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SHORT COMMUNICATION

# Studies of *Colletotrichum* species causing cowpea anthracnose in Nigeria reveal two first-time reports globally

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## Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important multipurpose crop in various countries in sub-Saharan Africa. However, cowpea production is affected by cowpea anthracnose disease (CAD). In Nigeria, *Colletotrichum lindemuthianum* and *C. destructivum* have been described as causal agents of CAD based on morphological features. Such characterization is unreliable because many similarities among and within *Colletotrichum* spp. exist. In the current study, *Colletotrichum* spp. were isolated from leaves showing CAD symptoms collected in farmers' fields across four states in Nigeria. Isolates were characterized using morphological keys, severity scores in detached leaf assays, and sequencing of the ITS, ACT, GADPH, TUB, ApMat, and CAL genes. Two species, *C. chrysophilum* and *C. siamense*, were identified after comparing multigene sequences. Morphological characteristics and disease symptoms were very similar for both species. However, severity scores varied among and within species. Both *C. chrysophilum* and *C. siamense* are reported for the first time as causal agents of CAD across the globe. The accurate diagnosis of organisms causing CAD in the studied region will allow developing effective management strategies.

**Keywords** Anthracnose · *Colletotrichum* · Cowpea · Diagnosis · Phylogeny

Cowpea ([*Vigna unguiculata* (L.) Walp.)] is an important legume for household nutrition in sub-Saharan Africa (SSA) and a source of income and employment for many people in rural Nigeria (Kebede and Bekeko 2020). Many farmers prefer growing cowpea because it thrives under low input production systems and fixes atmospheric nitrogen in the soil that benefits subsequent crops (Nyaga and Njeru 2020). The grains, rich in protein, carbohydrates, vitamins, and minerals, are an excellent option for diet diversification, especially in areas relying heavily on cereal-based diets (Boukar et al. 2015).

Nigeria is the largest cowpea producer worldwide (FAO-STAT 2020). However, cowpea anthracnose disease (CAD) caused by *Colletotrichum* spp. significantly limits the crop's production potential (Dania and Gbadamosi 2019). Several *Colletotrichum* spp. cause anthracnose in almost all crops. In the case of cowpea, CAD pathogens can survive in soils, dead plants, and in seeds for few years (Awurum and Ucheagwu 2013). CAD can begin in the seedling stage and spread within and between fields through rain splash, irrigation, and wind (Ntahimpera et al. 1997). Symptoms include reddish-brown streaks on leaves and round to irregular necrotic lesions on leaves, stems, and pods (Enyiukwu et al. 2020).

CAD can be effectively managed with some synthetic fungicides, plant extracts, biocontrol agents, and resistant varieties (Masangwa et al. 2013; Shiny et al. 2015; Ganiyu et al. 2018; Dania and Gbadamosi 2019). However, difficulties in using taxonomic characters to identify *Colletotrichum* spp. can be a limiting factor to recommend effective management methods and, therefore, molecular characterization is a must (Weir et al. 2012). Comparing sequences of at least three genes is needed for proper *Colletotrichum* spp. identification (Liu et al. 2016; Giacomin et al. 2021).

Several *Colletotrichum* spp. have been identified as causal agents of CAD globally, including *C. dematium* (Banyal and

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Kumar 2012), *C. fructicola* (Atghia et al. 2015), *C. lindemuthianum* (Pradhan et al. 2018) and *C.destructivum* (Satpathy and Beura 2021). Both *C. lindemuthianum* and *C. destructivum* were reported to cause CAD in Nigeria (Onesirosan and Barker 1971; Williams 1975; Enyiukwu et al. 2020) but no molecular characterization was done. Here we report causal agents of CAD in the middle belt region of Nigeria. Morphological features, pathogenicity, and sequences of six genes were compared. The results can contribute to the development of effective strategies to manage CAD in Nigeria.

In September and October 2018, farmers' fields were surveyed in the states of Niger, Kogi, and Nasarawa, and the Federal Capital Territory (FCT (Suppl. Fig. 1; hereinafter referred as the four states, even though FCT is not a state per se). Three fields were surveyed in each of three local government areas per state. Ten leaves with CAD symptoms were randomly collected in each field, placed in brown bags, labeled, and taken to the laboratory at IITA-Ibadan for pathogen isolation and characterization. About 3-mm at the advancing edges of lesions were cut and surface sterilized in 1% NaOCl for 1 min. Leaf pieces were rinsed in three changes of sterile water for 1 min before drying using sterile paper towel. Dried leaf pieces were plated on potato dextrose agar (PDA) and incubated at 28 °C (3–5 days). Isolates morphologically similar to *Colletotrichum* spp. were sub-cultured on PDA for 7 days. Initial identification was done based on cultural and spore descriptions (Damm et al. 2010). Seventy-two isolates were obtained, single-spored, and stored at -80 °C in 15% glycerol for further analysis. Morphological characterization included radial growth rate on PDA, malt extract agar (MEA), and Sabouraud agar, the colony color, spore type, and shape.

