Three new species of *Inosperma* (Agaricales, Inocybaceae) from Tropical Africa

Hyppolite L. Aïgnon¹, Sana Jabeen², Arooj Naseer³, Nourou S. Yorou¹, Martin Ryberg⁴

¹ Research Unit Tropical Mycology and Plant-Soil Fungi Interactions, Faculty of Agronomy, University of Parakou, 03 BP 125, Parakou, Benin  
² Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan  
³ Department of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan  
⁴ Systematic Biology Programme, Department of Organismal Biology, Uppsala University, Norbyvägen 18D, 752 36, Uppsala, Sweden

**Corresponding author:** Hyppolite L. Aïgnon (hyppoliteaignon@yahoo.com)

**Academic editor:** Zai-Wei Ge  |  Received 28 October 2020  |  Accepted 11 January 2021  |  Published 28 January 2021

**Citation:** Aïgnon HL, Jabeen S, Naseer A, Yorou NS, Ryberg M (2021) Three new species of *Inosperma* (Agaricales, Inocybaceae) from Tropical Africa. MycoKeys 77: 97–116. https://doi.org/10.3897/mycokeys.77.60084

**Abstract**

Here, we describe three new species of *Inosperma* from Tropical Africa: *Inosperma africanum*, *I. bulbomarginatum* and *I. flavobrunneum*. Morphological and molecular data show that these species have not been described before, hence need to be described as new. The phylogenetic placements of these species were inferred, based on molecular evidence from sequences of 28S and RPB2. Additional analysis using ITS dataset shows interspecific variation between each species. Phylogenetic analyses resolve *I. flavobrunneum* in Old World Tropical clade 1 with weak support, *I. bulbomarginatum* is sister of Old World Tropical clade 1 and *I. africanum* is indicated as sister to the rest of *Inosperma*. Complete description and illustrations, including photographs and line drawings, are presented for each species. A new combination of *Inocybe shawarensis* into *Inosperma* is also proposed.

**Keywords**

Ectomycorrhizal, molecular systematics, phylogeny, taxonomy, West Africa
Introduction

Inocybaceae Jülich (Basidiomycota, Agaricales) is a family of ectomycorrhizal species, forming symbiotic association with more than 23 families of vascular plants (Matheny et al. 2020). The family is diverse with an estimated 1050 species distributed worldwide (Matheny and Kudzma 2019; Matheny et al. 2020). The number of species described will continue to increase as new habitats are explored (Matheny and Watling 2004; Esteve-Raventós 2014; Latha and Manimohan 2015, 2016; Matheny et al. 2017; Naseer et al. 2018; Jabeen and Khalid 2020).

Recently, Inocybaceae was revised to include seven genera, *Auritella* Matheny & Bougher, *Inocybe* (Fr.) Fr., *Inosperma* (Kühner) Matheny & Esteve-Rav., *Mallocybe* (Kuyper) Matheny, Vizzini & Esteve-Rav., *Nothocybe* Matheny & K.P.D. Latha, *Pseudoperma* Matheny & Esteve-Rav. and *Tubariomyces* Esteve-Rav. & Matheny (Matheny et al. 2020). *Inosperma* is represented by more than 55 known species that are distributed in Africa, Asia, Australasia, Europe, North America and northern South America (Matheny et al. 2020). Typically, the species of the genus are characterised by a radially fibrillose and rimose or squamulose pileus; smooth, ellipsoid or phaseoliform basidiospores; and absence of metuloid hymenial cystidia. In addition, many species of *Inosperma* have odours that are fruity, pleasant, like honey, fishy, pelargonium or otherwise distinct (Matheny et al. 2020). Phylogenetically the genus is monophyletic with four major clades: the Maculata clade (Larsson et al. 2009), *I.* sect. *Inosperma* and two clades from the Old World tropics (Pradeep et al. 2016; Matheny et al. 2020).

In this study, we describe three new species of *Inosperma* from West Africa, based on morphological characters, as well as analysing their phylogenetic position using multigene molecular analysis of 28S and RPB2 sequences data.

Material and methods

Study area and specimen sampling

Specimens were collected in Benin in Okpara Forest (9°15.13'N, 2°43.05'E), N'dali Forest Reserve (09°45.73'N, 2°19.93'E), Toui-Kilibo Forest Reserve (8°32.74'N, 2°40.42'E) and Alibori Superieur Forest Reserve (10°23.76'N, 2°5.15'E). Additionally, specimens were collected in, Burkina Faso in the Forest Reserve of Kou (10°55.86'N,4°51.83'W); Ivory Coast in Gbeke Region (7°40.52'N, 4°54.48'W), Guinea in National Park of Haut Niger (10°30.76'N, 9°57.68'W) and Togo in Central Region (09°20.38'N, 1°14.44'E).

The habitats are woodland dominated by *Isoberlinia doka* Craib & Stapf, *I. tomentosa* (Harms) Craib et Stapf, *Uapaca togoensis* Pax or gallery forest dominated by *Berlinia grandiflora* (Vahl) Hutch. Specimens were preserved by drying on an electric dryer (type Stöckli Dörrex) for 24 hours at 45 °C. All studied materials are deposited at the Mycological Herbarium of Parakou University (UNIPAR).
Inosperma of Africa

Morphological analyses

Specimens were photographed in the field with a digital camera Sony FE. Colour codes are described using Kornerup and Wanscher (1978). For anatomical analyses, samples of specimens were rehydrated and examined directly in 3% potassium hydroxide (KOH) and Congo red. Drawings of microscopic characters were made with the aid of a drawing tube attached to a Leica DM2700. Microscopic characters were drawn at magnification 1000×. Spore measurements were made from 40 spores for each species. We measured length (L) and width (W) of the basidiospores and calculated the ratio \( Q = \frac{L}{W} \). Measurements of basidiospores and basidia excluded the apiculus and sterigmata, respectively and are given as \((a–)b–c(–d)\), where \((a) = \text{extreme minimum value, range } b–c \text{ contains minimum of 90% of the calculated values and } (d) = \text{extreme maximum value as used in Aignon et al. (2021).}\)

