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CHAPTER 3

PENICILLIUM VIRIDIGENUM PROV. NOM., A NEW SPECIES ISOLATED FROM MAIZE FARMS OF THE EASTERN CAPE OF SOUTH AFRICA.

FIGURES AND TABLES

Table 3.1. Primers for sequence amplification used in the PCR reaction and sequencing.

| Locus | PCR amplification profile | Primer pairs | Direction | Primer sequence (5'-3') | Reference |
|--|--|--------------|-----------|-----------------------------|---------------------------|
| RNA polymerase II second largest subunit (<i>RPB2</i>) | Denaturation 94°C 5min; 30 cycles of 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72 °C 7min. | 5F | Forward | GAY GAY MGW GAT CAY TTY GG | Liu <i>et al.</i> , 1999 |
| | | 7CR | Reverse | CCC ATR GCT TGY TTR CCC AT | Liu <i>et al.</i> , 1999 |
| Calmodulin (<i>CaM</i>) | Denaturation 94°C 5 min; 35 cycles of 94 °C 45 sec, annealing 55 °C 45 sec, 72°C 60sec; 72 °C 7 min. | CMD5 | Forward | CCG AGT ACA AGG ARG CCT TC | Hong <i>et al.</i> , 2006 |
| | | CMD6 | Reverse | CCG ATR GAG GTC ATR ACG TGG | Hong <i>et al.</i> , 2006 |

| | | | | | |
|--|--|-------|---------|---------------------------------|---|
| Internal Transcribed spacer 5.8S rDNA (<i>ITS</i>) | Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing 55°C 45sec; 72°C 60sec; 72°C 7 min. | V9G | Forward | TTA CGT CCC TGC CCT TTG TA | De Hoog and Gerrits van den Ende (1998) |
| | | LS266 | Reverse | GCA TTC CCA AAC AAC TCG ACT C | Masclaux <i>et al.</i> , (1995) |
| Internal Transcribed Spacer (<i>ITS</i>) | Denaturation 94°C 3 min; 35 cycles 94°C 60 sec; annealing at 55°C for 60 sec; 72°C for 60 sec; 72°C for 7 min. | ITS1 | Forward | TCC GTA GGT GAA CCT GCG G | White <i>et al.</i> , 1990 |
| | | ITS4 | Reverse | TCC TCC GCT TAT TGA TAT GC | White <i>et al.</i> , 1990 |
| β -tubulin (<i>BenA</i>) | Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72°C 7 min. | Bt2a | Forward | GGT AAC CAA ATC GGT GCT GCT TTC | Glass & Donaldson (1995) |
| | | Bt2b | Reverse | ACC CTC AGT GTA GTG ACC CTT GGC | Glass & Donaldson (1995) |

Table 3.2. NCBI GenBank accession numbers for *Penicillium* species in the section *Canescentia* series *Atroveneta* used for the phylogenetic analyses. ^T: ex-type strains.

