences. The tree was rooted with *Inonotus micantissimus* MUCL52413. Thickened branches represent bootstrap/BPP value greater than 0.90. Abbreviations used. AG = Argentina; AU = Australia; BR = Brazil; CH = China; CU = Cuba; RDC = Democratic Republic of Congo; EC = Ecuador; FG = French Guiana; FR = France; GA = Gabon; GE = Germany; KE = Kenya; MX = Mexico; PA = Pakistan; TU = Turkmenistan; UG = Uganda; UZ = Uzbekistan; (I)T = (Iso)Type; ME = Morpho-ecological type. ►





— 10 changes

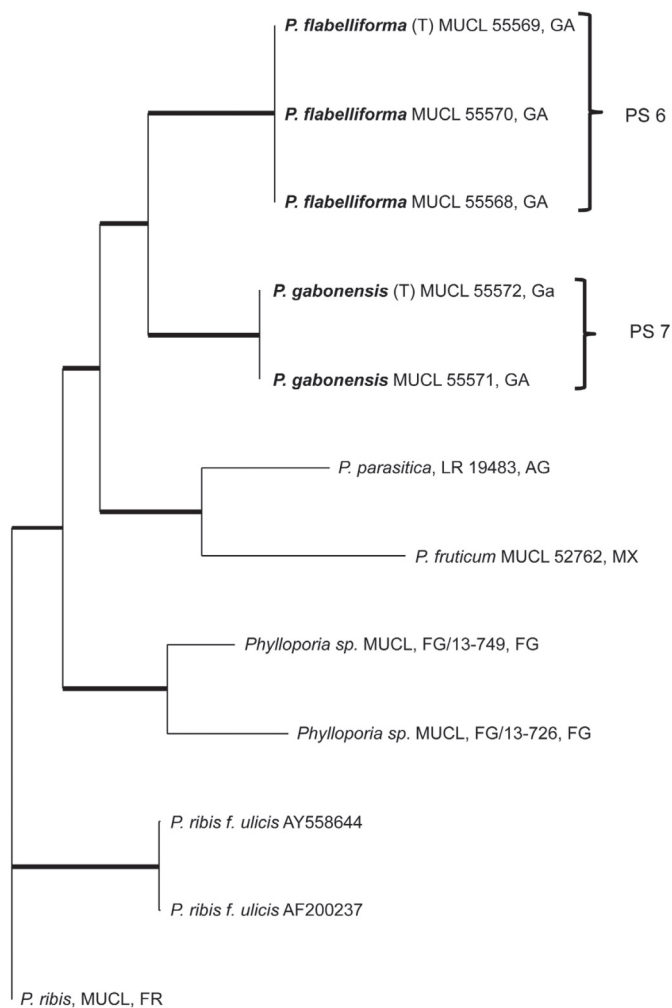


Fig. 2. Phylogenetic relationships of *Phylloporia* species inferred from ITS DNA sequences. The tree was rooted with *Phylloporia ribis* MUCL. Thickened branches represent bootstrap value greater than 0.90. Abbreviations used. AG = Argentina; FG = French Guiana; FR = France; GA = Gabon; MX = Mexico; T = Type.

to broadly ellipsoid basidiospores, averaging respectively  $3.4 \times 2.6 \mu\text{m}$  and  $3.9 \times 2.9 \mu\text{m}$ . We conclude that they represent two distinct phylogenetic units, or phylogenetic species, which are described below as two distinct species, respectively, as *Phylloporia flabelliforma* and *Phylloporia gabonensis*.

