**Article**

**Novel Botrytis and Cladosporium Species Associated with Flower Diseases of Macadamia in Australia**

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**Abstract:** Macadamia (*Macadamia integrifolia*) is endemic to eastern Australia and produces an edible nut that is widely cultivated in commercial orchards globally. A survey of fungi associated with the grey and green mold symptoms of macadamia flowers found mostly species of *Botrytis* (Sclerotinia-ceae, Leotiomycetes) and *Cladosporium* (Cladosporiaceae, Dothideomycetes). These isolates included *B. cinerea*, *C. cladosporioides*, and unidentified isolates. Amongst the unidentified isolates, one novel species of *Botrytis* and three novel species of *Cladosporium* were delimited and characterized by molecular phylogenetic analyses. The new species are *Botrytis macadamiae*, *Cladosporium devikae*, *C. macadamiae*, and *P. proteacearum*.

**Keywords:** Botrytis blight; Cladosporium blight; fungal ecology; raceme blight; taxonomy; tree nut

1. Introduction

*Macadamia* species and hybrids (*M. integrifolia × M. tetraphylla*) are native to Australia and are now grown worldwide in tropical and subtropical regions for their nuts that have edible kernels [1]. The expansion of macadamia orchards into new regions has led to an increase in the number and severity of diseases caused by fungi and oomycetes [2–4]. Flower and fruit diseases reduce the nut set and can cause significant yield losses in commercial macadamia orchards [5–7]. A mature macadamia tree can produce more than 10,000 racemes (inflorescences) during the flowering period, with 100–300 flowers per raceme [8,9]. Fruit and flower diseases often cause poor pollination that can reduce the nut set by 99% [10]. Diverse fungal pathogens are associated with flower blights of macadamia including *Botrytis cinerea* [11], *Cladosporium cladosporioides* [12], *Neopestalotiopsis macadamiae*, and *Peelotiopsis macadamiae* [7].

Under high humidity and moisture, *B. cinerea* causes grey mold (Botrytis blight) that covers infected macadamia flowers with mycelia (Figure 1a) [11]. Index Fungorum accepted 71 *Botrytis* species (http://www.indexfungorum.org accessed on 17 September 2021), most of which are important pathogens of a wide range of host plants, including the grapevine, tomato, strawberry, bulbous crops, and cut flowers, causing devastating diseases during the preharvest and postharvest stages [13]. Among them, *B. cinerea* is one of the most important plant pathogens with wide-reaching economic and scientific impacts [14,15]. Many new species of *Botrytis* have been proposed [16] since Staats et al. [17] used molecular phylogenies to recognize *Botrytis* spp.

The genus *Cladosporium* (Cladosporiaceae, Dothideomycetes) was introduced by Link [18] with *C. herbarum* (Pers.) Link as the type species. *Cladosporium cladosporioides* causes flower blight known as green mold (Cladosporium blight) that manifests as olive-
grey-colored mycelial patches with abundant conidia on macadamia racemes that later become necrotic (Figure 1b) [12]. *Cladosporium* spp. include endophytes, pathogens, and saprobes, and have a worldwide distribution across a range of substrates [19–23]. *Cladosporium* spp. are well-known as plant pathogens [19,24–26], and some can cause animal and human diseases [27–29]. Some pathogenic isolates of *Cladosporium* may have been wrongly classified as saprophytes, emphasizing the importance of the phylogenetic relationships for the identification of specialized lineages and cryptic species [24,28,30]. Some common species, *C. cladosporioides, C. herbarum,* and *C. sphaerospermum,* represent species complexes that await resolution as new isolates are collected from diverse ecosystems and geographical regions [19]. For example, *C. polonicum* and *C. neapolitanum* were described from within the *C. cladosporioides* species complex based on isolates recovered from galled flowers formed by midges on several species of Lamiaceae in Poland and Italy [31]. A phylogenetic analysis based on informative protein-coding genes is essential for the identification of species within *Botrytis* and *Cladosporium* genera [17,31].

![Figure 1. Macadamia racemes with symptoms of (a) grey mold, and (b) green mold. Scale bars: (a) = 5 mm; (b) = 10 mm.](image)

Macadamia is a recently domesticated tree nut crop, with only *B. cinerea* and *C. cladosporioides* in their respective genera, reported as flower blight pathogens [11,12]. However, several unidentified isolates of *Botrytis* and *Cladosporium* were obtained from macadamia racemes with grey and green mold symptoms. Therefore, this study was aimed to determine the identity of the species of *Botrytis* and *Cladosporium* that are associated with flower diseases of macadamia in Australia.