Molecular analyses

DNA extraction, PCR and sequencing

Genomic DNA was extracted from dried specimens by QIAGEN® plant mini kit following the manufacturer’s instructions and PCR products were cleaned using ExoSAP-IT (Bell 2018). The internal transcribed spacer regions (ITS), portions of the nuclear large subunit ribosomal RNA gene (28S) and DNA-directed RNA polymerase II subunit (RPB2) were amplified. For sequencing of the ITS region, we used the primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993), for LSU we used LR0R, LR7 and internal primers LR5 and LR3R (Vilgalys and Hester 1990; Cubeta et al. 1991; Rehner and Samuels 1995) and for RPB2, we used primer pairs b6F and b7.1R (Matheny 2005). PCR products were cleaned and sequenced at Macrogen Inc. (Macrogen Europe B.V., Amsterdam, Netherlands) using the same primers as those used for PCR.

Sequence alignments and phylogenetic analyses

Nineteen new sequences were generated (Table 1). Sequences were BLAST searched against NCBI and similar sequences were retrieved from GenBank (Benson et al. 2010). The sequences of ITS, 28S and RPB2 were aligned separately in MAFFT V7.464 (Katoh et al. 2019). Alignment is available online in TreeBase under accession number 27445 ([http://purl.org/phylo/treebase/phylows/study/TB2:S27445](http://purl.org/phylo/treebase/phylows/study/TB2:S27445)).

For phylogenetic analysis, the dataset of 28S and RPB2 was generated using Geneious 7.0.2 (Drummond et al. 2010) and partitioned in 28S, RPB2 codon position 1, RPB2 codon position 2, RPB2 codon position 3 and the intron in RPB2 separately (Suppl. material 1). We tested for the best partitioning scheme and best model for each partition using ModelFinder (Kalyaanamoorthy et al. 2017). It was indicated that keeping all the
### Table 1. List of species, geographic origin and GenBank accession numbers of ITS, 28S and RPB2 sequences used in the molecular analysis; the new species and new combinations are in bold.