The pathogenicity of the isolates was tested in a detached leaf assay. Plants (76 total) of CAD susceptible variety, 'Ife Brown', were grown in the screenhouse for three weeks with adequate watering. Inocula were prepared from 18 representative isolates grown in PDA for 7 days. Cultures were scrapped off the medium surface with sterile water into sterile beakers and the suspensions filtered through sterile cheesecloth. Spore count was done using an hemocytometer, adjusted to $10^6$ spores/ml, and supplemented with 1 ml/l 20% TWEEN®20.

Crisper boxes (31 × 23 × 10 cm) were sterilized with 2% NaOCl, rinsed with two changes of sterile water, and drained under a biosafety cabinet. Cotton wool and paper towels wrapped in aluminum foil were autoclaved (121 °C, 15 min) and then laid in equal proportion in the boxes. Fifteen ml sterile water were added to each box before placing three surface sterilized leaves with the abaxial side exposed. Leaves were inoculated with 40 µl spore suspensions, using one isolate per box. The process was repeated for all 18 isolates. Healthy leaves in the control box were treated with sterile water. The experiment was laid in a completely randomized design and replicated thrice. CAD symptoms were monitored, and disease severity was recorded from second day after inoculation (DAI). A disease severity scale of 1 to 5 where 1 = 0–5% leaf area affected and 5 = more than 50% leaf area affected was used, as described by Nwadili et al. (2017).

To extract genomic DNA, mycelia from 7-day-old PDA cultures of the 18 isolates were independently transferred into 15 ml vials with potato dextrose broth and placed on an Edmund Bühler KL2 shaker for 72 h at 28 °C. DNA was extracted with the Zymo Quick-DNA/RNA™ Miniprep Kit (Zymo Research Corp., Irvine, CA, USA). The internal transcribed spacer (ITS) region, and actin (ACT), glyceraldehyde-3-phosphate (GADPH), beta-tubulin (TUB), calmodulin (CAL), and intergenic spacing and partial mating-type (mat 1–2) (ApMat) genes were studied. The primers used were ITS4/ITS5 (White et al. 1990), ACT512F/ACT783R (Carbone and Kohn 1999), GDF/GDR (Templeton et al. 1992), BT1/BT2B (Glass and Donaldson 1995), CL1C/CL2C (Weir et al. 2012), and CgDL-F6/CgMAT1F2 (Rojas et al. 2010). Each PCR tube had 2 µl DNA and 10.5 µl cocktail mix (0.25 µl 10 mM dNTPs, 0.75 µl 25 mM $MgCl_2$, 0.25 µl 10 pMol primer pair, 0.06 µl Taq polymerase, 2.5 µl green buffer mix, and 6.44 µl RNase-free sterile water). PCR conditions included 5 min of initial denaturation at 94 °C, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s primer annealing temperatures at 55 °C for ITS, 58 °C for ACT, 59 °C for both CAL and TUB, 56 °C for GADPH, and 53 °C for ApMat, followed by 1 min extension at 72 °C, and a 5 min final extension at 72 °C. Electrophoresis was done on a 1.5% agarose gel (100 V, 1 h).

After ethanol purification, all amplicons were sequenced bidirectionally with an ABI 3130xl Genetic Analyser (Applied Biosystems) by the Bioscience Centre, IITA-Ibadan. Sequences were assembled using BioEdit software and verified via the BLAST search tool (ncbi.nlm.nih.gov/). Sequences of the 18 isolates were aligned alongside those of 17 reference *Colletotrichum* spp. (Table 1). The 35 sequences were trimmed to equal lengths. Polymorphic regions were marked in each region across the species. Trimmed sequences were concatenated (2,889 positions in the final dataset), and a single phylogenetic tree inferred using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) was obtained using the Molecular Evolutionary Genetics Analysis (MEGA) software v11.