2. Materials and Methods

2.1. Sample Collection and Isolation

The isolates included in this study were obtained from macadamia racemes with symptoms of grey and green mold diseases (Table 1). Samples were collected from commercial macadamia orchards in Queensland and New South Wales, Australia in 2019 and 2020. The specimens were surface sterilized and incubated, as described by Akinsanmi et al. [7]. Monoconidial cultures of the isolates were established, as described by Akinsanmi et al. [32], and preserved in a sterile 15% glycerol solution at -80 °C. Living cultures of the isolates were deposited in the Queensland Plant Pathology Herbarium (BRIP), Dutton Park, Australia.
Table 1. Details of Botrytis and Cladosporium isolates obtained from macadamia racemes with flower blight symptoms included in this study.

| Isolate ¹ | Species                  | Cultivar | Flower Growth Stage | Location ² |
|-----------|--------------------------|----------|---------------------|------------|
| BRIP 72259a | Botrytis macadamiae     | HAES 246 | 3                   | Alstonville, NSW |
| BRIP 72261a | B. macadamiae           | HAES 246 | 3                   | Alstonville, NSW |
| BRIP 72276a | B. macadamiae           | HAES 344 | 3                   | Fernleigh, NSW |
| BRIP 72295a | B. macadamiae           | A16      | 3                   | Knockrow, NSW |
| BRIP 72278a | Cladosporium devikae    | HAES 344 | 1                   | Fernleigh, NSW |
| BRIP 72269a | C. macadamiae           | HAES 792 | 4                   | Nambour, QLD |
| BRIP 72287a | C. macadamiae           | A16      | 3                   | Maleny, QLD |
| BRIP 72301a | C. proteacearum         | HAES 344 | 1                   | Rosebank, NSW |

¹ BRIP: Queensland Plant Pathology Herbarium (BRIP) accession numbers. ² NSW: New South Wales, Australia; QLD: Queensland, Australia.

2.2. Colony Characteristics and Morphological Studies

Colony characteristics of cultures on a 1/2-potato dextrose agar (PDA; Difco Laboratories, Franklin Lakes, NJ, USA) medium were photographed after 14 d of incubation at 25 °C. The fungal morphology was recorded from colonies grown in the dark for 14 d at 25 °C on PDA. Fungal structures were examined in lactic acid on slide mounts under a Leica DM5500B compound microscope (Wetzlar, Germany) with Nomarski differential interference contrast illumination, and images were captured with a Leica DFC 500 camera. Measurements of at least 30 conidia and other fungal structures were taken at 1000× magnification. Novel species were registered in MycoBank [33].

2.3. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from approx. 40 mg of mycelium from colonies grown on PDA for 14 d. The mycelium was homogenized using TissueLyser (Qiagen, Chadstone, Australia) for 2 min at 30 Hz, and DNA was extracted using the BioSprint 96 DNA Plant Kit on a robotic platform (Qiagen, Chadstone, Australia). The DNA concentration was determined with a BioDrop Duo spectrophotometer (BioDrop, Cambridge, UK) and adjusted to 10 ng µL⁻¹. For Botrytis isolates, partial sequences of the glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene with primers G3PDrfor+ and G3PDHrev+ [17], DNA-dependent RNA polymerase subunit II (RPB2) gene with primers RPB2for+ and RPB2rev+ [17], and heat shock protein 60 (HSP60) gene with primers HSP60for+ and HSP60rev+ [17] were amplified. For Cladosporium isolates, amplification was carried out using primers ITS4 and ITS5 [34] for the internal transcribed spacer (ITS) region of rDNA, primers EF1-526F and EF1-1567R [35] for partial sequences of the translation elongation factor 1-alpha (TEF1α) gene, and primers ACT-512F and ACT-783R [36] for the actin (ACT) gene sequences. The DNA of each isolate served as the template for the PCR amplification. Each reaction was performed in a 25 µL reaction mixture containing 5 µL of 5 × reaction buffer (Bioline, Eveleigh, Australia), 1.5 µL of 25 mM MgCl₂, 0.5 µL of 10 mM dNTPs, 1 µL each of 10 µM forward and reverse primers, 0.125 µL of MangoTaq DNA polymerase (5 U/µL; Bioline, Eveleigh, Australia), 13.875 µL of nuclease free water, and 2 µL of DNA template. Amplification was performed in a SuperCycler Thermal Cycler (Kyratec, Wembley, Australia) with initial denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, an annealing step at 55 °C for 30 s, and elongation at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. The quality of PCR amplicons was checked on 1% agarose gel electrophoresis stained with GelRed (Biotium, Melbourne, Australia) under UV light by Molecular Imager GelDoc (Bio-Rad Laboratories Inc., Gladesville, Australia). The targeted PCR products were purified and sequenced in both directions at Macrogen Inc. (Seoul, Korea).
2.4. Phylogenetic Analyses