| Species                        | Voucher         | Country     | ITS        | 28S        | RPB2       | References                          |
|-------------------------------|-----------------|-------------|------------|------------|------------|-------------------------------------|
| *Auritella brunescens* Matheny & Bougher | PBM3174         | Australia   | KJ702344   | JQ313571   | KJ702349   | Matheny et al. (2017)               |
| *Auritella dolichoventris* Matheny, Trappe & Bougher | Trappe 24844     | New South Wales | AY380371   | AY337371   |            | Matheny (2005)                      |
| *Auritella frequentis* Matheny & Bougher | BRI14Q669485     | Australia   | KJ702355   | KJ702353   | KJ702357   | Matheny et al. (2017)               |
| *Auritella hispida* Matheny & T.W. Henkel | TH1009, TH10379  | Cameroon    | KT378203   | KT378208   | KT378215   | Matheny et al. (2017)               |
| *Auritella serpentinocystis* Matheny, Trappe & Bougher ex Matheny & Bougher | PBM3188         | Australia   | KJ729858   | KJ313559   | KJ756402   | Matheny et al. (2017)               |
| *Auritella spiculosa* Matheny & T.W. Henkel | MCA7031, TH9866  | Cameroon    | MF374763   | KT378206   | KT378214   | Matheny et al. (2017)               |
| *Inosperma adaequatum* (Britzelm.) Matheny & Esteve-Rav. | JV 16501F, JV11290F | Finland    | JQ801381   | JQ815407   | AY333771   | Matheny et al. (2020)               |
| *I. africanum* Aïgnon, Yorou & Ryberg | MR00387         | Togo        | MN096189   | MN097881   | MT770739   | This study                          |
|                               | HLA0361         | Benin       | MT534295   | MT560735   |            |                                     |
|                               | HLA0383         | Benin       | MT534298   | MT560733   |            |                                     |
|                               | HLA0353         | Benin       | MT534299   |            |            |                                     |
|                               | BRF4157         | Benin       | MK908843   |            |            | Unpublished                         |
| 1. *akirnum* (K.P.D. Latha & Manimohan) Matheny & Esteve-Rav. | CAL 1358        | India       | NG_057279  | KY553236   | Latha and Manimohan (2016)          |
| 1. *apiosmotum* (Grund& D.E. Stuntz) Matheny & Esteve-Rav. | AU10560, TENN:062779 | Canada, USA | HQ201336 | JN975022 | JQ846463 | Ryberg and Matheny (2012) |
| 1. *bongardii* (Weinm.) Matheny & Esteve-Rav. | EL9406          | Sweden      | FN550943   | FN550943   |            | Unpublished                         |
| 1. *bulbomarginatum* Aïgnon, Yorou & Ryberg | MR00357         | Benin       | MN096190   | MN097882   | MN200775   | This study                          |
|                               | HLA0357         | Benin       | MT534301   |            |            |                                     |
|                               | HLA0389         | Benin       | MT534302   |            |            |                                     |
|                               | HLA0417         | Benin       | MT534300   | MT560734   |            |                                     |
|                               | PC96082         | Zambia      | JQ801412   | JN975027   |            | Ryberg and Matheny (2012)           |
| 1. *catenistratioides* (E. Horak) Matheny & Esteve-Rav. | PBM3384         | Australia   | JQ815415   | KJ729949   | Latha and Manimohan (2016)          |
| 1. *catenistratum* (Fr.) Matheny & Esteve-Rav. | PBM1105         | USA         | JQ815409   | JQ846466   | Paddeep et al. (2016)               |
| 1. *carnosulosum* (C.K. Pradeep & Matheny) Matheny & Esteve-Rav. | TBGT:12047      | India       | KT329444   | KT329445   | KT329444   | Paddeep et al. (2016)               |
| 1. *criticulum* (Pers.) Matheny & Esteve-Rav. | SJ04024, TURA:4761 | Sweden, Finland | AM882939 | AM882939 | JQ846474 | Ryberg et al. (2008)               |
| 1. *cruzi* (Bres.) Matheny & Esteve-Rav. | EL70A03         | Sweden      | AM882953   | AM882953   |            | Ryberg et al. (2008)               |
| 1. *cyanoschizium* (Matheny, Bougher & G.M. Grant) Matheny & Esteve-Rav. | TENN:065729       | Australia  | JQ815418   | KJ729948   | Unpublished                         |
| 1. *flavobrunneum* Aïgnon, Yorou & Ryberg | HLA0367         | Benin       | MN096199   | MT36754    |            | This study                          |
|                               | HLA0372         | Benin       | MT534290   | MT536756   |            |                                     |
| 1. *graniosorum* (J. Favre) Matheny & Esteve-Rav. | EL10606         | Sweden      | FN530945   | FN530945   |            | Latha and Manimohan (2016)          |
| 1. *gregarium* (K.P.D. Latha & Manimohan) Matheny & Esteve-Rav. | CAL 1309        | India       | KX852305   | KX852306   | KX852307   | Latha and Manimohan (2016)          |
| 1. *kranatodiscum* (Kaufman) Matheny & Esteve-Rav. | PBM2451         | USA         | JQ108759   | JQ319688   | JQ846483   | Latha and Manimohan (2016)          |
| 1. *maculatum* (Boud.) Matheny & Esteve-Rav. | MR00020         | Sweden      | AM882958   | AM882958   |            | Ryberg et al. (2008)               |
| 1. *maximum* (A.H. Sm.) Matheny & Esteve-Rav. | PBM 2222, UBC F33244 | USA, Canada | MG955983  | EU569854   |            | Matheny et al. (2009)               |
| 1. *misarea* (Matheny & Watling) Matheny & Esteve-Rav. | PBM3174         | Zambia      | JQ801409   | EU569874   | EU569873   | Paddeep et al. (2016)               |
| Species | Voucher | Country | ITS | 28S | RPB2 | References |
|---------|---------|---------|-----|-----|------|-------------|
| *I. mutatum* (Peck) Matheny & Esteve-Rav. | PBM4108, PBM2953 | USA | MG773837 | JQ994476 | JQ846488 | Matheny et al. (2020) |
| *I. nobilis* (Grund & D.E. Stuntz) Matheny & Esteve-Rav. | PBM 2452 | USA | EUS69868 | EUS69867 | Matheny et al. (2009) |
| *I. quirubronces* (Bon) Matheny & Esteve-Rav. | PAM01091310, PAM00090117 | France | FJ936168 | FJ936168 | Larson et al. (2009) |
| *I. nivinoside* (Peck) Matheny & Esteve-Rav. | PBM 2459, PBM3311 | USA | JQ801414, JQ815426 | DQ385884 | Latha and Manimohan (2016) |
| *I. rubriflorum* (Matheny & Bougher) Matheny & Esteve-Rav. | PAM00090117 | France | FJ904176 | FJ904176 | Larson et al. (2009) |
| *I. rubriflorum* (Matheny & Bougher) Matheny & Esteve-Rav. | PBM3784 | Austria | NG, 057260 | KM406230 | Horak et al. (2015) |
| *I. rubricosum* (Matheny & Esteve-Rav.) | | | | | |
| *I. rubroinum* (Naseer & Khalid) Aïgnon & Naseer | FLAS-FS9456 | Pakistan | KY616965, KY616966 | Nase et al. (2018) |
| *I. shawarense* | | | | | |
| *I. vinaceobrunneum* (Matheny, Ovrebo & Kudzma) Haelew. | TENN:062709, PBM 2951 | USA | FJ601813, NG_067775, JQ846478 | Matheny and Kudzma (2019) |
| *I. viridipes* (Matheny, Bougher & G.M. Gates) Matheny & Esteve-Rav. | PBM3767 | Australia | NR, 153168, KP171094, KM656138 | Latha and Manimohan (2016) |
| *I. virosum* (K.B. Vrinda, C.K. Pradeep, A.V. Joseph & T. K. Abraham ex C.K. Pradeep, K.B. Vrinda) Matheny & Esteve-Rav. | TBGT:753 | India | KT329452, KT329458, KT329446 | Pradeep et al. (2016) |
| *Malloocybe myriadophylla* (Vauras & E. Larss.) Matheny & Esteve-Rav. | JV19652F | Finland | DQ221106, AT700196, AY835751 | Matheny et al. (2007) |
| *M. subdecurrens* (Ellis & Everh.) Matheny & Esteve-Rav. | PAM00090117 | France | FJ904176 | FJ904176 | Larson et al. (2009) |
| *M. tomentosula* Matheny & Esteve-Rav. | PBM4138 | USA | MG773814, MK421969, MH577506 | Matheny et al. (2020) |
| *Pseudosperma* | TENN062709, PBM 2951 | USA | FJ601813, NG_067775, JQ846478 | Matheny and Kudzma (2019) |
| *P. pluviorum* (Matheny & Bougher) Matheny & Esteve-Rav. | PBM3767 | Australia | NR, 153168, KP171094, KM656138 | Latha and Manimohan (2016) |
| *Tubariomyces inexpectatus* (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny | AH25500, AH20390 | Spain | GU907095, EU569855, GU907088 | Matheny et al. (2009), Alvarado et al. (2010) |
| *T. similis* Della Magg., Tolaini & Vizzini | AH25500, AH20390 | Spain | GU907095, EU569855, GU907088 | Alvarado et al. (2010) |
| *T. limata* | AH25500, AH20390 | Spain | GU907095, EU569855, GU907088 | Alvarado et al. (2010) |
| *T. hymenophoroides* Esteve-Rav., P.A. Moreau & C.E. Hermos. | P05112008 | France | GU907097, GU907094, GU907090 | Pradeep et al. (2016) |
Figure 1. ML tree of 28S and RPB2 sequences showing the placement of *Inosperma africanum*, *I. bulbomarginatum* and *I. flavobrunneum*. Values above or below branches indicate bootstrap proportions SH-aLRT support ≥ 80% / ultrafast bootstrap support ≥ 95% / Bayesian posterior probabilities > 0.95 as shown. Origin of species is given after the name of each taxon. The new species are in red.

