New species and a new record of Phylloporia from Benin

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Species of the wood-decay genus Phylloporia (Hymenochaetaceae, Hymenochaetales, Basidiomycota) are widely distributed in the tropics. Phylloporia species are, however, morphologically and ecologically diverse, which makes morphology-based species identification challenging. In this study, we re-examined species of Phylloporia reported from Benin (West Africa). Using an integrative approach combining morphology, ecology, and phylogenetic analyses, we describe Phylloporia beninensis sp. nov. and report Phylloporia littoralis for the first time outside of its type locality. Phylloporia beninensis sp. nov. is characterized by its annual and imbricate basidiomata, duplex context with a black zone separating the upper context from the lower one, dimitic hyphal system, presence of cystidioles, basidia of 9–12 × 4–5 μm, and subglobose to ellipsoid basidiospores measuring 3–4.6 × 2.1–3.6 μm. Detailed descriptions with illustrations for the new species are provided. With the addition of the new species, 15 Phylloporia species are now known to occur in tropical Africa. Our discovery of a new Phylloporia species in Benin should stimulate further mycological investigations in tropical African ecosystems to discover other new polypore species. To facilitate further taxonomy studies on tropical African Phylloporia taxa, a key to the known tropical African species is provided.

Abbreviations

ABGD  Automatic barcode gap detection
BS  Bootstrap values
BY  Bayesian
ITS  Internal transcribed spacer
KAS  Mycological Herbarium of the University of Kassel
L  Length
LSU  Large subunit
m a.s.l.  Meters above sea level
ML  Maximum likelihood
nrDNA  Nuclear ribosomal DNA
PP  Posterior probabilities
PTP  Poisson tree process
PTP_Bs  Poisson tree process Bayesian solution
PTP_Mls  Poisson tree process maximum likelihood solution
Q  Length to width ratio
UNIPAR  Mycological Herbarium of the University of Parakou, Benin
W  Width

Phylloporia Murrill is a widely distributed polypore genus in Hymenochaetaceae (Hymenochaetales, Agaricomycetes, Basidiomycota) typified by P. parasitica Murrill1. Species of Phylloporia occur mainly in the tropics but are also known from higher latitude ecosystems well1-4. Phylloporia sensu Murrill was characterized by resupinate and annual basidiomata with the ability to grow on the underside of living leaves, monomitic hyphal system, and absence of the setae which characterize most members of the Hymenochaetaceae1. Although the genus was erected in 1904, it remained undocumented until Ryvarden6 re-examined the type material and recognized four additional species: P. bibulosa (Lloyd) Ryvarden, P. chrysites (Berk.) Ryvarden, P. fruticum (Berk. & M. A. Curtis) Ryvarden, and P. weberiana (Bres. & Henn. ex Sacc.) Ryvarden, each sharing the microscopic features characterizing the type species. Subsequently, with the advent of DNA barcoding, Phylloporia taxonomy was revisited7.

Based on phylogenetic analysis inferred from nuc 28S rDNA sequences as well as morphological and anatomical features, Wagner and Ryvarden7 demonstrated that Phylloporia is monophyletic, with Fulvifomes Murrill as a
sister genus. From this pioneering molecular work, *Phylloporia* received much more attention, resulting in the current recognition of 61 species worldwide\(^9\)-\(^13\). The current morphological concept for *Phylloporia* species includes annual to perennial basidiomata with resupinate, pileate-sessile, or pileate-stipitate habits, homogenous to two-layered context, monomitic to dimitic hyphal system, presence or absence of cystidioles, and subglobose to ellipsoidal basidiospores\(^8\),\(^10\),\(^13\),\(^14\). The presence of setae in *Phylloporia* was not noted until reported by Wu et al.\(^10\). Ecologically, some *Phylloporia* species are putatively host-specific parasites on living leaves, bushes, branches, and trees\(^5\),\(^13\),\(^15\)-\(^17\), others are saprotrophs on wood\(^14\)-\(^20\), with the trophic status of others still unknown\(^10\).

Despite the reasonably well-defined generic diagnostic features, *Phylloporia* remains heterogeneous and the species can be difficult to separate morphologically from those of related hymenochaetoid genera. As an example, Douauala-Meli et al.\(^21\) described a new species from Cameroon as *P. resupinata* Douauala-Meli and Ryvarden, but subsequent molecular phylogenetic analysis placed *P. resupinata* within the hymenochaetoid *Fomitiporella* clade and the species was transferred to that genus\(^22\).

Currently 14 species have been reported from tropical Africa with eight of these described from type material collected in the region\(^2\),\(^7\),\(^9\),\(^12\),\(^18\),\(^23\). These species are mainly Central or East African and to our knowledge, only *P. weberiana* was known from West Africa\(^7\) until Olou et al.\(^24\) reported two species identified as *Phylloporia* sp. However, since species of *Phylloporia* can be host specific, we have since re-examined both of the species of Olou et al.\(^24\). Utilizing morphological and molecular phylogenetic analyses, we found that one of these species is *P. littoralis* Decock & Yombiyeni, previously known only from Gabon, while the second is new to science. Here we describe the new species and provide a key to the known tropical African *Phylloporia* species.