The cases of PS 4 and PS 5 are more critical. Their voucher specimens are mainly characterized by perennial, hard corky, triquetrous basidiomata. Microscopically, they are characterized by a dimitic hyphal system with short skeletal hyphae and subglobose basidiospores. The basidiomata are gregarious, emerging in clusters from living trunks of small-stemmed trees. These morphological, biological and ecological characteristics are found identical in *P. pectinata*, to which they were tentatively identified in the field. However, PS 4 and PS 5 are phylogenetically distant from the reference *P. pectinata* lineage (*viz.* *P. pectinata* sensu Wagner and Ryvarden 2002) (Fig. 1), what indicates two distinct species. In

that sense, they both pertain to the *P. pectinata* morpho-ecological group as defined by Yombiyeni *et al.* (2015). Nevertheless, we refrain from naming these two species for the moment; this would require a more in-depth analysis of the current morphological concept of *P. pectinata* and the revision of some its taxonomic synonyms.

## TAXONOMY

*Phylloporia flabelliforma* Decock & Yombiyeni **sp. nov.**

Figs 3a-b, 4a-e

*Mycobank*: MB815277

*Etymology*: “*flabelliforma*” (L.) in reference to the flabelliform shape of the pileus.

**Basidiomata** annual, pileate, sessile, gregarious, emerging simultaneously in clusters of up to > 100 individual basidiomata, mostly superposed; individual **basidiomata** spatulate to flabelliform, rarely clavate, attached to the substrate by a narrow, discoid basal area, occasionally laterally fused, projecting horizontally (3-) 10-15 (-20) mm, 5-15 mm wide, from 0.5 mm thick at the very margin up to 1.5 mm the thickest part; **pileus surface** shiny, smooth, radially faintly wrinkled on drying, mostly uniformly grayish orange to brownish orange (cork-colored) when fresh (5[B-C] [4-5], golden blonde) or faintly concentrically zonate with narrow, slightly darker bands, on drying yellowish (4A[5-6], maize yellow) toward the margin and



Fig. 3. Basidiomata of *Phylloporia flabelliforma* (a, b, MUCL 55569) and *P. gabonensis* (c, d, MUCL 55572) *in situ* (scale bar = 20 mm).

darkening to brownish orange, light brown toward the base (5[C-D]6, honey yellow); **margin** thin, entire, acute, mostly regular in outline, rarely slightly wavy, white, whitish when fresh contrasting with the pileus surface, pale yellowish grey on drying; **pore surface** plane, the pore field starting at about 0.5-1 mm behind the very margin, leaving a pale grayish yellow sterile zone, the pore field mostly grayish to pale grayish orange when fresh, drying pale grayish orange to yellowish brown (5[E]6, honey yellow, oak brown, mustard brown), discoloring rather abruptly to olive brown (4D6, honey yellow) toward the base; **pores** irregular, mostly round to angular, overall 5-6/mm, (85-) 100-175 (-190)  $\mu\text{m}$  wide (ave = 122  $\mu\text{m}$  wide), occasionally radially ellipsoid to oblong, 160-250  $\times$  90-160  $\mu\text{m}$ , or multilobed up to 400  $\times$  350  $\mu\text{m}$ ; **dissepiments** thin, 20-60  $\mu\text{m}$  thick (ave = 32.5  $\mu\text{m}$ ), not agglutinated, with free hyphal tips, appearing slightly plumose under the lens; **context** homogeneous, without black line, very thin to the margin, up to 0.5-1.0 mm thick at the base, grayish orange (cork-colored) to grayish brown; **tube layer** up to 0.5 mm deep, pale whitish to grayish and contrasting with the context; context and tube layer briefly discoloring to reddish brown in 3 % KOH, then pale brown.

**Hyphal system** monomitic both in the context and hymenophoral trama; **generative hyphae** simple septate, thin- to slightly thick-walled, hyaline, yellowish to light golden brown, darker, brownish in KOH, scarcely ramified, the branches constricted at their emergence point, soon growing parallel to mother' hyphae; **in the context** hyphae with a parallel to subparallel (synclinal) orientation, mostly moderately thick-walled with the lumen widely open, septate, but with long aseptate segments, (3.5-) 4.0-5.0 (-5.8)  $\mu\text{m}$  diam (ave = 4.6  $\mu\text{m}$ ); **pileus surface** with prostrate hyphae, mostly unbranched, identical to the contextual hyphae; in the **hymenophoral trama** hyphae with a subparallel disposition, thin- to slightly thick-walled, the lumen widely open, septate, but with long aseptate segments or with occasional with secondary septa, (2.3-) 2.5-3.7 (-4.0)  $\mu\text{m}$  diam (ave = 2.9  $\mu\text{m}$ ).