Partitions was the best way to proceed. Maximum Likelihood (ML) analysis was performed with IQTREE 1.6.12 (Nguyen et al. 2015). Branch support was assessed with 1000 replicates of ultrafast bootstrap replicates and approximate likelihood ratio test [aLRT] and Shimodaira-Hasegawa [SH]-aLRT (SH-Alrt) test with 1000 replicates (Hoang et al. 2017).

For Bayesian Inference (BI) analyses, GTR models with gamma-distributed rate heterogeneity and a proportion of invariant sites parameter were assigned to each
partition as indicated above, using MrBayes 3.2.7 (Ronquist et al. 2012), set as follows: lset applyto = (all), nst = 6, rates = invgamma, ngammacat = 4, sampling frequency = 1000 and the command “unlink” was used to unlink parameters across characters on partitioned datasets. Two independent Markov Chain Monte Carlo (MCMC) processes were executed, each in four chains for 20 million generations. Posterior probabilities (BPP) were calculated after burning the first 25% of the posterior sample and ensuring that this threshold met the convergence factors described above. The sequences from *Pseudosperma lepidotellum* (Matheny & Aime) Matheny & Esteve-Rav., *P. pluviorum* (Matheny & Bougher) Matheny & Esteve-Rav., *Pseudosperma* sp. PBM3751 and *Pseudosperma* sp. TR194-02 were used as outgroup taxa. We also produced trees using ITS database only to show interspecific variation between each species.

**Results**

**Phylogenetic analyses**

*Inosperma* is indicated as monophyletic with full bootstrap support. All three of the species described here, *Inosperma africanum* *I. bulbomarginatum* and *I. flavobrunneum*, are members of this genus. Phylogenetically, *I. africanum* is indicated as sister to the rest of *Inosperma*, with full support (99.9% SH-aLRT values, 100% ML ultrafast bootstrap, 1 BPP). The Old World Tropical clade 1 is retrieved with strong support (93.8% SH-aLRT values, 99% ML bootstrap, 1 BPP) and *I. bulbomarginatum* is indicated as the sister of Old World Tropical clade 1 with full bootstrap support (100% SH-aLRT values, 100% ML Ultrafast bootstrap, 1 BPP). The sequences of collection PC96082 are very similar to the sequences of *I. bulbomarginata* that we infer to be of the same species. *Inosperma flavobrunneum* is nested in Old World Tropical clade 1 as sister species to three undescribed collections, BB3233, G1842 and PC96013, all from Zambia with weak support.

**Taxonomy**

1. *Inosperma africanum* Aïgnon, Yorou & Ryberg, sp. nov.
   
   MycoBank No: 836199
   
   Figs 2a, 3

**Diagnosis.** *Inosperma africanum* is distinct from all species of *Inosperma* and truly outstanding by its vinaceous to red colouration.

**Type.** *Holotype.* BENIN, Collines Region, Kilibo: 8°32.74’N, 2°40.42’E, on soil in Forest Reserve of Toui-Kilibo in Woodland dominated by *Isoberrinia doka* and *I. tomentosa*, 11 August 2017, leg. AIGNON L.H, Voucher (HLA0383) GenBank accession: ITS (MN096193); LSU (MN0977885) and RPB2 (MT770739).

**Description.** Pileus 8.5–15 mm diam., convex to plane, uniform, surface fibrillose, vinaceous to red (8F8), surface rimose, dry. Lamellae moderately close, subven-
Figure 2. Macromorphology of: A *Inosperma africanum* (HLA0383) B *Inosperma bulbomarginatum* (MR00357) C, D *Inosperma flavobrunneum* (HLA0367). Scale bar: 1 cm.

Tricose, narrowly attached, 0.5–1 mm deep; vinaceous, sometimes light pinkish (8F8), edges fimbriate, vinaceous (8B8). Stipe 15–23 × 0.5–1 mm, cylindrical, central, fibrilllose, swollen, bulbous at the base, veil none with the lower two thirds pinkish-white (8A3) and the upper third light vinaceous (8A5). Odour and taste not distinctive. Basidiospores (6.2) 8–10 (10.3) × (3.8) 4–6.8 (7) μm, avl × avw = 8.3 × 5.3 μm, Q = (1.2) 1.1–2.1 (2.2), avQ = 1.6, smooth, (sub) globose to cylindrical, sometimes ellipsoid. Basidia 18–47 × 7–10 μm, clavate, 3–4 sterigmate, hyaline. Cheilocystidia 22–54 × 8–12 μm, cylindrical to clavate, thin-walled, hyaline. Pleurocystidia absent. Pileipellis a cutis with cylindrical, smooth, thin-walled hyphae, 6–20 μm diam., negative reaction of pileus surface in KOH. Stiopitpellis a cutis radially arranged, hyphae 5–13 μm diam., parallel, sometimes septate, filamentous. Caulocystidia 22–63 × 8–13 μm, fusiform sometimes utriform, observed on the upper third of the stipe. Clamp connections present.

