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New species of *Gondwanamyces* from dying *Euphorbia* trees in South Africa

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Abstract: *Gondwanamyces* and its *Custingophora* anamorphs were first described from *Protea* infructescences in South Africa. Subsequently these unusual fungi were also found on *Cecropia* in Central America. During an investigation into the decline and death of native *Euphorbia* trees in South Africa, several fungal isolates resembling the anamorph state of *Gondwanamyces* were obtained from diseased tissues. In this study these isolates are identified based on morphology and comparisons of DNA sequences. Two previously unknown *Gondwanamyces* species were identified, both were associated with damage caused by beetles (*Cossonus* sp.). Inoculation studies showed that the new species of *Gondwanamyces* are pathogenic on *Euphorbia ingens* and may contribute to the decline of these trees.

Key words: *Cossonus*, *Custingophora*, *Euphorbia ingens*, *Euphorbia tetragona*, insect-fungus interactions, *Knoxdavesia*, tree diseases

Introduction

The genus *Gondwanamyces* Marais & M.J. Wingf was established in 1998 for two species of fungi collected from the infructescences of native *Protea* plants in the Cape Floristic Region (CFR) of South Africa (Marais et al. 1998). These fungi were described as having anamorphs in a new genus, *Knoxdaviesia* (Wingfield et al. 1988, Wingfield and van Wyk 1993), but DNA sequence data showed that they could be accommodated in *Custingophora* (Kolařík and Hulcr 2009). *Custingophora* species have mononematous conidiophores that terminate in obovoid conidiogenous cells with distinct collarettes and conidia. The *Gondwanamyces* teleomorphs are characterized by ascomata similar to those of species of *Ceratocystis* and *Ophiostoma*, with globose ascomatal bases and long necks bearing ascospores in slimy masses (Marais et al. 1998). Phylogenetic studies based on DNA sequences have shown that these fungi reside in the Microascales and are closely related to but distinct from species of *Ceratocystis* (Marais et al. 1998, Zhang et al. 2006).

Until recently *Gondwanamyces* spp. was known only from southern Africa, however Kolařík and Hulcr (2009) described two more species, *Custingophora cecropiae* M. Kolařík (no sexual state found) and *G. scolytodis* M. Kolařík from *Cecropia angustifolia* Trécul in Costa Rica. The discovery of *Gondwanamyces* on native trees in the Neotropics in Central America calls to question a hypothesis that these fungi are specific to the southern hemisphere (Roets et al. 2009a).

Species of *Gondwanamyces* are the dominant fungi in the infructescences of many *Protea* spp. in the CFR. One species, *Gondwanamyces proteae* Marais & M.J. Wingf., is specific to the infructescences of *Protea repens* L. (Wingfield et al. 1988). In contrast *Gondwanamyces capensis* Marais & M.J. Wingf is found in the floral parts of many *Protea* spp. (Wingfield and van Wyk 1993). It has been shown that *Ophiostoma* spp. occurring on *Protea* are vectored by mites and that *Gondwanamyces* might also be dispersed by the same vectors in a complex association with *Protea* spp. in the CFR (Roets et al. 2007, 2009b, 2011). *C. cecropiae* from Costa Rica is associated with a scolytine beetle, *Scolytus unipunctatus* Blandford, but whether it also is associated with mites is unknown.

Diseased and dying *Euphorbia ingens* E. Meyer: Boissier trees were observed in Limpopo Province in the 1990s (Malan 2006; Roux et al. 2008, 2009). These trees are native to Africa and represent one of the largest succulent *Euphorbia* species. The species consists of a main woody stem supporting cactus-like succulent branches (van Wyk and van Wyk 1997, Palgrave 2002, Gildenhuys 2006). The succulent branches can reach up to 10 m and contain a milky latex that has caustic and toxic properties (van Wyk and van Wyk 1997, Palgrave 2002, Gildenhuys 2006). During investigations to determine the cause of death of these trees, fungal fruiting bodies resembling those of species of *Gondwanamyces* were observed on diseased plant material as well as in the tunnels of

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weevils (Scolytinae: Curculionidae) infesting these trees (Roux et al. 2009). The aim of this study was to identify these fungi with DNA sequence comparisons and morphology. Inoculation experiments on *E. ingens* were also undertaken to consider the possible role of these fungi in the decline and death of these trees in South Africa. We also included isolates from diseased *Euphorbia tetragona* Haw trees in Eastern Cape Province, which were collected from insect tunnels in diseased plant material.