**Hymenium:** **basidioles** slightly pyriform to broadly clavate, 6.0-9.0  $\times$  3.5-4.5  $\mu\text{m}$ ; mature **basidia** broadly clavate, with four sterigmata, 9-11  $\times$  4-5  $\mu\text{m}$ ; **cystidioles** few, fusoid, thin-walled; **basidiospores** ellipsoid to broadly ellipsoid to subglobose, appearing somewhat angular on drying, thick-walled, smooth, pale yellowish in KOH, without reaction in Melzer's reagent, (3.0-) 3.3-4.0  $\times$  (2.2-) 2.5-2.8 (-3.0)  $\mu\text{m}$  (ave = 3.4  $\times$  2.6  $\mu\text{m}$ ),  $R = 1.2-1.4 (-1.7)$  (ave<sub>Q</sub> = 1.37).

**Ecology (substrate, host, habitat):** base of living, small-stemmed trunks, *Dichostemma glaucescens* Pierre (locally known as "Ka") and *Anthostema aubryanum* Baill. (Euphorbiaceae), understory compartment, lower Guineo-Congolian rainforest.

**Distribution:** currently known from southwestern Gabon.

**Specimens examined:** GABON, OGOOUE MARITIME. Gamba complex, CTFS-ForestGEO Rabi forest monitoring Plot, approx. 0.597988 S-9.786291 E, elevation approx. 30-60 m, lower part of small stemmed, living trunk, *Dichostemma glaucescens* (Euphorbiaceae), Sep 2014, *P. Yombiyeni* w/o number, NY (holotype), MUCL 55569 (isotype); *ibid.*, lower part of small stemmed, living trunk, *Dichostemma glaucescens* (Euphorbiaceae), Sep 2014, *P. Yombiyeni* w/o number, MUCL 55568; *ibid.*, base of living trunk of *Anthostema aubryanum* (Euphorbiaceae), Sep 2014, *P. Yombiyeni* w/o number, MUCL 55570.

***Phylloporia gabonensis*** Decock & Yombiyeni **sp. nov.**

Figs 3c-d, 5a-d

Mycobank: MB815278

**Etymology:** "gabonensis" (L.) in reference to the country of origin, Gabon.

**Basidiomata** annual, pileate, sessile, gregarious, emerging simultaneously in clusters of up to ~ 100 individual basidiomata, mostly superposed, occasionally