| Species name | Strains | GenBank:Ben A | GenBank:Ca M | GenBank:/ TS | GenBank: RPB2 |
|--------------------------------|---|------------------|-----------------|-----------------|------------------|
| <i>P. antarcticum</i> | CBS100492 ^T | MN969371 | MN969236 | KJ34503 | JN406653 |
| <i>P. antarcticum</i> | CBS116938 | KP016925 | KP016827 | KP016845 | KP016848 |
| <i>P. antarcticum</i> | CBS116939 | KP016921 | JX157255 | KP016829 | KP016849 |
| <i>P. atrovenetum</i> | CBS241.56 ^T | JX140944 | KJ867004 | AF033492 | JN121467 |
| <i>P. atrovenetum</i> | CBS243.56 | JX140945 | MN969241 | KP016835 | JN121467 |
| <i>P. atrovenetum</i> | NRRL2571 | KJ775171 | KJ775405 | KJ775678 | MN969116 |
| <i>P. janczewskii</i> | CBS221.28 ^T | KJ866967 | KJ866998 | KC411682 | KP016853 |
| <i>P. coralligerum</i> | CBS114.69 | KJ866970 | KJ866991 | KP016836 | KP016847 |
| <i>P. coralligerum</i> | CBS123.65 ^T | MN969378 | MN969248 | JN617667 | JN406632 |
| <i>P. novae-zeelandiae</i> | CBS137.41 ^T | MN969390 | MN969279 | JN617688 | JN406628 |
| <i>P. novae-zeelandiae</i> | CV0042 | JX140956 | JX157352 | JX140853 | KP016864 |
| <i>P. nucicola</i> | KAS2101 | KT887807 | KT887768 | KT887846 | OR146006 |
| <i>P. nucicola</i> | KAS2203 ^T | KT887821 | KT887782 | KT887860 | MN969171 |
| <i>P. nucicola</i> | CBS140987 ^T | KT887821 | KT887782 | KT887860 | MN969171 |
| <i>P. pole-evansii</i> | CBS138946 ^T | JX141005 | JX157412 | JX140831 | KP016911 |
| <i>Penicillium viridigenum</i> | CN069H6=C MW59679=C MWIA003483 ^T | n.a. | n.a | n.a | n.a |

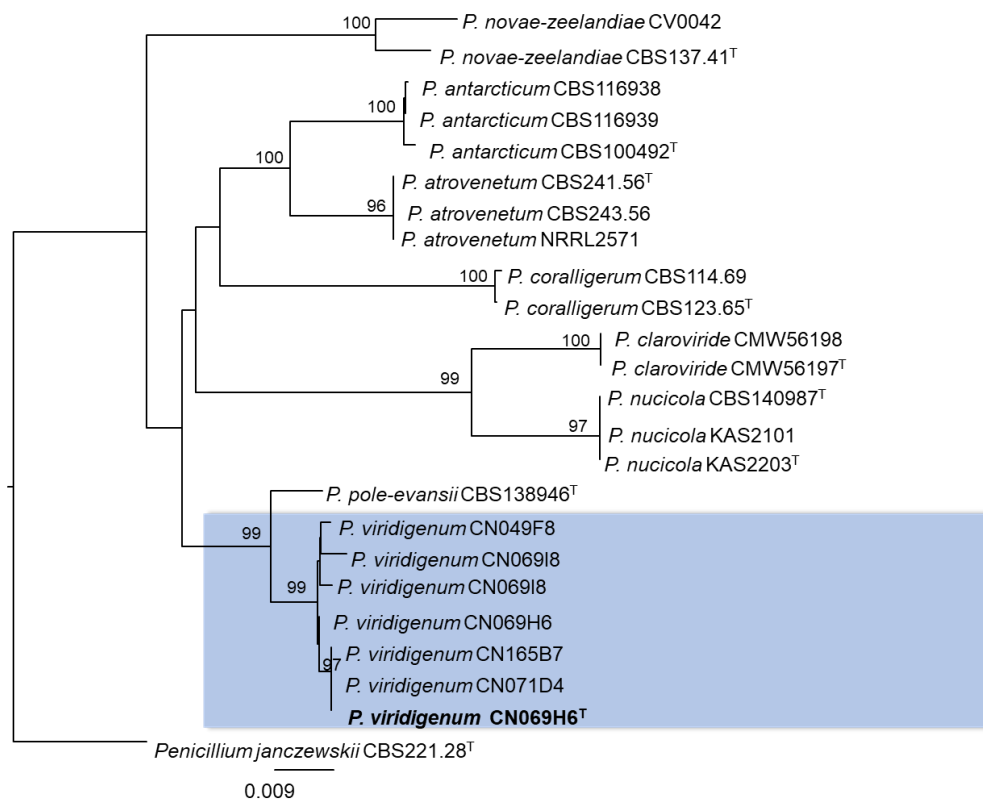


Figure 3.1. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a concatenated dataset of *BenA*, *CaM*, ITS and *RPB2*. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^T= ex-type).