MATERIALS AND METHODS

Collection of samples and isolations.—Isolates were obtained from two different *Euphorbia* spp. growing in climatically different areas of South Africa. Isolates from *E. ingens* trees were collected during summer and winter 2009 and summer 2010 at four sites in Limpopo Province. Isolates from *E. tetragona* were collected Mar 2010 in the Great Fish River Nature Reserve near Grahamstown in Eastern Cape Province. All isolates were obtained from diseased plant material, insect tunnels or directly from insects associated with dying trees.

Isolations were made directly from fungal fruiting bodies observed on wood placed in moist chambers. Freshly produced spore drops were removed from conidiophores and ascomata with a dissection needle and placed on 2% malt extract agar (MEA; 15 g agar and 20 g malt extract per 1000 mL distilled water; Biolab, Merck, Midrand, RSA) containing streptomycin sulphate (0.4 g/L; Sigma-Aldrich, St Louis, Missouri). For isolations insects were crushed in 100 μL sterile distilled water with a plastic pestle, after which a series of dilutions were made (10^{-2} and 10^{-3}) and streaked onto water agar (WA; 15 g per 1000 mL distilled water; Biolab). Petri plates were incubated at 20 C for up to 6 d, and single hyphal tips or spore drops of resultant fungi were transferred to MEA. Isolates were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), and representative isolates were deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS) Utrecht, the Netherlands. Herbarium material of the new species was deposited with the National Fungal Collection (PREM), Pretoria, South Africa.

Morphological characteristics.—Pure cultures of *Gondwanamyces*-like isolates were incubated on MEA at 20 C under near-ultraviolet light to stimulate growth and fruiting body production. After 10 d cultures were examined and fruiting bodies mounted on glass microscope slides in 75% lactic acid. A Zeiss microscope (Carl Zeiss Ltd., Germany) was used to examine and study the fungal structures, and digital images of structures were taken with an Axiocam digital camera, with Axiovision 3.1 software, mounted on the microscope. Colonies and morphological characters were determined with the color notations of Rayner (1970). Fifty measurements were made of each fungal character and are presented as (min–)av. ± std. dev.(–max) for the length and width of the structures (l × w) representing the minimum, maximum, standard deviation (SD), mean values and the length/width (l/w) ratios.

Growth studies.—These were conducted to determine optimum growth temperatures. The study was conducted at 10–35 C at 5 C intervals. Five millimeter disks from 6 d old colonies on MEA were placed at the center of 90 cm Petri plates containing MEA, with the mycelium side of the disks placed flat on the agar. The plates were incubated in the dark 10 d with growth measured at 24 h intervals. For each plate two diameter measurements perpendicular to each other were made, resulting in a total of 10 measurements for each isolate at each temperature. The experiment was repeated once under the same conditions. A student *t*-test was conducted, $P < 0.05$ designated as significant, to determine significant differences in growth between the fungi at each temperature.

DNA extraction and PCR.—Cultures were grown 14 d on MEA to obtain material for DNA extraction with the protocol described by Möller et al. (1992). Conidiophores were removed from the surfaces of MEA plates with a sterile scalpel and placed in 2 mL Eppendorf tubes and freeze dried 24 h. DNA was extracted and concentrations measured with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware).

The internal transcribed spacer (ITS) regions (ITS1, ITS2) and the 5.8S gene were amplified with primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) with an Applied Biosystems Veriti thermo-cycler (Applied Biosystems, Foster City, California). The 25 μL PCR reactions consisted of 0.2 μL Super-therm polymerase (5 u/μL) (Hoffmann-La Roche, Nutley, New Jersey), 2.5 μL (2.5 mM) dNTPs (Fermentas, Vilnius, LIT), 0.5 μL (25 mM) $MgCl_2$ (Roche Diagnostics, Mannheim, DE), 2.5 μL 10 × PCR buffer with $MgCl_2$ (Roche Diagnostics), 0.5 μL (10 mM) of each primer (InqabaBiotec, Pretoria, RSA), 16.3 μL sterile distilled water (Adcock Ingram, Bryanston, RSA) and 2.0 μL DNA at a concentration of 10 ng/μL. Confirmation of amplified products was made on 2% agarose gels (Whitehead Scientific, Cape Town, RSA) loaded with GelRed (Anatech, USA) under UV illumination. To estimate the size of the PCR products a 100 bp DNA molecular marker ladder was used (O'RangeRuler™ 100 bp DNA ladder, Fermentas Life Sciences). Amplified products were purified for sequencing with Sephadex G-50 columns (1 g in 15 mL distilled water; SIGMA, Steinheim, Germany) following the manufacturer's instructions.