### Material and methods

**DNA extraction, amplifications, and sequencing.** We extracted DNA from dried specimens using the microwave method\(^25\). Although previous studies involving *Phylloporia* species have used primarily the nuclear ribosomal large subunit, here we amplified two nuclear ribosomal DNA regions (nrDNA), the internal transcribed spacer (ITS) and the D1–D4 domain of large subunit (LSU). The primer pairs ITS-1F/ITS4\(^26\)-\(^27\) and LR0R/LRS\(^28\) were used to amplify both target DNA regions. For the polymerase chain reaction (PCR) procedure, the PCR products purification, and Sanger sequencing, we followed Olou et al.\(^24\). A total of six sequences, composed of two ITS and four LSU, were generated in this study and deposited in GenBank. Table 1 gives the accession numbers for all taxa included in this study.

**Sequence alignment and species delimitation.** To place our newly generated sequences accurately in the phylogenetic tree, we aligned them in addition to 126 LSU sequences retrieved from GenBank and used by previous studies on *Phylloporia*\(^29\). Sequences were aligned using the online mode of MAFFT version 7\(^30\), with the algorithm FFT-NS-i as the most suitable. The resulting multiple sequences alignment was checked in Geneious 5.6.7 (https://www.geneious.com)\(^31\), where the ends rich in gaps were manually trimmed. Further, the multiple sequences alignment was inspected and some bases were manually adjusted using AliView\(^32\). Two model-based methods for species delimitation namely the Automated Barcode Gap Discovery (ABGD)\(^33\) and the Poisson Tree Process (PTP)\(^34\) were performed. The ABGD analysis detect potential barcode gap and use the identified barcode gap to sort the datasets into a hypothetical species. This analysis was performed on ABGD web interface using the Jukes-Cantor (JC69) and Kimura two-parameter (K2P). The relative gap width was set to 1.0 because if the gap is too large, the model will sort the dataset into a single species. We kept all other parameters as default. Like the ABGD method, the PTP is another species delimitation method that inferred putative species boundaries on a given phylogenetic input tree. To run the PTP analysis, we first built a single phylogenetic tree using IQ-tree 1.6.12 (http://www.iqtree.org/) in command line mode. The resulted tree without annotations in Newick format was used as the input tree to run the PTP analysis on a web server (http://species.h-its.org/ptp/) for 500,000 generations and 25% were discarded as burn-in. To compare both species delimitation models to the phylogenetic analysis, Maximum likelihood (ML) analysis under the Ultrafast Bootstrap with 5000 replicates was performed on the dataset using IQ-tree 1.6.12 (http://www.iqtree.org/) in command line mode with TM3 + F + I + G4 as the best substitution model selected using the command TESTONLY.

**Phylogenetic analyses.** For phylogenetic analyses, 73 sequences from the LSU region out of the 126 sequences previously used to inform species delineation in *Phylloporia* were selected and aligned with the 4 newly generated sequences in this study. In addition, 34 sequences from the ITS region including the type material of the genus were downloaded from GenBank and aligned together with the sequences newly generated in this study. *Inonotus andersonii* (Ellis & Everh.) Nikol. and *I. hispidus* (Bull.) P. Karst. were chosen as outgroup for both regions. Each region was aligned separately using the online mode of MAFFT version 7\(^30\), with the algorithm L-INS-i. The multiple sequences alignments were checked and concatenated in Geneious 5.6.7 (https://www.geneious.com)\(^31\).