The DNA sequences were assembled in Geneious Prime v. 2021.0.3 (Biomatters Ltd., San Diego, CA, USA), manually trimmed, and aligned to produce consensus sequences for each locus. The consensus sequences generated in this study were deposited in GenBank (Tables 2 and 3). The sequences were compared against the NCBI GenBank nucleotide database using BLASTn to determine the closest phylogenetic relatives. The sequences of the reference isolates of the *Botrytis* (Table 2) and *Cladosporium* (Table 3) species were retrieved from GenBank and aligned with the sequences generated from our isolates using MAFFT v.7.3.8.8 [37] in Geneious. Ambiguously aligned positions in each multiple alignment were excluded using Gblocks v. 0.91b [38]. The concatenated three-locus sequence dataset (RPB2 + HSP60 + G3PDH) of *Botrytis* consisted of 42 taxa, with the outgroup taxon *Sclerotinia sclerotiorum* 484 (Table 2). The combined ITS, *TEF1α*, and *ACT* sequences of isolates belonging to the *C. cladosporioides* species complex comprised 72 taxa, with the outgroup taxon *C. herbarum* CBS 121,621 (Table 3). The combined sequence datasets were manually improved with BioEdit v. 7.2.5 [39], and gaps were treated as missing data. Phylogenetic trees were generated from Maximum Likelihood (ML), Bayesian Inference (BI), and Maximum Parsimony (MP) analyses.

| Species                  | Isolate          | GenBank Accession Numbers 1 |
|--------------------------|------------------|-----------------------------|
|                          |                  | G3PDH          | HSP60          | RPB2            |
| *Botrytis aclada*        | MUCL8415         | AJ704992       | AJ716050       | AJ745664       |
| *B. allii*               | MUCL403          | AJ704996       | AJ716055       | AJ745666       |
| *B. byssaroides*         | MUCL94           | AJ704998       | AJ716059       | AJ745670       |
| *B. californica*         | X655             | KJ937069       | KJ937059       | KJ937049       |
| *B. calthae*             | MUCL1089         | AJ705000       | AJ716061       | AJ745672       |
| *B. caroliniana*         | CB15             | JF811584       | JF811587       | JF811590       |
| *B. cinerea*             | MUCL87           | AJ705004       | AJ716065       | AJ745676       |
| *B. convoluta*           | MUCL11595        | AJ705008       | AJ716069       | AJ745680       |
| *B. croci*               | MUCL436          | AJ705009       | AJ716070       | AJ745681       |
| *B. deweyae*             | CBS 134649       | HG799521       | HG799519       | HG799518       |
| *B. ellipitca*           | BE9714           | AJ705012       | AJ716073       | AJ745684       |
| *B. eucalypti*           | CERC 7170        | KX301020       | KX301024       | KX301028       |
| *B. euroamericana*       | B83              | KC191677       | KC191678       | KC191679       |
| *B. fabae*               | MUCL98           | AJ705014       | AJ716075       | AJ745686       |
| *B. fabiopsis*           | BroadbeanBC–2    | EU519211       | EU514482       | EU514473       |
| *B. ficariarum*          | MUCL376          | AJ705016       | AJ716077       | AJ745688       |
| *B. fragariae*           | U14_P1           | KX429699       | KX429692       | KX429706       |
| *B. galanthina*          | MUCL435          | AJ705018       | AJ716079       | AJ745689       |
| *B. gladiolorum*         | MUCL3865         | AJ705020       | AJ716081       | AJ745692       |
| *B. globosa*             | MUCL444          | AJ705022       | AJ716083       | AJ745693       |
| *B. hyacinthi*           | MUCL442          | AJ705024       | AJ716085       | AJ745696       |
| *B. macadamiae*          | BRIP 72259a      | MZ344223       | MZ344234       | MZ356230       |
|                         | BRIP 72261a      | MZ344224       | MZ344235       | MZ356231       |
|                         | BRIP 72276a      | MZ344225       | MZ344236       | MZ356232       |
|                         | BRIP 72295a      | MZ344226       | MZ344237       | MZ356233       |
| *B. medusae*             | B–555            | MH732861       | MH732866       | MH732870       |
| *B. narcissica*          | MUCL2120         | AJ705026       | AJ716087       | AJ745697       |
| *B. paonae*              | MUCL16084        | AJ705028       | AJ716089       | AJ745700       |
| *B. pelargonii*          | CBS497.50        | AJ704990       | AJ716046       | AM087030       |
| *B. polyblastis*         | CBS287.38        | AJ705030       | AJ716091       | AJ745702       |
Table 3. Cladosporium species and isolates used in the phylogenetic analysis with GenBank accession numbers.