**Distribution.** Currently known from Benin, Burkina Faso, Guinea, Ivory Coast, Togo.
Figure 3. Microscopic structures of *Inosperma africanum* (HLA0383) **A** basidiospores **B** basidia **C** cheilocystidia **D** caulocystidia **E** pileipellis **F** stipitpellis. Scale bars: 3 μm (**A**); 5 μm (**B**); 10 μm (**C–F**).
Ecology. Scattered in Tropical Woodlands dominated by *Isoberlinia doka* and *I. tomentosa* or gallery forests dominated by *Berlinia grandiflora*.

Etymology. *africanum*, referring to the distribution in Africa.

Additional specimens examined. **Benin**, Borgou Province, N’dali Region: 8°32.74’N, 2°40.42’E, on soil in Woodland dominated by *Isoberlinia doka*, 30 August 2017 in Forest Reserve of N’dali, Leg. Aïgnon HL., Voucher (HLA0461) GenBank accession: ITS (MT534297) and LSU (MT560732). **Benin**, Borgou Province, Tchaorou Region: 9°15.28’N, 2°43.38’E, on soil in forest of Okpara in woodland dominated by *I. doka*, 7 June 2017, leg. Aïgnon HL., Voucher (HLA0353) GenBank accession: ITS (MT534299). **Benin**, Borgou Province, N’dali Region: 8°45.73’N, 2°19.93’E, on soil in Woodland dominated by *Isoberlinia doka*, 7 August 2017 in Forest Reserve of N’dali, Leg. Aïgnon HL., Voucher (HLA0557/). **Benin**, Province, Boukoumbe, North Region: 10°14.45’N, 1°7.00’E, on soil in Woodland dominated by *Isoberlinia doka*, 25 July 2020 in Koussoukouangou waterfall, Leg. Aïgnon HL., Voucher (HLA0736). **Burkina Faso**, Kenedougou Province, Toussiambandougou Region: 10°55.86’N, 4°51.83’W, on soil in gallery forest dominated by *Berlinia grandiflora*, 27 June 2018, leg. Aïgnon HL., Voucher (HLA0355). **Ivory Coast**, Kekrekouakoukro Province, Bouake, Gbeke Region: 7°40.52’N, 4°54.48’W, on soil in Woodland dominated by *B. grandiflora*, 11 July 2018, leg. Aïgnon HL., Voucher (HLA0562). **Guinea**, Faranah Province, Upper Guinea Region, National Park of Haut Niger: 10°30.76’N, 9°57.68’W, on soil in Woodland dominated by *B. grandiflora*, 4 July 2018, leg. Aïgnon HL., Voucher (HLA0532). **Togo**, Central Region, Prefecture of Assoli, on the road between Bafilo and Aledjo: 09°20.38’N, 1°14.44’E in Woodlands dominated by *I. tomentosa*, 7 August 2013, leg. Martin Ryberg, Voucher (MR00387) GenBank accession: ITS (MN096189); LSU (MN097881), RPB2 (MT770739).

Notes. *Inosperma africanum* is nested in *Inosperma* and indicated as sister to the rest of the genus in our phylogenetic analyses and is very distinct by its small size and a vinaceous to red pileus. It has a wide distribution in West Africa.

2. *Inosperma bulbomarginatum* Aïgnon, Yorou & Ryberg, sp. nov.
MycoBank No: 836198
Figs 2b, 4

Diagnosis. *Inosperma bulbomarginatum* differs from *I. flavobrunneum* by the smaller size of its basidiomata and larger basidiospores. It is phylogenetically distinct from all other undescribed African *Inosperma* in Old World Tropical clade 2

Type. **Holotype.** **Benin**, Borgou Province, N’dali Region: 09°45.73’N, 2°19.93’E, on soil in Woodland dominated by *Isoberlinia doka* and *I. tomentosa*, 8 July 2013, leg. Martin Ryberg, Voucher (MR00357), GenBank accession: ITS (MN096190); LSU (MN097882) and RPB2 (MN200775).

Description. Pileus 13–18 mm diam., undulating plane, fibrillose, margin rimose, orange-brown to somewhat cinnamon, greyish-white (8E5), splitting at edge. Lamellae
Figure 4. Microscopic structures of *Inosperma bulbomarginatum* (MR00357) A basidiospores B basidia C cheilocystidia D caulocystidia E pileipellis F stipitipellis. Scale bars: 3 μm (A); 5 μm (B); 10 μm (C–F).
2–2.5 mm deep, moderately close, narrowly attached, pale grey brown (9B5) to dark brown (9D5), sinuate. Stipe 10–22 × 2–2.5 mm, central, equal, marginate bulb, white to pinkish-buff (7A2), velar remnants. Odour and taste not distinctive. Basidiospores (7.1) 8–12.1 (14) × (4) 4.2–6.7(7) μm, avl × avw = 9.6 × 5.4 μm, Q = (1.3) 1.2–2.3(2.6), avQ = 1.8, smooth, elongate, thick-walled. Basidia (25) 28–40 × 6–12 μm, tetrasporic. Cheilocystidia 20–25 × 10–12 μm, clavate, thin-walled hyaline. Pleurocystidia absent. Pileipellis a cutis, thin-walled hyphae, 3–12 μm diam., cylindrical. Stipitipellis a cutis with subparallel hyphae 3–15 μm diam., septate, filamentous, subhymenium of compact hyphae, any reaction of pileus surface in KOH not observed. Caulocystidia 25–60 × 7–20 μm, ovoid to obvoid, sometimes utriform, observed on the upper third of the stipe.