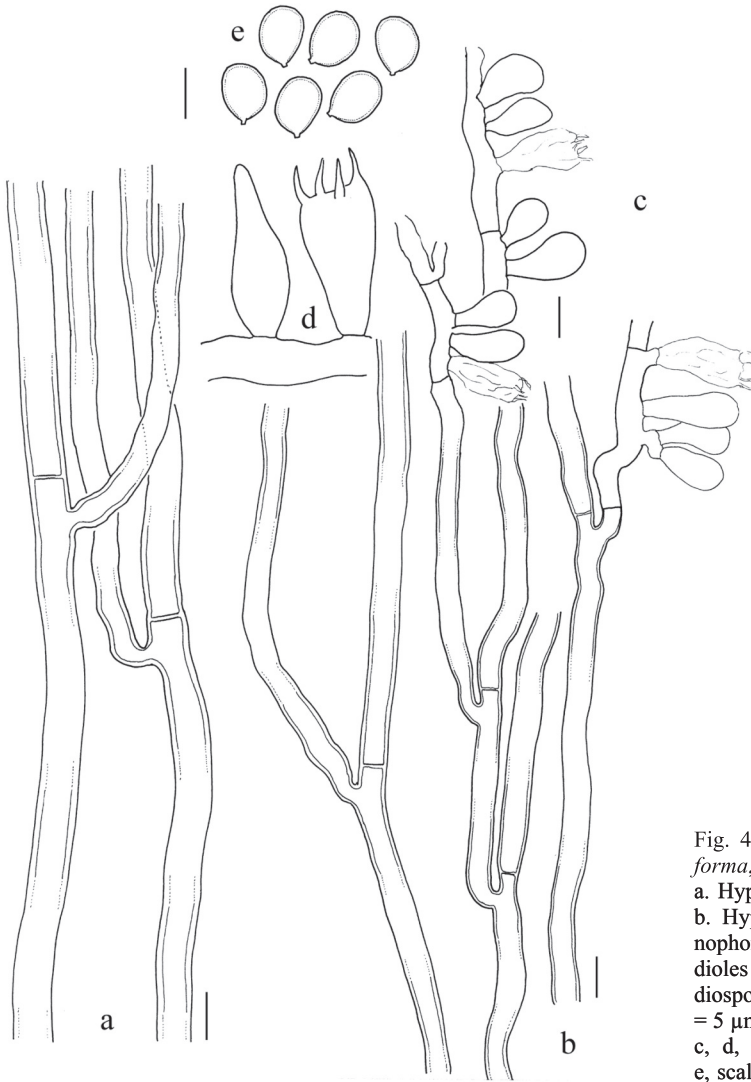


Fig. 4. *Phylloporia flabelliforma*, MUCL 55569 (type). a. Hyphae from the context. b. Hyphae from the hymenophoral trama. c, d. Basidioses and basidia. e. Basidiospores (type) (a, scale bar = 5  $\mu\text{m}$ ; b, scale bar = 4  $\mu\text{m}$ ; c, d, scale bar = 3.0  $\mu\text{m}$ ; e, scale bar = 2.5  $\mu\text{m}$ ).

laterally fused; individual **basidiomata** mostly spatulate to flabelliform, attached to the substrate by a narrowly discoid basal area, projecting horizontally 15-25 mm, 10-15 mm wide, from 0.5 mm thick at the very margin up to 1.5-2.5 mm at the thickest part, the margin enrolling downward on drying; **pileus surface** dull, smooth, radially faintly wrinkled on drying, mainly cork-colored, very pale toward the margin (4B([5-6], grayish yellow) progressively darkening toward the base, grayish orange (5[B-C] ([5-6], reddish blond), yellowish brown (5D6 to 5E6, honey yellow to mustard yellow, bronze), faintly concentrically zonate with narrow, darker band; **margin** thin, irregular in outline, dentate to lobed, pale yellow to pale grayish orange when fresh, drying pale grayish orange; **pore surface** plane, the pore field starting at the very margin, yellowish brown when dry (5E[5-6], soot brown, tobacco brown)