| Species                        | Isolate | ITS          | TEF1α         | ACT          |
|--------------------------------|---------|--------------|---------------|--------------|
| Cladosporium acalyphae         | CBS 125982 t | HM147994     | HM148235      | HM148481     |
| C. alboflavescens              | CBS 140690 t | LN834420     | LN834516      | LN834604     |
| C. angulorum                   | CBS 140692 t | LN834425     | LN834521      | LN834609     |
| C. angustisporum               | CBS 125983 t | HM147995     | HM148236      | HM148482     |
| C. angustiterminale            | CBS 140480 t | KT600379     | KT600476      | KT600575     |
| C. anthropophilum              | CBS 140685 t | LN834437     | LN834533      | LN834621     |
| C. arenosum                    | CHFC–EA 566 | MN879328     | MN890011      | MN890008     |
| C. asperulatum                 | CBS 126340 t | HM147998     | HM148239      | HM148485     |
| C. australiensae               | CBS 125984 t | HM147999     | HM148240      | HM148486     |
| C. austroafricanum             | CBS 140481 t | KT600381     | KT600478      | KT600577     |
| C. chalastosporoides           | CBS 125985 t | HM148001     | HM148242      | HM148488     |
| C. chasmanthicola              | CBS 142612 t | KY646221     | KY646227      | KY646224     |
| C. chubutense                  | CBS 124457 t | FJ936158     | FJ936161      | FJ936165     |
| C. eladosporioides             | CBS 112388 t | HM148003     | HM148244      | HM148490     |
| C. colosasa                    | CBS 386.64 t | HM148067     | HM148310      | HM148555     |
| C. colombiae                   | CBS 274.808 t | FJ936159     | FJ936163      | FJ936166     |
| C. crousii                     | CBS 140686 t | LN834431     | LN834527      | LN834615     |
| C. cucumerinum                 | CBS 171.52 t | HM148072     | HM148316      | HM148561     |
| C. devikae                     | BRIP 72278a t | MZ303808     | MZ344193      | MZ344212     |
| C. endoviticola                | JZB390018 t | MN654960     | MN984228      | MN984220     |
| C. europaeum                   | CBS 134914 t | HM148056     | HM148298      | HM148543     |
| C. exasperatum                 | CBS 125986 t | HM148090     | HM148334      | HM148579     |
| C. exile                       | CBS 125987 t | HM148091     | HM148335      | HM148580     |
| C. flabelliforme               | CBS 126345 t | HM148092     | HM148336      | HM148581     |
| C. flavovirens                 | CBS 140462 t | LN834440     | LN834536      | LN834624     |
| C. funiculorum                 | CBS 122129 t | HM148094     | HM148338      | HM148583     |
| C. gamsiatum                   | CBS 125989 t | HM148095     | HM148339      | HM148584     |
| C. globisporum                 | CBS 812.96 t | HM148096     | HM148340      | HM148585     |
| C. grevilleae                  | CBS 114271 t | JF770450     | JF770472      | JF770473     |
| C. herbarum                    | CBS 121621 t | EF679363     | EF679440      | EF679516     |
| C. hillianum                   | CBS 125988 t | HM148097     | HM148341      | HM148586     |
| C. inversicolor                | CBS 401.80 t | HM148101     | HM148345      | HM148590     |
| C. iperiacae                   | CBS 140483 t | KT600394     | KT600491      | KT600589     |
| C. iranicum                    | CBS 126346 t | HM148110     | HM148354      | HM148599     |

1 G3PDH: glyceraldehyde 3-phosphate dehydrogenase; HSP60: Heat shock protein 60; RPB2: DNA-dependent RNA polymerase subunit II. The name and isolates of the new species, and newly generated sequences, are shown in bold font.
| Fungi isolate | CBS (or other accession) | KY646222 | KY646228 | KY646225 |
|---------------|--------------------------|-----------|-----------|-----------|
| C. kenpeggi   | CBS 142613^t             |           |           |           |
| C. licheniphilum | CBS 125990^t         |           |           |           |
| C. longicatenatum | CBS 140485^t        |           |           |           |
| C. macadamiae | BRIP 72269a^t          |           |           |           |
| C. montecillanum | CBS 140486^t       |           |           |           |
| C. myrtaceaerum | CBS 126350^t          |           |           |           |
| C. needhamense | CBS 143359^t          |           |           |           |
| C. neerlandicum | CBS 143360^t          |           |           |           |
| C. neopsychochotolerans | CGMCC3.18031^t |           |           |           |
| C. oxyporum | CBS 125991             |           |           |           |
| C. paracladosporioides | CBS 171.54^t     |           |           |           |
| C. parapenidielloides | CBS 140487^t      |           |           |           |
| C. perangustum | CBS 125996^t          |           |           |           |
| C. phaeoconaeae | CBS 128769^t         |           |           |           |
| C. phylactinicoila | CBS 126355^t        |           |           |           |
| C. phyllophilum | CBS 125992^t          |           |           |           |
| C. pini-ponderosaes | CBS 124456^t      |           |           |           |
| C. proteacearum | BRIP 72301a^t         |           |           |           |
| C. pseudochalastosporioides | CBS 140490^t   |           |           |           |
| C. pseudocladosporioides | CBS 125993^t       |           |           |           |
| C. rectoides | CBS 125994^t          |           |           |           |
| C. rugulovarians | CBS 140495^t         |           |           |           |
| C. scabrellum | CBS 126358^t          |           |           |           |
| C. silens | CBS 109082^t           |           |           |           |
| C. sinuatum | CGMCC3.18096^t        |           |           |           |
| C. subuliforme | CBS 126500^t          |           |           |           |
| C. tenuissimum | CBS 125995^t          |           |           |           |
| C. tianshanense | CGMCC3.18033^t     |           |           |           |
| C. uredinicola | CPC 5390              |           |           |           |
| C. uwebraunianum | CBS 143365^t       |           |           |           |
| C. varians | CBS 126362^t          |           |           |           |
| C. verrucocladosporioides | CBS 126363^t    |           |           |           |
| C. vicinum | CBS 143366^t          |           |           |           |
| C. vignae | CBS 121.25             |           |           |           |
| C. welwitschiiola | CPC 1864^t         |           |           |           |
| C. westerdijkiae | CBS 113746^t       |           |           |           |
| C. xanthochromaticum | CBS 140691^t     |           |           |           |
| C. xylophilum | CBS 125997^t          |           |           |           |

^1 ITS: internal transcribed spacer; TEF1α: translation elongation factor 1-α; ACT: actin. ^t Ex-type isolates. The name and isolates of the new species, and newly generated sequences, are shown in bold font.