After assignment to their corresponding species, the isolates were subjected to pathogenicity tests. A total of 76 plants of 'Ife Brown' variety were grown as above. Spore suspensions of the 18 isolates were prepared as above and

**Table 1** Details and GenBank sequence accessions of reference *Colletotrichum* isolates used to determine phylogenetic relatedness of *Colletotrichum* species causing cowpea anthracnose disease in middle belt states of Nigeria

| Isolate/Strain ID | Species | DNA region[a] and NCBI GenBank accession number | | | | | |
|---|---|---|---|---|---|---|---|
| | | ITS | TUB | ACT | CAL | GAPDH | ApMat |
| NFUCF-214 | *C. fructicola* | OR056217 | OR073852 | OR096466 | OR096539 | OR069501 | OR105838 |
| NFUCF-118 | *C. fructicola* | OR056214 | OR073849 | OR09646 | OR096536 | OR069498 | OR105835 |
| NFUCF-179 | *C. fructicola* | OR056216 | OR073851 | OR096465 | OR096538 | OR069500 | OR105837 |
| NFUCF-95 | *C. fructicola* | OR056213 | OR073848 | OR096462 | OR096535 | OR069501 | OR105834 |
| NFUCF-74 | *C. fructicola* | OR056212 | OR073847 | OR096461 | OR096534 | OR069497 | OR105833 |
| W-1/GS03 | *C. siamense* | OQ771887 | OQ759587 | OQ759555 | OQ759564 | OQ759579 | KC790676 |
| G1-3/Col-1834 | *C. siamense* | OQ771888 | OQ759588 | OQ759556 | OQ759563 | OQ759580 | MZ568781 |
| CBS:125,378/ITCC6152/C1316.5 | *C. siamense* | JX010278 | JX010410 | JX009441 | JX009709 | JX010019 | KC790703 |
| ICMP:17,785/ b120/C1259.2 | *C. siamense* | JX010272 | JX010391 | JX009446 | JX009706 | JX010058 | MW14222 |
| DAR:76,934/Col-1929/SYCo2 | *C. siamense* | JX010270 | MW583638 | JX009535 | JX009707 | JX010002 | MZ568783 |
| AFK17 | *C. chrysophilum* | MN625458 | MN622860 | MN622831 | MN622859 | MN632506 | MN62287 |
| AFK31 | *C. chrysophilum* | MN625452 | MN622863 | MN622837 | MN622852 | MN653161 | MN62281 |
| C95/CPO 27.845 | *C. chrysophilum* | MZ562291 | MN848366 | MN741052 | MZ562255 | MN737345 | MZ562282 |
| C86/AREC3a | *C. chrysophilum* | MZ562288 | MT513054 | MN741048 | MZ562252 | MT512986 | MZ562279 |
| C53/CMS-6760 | *C. chrysophilum* | MZ562285 | MN741068 | MN741045 | MZ562249 | MN741090 | MZ562276 |
| Ninomiya_5/GD-LO3 | *C. gloeosporioides* | LC811975 | LC812149 | LC812144 | HM575330 | LC812147 | LC812146 |
| LF916 | *C. gloeosporioides* | KJ955226 | KJ955371 | KJ954493 | KJ954777 | KJ954927 | KJ954629 |
| CC1 | *C. chrysophilum* | OQ254724 | OQ259589 | OQ259537 | OQ259564 | OQ259570 | PQ570534 |
| CC2 | *C. chrysophilum* | OQ254725 | OQ259588 | OQ259538 | OQ259552 | OQ259571 | PQ570535 |
| CC3 | *C. chrysophilum* | OQ254726 | - | - | OQ259553 | OQ259572 | PQ570536 |
| CC4 | *C. chrysophilum* | - | OQ259590 | OQ259539 | OQ259554 | OQ259573 | PQ570537 |
| CC5 | *C. chrysophilum* | OQ254727 | OQ259591 | OQ259540 | OQ259555 | OQ259574 | PQ570538 |
| CC7 | *C. chrysophilum* | OQ254728 | OQ259592 | OQ259541 | OQ259556 | OQ259575 | PQ570539 |
| CC10 | *C. chrysophilum* | OQ254729 | OQ259593 | OQ259546 | OQ259565 | OQ259576 | PQ570540 |
| CC11 | *C. chrysophilum* | OQ254730 | OQ259594 | OQ259542 | OQ259557 | OQ259577 | PQ570541 |
| CC12 | *C. chrysophilum* | OQ254731 | OQ259595 | OQ259543 | OQ259558 | OQ259578 | PQ570542 |
| CC13 | *C. chrysophilum* | OQ254732 | OQ259596 | OQ259544 | OQ259559 | OQ259579 | PQ570543 |
| CC14 | *C. chrysophilum* | OQ254733 | OQ259597 | OQ259547 | OQ259560 | OQ259580 | PQ570544 |
| CC15 | *C. chrysophilum* | OQ254734 | OQ259598 | OQ259548 | OQ259561 | OQ259581 | PQ570545 |
| CC16 | *C. chrysophilum* | OQ254735 | - | OQ259545 | OQ259566 | OQ259582 | PQ570546 |
| CC22 | *C. chrysophilum* | OQ254736 | OQ259599 | OQ259549 | OQ259562 | OQ259583 | PQ570547 |
| CC23 | *C. chrysophilum* | OQ254737 | OQ259600 | OQ259550 | OQ259563 | OQ259584 | PQ570548 |
| CC6 | *C. siamense* | OQ254739 | OQ259601 | OQ259551 | OQ259567 | OQ259585 | PQ570549 |
| CC8 | *C. siamense* | OQ254740 | - | - | OQ259568 | OQ259587 | PQ570550 |
| CC9 | *C. siamense* | OQ254741 | OQ259602 | - | OQ259569 | OQ259586 | PQ570551 |