Sequencing and phylogenetic analyses.—Amplification products were sequenced with Big Dye Cycle Sequencing Kit 1.1 (Applied Biosystems) and an ABI3700 DNA analyzer (Applied Biosystems) following the instructions provided by the manufacturer. Forward and reverse sequences were obtained and compared with MEGA4 (molecular evolutionary genetics analysis) (Tamura et al. 2007). BLASTN (www.ncbi.nih.nlm.gov) was used to confirm gene identity and to obtain related sequences from GenBank for phylogenetic analyses. Sequences generated in this study and those of closely related species obtained from GenBank were aligned with the online platform of MAFFT 5.851 (method for rapid

sequence alignment based on fast Fourier transform) (Katoh et al. 2002). Aligned sequences were compared with PAUP* 4.0b10 (phylogenetic analysis using parsimony) (Swofford 2002). Heuristic searches, with random stepwise addition and tree bisection and reconstruction as branch swapping algorithms, were used to produce most parsimonious trees. Maximum parsimony and bootstrap analysis, with 1000 replicates (Felsenstein 1985), were determined for all datasets. *Ceratocystis fimbriata* Ellis & Halsted sensu stricto was used as outgroup taxon for the analyses and was treated as a monophyletic sister group to the ingroup.

Bayesian analyses, using the Monte Carlo Markov chain (MCMC) method (parameters set at four chains producing 5 000 000 generations recording trees every 100 generations), was used to determine the posterior probabilities. MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) was used to run the Bayesian analysis with the appropriate nucleotide substitution model that was determined with jModelTest 0.1.1 (Posada 2008). The model used for ITS, according to Akaike information criterion (AIC), was determined as the TPM2uf model. Graphical analysis (Tracer 1.5) was used to determine the burn-in value at the point where values converged. Posterior probabilities were determined from the trees produced in MEGA4 (Tamura et al. 2007).

Pathogenicity studies.—Two isolates (CMW36767, CMW36768, CMW36769, CMW36770) of each of the species identified in this study were grown on MEA 10 d. Material for inoculation was prepared by soaking wooden toothpicks in sterile MEA and letting the test fungi colonize them for 2 wk. Colonized toothpicks as well as control toothpicks that had been soaked in MEA were inserted into succulent branches of healthy *E. ingens* trees to a standard depth of 3 mm for each treatment. Each species was inoculated into branches of five trees that were in their natural environment in North West Province. After 6 wk inoculated branches were examined for lesions and lesion lengths were measured. Where lesions were observed branch material was incubated in moist chambers and examined for the presence of fruiting bodies resembling species of *Gondwanamyces*. To complete Koch's postulates pieces from the diseased tissue were placed on MEA to re-isolate the fungi. Data were analyzed by first determining the distribution and normality of the data, after which variation in lesion lengths were compared with a one-way analysis of variance (ANOVA), with $P < 0.05$ as significant. Because there was no variance in the control (all replicates showed zero lesion length) we compared each isolate against zero using independent, one sided t-tests, Bonferroni-corrected for multiple comparisons ($\alpha = 0.05$). All tests were conducted with JMP 9.0.2 (SAS institute 2011).

RESULTS

Collection of samples and isolations.—Ten isolates of *Gondwanamyces* were obtained from diseased *E. ingens* in Limpopo Province (FIG. 1A), and two isolates were obtained from diseased *E. tetragona* in Eastern Cape Province. The isolates from Limpopo Province were collected at three localities, the Capricorn Toll Plaza region, Last Post Private Game Reserve (Bandelierskop) and Euphorbia Drive at the National Game Breeding Centre in Mokopane. These isolates were obtained from *Cossonus* Clairville (Coleoptera: Curculionidae, Cossoninae) beetles as well as from discolored plant material found in the brown internal parts of succulent branches on diseased trees (FIG. 1B, C, D). The isolates from Eastern Cape Province were obtained from insect tunnels in dying stems of one tree in the Great Fish River Nature Reserve (Grahamstown).