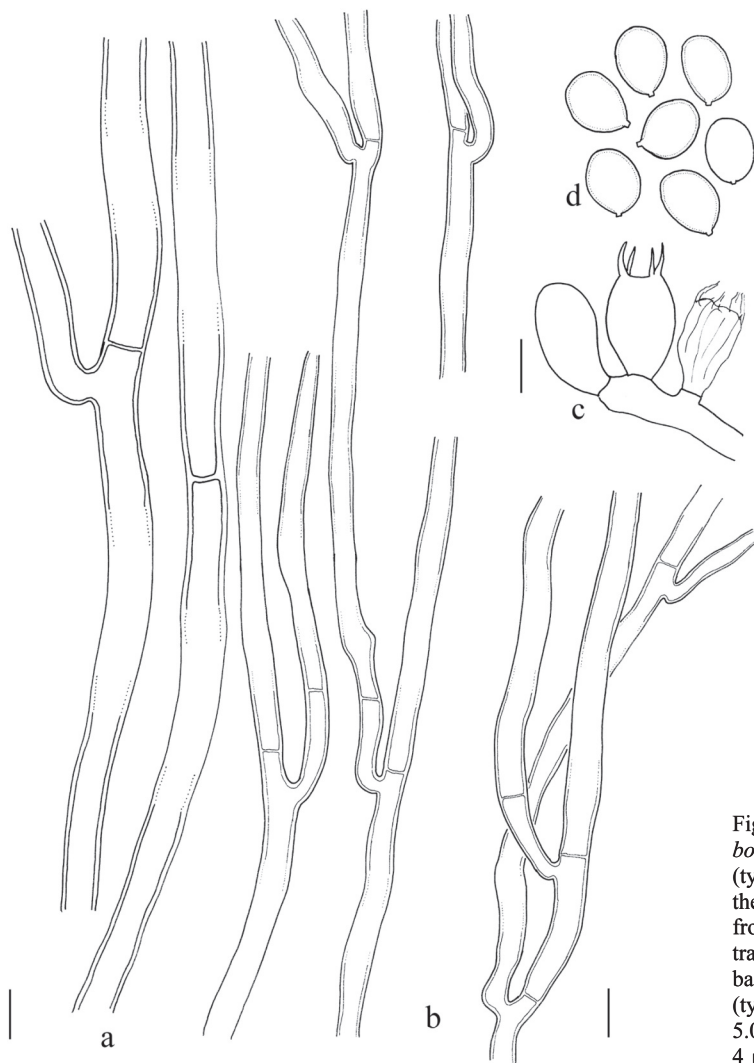


Fig. 5. *Phylloporia gabonensis*, MUCL 55572 (type). a. Hyphae from the context. b. Hyphae from the hymenophoral trama. c. Basidioles and basidia. d. Basidiospores (type) (a, b, scale bar = 5.0  $\mu\text{m}$ ; c, d, scale bar = 4  $\mu\text{m}$ ).

(no data on the fresh state, probably paler, toward olive brown); **pores** mostly round to angular, overall 5-6/mm, (50-) 70-205 (-225)  $\mu\text{m}$  wide (ave = 133  $\mu\text{m}$  wide), occasionally radially ellipsoid to oblong, or fused and multilobed; **dissepiments** thin, entire to slightly lacerated, 15-55  $\mu\text{m}$  thick (ave = 30.5  $\mu\text{m}$ ), not agglutinated, with free hyphal tips, appearing slightly plumose under the lens; **context** homogeneous, without black line, soft corky, with a slightly fibrous texture, 0.25 mm thick at the margin up to 1 mm in the middle thickest part, grayish orange; **tube layer** 0.25-1.5 mm deep, concolorous with the context; context and tube layer briefly discoloring to reddish brown in 3 % KOH, then pale brown.

**Hyphal system** overall monomitic both in the context and hymenophoral trama; **generative hyphae** simple septate, thin- to slightly thick-walled, the lumen widely open, hyaline, yellowish to light golden brown, darker, brownish in KOH,

scarcely ramified, the branches constricted at their emergence point, soon growing parallel to the mother hyphae; in the **context**, hyphae with a parallel to sub-parallel (synclinal) orientation, regularly septate but with long aseptate segments, (3.3-) 3.5-5.5 (-6.0)  $\mu\text{m}$  diam (ave = 4.6  $\mu\text{m}$ ); **pileus surface** with prostrate hyphae, mostly unbranched, identical to the contextual hyphae; in the **hymenophoral trama**, hyphae with a subparallel disposition, septate, but with long aseptate segments or with occasional with secondary septa, (2.5-) 2.5-3.5 (-3.8)  $\mu\text{m}$  diam (ave = 3.0  $\mu\text{m}$ ).