**Distribution.** Currently known from Benin and Zambia.

**Ecology.** Scattered in Woodland dominated by *Isoberlinia doka* and *I. tomentosa*.

**Etymology.** *bulbomarginatum* referring to the presence of a marginate bulb at the base of the stipe.

**Additional specimens examined.** **Benin,** Collines Province, Kilibo Region: 8°32.74’N, 2°40.42’E, on soil in Woodland dominated by *Isoberlinia doka*, 22 June 2017 in the Forest Reserve of Toui-Kilibo, leg. Aïgnon HL., Voucher (HLA0389); GenBank accession: ITS (MT534302).

**Benin,** Tchaorou, Borgou Prov, Okpara Forest: 9°15.28’N, 2°43.38’E, on soil in Woodland dominated by *Isoberlinia doka*, 13 June 2017, leg. Aïgnon HL., Voucher (HLA0373); GenBank accession: ITS (MT534301).

**Benin,** Alibori Borgou Prov, Alibori Superieur Forest Reserve: 10°23.76’N, 2°5.15’E on soil in Woodland dominated by *Isoberlinia doka*, 11 July 2017, in Forest Reserve of Alibori Supérieur leg. Aïgnon HL., Voucher (HLA0417); GenBank accession: ITS (MT534300) and LSU (MT534301) and LSU (MT536734).

**Notes.** *Inosperma bulbomarginatum* is similar to *Inosperma cervicolor* (Pers.) Matheny & Esteve-Rav., by its orange-brown pileus, but differs from it by the smaller size of the basidiomata and basidiospores, as well as its ecological association with Fabaceae Lindley and/or Phyllanthaceae Martynov and extensive distribution in Tropical Africa. *I. cervicolor* is associated with Pinaceae Spreng. ex F. Rudolphi and distributed in Europe and North America.

**3. Inosperma flavobrunneum** Aïgnon, Yorou & Ryberg, sp. nov.

Mycobank No: 836197
Figs 2c, d, 5

**Diagnosis.** Characterised by yellow to orange-brown pileus, 7–12 × 4–7 μm smooth, thick-walled, ellipsoid basidiospores with cheilocystidia measuring 23–41 × 7–10 μm, clavate, thin-walled.

**Type. Holotype.** **Benin,** Borgou Province, Tchaorou, Okpara Forest: 9°15.13’N, 2°43.05’E on soil in Woodland dominated by *Isoberlinia doka* 12 June 2017, leg. AIGNON L.H, Voucher (HLA0367); GenBank accession: ITS (MN096199); LSU (MT536754).
Figure 5. Microscopic structures of *Inosperma flavobrunneum* (HLA0367) **A** basidiospores **B** basidia **C** cheilocystidia **D** caulocystidia **E** pileipellis and **F** stipitipellis. Scale bars: 3 μm (**A**); 4 μm (**B**); 10 μm (**C–F**).
Description. Pileus 28–38 mm diam., umbonate, yellow (5A3) to orange brown (5C5), dark brown in middle, convex when young, plane at maturity, hard, surface rimose, dry. Lamellae emarginated, adnexed and decurrent, yellow brown (5B5). Stipe 27–39 × 5–6 mm, central, cylindrical, uniform; white, equal, solid, hard, base slightly swollen to bulbous, pruinose at the apex. Basidiospores (7.1) 9.2–11.2 (12) × (4.1) 5.7–7 (7.2) μm, avl × avw = 9.2 × 5.7 μm, Q = (1.2) 1.6–2.1 (2.5), avQ = 1.6, smooth, ellipsoid. Basidia 24–40 × 6–14 μm, clavate, 2–4 spored. Cheilocystidia 23–41 × 7–10 μm, clavate, thin walled. Pleurocystidia absent. Pileipellis a cutis thin-walled hyphae 4–16 μm diam., subparallel, compact hyphae, negative reaction of pileus surface in KOH. Stipitipellis a cutis hyphae 5–10 μm diam., septate, filamentous, thick, subparallel, compact. Caulocystidia 23–52 × 9–10 μm, utriform, rare, observed on the upper third of the stipe.

Distribution. Currently known only from Benin in Soudano-Guinean zone.

Ecology. Gregarious under Woodland dominated by Isoberlinia doka, I. tomentosa and Monotes kerstingii Gilg.

Etymology. flavobrunneum referring to yellow to dark brown pileus.

Additional specimens examined. BENIN, Tchaorou, Borgou Province, Okpara Forest: 9°15.27'N, 2°43.40'E on soil in Woodland dominated by Isoberlinia doka, I. tomentosa 13 June 2017, leg. AIGNON L.H, HLA0372, GenBank accession: ITS (MT534290); LSU (MT536756).

Notes. In the phylogenetic tree (Figure 1), Inosperma flavobrunneum is a sister of Inosperma sp. PC96013, an undescribed species from Zambia in Miombo woodland. Morphologically, I. flavobrunneum is similar to I. lanatodiscum by its yellow to orange-brown pileus, but differs from it by the smaller size of the basidiomata, larger basidiospores, ecological association with Fabaceae / Dipterocarpaceae Blume and distribution in West Africa. I. lanatodiscum is associated with a variety of hardwoods and conifers and is widely distributed from Europe to North and Central America (Kropp et al. 2013). The other related taxa are all African taxa not yet described, such as Inosperma sp. BB3233 from Zambia and the Democratic Republic of Congo, as well as Inosperma sp. G1842 distributed in south-eastern Africa, while I. flavobrunneum is distributed in West Africa.

Taxonomic key to species of Inosperma from West Africa

1 Basidiomata large, pileus 28–38 mm diam., yellow to orange-brown, surface clearly rimose, lamellae adnexed and decurrent, subdistant .......................... Inosperma flavobrunneum

– Basidiomata small, pileus 8.5–15 mm diam., fibrillose, lamellae close........... 2

2 Pileus vinaceous to red, basidiospores 8–10 × 4–7, (sub) globose to cylindrical, sometimes ellipsoid..................................................... I. africanaum

– Pileus orange-brown to somewhat cinnamon, greyish-white, basidiospores 8–14 × 4–7 μm, elongate................................................. I. bulbomarginatum
New combination

For an evolutionarily-consistent taxonomy, we propose the following combination:

*Inosperma shawarense* (Naseer & Khalid) Aignon & Naseer, comb. nov.
MycoBank No: 836296

*Inocybe shawarensis* Naseer & Khalid, Mycotaxon 132: 912. 2018. Basionym.