The ML analysis was implemented using RAxML v. 8.2.11 [40] in Geneious. The search option was set to rapid bootstrapping, and the analysis was run using the GTR + G + I substitution model with 1000 bootstrap iterations. The BI analysis was conducted with MrBayes v. 3.2.1 [41] in Geneious to calculate posterior probabilities by the Markov Chain Monte Carlo (MCMC) method. The GTR + G + I evolution model was applied in the BI analysis. Four MCMC chains were run simultaneously, starting from random trees for 1,000,000 generations. The temperature of the heated chain was set to 0.25, and trees were sampled every 200 generations until the average standard deviation of split frequencies reached 0.01 (stop value). Burn-in was set at 25%, after which the likelihood values were stationary. The MP analysis was performed with PAUP v. 4.0b10 [42]. Trees were inferred
using a heuristic search strategy with a 100 random stepwise addition and tree-bisection-reconnection (TBR) branch swapping. Max-trees were set to 5000, and bootstrap support values were evaluated for tree branches with 1000 replications [43]. Phylograms were visualized in FigTree v. 1.4.4 [44] and annotated in Adobe Illustrator 2021.

3. Results

3.1. Phylogenetic Analyses

The concatenated sequence data matrix of Botrytis comprised 2950 base pairs (bp) (1093 for RPB2, 971 for HSP60, and 886 for G3PDH), of which 2240 bp were constant, 296 bp were parsimony uninformative, and 414 bp were parsimony informative. The ML analysis resulted in a best scoring tree with a final ML optimization value of -11,930.57 and the following model parameters: alpha=0.561, Π(A)=0.268, Π(C)=0.241, Π(G)=0.237, and Π(T)=0.254.

The combined sequence dataset of Cladosporium consisted of 1000 bp (494 for ITS, 297 for TEF1α, and 209 for ACT), of which 678 bp were constant, 73 bp were parsimony uninformative, and 249 bp were parsimony informative. The ML analysis resulted in a best scoring tree with a final ML optimization value of -10,089.02 and the following model parameters: alpha=0.675, Π(A)=0.212, Π(C)=0.311, Π(G)=0.250, and Π(T)=0.227.

The tree topology generated by the ML analysis was similar to that of the BI and MP analyses. The best scoring ML phylograms of Botrytis and Cladosporium are shown in Figures 2 and 3, respectively. ML bootstrap values, BI posterior probabilities, and MP bootstrap values (MLBS/BIPP/MPBS) are given at nodes of the phylogenetic trees (Figures 2 and 3). The phylogenetic tree inferred from the concatenated alignment resolved the four Botrytis isolates associated with the grey mold symptoms into an independent monophyletic clade with high support that represents a novel species within the Botrytis genus (Figure 2). The phylogram inferred from the combined sequence data assigned four Cladosporium isolates associated with the green mold symptoms into three well-supported monophyletic clades that represent novel species within the Cladosporium genus (Figure 3).
Figure 2. Maximum Likelihood tree topology of *Botrytis* based on a concatenated multi-locus alignment (RPB2 + HSP60 + G3PDH). *Sclerotinia sclerotiorum* 484 was used as an outgroup taxon. Maximum Likelihood bootstrap support values (>50%), Bayesian Inference posterior probabilities (>90%), and Maximum Parsimony bootstrap proportions (>50%) are displayed at the nodes, respectively. Isolates of the newly described species are depicted in red.
**Figure 3.** Maximum Likelihood tree topology of *Cladosporium* based on a combined multi-locus alignment (ITS + TEF1α + ACT). *Cladosporium herbarum* CBS 121621 was used as an outgroup taxon. Maximum Likelihood bootstrap support values (>50%), Bayesian Inference posterior probabilities (>90%), and Maximum Parsimony bootstrap proportions (>50%) are displayed at the nodes, respectively. Isolates of the newly described species are depicted in red.

### 3.2. Taxonomy

**Botrytis macadamiae** Prasannath, Akinsanmi & R.G. Shivas, sp. nov. (Figure 4).

**MycoBank:** MB841218.

**Etymology:** Named after *Macadamia*, from which the type was first isolated.

**Type:** AUSTRALIA, New South Wales, Knockrow, from flower blight of *Macadamia integrifolia*, 25 October 2019, J. Coates (Holotype BRIP 72295a, includes ex-type culture). GenBank: MZ344226 (G3PDH); MZ344237 (HSP60); MZ356233 (RPB2).