[a]ITS: internal transcribed spacer; TUB: beta-tubulin; ACT: actin; CAL: calmodulin; GADPH: glyceraldehyde-3-phosphate; ApMat: intergenic spacing and partial mating-type

sprayed on all plant parts using a high-pressure sprayer on 2-week-old plants. Control plants were sprayed with sterile water. The experiment was laid in a randomized complete block design (RCBD) with four replicates per treatment. All plants were covered with transparent plastic bags for 24 h to increase humidity and aid in the establishment of infection. Disease severity was scored each 7th day from the first DAI up to 12 weeks after inoculation. A modified scale of Latunde-Dada (1990) was used where 0 = no infection; 0.5 = hypersensitive spots on main stem only; 1 = small lesions on main stem and petioles of lower leaves; 2 = lesions on stem, petioles, and branches; 3 = advanced lesions on stem, petioles, branches, and veins on the abaxial surfaces of leaves; 4 = severe infection with lesions on stem, petioles, branches, leaf veins, and peduncles; and 5 = advanced lesions on stem, petioles, branches, and leaf veins, spreading lesions on peduncles and pods. Yield data was collected on weight of pods, average length of five longest pods, number, and grain weight. Grains were weighed using a Ohaus SP202 balance (OHAUS Instruments, Shanghai, China). Pod length was measured using a standard meter rule.

Mean severity scores were used to calculate the area under disease progress curve (AUDPC) using the formula $AUDPC = \sum_{i=1}^{n-1} 0.5\,(y_{i+1} + y_i)\,(t_{i+1} + t_i)$ (Campbell and Madden 1990) where "$t_i$" is the time (days after planting) at ith observation, "$y_i$" is the percent of an assessment of

the disease at the ith observation and "*n*" is the total number of observations. Analysis was carried out using SAS v9.2 (SAS Institute, Cary, NC, USA). For yield data, means were subjected to analysis of variance (ANOVA) and separated using Duncan's Multiple Range Test (DMRT; $\alpha = 0.05$). Relationships between AUDPC and yield were determined using Pearson Correlation test.

All isolates exhibited morphology typical of the *C. gloeosporioides* species complex, characterized by colonies that were either fluffy white or dense black with concentric ring patterns on PDA. The colonies' edges were either smooth or irregular and sometimes had orange or dark acervuli, with sectoring present in some isolates (Fig. 1). The spores were single-celled with a central mark that sometimes looked double-celled (Suppl. Fig. 2). Dark setae were seen in few isolates. The colony characteristics changed with sub-culturing and radial growth varied across the different media (Suppl. Table 1).

CAD symptoms on inoculated leaves were reddish brown necrotic lesions (Fig. 2) that appeared from the second DAI in all the leaves. The lesion size on the leaves progressed for each isolate daily until the experiment was terminated at day 7.

Initially, a BLAST search conducted with sequences of ITS, ACT, GADPH, TUB, and CAL revealed 15 isolates with >95% identity to *C. chrysophilum/C. fructicola* and three with >95% identity with *C. siamense*. Phylogenetic analysis using these five loci yielded inconclusive results in differentiating *C. fructicola* from *C. chrysophilum* (tree not shown). To resolve this, we sequenced the ApMat locus for the 15 unassigned isolates and those belonging to *C. siamense*. In the phylogenetic analysis using all six loci, all 15 ambiguous isolates grouped within the *C. chrysophilum* clade. Both *C. chrysophilum* and *C. siamense* isolates formed distinct, well supported clades with bootstrap values of 74% and 100%, respectively (Fig. 4) and both clearly separated from *C. gloeosporioides* isolates, which served as an outgroup. In our study, the ApMat marker was critical to distinguishing isolates in the *C. gloeosporioides* species complex, as in other studies (Silva et al. 2012; Doyle et al. 2013). Overall, the ApMat locus yielded the largest number of polymorphisms among all the loci examined (Table 2). The *C. chrysophilum* isolates were present in all four states: five isolates each from Niger and FCT, three from Nasarawa, and two from Kogi. The *C. siamense* isolates were from Kogi, one isolate, and Niger, two isolates. Similarity index revealed 81% similarities between *C. siamense* and *C. chrysophilum* isolates (Fig. 3).