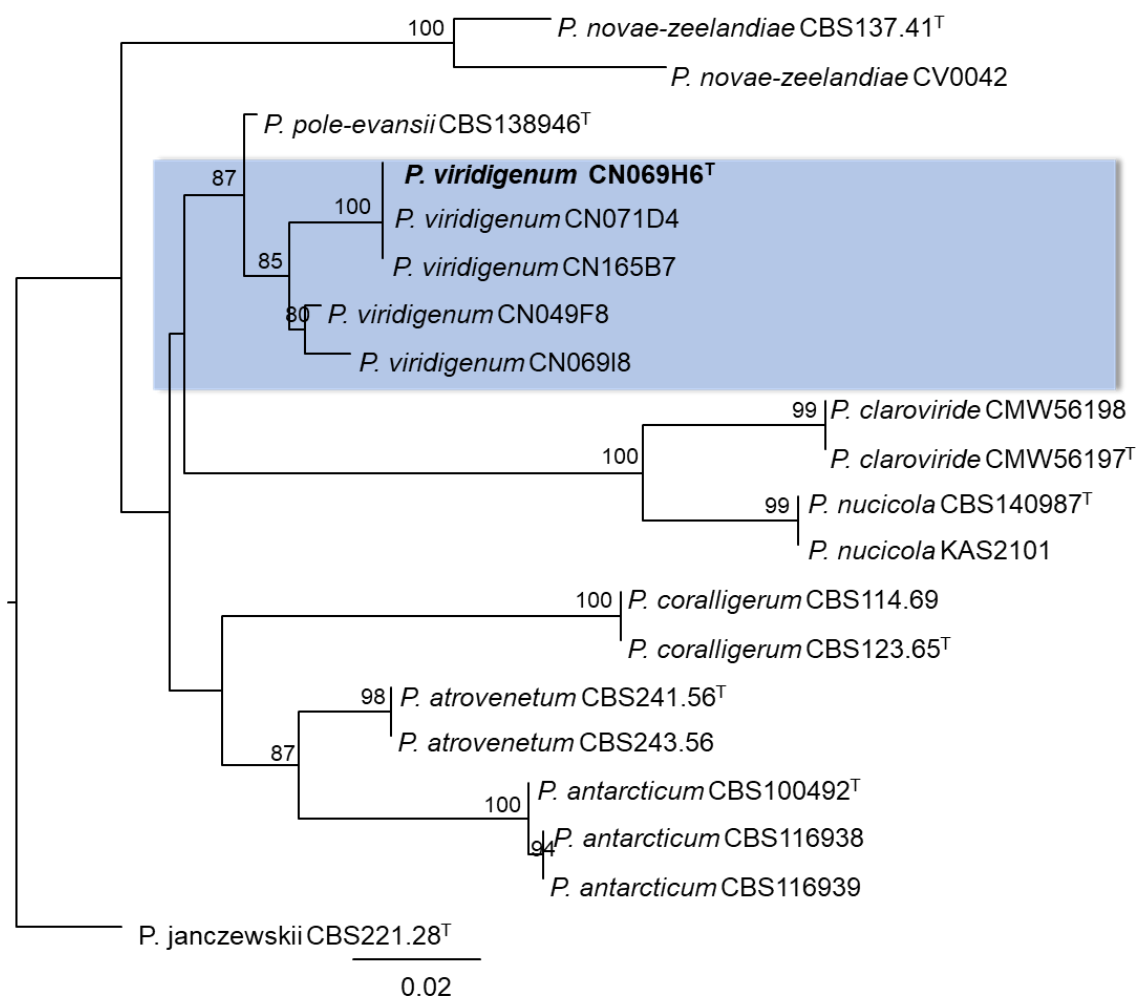


Figure 3.2. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on dataset of *BenA* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^T= ex-type).