**Description:** *Hyphae* hyaline to pale brown, septate, 3–8 μm wide. *Sclerotia* single, sparse, dark grey to black, irregular to spherical, immersed, scattered, 0.2–2 mm diam. *Conidiophores* branched at top, erect, septate, subhyaline to pale brown, 1020–2050 × 10–20 μm. *Conidiogenous* cells swollen at the apex, 10–12 × 12–14 μm. *Conidia* in botryose clusters, elliptical to ovoid, unicellular, hyaline to pale brown, 9–11 × 6–7.5 μm.

**Culture characteristics:** Colonies on PDA at 25 °C after 14 d cover the plate, pale grey, abundant aerial mycelium in dark grey irregular tufts that cover most of the surface; reverse pale grey to buff brown.

**Habitat and distribution:** Racemes of *Macadamia integrifolia* (Proteaceae); Australia.

**Other material examined:** AUSTRALIA, New South Wales, Alstonville, from flower blight of *Macadamia integrifolia*, 17 Aug. 2019, K. Prasannath (living cultures, BRIP 72259a...
and BRIP 72261a); AUSTRALIA, New South Wales, Fernleigh, from flower blight of *Macadamia integrifolia*, 23 Sep. 2019, S. Hill (living culture, BRIP 72276a).

**Notes:** *Botrytis macadamiae* was placed in a strongly supported clade with *B. cinerea*, *B. eucalypti*, and *B. pelargonii*. BLASTn searches in GenBank showed that *B. macadamiae* (BRIP 72295a) differed from *B. cinerea* (MUCL87) in *RPB2* (Identities 1070/1075, 0 gaps); from *B. eucalypti* (CERC 7170) in *HSP60* (Identities 934/935, 0 gaps) and *RPB2* (Identities 1071/1075, 0 gaps); from *B. pelargonii* (CBS497.50) in *RPB2* (Identities 1071/1075, 0 gaps).

![Figure 4. Botrytis macadamiae (BRIP 72295a).](image)

(a) Two-week-old colony on PDA, (b) sclerotia, (c) hyphae, (d) conidiophore, and (e) conidia. Scale bars: (a) = 1 cm; (b) = 1 mm; (c,e) = 10 μm; (d) = 50 μm.

**Cladosporium devikae** Prasannath, Akinsanmi & R.G. Shivas, sp. nov. (Figure 5).

![Figure 5. Cladosporium devikae (BRIP 72278a).](image)

(a) Two-week-old colony on PDA (upper surface and lower surface), (b) conidiophore, (c) ramoconidia, and (d) terminal conidia. Scale bars: (a) = 1 cm; (b) = 25 μm; (c,d) = 10 μm.
MycoBank: MB841219.

Etymology: Named after Devika Malkanthi De Costa, for her guidance and mentorship to the senior author.

Type: AUSTRALIA, New South Wales, Fernleigh, from flower blight of *Macadamia integrifolia*, 23 Sep. 2019, S. Hill (Holotype BRIP 72278a, includes ex-type culture). GenBank: MZ303808 (ITS); MZ344193 (TEF1α); MZ344212 (ACT).

Description: *Mycelium* composed of branched, septate, smooth to finely roughened, brown, 3–4.5 μm diam. hyphae. *Conidiophores* erect, flexuous, subcylindrical, branched and unbranched, 200–700 × 2.5–4 μm, multisepa, giving rise to an apical conidiogenous apparatus with chains of branched conidia. *Primary ramoconidia* subcylindrical, 11–30 × 3–5 μm, pale brown, smooth to finely roughened, 0–1-septate; hila thickened, darkened, refractive, 1.5–3.0 μm diam. *Secondary ramoconidia* subcylindrical to fusoid to ellipsoidal, 5–11 × 2–4 μm, pale brown, smooth to finely roughened, aseptate; hila thickened, darkened, refractive, 0.5–1.5 μm diam. *Intercalary and terminal conidia* in branched chains (~10), ellipsoidal, 3.5–7 × 2–3 μm, subhyaline to pale brown, smooth, aseptate; hila thickened, darkened, refractive, 0.5 μm diam.

Culture characteristics: Colonies on PDA 70 mm diam. after 14 d at 25 °C, flat, olivaceous, with sparse aerial mycelium, margins even and smooth; reverse black.

Habitat and distribution: Racemes of *Macadamia integrifolia* (Proteaceae); Australia.

Notes: *Cladosporium devikae* belongs to the *C. cladosporioides* species complex. *Cladosporium devikae* was a sister species to *C. anthropophilum* in the phylogeny. BLASTn searches in GenBank showed that *C. devikae* (BRIP 72278a) differed from *C. anthropophilum* ex-type (CBS 140685) in ACT (Identities 189/198, 0 gaps) and TEF1α (Identities 208/217, 1 gap).

*Cladosporium macadamiae* Prasannath, Akinsanmi & R.G. Shivas, sp. nov. (Figure 6).