In the pathogenicity assay, CAD symptoms occurred on all inoculated plants. Symptoms included reddish brown vein streaks on leaves and stems, white to slight pink/orange necrosis with reddish brown halo on leaves, and brown blotch on pods (Suppl. Fig. 3). All isolates produced similar symptoms, but severity varied among isolates in the same species (Suppl. Table 2). Similarly, the two species have been reported to produce similar symptoms in papaya (Zhang et al. 2021; Pacheco-Esteva et al. 2022), and avocado (Fuentes-Aragón et al. 2020). As expected, control plants had the highest yield parameters (Suppl. Table 2) and disease severity scores were negatively correlated with yield (Suppl. Table 3). The yield parameters had a significant positive correlation (>70%) with each other. The negative relationship between the disease severity and yield suggests that both *C. chrysophilum* and *C. siamense* can

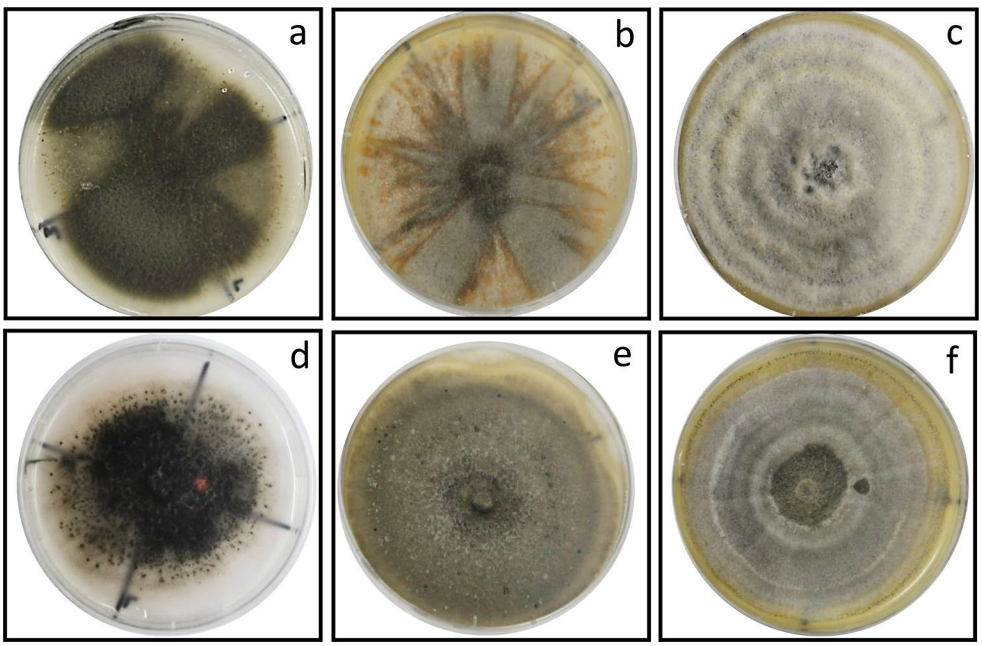


**Fig. 1** Cultures grown on potato dextrose agar (PDA), malt extract agar (MEA), and Sabouraud agar of *Colletotrichum siamense* isolate CC6 (**a**, **b**, **c**, respectively) and of *C. chrysophilum* isolate CC1 (**d**, **e**, **f**, respectively)