Given the gap in terms of number of sequences between the ITS and LSU regions (36 vs. 77), the concatenated alignment was considered as a single region and the best-fit evolutionary model was estimated as GTR + I + G using IQ-tree 1.6.12 (http://www.iqtree.org/) and the command TESTONLY. Following this substitution model, two phylogenetic tree inference methods, ML and Bayesian inference (BI) were performed. The ML was run using RAxML 8.2.10\(^15\) under standard bootstrap at the Cipres Science Gateway V3.\(^3\)\(^36\). The BI was executed using MrBayes 3.2.7 in command line mode (https://github.com/NBISweden/MrBayes)\(^37\) for five million generations until the standard deviation of split frequencies reached 0.01. Chain convergence was determined using Tracer, v1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/) and the first 25% (5000) trees was discarded as burn-in. The remaining trees were used to build the consensus tree using the Phylogenetic Tree Summarization (SumTrees) program within DendroPy 4.3.0 (https://github.com/jeetsukumaran/DendroPy)\(^38\). The topology of the ML tree...
| Species name                  | Voucher or strain | Origin       | GenBank N° | TS | LSU | References |
|------------------------------|-------------------|--------------|------------|----|-----|------------|
| Fomitiporella resupinata     | Cameroon          | Douala-Meli 476 | KJ787822   | JF712935 | 44 |
| Fomitiporella sinica         | China             | LWZ 20130809-9 | KJ787819   | KJ787810 | 44, 45 |
| Fomitiporella kemissima      | China             | Dai 12245     | KC456242   | KC999902 | 45, 46 |
| Fomitiporella ambridina      | USA               | JV 0509/114   | KX181314  | KX181336 | 45 |
| Fulvifomes fuscibus          | Philippines       | CBS 213.36    | AY55865    | AY059057 | 7 |
| Fulvifomes robiniiae         | USA/Arizona       | CFMR 2693     | KX065961   | KX065995 | Unpublished |
| Fulvifomes yorou             | Benin             | OAB0097       | MN017126   | MN017120 | 47 |
| Inonotus andersonii          | JV1209_66         | USA           | MN318443   | MN318443 | Unpublished |
| Inonotus hispidus            | 92–829            |              | AY624993   | AE311014 | 49 |
| Phylloporia afrospathulata   | MUCL 54511        |              | KJ743248   |        | 50 |
| Phylloporia afrospathulata   | MUCL 53983        |              | KJ743249   |        | 50 |
| Phylloporia alyxiae          | GC 1604-28        | Taiwan       | LC514408   |        | 50 |
| Phylloporia alyxiae          | Chen 1182         | Taiwan       | LC514407   |        | 50 |
| Phylloporia atlantica        | JRF151            | Brazil       | MG738814   |        | 5 |
| Phylloporia atlantica        | JRF142            | Brazil       | MG738813   |        | 5 |
| Phylloporia bicallosa        | Ahmad 27088       |              | AF411824   |        | 5 |
| Phylloporia boldo            | CIEFAPcc532       | Chile        | MK193759   |        | 5 |
| Phylloporia boldo            | CIEFAPcc584       | Chile        | MK193758   |        | 5 |
| Phylloporia caucasina        | Robledo 1610      | Argentina    | KJ651919   |        | Unpublished |
| Phylloporia fruticola        | MUCL 52762        | Mexico       | HM635668   |        | 5 |
| Phylloporia fruticola        | ENCR TR&RV 858    | Mexico       | HM635669   |        | 5 |
| Phylloporia chrysites        | MUC 52862         | Mexico       | HM635667   |        | 5 |
| Phylloporia chrysites        | MUC 52764         | Mexico       | HM635666   |        | 5 |
| Phylloporia chrysites        | MUC 52862         | Mexico       | HM635666   |        | 5 |
| Phylloporia clausenae        | Yuan 3528         | China        | KJ787795   |        | 5 |
| Phylloporia clausenae        | Cui8463           | China        | MH151186   | MH156888 | 5 |
| Phylloporia crustagii         | Dai18133          | China        | MH151191   | MH156856 | 5 |
| Phylloporia crustagii         | Dai 11016         | China        | JF712923   |        | 5 |
| Phylloporia cystidolophora   | Dai13953          | China        | MG738798   |        | 5 |
| Phylloporia cystidolophora   | Dai13945          | China        | MG738798   |        | 5 |
| Phylloporia dependens        | Cui13763          | China        | KX242353   | MH151190 | 5 |
| Phylloporia dependens        | Cui 13763         | China        | KX242353   | MH151190 | 5 |
| Phylloporia elegans          | FLOR:51179        | Brazil       | KJ631409   |        | 5 |
| Phylloporia elegans          | FLOR:51178        | Brazil       | KJ631408   |        | 5 |
| Phylloporia ephedrae         | MUCL 54472        |              | AF411826   |        | 5 |
| Phylloporia flabelliforma    | MUCL 55570        | Gabon        | NR_154332  | KU198350 | 5 |
| Phylloporia flabelliforma    | MUCL 55569        | Gabon        | KU198356   | KU198349 | 5 |
| Phylloporia flaccitae        | Yuan 6362         | China        | KJ787801   |        | 5 |
| Phylloporia flaccitae        | Yuan 6360         | China        | KJ787800   |        | 5 |
| Phylloporia fontanesae       | Cui12356          | China        | MH151188   | MH156871 | 5 |
| Phylloporia fontanesae       | Li 199            |              | JF712925   |        | 5 |
| Phylloporia fulva            | MUCL 54472        |              | KJ743247   |        | 5 |
| Phylloporia gabonensis       | MUCL 55572        | Gabon        | KU198354   | KU198352 | 5 |
| Phylloporia gabonensis       | MUCL 55571        | Gabon        | KU198351   | KU198353 | 5 |
| Phylloporia gutta            | Dai16070          | China        | MH151183   | MH156863 | 5 |
| Phylloporia gutta            | Dai 4197          | China        | JF712927   |        | 5 |
| Phylloporia hainanensis      | Dai 9460          |              | JF712928   |        | 5 |
| Phylloporia homocarnica      | Yuan 5766         | China        | KJ787804   |        | 5 |
| Phylloporia homocarnica      | Yuan 5750         | China        | MH151195   | KJ787803 | 5 |
| Phylloporia inonotoides      | MUCL 54468        | China        | KJ743242   |        | 5 |
| Phylloporia lespedae         | Dai17065          | China        | MH151179   | KY242602 | 5 |
| Phylloporia lespedae         | Dai17067          | China        | MH151180   | KY242603 | 5 |
| Phylloporia littoralis       | MUCL 56145        | Gabon        | KY349141   |        | 5 |