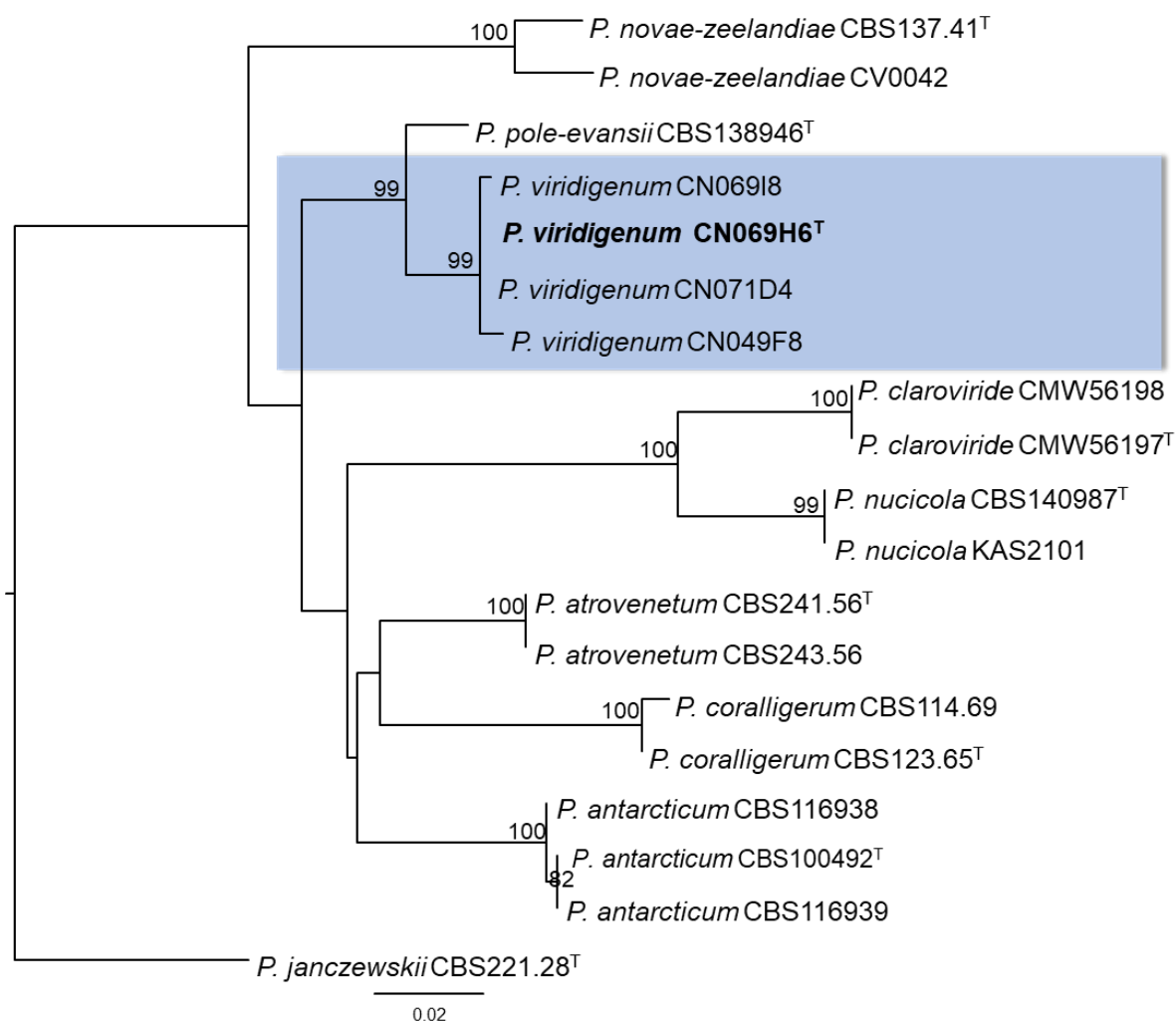


Figure 3.3. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on dataset of *CaM* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^T= ex-type).

Figure 3.4. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a dataset of *ITS* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^T= ex-type).