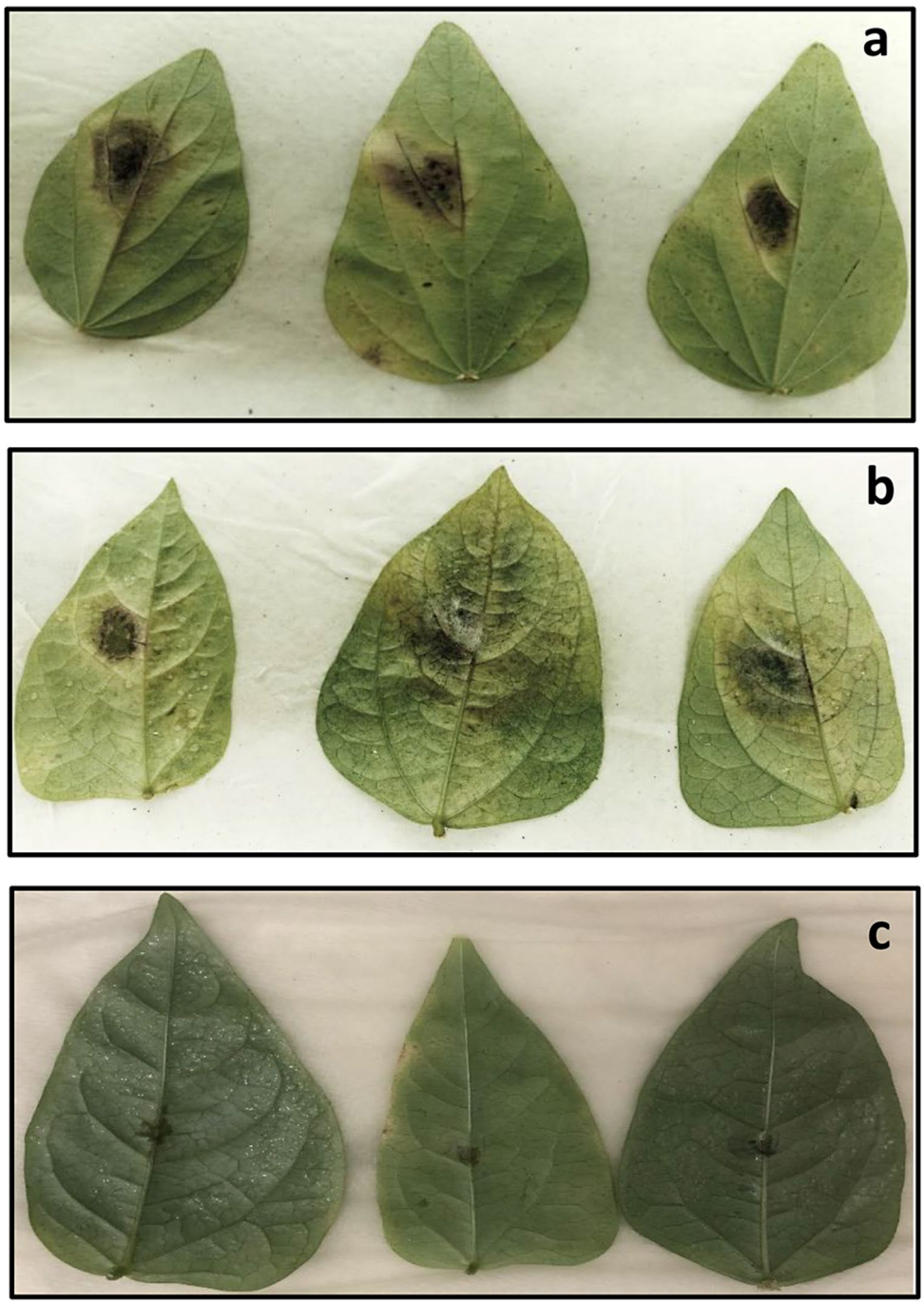


**Fig. 2** Anthracnose symptoms on cowpea leaves inoculated with *Colletotrichum siamense* isolate CC6 (**a**) and *C. chrysophilum* isolate CC1 (**b**) on the 5th day after inoculation. Sterile distilled water was used to inoculate the negative control leaves (**c**)

significantly reduce yield in susceptible cowpea varieties. Both species have been reported to cause significant damage in apple (Khodadadi et al. 2020; Chen et al. 2022) and avocado (Fuentes-Aragón et al. 2020).

The current study reports for the first time *C. chrysophilum* and *C. siamense* causing CAD worldwide. This report is based on morphological keys, pathogenicity assessment, and molecular tools. There were no isolates belonging to either *C. lindemuthianum* or *C. destructivum*, previously reported to cause CAD in Nigeria. Our report provides valuable information for the design of CAD control strategies in Nigeria.

In Nigeria, CAD was initially attributed to *C. lindemuthianum* (Onesirosan and Barker 1971; Williams 1975). However, Bailey et al. (1990) reported a CAD pathogen unrelated to *C. lindemuthianum* and Latunde-Dada et al. (1996) reported a CAD pathogen having close similarities with *C. destructivum*, with ovoid, slightly pointed apex, and truncate base spores. Those features were not detected in isolates from the present study, which have ovoid spores