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| Species name             | Voucher or strain | Origin               | GenBank N°            | References |
|-------------------------|-------------------|----------------------|-----------------------|------------|
|                         |                   |                      | TS                    | LSU        |          |
| Phylloporia littoralis   | MUCL: 56144       | Gabon                | KY349140              | 17         |
| Phylloporia lonicerae    | Dai17900          | China                | MH151175              | MG738802   | 15        |
| Phylloporia lonicerae    | Dai17899          | China                | MH151174              | MG738801   | 15        |
| Phylloporia lonicerae    | Dai17898          | China                | MH151173              | MG738800   | 15        |
| Phylloporia manglietiae  | Cui 13769         | China                | MF410324              | KX242358   | 11        |
| Phylloporia minuta       | FURB 55088        | Brazil               | NG_064479             |            |
| Phylloporia minutispora  | Dai16172          | China                | MH165873              | Unpublished|
| Phylloporia minutispora  | Ipute706          |                      |                       |            |
| Phylloporia minutispora  | MUCL 52865        | Democratic Republic of the Congo | HM635671 |            |
| Phylloporia montana      | BDNA2409          | Brazil               | MG738811              | 8          |
| Phylloporia montana      | BDNA2388          | Brazil               | MG738810              |            |
| Phylloporia mori         | Taiwan            | Wu 1105-2            | LC514412              |            |
| Phylloporia mori         | Taiwan            | Wu 1105-3            | LC514413              |            |
| Phylloporia mori         | Wu 1105-3         | Taiwan               | LC514413              |            |
| Phylloporia mori         | Wu 1105-2         | Taiwan               | LC514412              |            |
| Phylloporia murrayae     | Wu 1404-5         | Taiwan               | LC514410              |            |
| Phylloporia murrayae     | Wu 1404-4         | Taiwan               | LC514409              |            |
| Phylloporia nandiniae    | Dai 10625         |                      | JF712931              |            |
| Phylloporia nandiniae    | Dai 10588         |                      | JF712930              |            |
| Phylloporia nodostipitata| FLOR:51715        | Brazil               | KJ631413              |            |
| Phylloporia nodostipitata| FLOR:51713        | Brazil               | KJ639057              | KJ631412   | 20        |
| Phylloporia nouraguensis | MUCL/FG-11-409    | Guyana               | KC136224              |            |
| Phylloporia nouraguensis | MUCL/FG-11-404    | Guyana               | KC136223              |            |
| Phylloporia oblongospora | Zhou179           |                      | MH151197              | JF712932   | 20        |
| Phylloporia oreophila    | CUI2219           | China                | MH151196              | JF712933   | 20        |
| Phylloporia oreophila    | Cui 9503          | China                | JF712934              | 30         |
| Phylloporia osmantha     | Yuan 5655         | China                | KF729938              | 19         |
| Phylloporia parasitica   | Leaf Ryvarden 19843 | Argentina            | KU198361              |            |
| Phylloporia pectinata    | R. Coveny 113     |                      | AF411823              |            |
| Phylloporia pendula      | Cui 13691         | China                | KX242357              |            |
| Phylloporia pendula      | Cui 13876         | China                | KX901670              |            |
| Phylloporia perangusta   | Dai18139          | China                | MH151169              | MG738803   | 8          |
| Phylloporia pseudeceptina| Cui 13749         | China                | KX242356              |            |
| Phylloporia pseudeceptina| Cui 13746         | China                | KX242355              |            |
| Phylloporia pulla        | Dai 9627          | China                | KU904469              | 41         |
| Phylloporia pulla        | Cui 5251          | China                | KU904468              | 41         |
| Phylloporia radiata      | LNZ 20141122-5    |                      | KU904470              | 41         |
| Phylloporia rattiunicola | Dai18235          | China                | MH151172              | MG738808   | 8          |
| Phylloporia rattiunicola | Dai18233          | China                | MG738807              |            |
| Phylloporia resupinata   | Doual-la-Meli 476 | Cameroon             | KJ787822              | JF712935   | 30        |
| Phylloporia rubis (cited as Phellinus rubis) | 82-828            |                      | AF311040              | 52         |
| Phylloporia rinoreae (cited as Phylloporia sp.) | MUCL: 57328       | Gabon                | MN243146              | 9          |
| Phylloporia rinoreae (cited as Phylloporia sp.) | MUCL: 56283       | Gabon                | MN243144              | 9          |
| Phylloporia rubiacerum   | Chen 3584         | Taiwan               | LC514417              |            |
| Phylloporia rubiacerum   | Chen 3583         | Taiwan               | LC514416              |            |
| Phylloporia rzedowskii   | MUCL: 52860       | Mexico               | HM635674              | 40         |
| Phylloporia rzedowskii   | MUCL: 52859       | Mexico               | HM635673              | 40         |
| Phylloporia solcica      | JRF145            | Brazil               | MG738815              |            |
| Phylloporia sp.          | OAB0107           | Benin                | MW244097              | This study |
| Phylloporia sp.          | OAB0142           | Benin                | MW244094              | MW244099   |
| Phylloporia sp.          | OAB0204           | Benin                | MW244095              | MW244098   |
| Phylloporia sp.          | OAB0511           | Benin                | MW244096              |            |
| Phylloporia sp.          | FLOR:51258        | Brazil               | KJ631406              | unpublished|