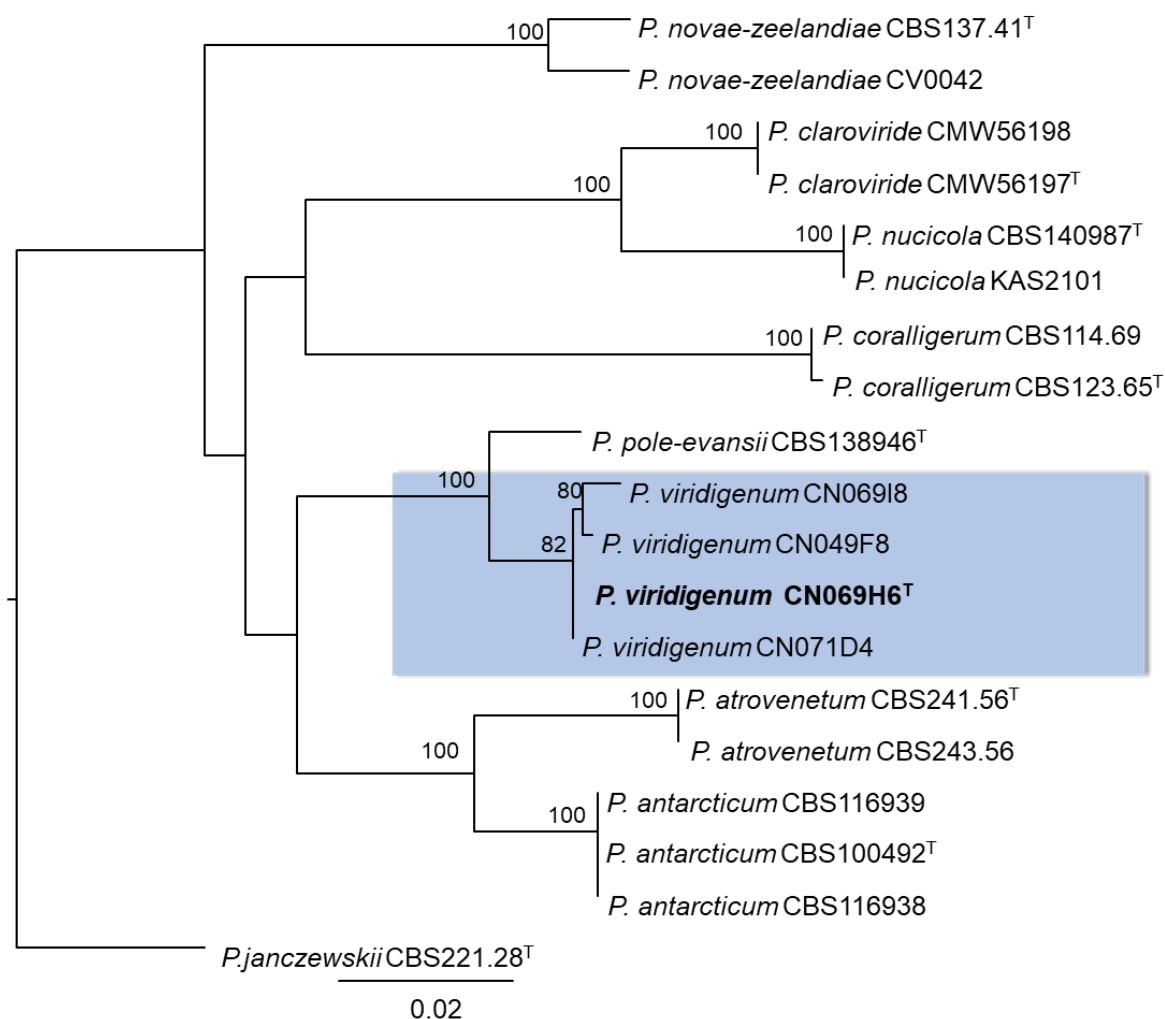


Figure 3.5. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a dataset *RPB2* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^T= ex-type).