**Table 2** Comparison of ITS, calmodulin (CAL), and glyceraldehyde-3-phosphate (GADPH) sequences of *Colletotrichum chrysophilum* and *C. siamense* isolates from Nigeria (with prefix CC) and isolates retrieved from NCBI GenBank[a]

| Isolate ID | ITS | | TUB | | | | | | | CAL | | | | | | | | | | | | GAPDH | | ACT | ApMat | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 39 | 60 | 39 | 70 | 84 | 110 | 112 | 250 | 370 | 150 | 262 | 326 | 358 | 365 | 366 | 369 | 465 | 475 | 476 | 490 | 616 | 174 | 182 | 77 | 1 | 6 | 7 | 11 | 12 | 14 | 15 | 16 | 17 | 20 | 22 | 24 | 25 |
| *C. chrysophilum* | C | T | A | G | A | T | G | C | G | C | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC1 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC2 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC3 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC5 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC10 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC11 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC15 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC16 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC22 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| CC23 | C | T | A | G | A | T | G | C | G | G | G | C | G | T | C | C | C | T | G | G | C | C | G | C | A | C | G | T | A | G | C | C | C | T | G | C | A |
| *C. siamense* | T | C | T | T | T | C | - | T | A | A | A | T | A | G | T | T | T | A | T | A | T | T | A | T | T | A | A | A | G | - | - | - | T | C | A | T | G |
| CC6 | T | C | T | T | T | C | - | T | A | A | A | T | A | G | T | T | T | A | T | A | T | T | A | T | T | A | A | A | G | - | - | - | T | C | A | T | G |
| CC8 | T | C | T | T | T | C | - | T | A | A | A | T | A | G | T | T | T | A | T | A | T | T | A | T | T | A | A | A | G | - | - | - | T | C | A | T | G |
| CC9 | T | C | T | T | T | C | - | T | A | A | A | T | A | G | T | T | T | A | T | A | T | T | A | T | T | A | A | A | G | - | - | - | T | C | A | T | G |

[a]In each loci, nucleotide position where polymorphisms occurred are indicated

| ID | CC1 | CC2 | CC3 | CC4 | CC5 | CC6 | CC7 | CC8 | CC9 | CC10 | CC11 | CC12 | CC13 | CC14 | CC15 | CC16 | CC22 | CC23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC1 | ID | | | | | | | | | | | | | | | | | |
| CC2 | 1 | ID | | | | | | | | | | | | | | | | |
| CC3 | 1 | 1 | ID | | | | | | | | | | | | | | | |
| CC4 | 1 | 1 | 1 | ID | | | | | | | | | | | | | | |
| CC5 | 1 | 1 | 1 | 1 | ID | | | | | | | | | | | | | |
| CC6 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | ID | | | | | | | | | | | | |
| CC7 | 1 | 1 | 1 | 1 | 1 | 0.81 | ID | | | | | | | | | | | |
| CC8 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | 1 | 0.81 | ID | | | | | | | | | | |
| CC9 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | 1 | 0.81 | 1 | ID | | | | | | | | | |
| CC10 | 1 | 1 | 0.99 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | ID | | | | | | | | |
| CC11 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | ID | | | | | | | |
| CC12 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 0.99 | 1 | ID | | | | | | |
| CC13 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | 1 | 1 | ID | | | | | |
| CC14 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | 1 | 1 | 1 | ID | | | | |
| CC15 | 1 | 1 | 1 | 1 | 1 | 0.81 | 0.99 | 0.81 | 0.81 | 0.99 | 1 | 1 | 1 | 1 | ID | | | |
| CC16 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | 1 | 1 | 1 | 1 | 1 | ID | | |
| CC22 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ID | |
| CC23 | 1 | 1 | 1 | 1 | 1 | 0.81 | 1 | 0.81 | 0.81 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ID |

**Fig. 3** Identity matrix of concatenated sequence of ITS, GADPH, beta-tubulin, calmodulin actin, and ApMat genes of *Colletotrichum siamense* (CC6, CC8, and CC9) and *C. chrysophilum* isolates (rest of the isolates) causing cowpea anthracnose in the sampled states in Nigeria

and straight round ends and fit the description of multiple *Colletotrichum* spp.: *C. gloeosporioides*, *C. siamense*, *C. fructicola*, *C. cliviicola*, *C. chrysophilum*, *C. lindemuthianum*, and *C. destructivum* (Gao et al. 2018; Sun and Liang 2018; Khodadadi et al. 2020; Pérez-Mora et al. 2021; Rodríguez-Palafox et al. 2021; Dada et al. 2022; Ma et al. 2023). Our results concur with observations made by Sharma and Shenoy (2014) that spore characteristics are not reliable to distinguish *C. gloeosporioides* species complex members.