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**Figure 6.** *Cladosporium macadamiae* (BRIP 72269a). (a) Two-week-old colony on PDA (upper surface and lower surface), (b) conidiophore, and (c) terminal conidia. Scale bars: (a) = 1 cm; (b) = 25 μm; (c) = 10 μm.
MycoBank: MB841220.

**Etymology:** Named after *Macadamia*, from which the type was first isolated.

**Type:** AUSTRALIA, Queensland, Nambour, from flower blight of *Macadamia integrifolia*, 22 Aug. 2019, O.A. Akinsanmi (*Holotype* BRIP 72269a, includes ex-type culture). GenBank: MZ303810 (ITS); MZ344195 (TEF1α); MZ344214 (ACT).

**Description:** *Mycelium* composed of branched, septe, smooth to finely roughened, brown, 3–4.5 μm diam. hyphae. *Conidiophores* erect, flexuous, subcylindrical, branched and unbranched, 200–500 × 2.5–5 μm, pale brown, multiseptate, giving rise to an apical conidiogenous apparatus with chains of branched conidia. *Primary ramoconidia* subcylindrical, 15–30 × 3–5 μm, pale brown, smooth, 0–1-septate; hila thickened, darkened, refractive, 1.5–3 μm diam. *Secondary ramoconidia* subcylindrical to fusoid to ellipsoidal, 7–18 × 3–4 μm, pale brown, smooth, aseptate; hila thickened, darkened, refractive, 0.5–1.5 μm diam. *Intercalary* and *terminal conidia* in branched chains (~10), ellipsoidal, 3–7 × 2–3 μm, subhyaline to pale brown, smooth, aseptate; hila thickened, darkened, refractive, 0.5 μm diam.

**Culture characteristics:** Colonies on PDA 70 mm diam. after 14 d at 25 °C, flat, olivaceous, with sparse aerial mycelium, margins even and smooth; reverse black.

**Habitat and distribution:** Racemes of *Macadamia integrifolia* (Proteaceae); Australia.

**Other material examined:** AUSTRALIA, Queensland, Maleny, from flower blight of *Macadamia integrifolia*, 20 Sep. 2019, O.A. Akinsanmi (living culture, BRIP 72287a).

**Notes:** *Cladosporium macadamiae* belongs to the *C. cladosporioides* species complex. BLASTn searches in GenBank showed that *C. macadamiae* (BRIP 72269a) differed from *C. croussii* ex-type (CBS 140686) in *ACT* (Identities 199/209, 0 gaps) and TEF1α (Identities 182/213, 2 gaps); from *C. endoviticola* ex-type (JZB390018) in *ACT* (Identities 153/170, 3 gaps) and TEF1α (Identities 248/266, 1 gap); from *C. pseudocladosporioides* ex-type (CBS 125993) in *ACT* (Identities 197/209, 0 gaps) and TEF1α (Identities 279/293, 0 gaps).

*Cladosporium proteacearum* Prasannath, Akinsanmi & R.G. Shivas, sp. nov. (Figure 7).

![Image](Figure 7. *Cladosporium proteacearum* (BRIP 72301a). (a) Two-week-old colony on PDA (upper surface and lower surface), (b) conidiophore, (c) ramoconidia, and (d) terminal conidia. Scale bars: (a) = 1 cm; (b) = 25 μm; (c,d) = 10 μm.)
MycoBank: MB841221.

**Etymology:** Named after Poteaceae, the family of the host plant from which the type was first isolated.

**Type:** AUSTRALIA, New South Wales, Rosebank, from flower blight of *Macadamia integrifolia*, 16 Oct. 2019, P. Fraser (Holotype BRIP 72301a, includes ex-type culture). GenBank: MZ303809 (ITS); MZ344194 (TEF1a); MZ344213 (ACT).

**Description:** *Mycelium* composed of branched, septate, smooth to finely roughened, brown, 3–4.5 μm diam. hyphae. *Conidiophores* erect, flexuous, subcylindrical, branched and unbranched, 150–500 × 2.5–4 μm, multiseptate, giving rise to an apical conidiogenous apparatus with chains of branched conidia. *Primary ramoconidia* subcylindrical, 12–48 × 3–5 μm, pale brown, smooth, 0–1-septate; hila thickened, darkened, refractive, 1.5–3 μm diam. *Secondary ramoconidia* subcylindrical to fusoid to ellipsoidal, 5–10 × 3–4 μm, pale brown, smooth, aseptate; hila thickened, darken, refractive, 0.5–1.5 μm diam. *Intercalary and terminal conidia* in branched chains (~10), ellipsoidal, 4–5 × 2–3 μm, subhyaline to pale brown, smooth, aseptate; hila thickened, darkened, refractive, 0.5 μm diam.

**Culture characteristics:** Colonies on PDA 70 mm diam. after 14 d at 25 °C, flat, olivaceous, with sparse aerial mycelium, margins even and smooth; reverse olivaceous.