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was better resolved than that of BI, so the ML tree was targeted. To add the posterior probabilities (PP) of BI on the ML tree, the Phylogenetic Tree Summarization (SumTrees) program within DendroPy 4.3.0 (https://github.com/jeetsukumaran/DendroPy) was used. Then, the bootstrap values were added to the ML best tree already having the posterior probabilities using IQ-tree. The resulting tree with (PP/BS) is presented in Fig. 3 and the support values ≥ 80% of PP and ≥ 70% of BS are indicated on each node. Alignment and phylogenetic tree generated in the study are deposited in TreeBASE: http://purl.org/phylo/treebase/study/TB2:S27303.

### Morphological examination.

Morphological descriptions were based on dried herbarium specimens. Macro-morphological characters were described with the aid of a stereomicroscope Leica EZ4 while micro-structures were described using a Leica DM500 light microscope. For the microstructures, fine sections through the basidiomata were prepared for observation using a razor blade under a stereomicroscope and mounted in distilled water and 5% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of phloxine. Melzer's reagent (to test for dextrinoid or amyloid reactions) and cotton blue (to test for cyanophilic reaction) were used and then examined at a magnification of 1000×. Leica Application Suite EZ V.3.4 software (Leica Microsystems Ltd., Switzerland) was used to capture images from the microscope. Measurements from captured images were done with the software “Makroaufmaßprogramm” from Jens Rüdigs (https://ruedig.de/tmp/messprogramm.htm) and analyzed with the software “Smaff” version 3.2.

### Results

#### Species delimitation.

The ABGD method with parameters JC69 and K2P gave identical results and partitioned the LSU dataset into 6 partitions. The first five partitions with interspecific priority divergence ranging from $P = 0.001$ to $P = 0.0077$ contained 83 groups each while the sixth partition with interspecific priority divergence $P = 0.0129$ contained only one group (Fig. 1). Each group within each partition represented a hypothetical species with one or several sequences (Supplementary Table S1). Given the congruence between the first five

| Species name       | Voucher or strain | Origin       | GenBank N° | TS | LSU | References |
|--------------------|-------------------|--------------|------------|----|-----|------------|
| *Phylloporia* sp.  | FLOR:51239        | Brazil       | KJ631407   |    |     | unpublished |
| *Phylloporia* sp.  | Robledo 1220      | Argentina    | KC136225   | 23 |     |            |
| *Phylloporia* sp.  | MUCL:KE_16_107    | Kenya        | KY349147   | 17 |     |            |
| *Phylloporia* sp.  | MUCL CU05_249     |             | KJ743282   | 12 |     |            |
| *Phylloporia* sp.  | MUCL FG-11-506    | Guyana       | KC136227   | 23 |     |            |
| *Phylloporia* sp.  | MUCL FG-11-462    | Guyana       | KC136231   | 23 |     |            |
| *Phylloporia* sp.  | MUCL 52864        | Ecuador      | KJ743276   | 12 |     |            |
| *Phylloporia* sp.  | MUCL 45062        | Cuba         | KJ743284   | 12 |     |            |
| *Phylloporia* sp.  | MUCL 43733        | Cuba         | KJ743278   | 12 |     |            |
| *Phylloporia* sp.  | LWZ 20150531-14   | China        | KJU904466  | 41 |     |            |
| *Phylloporia* sp.  | Dai 9257          | China        | KJU904464  | 41 |     |            |
| *Phylloporia* sp.  | ISA007            | Brazil       | KJ743265   | 11 |     |            |
| *Phylloporia* sp.  | MUCL 54295        | Brazil       | KJ743269   | 11 |     |            |
| *Phylloporia* sp.  | ISA_352           | Brazil       | KJ743267   | 11 |     |            |
| *Phylloporia* sp.  | MUCL FG12_523     | French Guiana| KJ743280 | 12 |     |            |
| *Phylloporia* sp.  | MUCL FG12_522     | French Guiana| KJ743259 | 12 |     |            |
| *Phylloporia* sp.  | MUCL FG11_506     | French Guiana| KJ743258 | 12 |     |            |
| *Phylloporia* sp.  | MUCL FG13_722     | French Guiana| KJ743264 | 12 |     |            |
| *Phylloporia* sp.  | MUCL FG13_721     | French Guiana| KJ743263 | 12 |     |            |
| *Phylloporia* sp.  | Chay456           |             | AF411822   | 7  |     |            |
| *Phylloporia* sp.  | Dai6282           | China        | MG738805   | 8  |     |            |
| *Phylloporia* sp.  | Cui8429           | China        | MG738804   | 8  |     |            |
| *Phylloporia* sp.  | Yuan 5738         | China        | KC778784   | 19 |     |            |
| *Phylloporia* sp.  | He2359            | China        | MH151189   | 19 |     |            |
| *Phylloporia* sp.  | Yuan 5491         | China        | KJ787805   | 13 |     |            |
| *Phylloporia* sp.  | MUCL 52867        | Mexico       | HM635678   | 49 |     |            |
| *Phylloporia* sp.  | MUCL 52866        | Mexico       | HM635677   | 49 |     |            |
| *Phylloporia* sp.  | Dai 9242          | China        | JF712936   | 30 |     |            |
| *Phylloporia* sp.  | YG 051            | Uzbekistan   | KM264325   | 53 |     |            |