Morphological characteristics.—Only the asexual states of *Gondwanamyces* were observed. These were characterized on MEA by light, abundant conidiophores with typical *Custingophora* morphology producing spore drops at the tops of erect, brown mononematous conidiophores. The cultures turned olivaceous buff (21‴d) with age and had no aerial mycelium. Isolates from Limpopo Province had longer conidiophores with a greater number of conidiogenous cells, producing shorter but wider conidia compared to those from Eastern Cape.

Growth studies.—Statistical analysis revealed highly significant differences between growth rates of the fungi at 10 C ($P = 0.022$, df = 6.22), 15 C ($P < 0.001$, df = 5.06), 20 C ($P < 0.001$, df = 8.00), 25 C ($P < 0.001$, df = 4.32) and 35 C ($P < 0.001$, df = 4.79). The fungi from Limpopo Province grew faster than those from Eastern Cape Province at 10–25 C and 35 C. Optimal growth was similar for the two groups, with the optimum temperature at 30 C.

DNA sequence analyses.—BLAST queries in GenBank supported initial identifications based on morphology, with sequences showing a high similarity to those of species of *Gondwanamyces*. The dataset consisted of 18 sequences of which four represented isolates from *E. ingens*, two from *E. tetragona* and 12 obtained from GenBank representing the previously described species of *Gondwanamyces* in the Microascales (TABLE I). Phylogenetic analysis revealed that these isolates clustered in two distinct groups, Group I including isolates from Limpopo and II including isolates from Eastern Cape Province (FIG. 2, TABLE II). These groups were also distinct from any previously described species of *Gondwanamyces*, suggesting that they represent novel species (TABLE III). The MP analysis (652 characters, 29% characters parsimony informative) generated one tree (TL = 645, CI = 0.859, RI = 0.885, RC = 0.760). In congruence with high bootstrap (BS) values the dataset was strongly supported statistically with values obtained from the Bayesian analysis (burn-in value: ITS 104) (TreeBase: http://purl.org/phylo/treebase/phylows/study/TB2:S11524).

FIG. 1. Symptoms of disease on *E. ingens* in the field and after inoculation with *Gondwanamyces serotectus*. A. Diseased *E. ingens* tree at the Capricorn Toll Plaza region in Limpopo Province showing graying and collapse of succulent branches (B) and (D) rotting and discoloration of the succulent branches on the exterior and internal core (C) insect tunnels in a succulent branch of *E. ingens*. E. Internal rot of succulent branch 6 wk after inoculation with *Gondwanamyces serotectus*. F. Control inoculation showing no internal rot.

TABLE I. Isolates used in phylogenetic analyses

Species[a]	Culture number[c]	Host	Location	Genbank accession number ITS
Ceratocytis fimbriata	CMW7765	*Eucalyptus* sp.	South Africa	DQ520635
Custingophora olivaceae	CBS335.68	Compost	Germany	AM267269
Gondwanamyces capensis	CMW1150	*Protea magnifica*	South Africa	EU660444
G. capensis	CMW1145	*P. coronata*	South Africa	EU660441
G. capensis	CMW978	*P. neriifolia*	South Africa	EU660443
G. capensis	CMW974	*P. coronata*	South Africa	EU660442
G. cecropiae	CCF3568	*Cecropia angustifolia*	Costa Rica	AM267266
G. cecropiae	CCF3565	*C. angustifolia*	Costa Rica	AM267267
G. proteae	CMW1042	*Protea repens*	South Africa	EU660436
G. proteae	CMW3757	*P. repens*	South Africa	EU660435
G. proteae	CMW1043	*P. repens*	South Africa	EU660434
G. scolytodis	CCF3569	*Cecropia angustifolia*	Costa Rica	AM267268
G. serotectus[a]	CMW36767	*Euphorbia ingens*	South Africa	JF947182
G. serotectus[a]	CMW34100	*E. ingens*	South Africa	JF947183
G. serotectus[ab]	CMW36768	*E. ingens*	South Africa	JF947184
G. serotectus[a]	CMW34101	*E. ingens*	South Africa	JF947185
G. ubusi[a]	CMW36769	*E. tetragona*	South Africa	JF947186
G. ubusi[ab]	CMW36770	*E. tetragona*	South Africa	JF947187

[a] Isolates collected and sequenced in this study.
[b] Ex-type strains.
[c] CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), South Africa; CBS = the Centraalbureau voor Schimmelcultures, the Netherlands; CCF = Culture Collection of Fungi, Czech Republic.