Because *C. lindemuthianum* was reported to cause CAD in Nigeria, we initially used two primer pairs specific to this species, CD1/CD2 (Chen et al. 2013) and C1F432/C1R533 (Wang et al. 2008). However, none of the 18 isolates amplified either primer pair. The 6-loci phylogenetic analysis employed allowed revealing that 15 isolates belong to *C. chrysophilum* and the other three to *C. siamense* (Fig. 4), both belonging to the *C. gloeosporioides* species complex. The previously reported CAD pathogens in Nigeria, *C. destructivum* and *C. lindemuthianum*, belong to the *C. destructivum* and *C. orbiculare* species complexes, respectively.

Methods to manage CAD in Nigeria have focused on *C. lindemuthianum* and *C. destructivum* (Ganiyu et al. 2018; Dania and Gbadamosi 2019; Enyiukwu et al. 2021). Those methods need to be tested against the species reported in the current study. The findings stress the need to investigate the prevalent *Colletotrichum* spp. causing CAD in other regions to develop appropriate control strategies in Nigeria. In addition, the large cowpea collection at IITA Genetics Resource Centre should be tested for their resistance to *C. chrysophilum*, *C. siamense*, and other *Colletotrichum* spp. that may be interacting with cowpea across Nigeria and elsewhere.

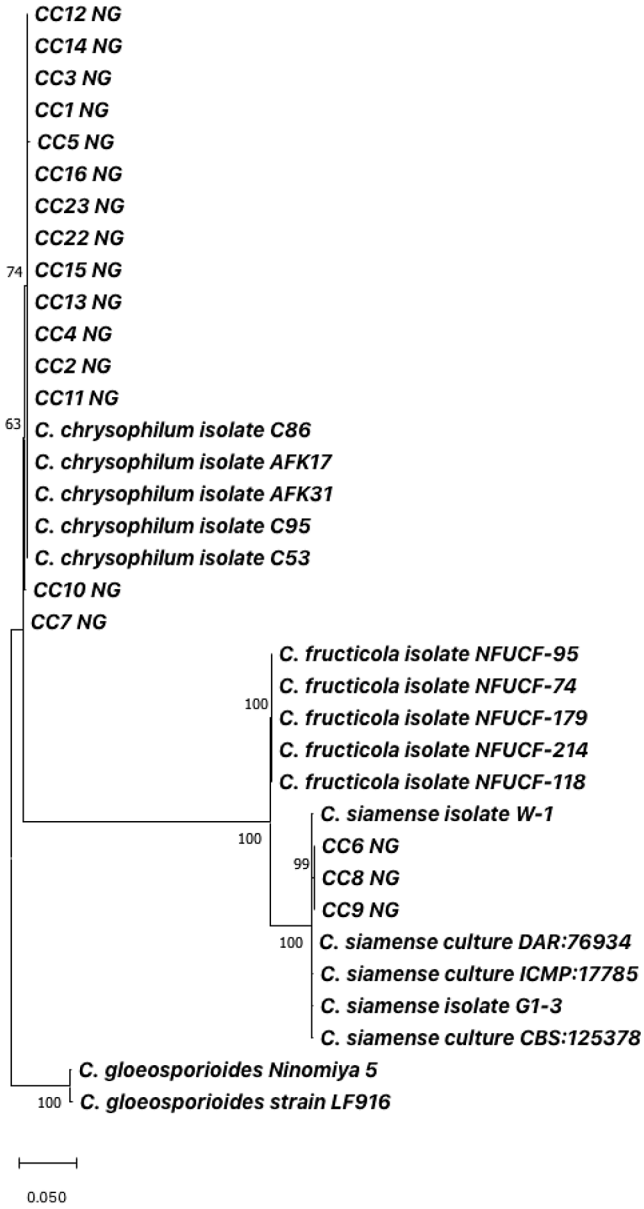


**Fig. 4** *Colletotrichum* species phylogeny inferred from a concatenated sequence (2889 nucleotide bases) of portions of the ITS, GADPH, beta-tubulin, calmodulin, actin, and ApMat genes. Isolates characterized in the current study have the CC NG codes

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**Author contributions** A.O.D., O.A.O., and M.A. designed the sampling strategy. A.O.D., V.O.D, and A.O.-B. designed the experiments. A.O.D. and O.A.O. performed the experiments. A.O.-B. and M.A. provided materials and supervision. A.O.D. wrote the manuscript. V.O.D., O.A.O., M.A., and A.O.-B. edited the manuscript. All authors gave final approval to publish the manuscript.

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**Data availability** The partial gene sequences from this study are available in NCBI-GenBank under the following accession numbers: ITS region OQ254724 to OQ254741; beta-tubulin OQ259588 to OQ259602; actin OQ259537 to OQ259551; calmodulin OQ259552 to OQ259569; GADPH OQ259570 to OQ259587; and ApMat PQ570534 to PQ570551.

## Declarations

**Competing interests** The authors declare no competing interests.



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