Table 1. Taxa names with collection details and GenBank accession numbers of all sequences of *Phylloporia* spp. used in this study.
partitions (83 groups in each), we have chosen one of them as the one that reflects well our dataset. Thus, all 130 sequences contained in our dataset represent 83 hypothetical species (Supplementary Table S1). The four newly generated LSU sequences in this study were sorted into two groups. The new sequence named *Phylloporia* sp. OAB0204 clustered together with other sequences of *P. littoralis* retrieved from GenBank. The other sequences (*Phylloporia* sp. OAB0107, *Phylloporia* sp. OAB0142, and *Phylloporia* sp. OAB0511) grouped together to form a distinct group (Supplementary Table S1).

The PTP species delimitation estimated that the number of species in LSU dataset was between 82 and 109, with the Mean of 97 species. The PTP species delimitation was supported by the maximum likelihood solution (PTP_Mls) and the Bayesian solution (PTP_Bs). Both solutions gave two different results in terms of the number of estimated species. The PTP_Mls yielded into 82 putative species (Supplementary Table S2) while PTP_Bs gave 100 putative species (Supplementary Table S3). Although the PTP_Mls and PTP_Bs yielded different results, the newly generated sequences formed two distinct species and are grouped identically in both outcomes (Supplementary Table S2, 3). Since species delimitation with PTP_Mls and PTP_Bs gave same results for our newly generated sequences with good support values, and considering the ML tree and ABGD results, we chose the results from PTP_Mls as the most suitable for our dataset. The Fig. 2 presents the ML tree with the putative species as found with ABGD and PTP_Mls.

**Phylogenetic analyses.** The combined ITS-LSU alignment contained 78 sequences with 2397 characters, of which 711 were parsimony-informative, 277 singleton sites, and 1409 constant sites. Four well supported major clades namely *Fomitiporella* (PP = 1.00/BS = 99), *Fulvifomes* (PP = 1.00/BS = 98), *Inonotus* (PP = 1.00/BS = 100), and *Phylloporia* (PP = 1.00/BS = 88) were recovered from the phylogenetic analyses inferred from the ITS-LSU (Fig. 3). *Phylloporia* appeared as a well-supported monophyletic clade, which split into two well-supported groups, here named A and B (Fig. 3). Group A (PP = 1.00/BS = 76) contained the sequences of the most *Phylloporia* species, including the generic type (*P. parasitica*), while group B (PP = 1.00/BS = 96) consisted of just three species of *Phylloporia*. The newly generated sequences nested within group A. The sequence OAB0204 clustered together with *P. littoralis* as it was found in the species delimitation analyses with high support (PP = 1.00/BS = 95). Sequences OAB0107, OAB 0142, and OAB0511 formed a distinct well-supported lineage (PP = 0.97/BS = 93) and had as a sister lineage an unidentified *Phylloporia* species from Kenya with high support (PP = 1.00/BS = 95). Since the sequences OAB0107, OAB 0142, and OAB0511 grouped together and had always formed a distinct lineage in all analyses (Figs. 2, 3; Supplementary Table S1–3), we proposed here as a new species and performed a detailed anatomical–morphological description on these specimens.