**Hymenium:** **basidioles** slightly pyriform to broadly clavate, 6.0-10.0  $\times$  3.0-5.0  $\mu\text{m}$ ; mature **basidia** slightly clavate, with four sterigmata; **cystidioles** few, fusoid to slightly lageniform, thin-walled; **basidiospores** ellipsoid (broadly ellipsoid), appearing somewhat angular on drying, thick-walled, smooth, hyaline to pale yellowish in KOH, without reaction in Melzer's reagent, 3.7-4.2 (-5.0)  $\times$  2.7-3.0 (-3.3)  $\mu\text{m}$  (ave = 3.9  $\times$  2.9  $\mu\text{m}$ ), R = 1.2-1.4 (-1.5) (ave<sub>Q</sub> = 1.35).

*Ecology (substrate, host, habitat):* small-stemmed living trunks, from the base up to 1 m high, *Dichostemma glaucescens* Pierre (Euphorbiaceae), understory compartment, lower Guineo-Congolian rainforest.

*Distribution:* currently known from Southwestern Gabon.

*Specimens examined:* GABON, OGOOUE MARITIME. Gamba complex, CTFS-ForestGEO Rabi forest monitoring plot, approx. 0.597802 S-9.786895 E, elevation approx. 30-60 m., on small stemmed, living trunk, *Dichostemma glaucescens* (Euphorbiaceae), Sep 2014, *P. Yombiyeni* w/o #, NY (holotype), MUCL 55572 (isotype); *ibid.*, on small stemmed, living trunk, *Dichostemma glaucescens* (Euphorbiaceae), Sep 2014, *P. Yombiyeni* w/o #, MUCL 55571.

## DISCUSSION

*Phylloporia flabelliforma* and *P. gabonensis* have mostly sessile, flabelliform to spatulate basidiomata, the pileus in grayish orange (cork-colored) to yellowish brown shades. Their context is homogeneous, pale cork-colored, briefly discoloring to reddish, reddish brown in alkali. Their hyphal system is identical, both in the context and in the hymenophoral trama; it is best described as monomitic. The basidiospores are ellipsoid to broadly ellipsoid and of a comparable size in both species, although slightly smaller in *P. flabelliforma* in comparison to those of *P. gabonensis*, averaging respectively 3.4  $\times$  2.6  $\mu\text{m}$  and 3.9  $\times$  2.9  $\mu\text{m}$ . Both species also share their reproduction strategy; their basidiomata are gregarious, emerging simultaneously in large number in dense clusters (Figs 3) and last one season.

In a phylogenetic perspective, both species are also very closely related, sister clades.

In an ecological context, *P. flabelliforma* and *P. gabonensis* share some important autecological features, including the habitat, substrate and hosts. They both occur sympatrically in the understory compartment of the lower Guinean rainforest at the CTFS-ForestGEO Rabi plot, growing from living trunks of two small-stemmed Euphorbiaceae, *Dichostemma glaucescens* (*P. flabelliforma* and *P. gabonensis*) and *Anthostema aubryanum* (*P. flabelliforma*), their basidiomata emerging at their lower part, from the soil level up to approx. 1 m high.

*Phylloporia flabelliforma* is specifically characterized by small-sized, thin basidiomata ( $\leq 1.5$  mm thick), a regular margin, white when fresh, contrasting with the grayish/brownish orange and a shiny pileus surface. *Phylloporia gabonensis*

have thicker basidiomata ( $\geq 1.5$  mm), an irregular, lobed, incised margin, a dull pileus darkening to yellowish brown toward the base, and slightly larger basidiospores, in which features it differs from *P. flabelliforma*.

In the lower Guinean rainforest of Gabon, these 2 species should be compared to *P. inonotoides* and *P. fulva*.