**Habitat and distribution:** Racemes of *Macadamia integrifolia* (Proteaceae); Australia.

**Notes:** *Cladosporium proteacearum* belongs to the *C. cladosporioides* species complex. *Cladosporium proteacearum* was a sister to *C. cucumerinum* in a well-supported clade. BLASTn searches in GenBank showed that *C. proteacearum* (BRIP 72301a) differed from *C. cucumerinum* ex-type (CBS 171.52) in *ACT* (Identities 198/209, 0 gaps); ITS (Identities 481/494, 1 gap); TEF1a (Identities 274/297, 3 gaps).

4. Discussion

*Botrytis macadamiae, Cladosporium devikae, C. macadamiae, and C. proteacearum* were isolated from macadamia inflorescences with grey and green mold symptoms and subsequently described. Each species formed a well-supported monophyletic clade in the phylogenetic analysis. The ITS region of the nuclear rDNA discriminates *Botrytis* from other genera in Sclerotiniaceae, although ITS is not useful for the delineation of the *Botrytis* species [45]. The three nuclear protein-coding genes, G3PDH, HSP60, and RP2B, have been used to characterize the *Botrytis* species [17]. To date, 40 species are phylogenetically recognized in *Botrytis* [16,46], including *B. macadamiae*. Whether *B. macadamiae* causes grey mold in macadamia has yet to be ascertained.

Grey mold is the most common disease caused by the *Botrytis* species affecting different plant organs, including flowers, fruits, leaves, and shoots [47]. Vegetables and small fruit crops such as the tomato, raspberry, grape, strawberry, blueberry, apple, and pear are among the most severely affected by these pathogens [47]. The genus *Botrytis* consisting of necrotrophic species has a very broad host range (e.g., *B. cinerea* and *B. pseudocinerea*) impacting more than 1400 different plant species [13]. On the contrary, other species have a narrow host range or are even host-specific, including *B. fabae* (broad bean) and *B. caltha* (marsh marigold) [48]. In some circumstances, multiple *Botrytis* species co-infect the same host plant; e.g., *B. squamosa, B. allii, and B. aclada* all cause significant economic risk to commercial onion production [15]. Interestingly, *B. squamosa* is family-specific and pathogenic on the onion, garlic, and leek (*Ailium* spp.), while the closely related sister species are restricted to the lily (*B. elliptica*) and daylily (*B. deweyae*) [49]. Diversity among the *Botrytis* species is shown by cultural characteristics, virulence, and host specificity. However, the unique feature among all grey mold fungi is their necrotrophic lifestyle in which they kill host cells via the secretion of effector proteins to induce cell death, obtain nutrients, and subsequently colonize dead plant tissue [49,50].

The *Cladosporium* species are known as common and abundant fungi in indoor and outdoor environments. The *Cladosporium* species are also economically important spoilage organisms of grains, fruits, and refrigerated meat [51–53]. Several *Cladosporium* species are pathogenic to a wide range of hosts [30]. Most *Cladosporium* species are saprobic, but some
have also been reported as endophytes, phylloplane fungi, and hyperparasites on other fungi [54–56]. Certain species show the ability to produce compounds of medical interest or are relevant as potential biocontrol agents for plant diseases [57,58]. Some species are pathogens to various crops and can cause economically important diseases, while others have only endemic importance [59]. These fungi can cause diseases of plants, often with different names, depending on the infected plants and the type of symptoms. Pathogenic species of *Cladosporium* are known to cause leaf mold of the tomato [60] and scab disease on leaves of the cucumber, the strawberry, and tea [61–63]. *Cladosporium cladosporioides* has been reported as a pathogen of scab in papaya [64], sooty mold in the persimmon [65], blossom blight in the strawberry [66], and leaf spot in the tomato [67].

Three major species complexes are recognized within the genus *Cladosporium*, viz. the *C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum* species complexes [30]. The species identification and delineation in *Cladosporium* require a multi-locus DNA sequence analysis of the ITS region of rDNA gene, partial ACT, and *TEF1α* gene sequences [30]. The molecular approach combined with morphological features allowed the recognition of more than 230 species within the genus *Cladosporium* [68]. Our phylogenetic analysis using these three loci placed *C. devikae*, *C. macadamiae*, and *C. proteacearum* in the *C. cladosporioides* species complex. These species were well-delineated from other species in the *C. cladosporioides* species complex.

The proper identification of species is essential for all biological studies. The present study found a high diversity of *Cladosporium* spp. on macadamia racemes with green mold symptoms. Future studies will determine whether *B. macadamiae*, *C. devikae*, *C. macadamiae*, and *C. proteacearum* are pathogens or saprobes on macadamia inflorescences. Living cultures of *B. macadamiae*, *C. devikae*, *C. macadamiae*, and *C. proteacearum* are preserved and accessible in BRIP as cryopreserved cultures for future research and study.

### 5. Conclusions

*Botrytis macadamiae*, *Cladosporium devikae*, *C. macadamiae*, and *C. proteacearum* were described and illustrated. These fungi were isolated from inflorescences of macadamia with grey and green mold symptoms in Australia. The pathogenicity of these novel species on macadamia racemes has yet to be examined. Cryopreserved isolates of these fungi are available in BRIP for future research.

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