**Taxonomy.** *Phylloporia beninensis* Olou & Langer, sp. nov.

*Mycobank No. MB839326*

Figures 4, 5, 6

*Phylloporia beninensis* differs from other known species of *Phylloporia* by the combination of the following characteristics: basidiomata imbricate; pileus projecting up to 3 cm, 5 cm wide, and 1 cm thick at base, surface concentrically sulcate and zonate; cystidioles present, variable in size and shape; basidiospores ellipsoid to subglobose, 3–4.6 × 2.1–3.6 μm.

**Figure 1.** LSU data partition from Automatic Barcode Gap Detection (ABGD).
Figure 2. Maximum likelihood tree of the LSU dataset of *Phyllopora* with rapid bootstrap values and species delimitation as recovered in ABDG and PTP analyses. The sequence names are followed by voucher or strain number and country of origin.
Holotype.  BENIN. Borgou province, Woodlands of Okpara/Parakou, 9° 15′ 36.62″ N, 2° 43′ 28.40″ E, altitude

Figure 3. Bayesian analysis (BY) and maximum likelihood (ML) analyses of the combined ITS-LSU dataset of *Phylloporia*. Branch support values given as PP/BS. Newly generated sequences are highlighted in red. The sequence names are followed by voucher or strain number and country of origin.
Figure 4. Macromorphology of Phyllopora beninensis. (A) Basidiomata on dead wood stump, (B) Basidiomata showing effused-reflexed attachment, (C) Basidiomata on dead part of living tree showing the margin of actively growing specimens, (D) poroid hymenophore, (E) Context showing the black line separating the tomentum and the lower context.
331 m.a.s.l., on dead stump of an unidentified angiosperm tree, leg. Boris A. Olou, sampling date: 11.09.2019, OAB0511 (dried specimen, holotype in UNIPAR and isotype in KAS). Holotype sequences: LSU, accession number: MW244096.

**Etymology.** *beninensis* (lat.): referring to the country of the type locality.

**Description.** Basidiomata annual, pileate, sessile, imbricate with overlapping pilei, broadly attached or effused-reflexed (Fig. 4a–c), hard when dried, without odour or taste, projecting up to 3 cm, 5 cm wide, and
Figure 6. Microstructures of the type specimen of *Phylloporia beninensis*. (A) Hyphae from tomentum, (B) generative hyphae from trama in KOH mixed with 1% phloxine, (C) Section through the hymenium in KOH mixed with 1% phloxine showing basidia, basidioles, and basidiospores, (D) section through the hymenium in KOH showing hyaline basidia and basidioles, some with several guttulae, (E) Basidiospores in KOH mixed with 1% phloxine, (F) Basidiospores with one or two guttulae in KOH.
Africa in comparison with Europe, where only in its perennial basidiomata; smaller pores (9–11 per mm), and cyanophilic basidiospores.

re-examination of herbarium specimens initially assigned to the genus Phellinus, it is likely that many more Phylloporia species share the pileate and imbricate basidiomata, velutinous pileus surface, two-layered context separated by a black line, presence of cystidioles, and dimitic hyphal system. However, knowing that a dimitic hyphal system suggests that the species may be more widely distributed in sub-Saharan Africa.

Phylogenetic analyses inferred from the LSU and ITS-LSU datasets, coupled with macro- and microscopic examinations and ecological analyses, support the recognition of P. beninensis as a new species. Phylloporia beninensis is morphologically distinguished from other Phylloporia species by its annual, sessile, pileate, and imbricate basidiomata, two-layered context with the layers separated by a black line, dimitic hyphal system, and presence of cystidioles that vary in size and shape.

Phylogenetically similar to P. rattanicola and P. minuti-pora, P. beninensis is macroscopically most similar to P. rattanicola. The species share the pileate and imbricate basidiomata, velutinous pileus surface, two-layered context separated by a black line, presence of cystidioles, and dimitic hyphal system. Phylloporia rattanicola differs from P. beninensis in its perennial basidiomata; smaller pores (9–11 per mm), and cyanophilic basidiospores. Phylloporia minutipora is also similar in its annual, sessile basidioma with velutinate pileus surface, duplex context, and a dimitic hyphal system.

However, P. minutipora can be easily differentiated from P. beninensis by its much smaller pore size (12–15 per mm), larger basidiomata that project up to 10 cm from the substratum, absence of cystidioles, and smaller basidiospores 2.5–3 × 1.5–2.5 μm. In addition to these morphological differences, P. beninensis clustered in a strongly supported and distinct lineage within Phylloporia clade in our molecular phylogenetic analyses (Figs. 2, 3). In these analyses P. beninensis has a strong phylogenetic relationship (PP = 1.00, BS = 95%) with an unidentified species of Phylloporia from Kenya (MUCLKE 16107, GenBank KY349147) and is phylogenetically distant from P. rattanicola and P. minutipora.