**Notes.** This species is placed in the old *Inosperma* clade which became the genus *Inosperma*, but the combination is not made in the study of Matheny et al. (2020). The new combination is based on molecular phylogenetic data and sequencing the type of *Inocybe shawarensis* (Naseer et al. 2018).

**Discussion**

The new species exhibit the overall characteristics often observed in *Inosperma*. These characters include; pileus radially rimose, fibrillose or squamulose and absence of pleurocystidia (Matheny et al. 2020). They can be distinguished from other *Inosperma* species by their remarkable characteristics. In addition, *I. africanum* is common in West Africa and *I. bulbomarginatum* presents a large distribution and was recognised in Zambia in the collections of Bart Buyck (Matheny et al. 2009). However, the low sequence divergences between the sequences (2.2%–2.5%) of ITS and 0.3% of 28S allows us to confirm the wide distribution of *I. bulbomarginatum*.

Phylogenetically, *I. africanum* is nested in *Inosperma* with full support (99% SH-aLRT values, 100% ML Ultrafast bootstrap, 1 BPP) and *I. bulbomarginatum* is indicated as the sister of Old World Tropical clade 1 with full support (100% SH-aLRT values, 100% ML bootstrap, 1 BPP). Sequences of *Inosperma bulbomarginatum* from West Africa and Zambia formed a subclade. *Inosperma flavobrunneum* is nested in Old World Tropical clade 1 and has sister species undescribed in a collection from Zambia, BB3233, G1842 and PC96013. ML and BI analysis, using 28S and RPB2 sequences data, shows most nodes well resolved; for example, the node uniting Old World Tropical clade 2 with the crown group of *Inosperma* is supported with 0.97 BPP, but with weak ML bootstrap as shown in Pradeep et al. (2016); based also on combined data of 28S and RPB2, this node is with weaker support < 50% ML bootstrap.

The position of each of these new species is confirmed by single data from ITS (Fig. 6). There are several collections from undescribed species in *Inosperma* (e.g. *Inosperma* sp. G1842, *Inosperma* sp. BB3233, *Inosperma* sp. PC 96073, *Inosperma* sp. PC96013, *Inosperma* sp. PC96082, *Inosperma* sp. PC96080 and *Inosperma* sp. Zam07) that are of African origin, thereby attesting the need for further studies of this genus on this continent. Previously, in *Inosperma*, only one species, *Inosperma*
Figure 6. ML phylogeny of *Inosperma africanum*, *I. bulbomarginatum* and *I. flavobrunneum* based on ITS dataset.

*misakaense*, has been described from Africa before this study (Matheny and Watling 2004). So, this study reinforces the diversity of *Inosperma* in Tropical Africa which now amounts to four described species.

Acknowledgements

We are grateful to the Rufford Small Grants Foundation (grant n° 30738-2) which allowed us to collect some additional samples analysed in this paper, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (grant
n° 226-2014-1109) for funding molecular analysis and the Deutscher Akademischer Austauschdienst (DAAD, grant n° PKZ 300499) for granting the University of Parakou with a microscope, type Leica DM5700, that enabled us to perform microscopic investigations. Anneli SVANHOLM, Bobby SULISTYO and Brandon FURNEAUX (Systematic Biology programme, Department of Organismal Biology, Uppsala University) are thanked for their assistance during molecular analyses. We also thank Kassim TCHAN ISSIFFOU and Evans CODJIA (MyTIPS Research Unit, University of Parakou) for their assistance during field data collection. P. Brandon MATHENY (Department of Ecology and Evolutionary Biology, University of Tennessee, USA) and an anonymous reviewer are thanked for their corrections and suggestions to improve our paper.

References

Aïgnon HL, Naseer A, Matheny PB, Yorou NS, Ryberg M (2021) Malloccybe africana (Inocybaceae, Fungi) the first species of Malloccybe described from Africa. Phytotaxa 478(1): 049–060. https://doi.org/10.11646/phytotaxa.478.1.3

Alvarado P, Manjón JL, Matheny PB, Esteve-Raventós F (2010) Tubariozymes, a new genus of Inocybaceae from the Mediterranean region. Mycologia 102: 1389–1397. https://doi.org/10.3852/10-041

Bauman D, Raspé O, Meerts P, Degreel J, Ilunga Muledi J, Drouet T (2016) Multiscale assemblage of an ectomycorrhizal fungal community: the influence of host functional traits and soil properties in a 10-ha miombo forest. FEMS Microbiology Ecology 92(10): fiw151. https://doi.org/10.1093/femsec/fiw151

Bell JR (2018) A simple way to treat PCR products prior to sequencing using ExoSAP-IT. BioTechniques 44(6): 834–834. https://doi.org/10.2144/000112890

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2010) GenBank. Nucleic Acids Research 38: 46–51. https://doi.org/10.1093/nar/gkp1024

Cubeta M, Echandi E, Albernethy T (1991) Characterization of anastomosis groups of binucleate Rhizoctonia species using restriction analysis of an amplified ribosomal RNA gene. Phytopathology 81: 1395–1400. https://doi.org/10.1094/Phyto-81-1395

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Baroni T, Wilson T (2010) Geneious v5.3. http://www.geneious.com/

Esteve-Raventós F (2014) Inocybe aureocitrina (Inocybaceae), a new species of section Rimosae from Mediterranean evergreen oak forests. Plant Biosystems 148: 377–383. https://doi.org/10.1080/11263504.2013.877532

Gardes M, Bruns T (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281
Horak E, Matheny PB, Desjardin DE, Soytong K (2015) The genus *Inocybe* (Inocybaceae, Agaricales, Basidiomycota) in Thailand and Malaysia. Phytotaxa 230: 201–238. https://doi.org/10.11646/phytotaxa.230.3.1

Jabeen S, Khalid AN (2020) *Pseudosperma flavorimosum* sp. nov. from Pakistan. Mycotaxon 135: 183–193. https://doi.org/10.5248/135.183

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166.