Pathogenicity tests.—Isolates of both groups produced lesions on healthy *E. ingens* branches. Lesions were found on the exterior of the succulent branches, in the cambium, and more significantly in the internal core of the succulent branches (FIG. 1E). Lesions were dark brown and circular on the exterior of the branch and extended upward and downward in the cambium from the points of inoculation. Lesions in the internal core represented an amorphous mass of rotten tissue. Control inoculations (FIG. 1F) produced only small external lesions (FIG. 5). Statistical analysis did not show significant differences in pathogenicity between groups with P values of 0.6380 (F ratio: 0.5707), 0.6627 (F ratio: 0.5329) and 0.4167 (F ratio: 0.9718) for the cambium lesions, internal lesion depths and internal lesion width data respectively (Degrees of freedom = 3). The calculated P values from the Bonferroni procedure were all significant with $P < 0.05$. Isolates of the same organisms used to inoculate the trees were re-isolated from the lesions on the inoculated trees. No fungi were isolated from the controls.

TAXONOMY

Based on differences in morphological characters and DNA sequence comparisons, the isolates clearly represent two undescribed species that phylogenetically belong in the genus *Gondwanamyces*. Although only the asexual states of these fungi were seen, the trend to provide a single name for fungi (Hawksworth 2005, McNeill et al. 2005) is followed here and we describe these fungi as species of *Gondwanamyces*.

Gondwanamyces serotectus van der Linde, Jol. Roux sp. nov. FIG. 3

MycoBank MB561550

Etymology: Name refers to the ability of the fungus to resist the poisonous latex of *E. ingens*.

Conidiophorae mononematae, macronematae in collo superiori cum usque ad 6 anfractibus, in superficie MEA pervulgatae, e rhizoideis 1–5 exorientes, cellulis conidiogenis 4–10 terminantes; etiam cum conidiophoris secondariis cellulas conidiogenas ferentibus. Cellulae conidiogenae cum colliculis distinctis, interdum cum cellulis conidiogenis secondariis inter sese colligatis, obovoideae olivaceo-brunneae. Conidia apice basique rotundata, oblonga hyalina non septata 8.4 × 4.2 μm.

Colonies olivaceous buff (21‴d) with age on MEA. Conidiophores sepia (17″m), mononematous, macronematous, apically sinuate up to six constrictions in the upper neck, abundant on the surface of MEA, arising from rhizoids, 1–5, (7.0–)11.4–23.4(–31.4) × (2.7–)5.0–8.1(–10.2) μm (average of 50 rhizoids 17.4 × 6.6 μm, l/w 2.6) and terminating in 4–10 conidiogenous cells, secondary conidiophores bearing conidiogenous cells, (49.2–)74.0–208.0(–345.0) × (5.6–)6.4–9.2(–11.8) μm (av. 50 conidiophores 141.0