We cannot yet confirm whether or not P. beninensis is saprotrophic even though it was mainly found on dead wood (Fig. 4a,b), because it is well evidenced, that the habit of a fungus to produce fruit body on dead wood does not necessarily indicate a saprotrophic lifestyle. However, although the lifestyle of P. beninensis is not yet well known, the fact that it was mainly found on dead wood we can reasonably say that the latter is saprotrophic. As saprotroph, P. beninensis is therefore ecologically different from P. minutipora and Phylloporia sp., which are mainly collected from living trees. Like P. beninensis, P. rattanicola is also saprotrophic because it was collected from dead rattan. However, knowing that Phylloporia species display a high level of host specificity, and that P. rattanicola is only collected on rattan while P. beninensis is collected on hardwood, we can safely say that P. beninensis and P. rattanicola do not belong to the same morpho-ecological group as stated above.

We also reported here P. littoralis Decock & Yombiyeni on the basis of molecular and morphological analyses, constituting the first record of the species from Benin (Figs. 2, 3, 7). The Benin P. littoralis specimen fits well morphologically and genetically to the Central African type specimen (see Fig. 2, in Yombiyeni and Decock 2017). To our knowledge, this is the first time P. littoralis has been reported outside its type locality Gabon, and suggests that the species may be more widely distributed in sub-Saharan Africa.

The recognition of P. beninensis brings the number of described Phylloporia species to 62 worldwide. Among these 62 species, nine were described from tropical Africa. Phylloporia are more diverse in tropical Africa in comparison with Europe, where only P. ribis (Schumach.) Ryvarden has been reported to date. Considering that tropical Africa remains poorly explored for wood-decay fungi, it is likely that many more Phylloporia species remain to be found. We are also confident that new investigations of new still unexplored habitats and re-examination of herbarium specimens initially assigned to the genus Phellinus will reveal more new species.
of *Phylloporia* from tropical Africa. Aside the nine species described with type specimens, six other *Phylloporia* species have been reported from tropical Africa\(^2\)–\(^7\), which brings the number of regional *Phylloporia* species to 15. To facilitate future taxonomic studies in the genus, we provide a dichotomous key for identification of tropical African *Phylloporia* species.

Identification key to African *Phylloporia* species

1. Basidiomata resupinate on the underside of living leaves… *P. parasitica*
2. Basidiomata sessile to stipitate…2
3. Basidiomata stipitate…5
4. Basidiomata sessile…5
5. Context homogenous, black line lacking… *P. minutispora*
6. Context duplex, black line present…4
7. Pores 7–9 per mm… *P. spathulata*
8. Pores 10–11 per mm… *P. afrospathulata*
9. Perennial, pore surface glancing… *P. pectinata*
10. Annual, pore surface not glancing…6
11. Basidiomata gregarious…7
12. Basidiomata solitary to imbricate…9
13. Clustered in more than 100 individuals, pileus shiny… *P. flabelliformis*
14. Clustered in a small groups of less than 100 individuals, pileus dull…8
15. Hyphal system monomitic, pores 5–6 per mm… *P. gabonensis*
16. Hyphal system dimitic, pores 9–11 per mm… *P. fulva*
17. Cystidioles present…10
18. Cystidioles absent…11
19. On living trees and bushes…12
20. On dead and living trees…14
21. Host specific, found on species of *Rinorea* (Violaceae)… *P. rinoreae*
22. Not host specific…13
23. Basidioma 0.5–3 cm in diam., 0.5–1 cm thick, basidia 8.5 × 5 μm… *P. littoralis*
24. Basidioma 1–5 cm in diam., up to 2 cm thick, basidia 8–10 × 3–4 μm… *P. fruticum*
25. Basidiospores 3–4.5 × 2.5–3.5 μm… *P. weberiana*
26. Basidiospores 2.5–3.5 × 2–2.5 μm… *P. pulla*

Data availability
Alignment and phylogenetic tree from the combined ITS-LSU dataset generated in this study are available in TreeBASE under this link: [http://purl.org/phylo/treebase/phylows/study/TB2:S27303](http://purl.org/phylo/treebase/phylows/study/TB2:S27303). Newly generated sequences are available in GenBank and the accession numbers are given in Table 1. Alignment, phylogenetic tree, and accession numbers of newly generated sequences will be public after the paper is published. Collected specimens are available at the mycological herbaria of the University of Parakou (UNIPAR) in Benin and University of Kassel (KAS) in Germany. Following the new requirement of MycoBank, the new species will be registered in MycoBank and the registration number will be given in the taxonomy section of this paper as soon as the paper is accepted.
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Author contributions
B.A.O. carried out the field work, molecular lab works, planned and performed the phylogenetic and species delimitation analyses, examined all specimens, performed microscopic investigation of the new species, drafted the description of the new species, and wrote the first draft of the manuscript. E.L. commented and improved the description of the new species. E.L. and N.S.Y. contributed to later versions of the manuscript. All authors read and approved the final manuscript.

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