Kornerup A, Wanscher JH (1978) Methuen Handbook of Colour. 3d ed. E. Methuen, London, 252 pp. https://doi.org/10.3852/12-185

Kropp BR, Matheny PB, Hutchison LJ (2013) *Inocybe* section *Rimosae* in Utah: phylogenetic affinities and new species. Mycologia 105: 728–747.

Larsson E, Ryberg M, Moreau PA, Mathiesen ÅD, Jacobsson S (2009) Taxonomy and evolutionary relationships within species of section Rimosae (*Inocybe*) based on ITS, LSU and mtSSU sequence data. Persoonia: Molecular Phylogeny and Evolution of Fungi 23: 86–98. https://doi.org/10.3767/003158509X475913

Latha KP, Manimohan P (2015) *Inocybe* griseorubida, a new species of *Pseudosperma* clade from Tropical India. Phytotaxa 221: 166–174. https://doi.org/10.11646/phytotaxa.221.2.6

Latha KPD, Manimohan P (2016) *Inocybe* gregaria, a new species of the *Inosperma* clade from Tropical India. Phytotaxa 286(2): 107–115. https://doi.org/10.11646/phytotaxa.286.2.5

Matheny P, Ammirati J (2003) *Inocybe* angustispora, *I. taedophila*, and *Cortinarius* aureifolius: an unusual inocyboid Cortinarius. Mycotaxon 88: 401–407.

Matheny P, Watling R (2004) A new and unusual species of *Inocybe* (*Inosperma* clade) from Tropical Africa. Mycotaxon 89: 497–503.

Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe, Agaricales*). Molecular Phylogenetics and Evolution 35(1): 1–20. https://doi.org/10.1016/j.ympev.2004.11.014

Matheny PB, Kudzma LV (2019) New species of *Inocybe* (Inocybaceae) from eastern North America. The Journal of the Torrey Botanical Society 146(3): 213–235. https://doi.org/10.3159/TORREY-D-18-00060.1

Matheny PB, Hobbs AM, Esteve-Raventós F (2020) Genera of Inocybaceae: New skin for the old ceremony. Mycologia 112: 83–120. https://doi.org/10.1080/00275514.2019.1668906

Matheny PB, Aime M, Smith ME, Henkel TW (2012) New species and reports of *Inocybe* (Agaricales) from Guyana. Kurtziana 37(1): 23–39.

Matheny PB, Henkel TW, Séné O, Korotkin HB, Dentinger BTM, Aime MC (2017) New species of *Auritella* (*Inocybaceae*) from Cameroon, with a worldwide key to the known species. IMA Fungus 8: 287–298. https://doi.org/10.5598/imagfungus.2017.08.02.06

Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Henrik Nilsson R, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL (2007) Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution 43: 430–451. https://doi.org/10.1016/j.ympev.2006.08.024
Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin DE, Horak E, Kropp BR, Lodge DJ, Sotyong K, Trappe JM, Hibbett DS (2009) Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. Journal of Biogeography 36: 577–592. https://doi.org/10.1111/j.1365-2699.2008.02055.x

Naseer A, Khalid AN, Smith ME (2018) Inocybe shawarensis sp. nov. in the Inosperma clade from Pakistan. Mycotaxon 132: 909–918. https://doi.org/10.5248/132.909

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Pradeep CK, Vrinda KB, Varghese SP, Korotkin HB, Matheny PB (2016) New and noteworthy species of Inocybe (Agaricales) from Tropical India. Mycological Progress 15: 1–25. https://doi.org/10.1007/s11557-016-1174-z

Rehner S, Samuels G (1995) Molecular Systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorph. Canadian Journal of Botany 73(Suppl 1): 816–823. https://doi.org/10.1139/b95-327

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Ryberg M, Matheny PB (2012) Asynchronous origins of ectomycorrhizal clades of Agaricales. Proceedings of the Royal Society B – Biological Sciences 279: 2003–2011. https://doi.org/10.1098/rspb.2011.2428

Ryberg M, Nilsson RH, Kristiansson E, Töpel M, Jacobsson S, Larsson E (2008) Mining metadatata from unidentified ITS sequences in GenBank: A case study in Inocybe (Basidiomycota). BMC Evolutionary Biology 8: 1–14. https://doi.org/10.1186/1471-2148-8-50

Tedersoo L, Põlme S (2012) Infrageneric variation in partner specificity: multiple ectomycorrhizal symbionts associate with Gnetum gnemon (Gnetophyta) in Papua New Guinea. Mycorrhiza 22: 663–668. https://doi.org/10.1007/s00572-012-0458-7

Tedersoo L, Bahram M, Jairus T, Bechem E, Chinoya S, Mpumba R, Leal M, Randrianjohany E, Razafimandimbison S, Sadam A, Naadel T, Koljalg U (2011) Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rainforests of Continental Africa and Madagascar. Molecular Ecology 20(14): 3071–3080. https://doi.org/10.1111/j.1365-294X.2011.05145.x

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
Supplementary material I

Partition for phylogeny analysis
Authors: Hyppolite L. Aignon, Sana Jabeen, Arooj Naseer, Nourou S. Yorou, Martin Ryberg
Data type: phylogeny data
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/mycokeys.77.60084.suppl1