*Phylloporia inonotoides* is sympatric with *P. gabonensis* and *P. flabelliforma* in the understorey compartment at the CTFS-ForestGEO Rabi plot. It also shares with the two latter taxa the substrate (living trunk of small-stemmed tree) and the host at family level (Euphorbiaceae). These three species also have the same monomitic hyphal system. *Phylloporia inonotoides* differs in having larger and thicker basidiomata, up to 20-30 mm wide, 6-8 mm thick, a darker, light brown to brown pileus, larger pores, 2-3/mm [(125-) 170-600 (-750) mm diam] and, microscopically, oblong to suballantoid and longer basidiospores [4.5-5.5 (-6.5)  $\mu\text{m}$  long, ave = 4.9  $\mu\text{m}$ ] (Yombiyeni *et al.* 2015). Furthermore, basidiomata of *P. inonotoides* emerge solitary and are known so far from *Crotonogyne manniana* Müll. Arg. (Euphorbiaceae).

*Phylloporia fulva* was described on the basis of a specimen collected at the Mont de Cristal National Park (approx. N 00°37,05'-E 010°24.6'), in northern Gabon (Yombiyeni *et al.* 2015). It shares with *P. gabonensis* and *P. flabelliforma* gregarious, small basidiomata and grayish orange pilei (when fresh). *Phylloporia fulva* is additionally characterized by a thin black line in the context, subtending a thin pileal trichoderm, 9-11 pores/mm [(70-) 80-100 (-105)  $\mu\text{m}$  diam], a dimittic hyphal system and distinctly subglobose basidiospores (Yombiyeni *et al.* 2015), in which features it differs from both *P. gabonensis* and *P. flabelliforma*.

*Phylloporia flabelliforma* and *P. gabonensis* could be compared also to two species occurring in the Neotropics and currently accepted in *Inonotus* Murrill, respectively *Inonotus pusillus* Murrill<sup>2</sup> and *Inonotus dentatus* Decock & Ryvardeen<sup>3</sup> (Murrill 1904, Ryvardeen 2004, 2005).

*Phylloporia flabelliforma* and *I. pusillus* share a set of macro- and micro-morphological characters, including thin, flabelliform basidiomata, 5-6 pores/mm, a monomitic hyphal system and ellipsoid basidiospores (type examined<sup>2</sup>; cf. also Ryvardeen 2005). These species also share their reproduction strategy and some ecological parameters: *Inonotus pusillus*, as *P. flabelliforma*, also produces gregarious basidiomata emerging simultaneously in large number, in dense clusters, from small branches of shrubby tree [*Jaquinia* L. (Theophrastaceae) for the type specimen<sup>2</sup>] (cf. also NYBG virtual herbarium [<http://sweetgum.nybg.org/vh/specimen.php?irn=884041>]). *Inonotus pusillus* differs from *P. flabelliforma* in having still smaller basidiomata (up to 2 mm wide), the pileus surface and the context darker, fulvous to rusty [ferruginous] brown (grayish orange in *P. flabelliforma*) and larger basidiospores, 4.5-6  $\times$  3.5-4.5  $\mu\text{m}$  (3.3-4.0  $\times$  2.5-2.8  $\mu\text{m}$  in *P. flabelliforma*). *Inonotus pusillus* is only known, as far as we have been able to ascertain, from Western Mexico (type locality) and Belize (Murrill 1904, Ryvardeen 2005).

*Phylloporia gabonensis* and *I. dentatus* also share a set of similar characters, of which pileate basidiomata, soft pilei, in grayish orange to yellowish brown tone, the context reddening in alkali, irregular pores and the same, monomitic

2. Type examined: MEXICO, STATE OF COLIMA. Manzanillo, emerging from lenticels of branch of *Jaquinia* L. [Theophrastaceae], 2-18 Mar 1891, E. Palmer # 1520, NY !

3. Type examined: FRENCH GUIANA, CAYENNE AREA. Matoury, Mont Grand Matoury Nature Reserve, *Sentier d'interprétation de la nature "Lamirande"*, on hanging stem, unidentified living liana, at about 2 m above soil level, 24 Jan 2000, C. Decock, FG-00-2142, MUCL 42641, Isotype NY and O !



hyphal system. The basidiomata of *I. dentatus* were also found emerging in (moderate) number from a living (unidentified) liana (C. Decock, pers. obs.). *Inonotus dentatus* differs from *P. gabonensis* in having larger pores (1-3/mm) and oblong and longer basidiospores  $4.5-5.5 (-6.0) \times 2.8-3.3 \mu\text{m}$  (Ryvarden 2005). It was described and, so far, is only known from the rainforest of French Guiana (Ryvarden 2005).