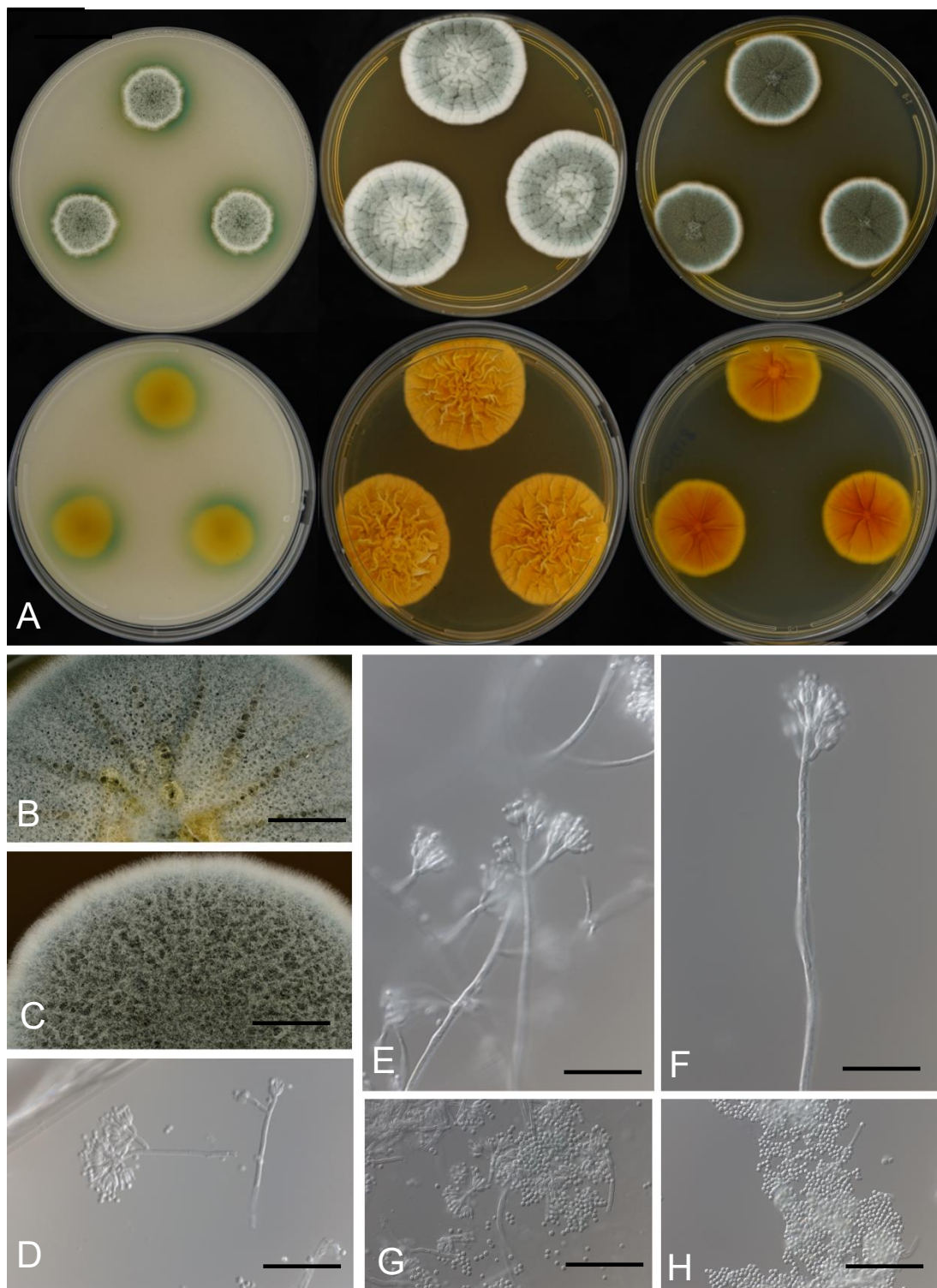


Figure 3.6. Morphological characters of *Penicillium viridigenum*; A. Colonies (top row, left to right: OA, MEA, CYA; bottom row, left to right: OA reverse, MEA reverse, CYA reverse); B, C. colony texture on MEA and CYA. D-F. conidiophores; G-H. conidia. Scale bars B-H = 10 μ m.