Many features of *I. pusillus* and *I. dentatus* point toward affinities with *Phylloporia* rather than with *Inonotus*. The reddish discoloration of the hyphae in alkali as observed in *I. dentatus* is a very unusual feature in Hymenochaetaceae. It is also known, as far as we have been able to ascertain, in *Inonotus splitgerberi* (Mont.) Ryvarden (Ryvarden 2005) but also in *Phylloporia oblongospora* (Cui *et al.* 2010) and, to a lesser degree, in *P. gabonensis* and *P. flabelliforma*. The affinities of *I. pusillus* and *I. dentatus* should be ascertained when DNA sequence data will become available.

With the addition of *P. gabonensis* and *P. flabelliforma*, 10 named *Phylloporia* species are now reported from the Guineo-Congolian phytogeographic region (Hjortstam *et al.* 1993, Ipulet and Ryvarden 2005, Núñez and Daniëls 1999, Roberts and Ryvarden 2006, Ryvarden 2000, Ryvarden and Johansen 1980, Wagner and Ryvarden 2002, Yombiyeni *et al.* 2015). Two unnamed species pertaining to the *P. pectinata* morpho-ecological group (PS 4 and PS 5, Fig. 1) are also known from this region (Yombiyeni *et al.* 2015). Both were found growing on two small-stemmed understorey species of Melastomataceae, *Warneckea* Gilg. and *Memecylon* L. (Yombiyeni *et al.* 2015) in Gabon.

*Phylloporia flabelliforma*, *P. gabonensis*, *P. inonotoides*, and *Phylloporia* PS 4 and PS 5 are all found sympatrically at the CTFS-ForestGEO Rabi plot. Locally, their known hosts (*Anthostema*, *Crotonogyne*, *Dichostemma*, *Memecylon*, *Warneckea*) are frequent. These hosts are also largely distributed in Gabon where they sometimes represent, at a local scale, an important component of the understorey compartment. For instance, *D. glaucescens* is reported forming dense stands (with about 50 stems/Ha) within the Haut Abanga Forest concession (approx. N 0°30'-E 11°0') (FAO 2002). Therefore, these *Phylloporia* species are expected occurring all over the sub-region.

The exact trophic relationships of *Phylloporia* species are uncertain. The species are for their vast majority growing on living plants; hence they have often been qualified as pathogens or parasites. However, their relevance as possible tree pathogens has been rarely raised or tested. For the vast majority, nothing is known about their potential pathogenicity and impact on the plant fitness. Corner (1991) discussed the case of *P. pectinata*, suggesting trunk and branch infections through dead snags, and wood decay down to the heartwood, nevertheless without killing trees. Corner (1991) also reported basidiomata of *P. bibulosa* (C.G. Lloyd) Ryvarden *sensu* Corner emerging from petioles and midribs of living leaves of *Ixora* sp. (Rubiaceae) but noted that “the leaves, however, did not seem to suffer as a whole”. Nonetheless, Esquivel and Carranza (1996) reported in Costa Rica a reduction in the growth rate of *Erythrochiton gymnanthus* Kallunki (Rutaceae) infected by *P. chrysites*, which basidiomata emerge from petioles of living leaves; this reduction could reach 52%. A high level of contamination could therefore impact the development and the dynamic of the small-stemmed or shrubby species. The host and trophic relationships of *Phylloporia* species and their potential impact on the tree fitness would be worth investigating also in the understorey compartment of the Guineo-Congolian rainforest in Gabon, to better understand the dynamic of the local small-stemmed trees.

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