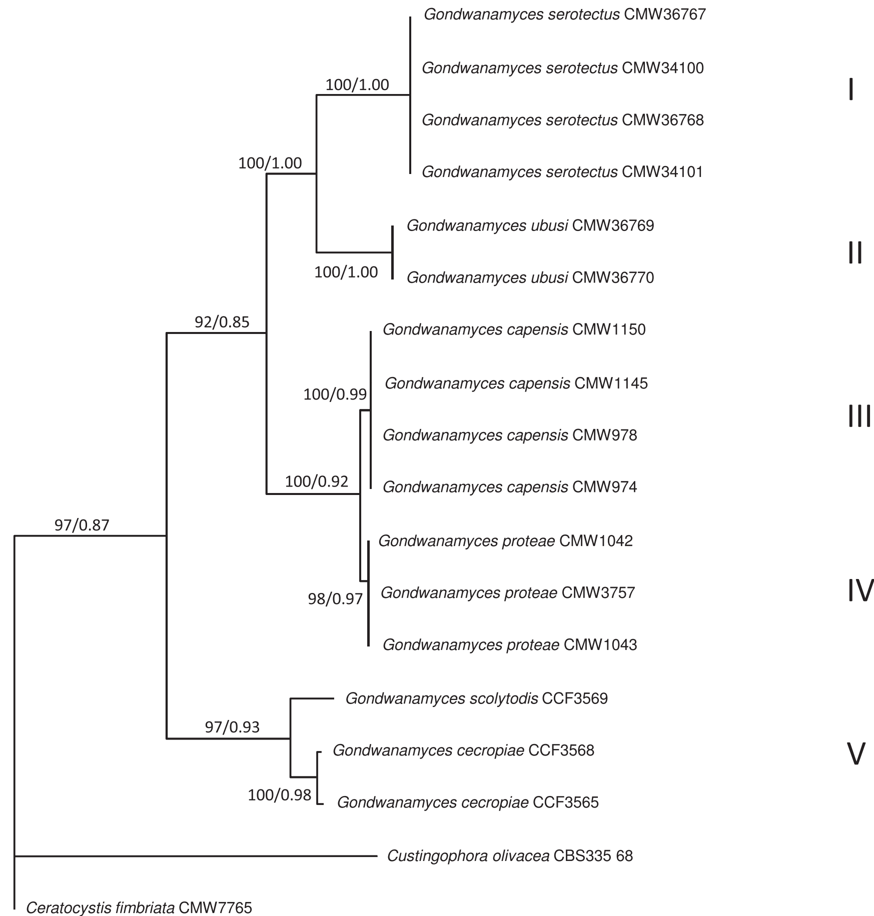

FIG. 2. The most parsimonious tree obtained from maximum parsimony analyses of ITS sequence data of the representative taxa of the genus *Gondwanamyces*. Numbers at the nodes indicate maximum likelihood bootstrap and Bayesian MCMC posterior probabilities.

× 7.8 μm, l/w 18.0) (FIG. 3A). Conidiogenous cells fawn (13‴) with distinct collarettes, occasionally giving rise to secondary conidiogenous cells, obovoid, (5.5–)7.2–10.6(–14.1) × (2.6–)3.0–4.0(–4.5) μm (av. 50 conidiogenous cells 8.9 × 3.5 μm, l/w 2.6) (FIG. 3D, E). Conidia vinaceous buff (17‴d), rounded at base and apex, oblong, hyaline, aspetate, (5.9–)7.4–9.5(–10.6) × (2.9–)3.7–4.7(–5.3) μm (av. 50 conidia 8.4 × 4.2 μm, l/w 2.0) (FIG. 3B, C). Optimum temperature for growth 30 C, growing at 9.5 mm/d, minimum growth 10 C and maximum growth at 35 C.

HOLOTYPE. SOUTH AFRICA, LIMPOPO PROVINCE: Last Post (S23 17.738 E29 55.467, 900–1000 m) isolated from *Cossonus* on diseased *Euphorbia ingens*, May 2009, van der Linde JA and Roux J, holotype (PREM 60566), dry culture on MEA CMW 36767 = CBS 129738; ex-type culture CMW 36768 = CBS 129739 (PREM 60567). Teleomorph not observed.

Additional specimens examined. SOUTH AFRICA, LIMPOPO PROVINCE: Last Post and Capricorn (S23 21.910 E29 44.621, 1000–1100 m) isolated from *Cossonus* on diseased *E. ingens*, May 2009, van der Linde JA and Roux J, CMW 34100 (CBS = 129740), CMW 34101 (CBS = 129741). Teleomorph not observed.

Gondwanamyces ubusi van der Linde, Jol. Roux sp. nov. FIG. 4

MycoBank MB561551

Etymology: Name derived from the Xhosa word for honey reflecting the fact that the host is commonly known as the honey tree.

Conidiophorae mononematae, macronematae in collo superiori cum usque ad 7 anfractibus, in superficie MEA pervulgatae, e rhizoideis 1–3 exorientes, cellulis conidiogenis 3–7 terminantes; etiam cum conidiophoris secondariis cellulas conidiogenas ferentibus. Cellulae conidiogenae cum colliculis distinctis, interdum cum cellulis conidiogenis secondariis inter sese colligatis, obovoideae olivaceo-brunneae. Conidia apice basique rotundata, oblonga hyalina non septata 10.0 × 3.5 μm.

Colonies olivaceous buff (21‴d) with age on MEA. Conidiophores sepia (17″m), mononematous, macronematous, sinuous at apex with up to seven constrictions (sinuae) in the upper part, abundant on the

TABLE II. Fixed base pair differences, and their positions, between *G. serotectus* and *G. ubusi* isolates

Identity	Culture number	ITS																											
		52	53	55	56	57	64	65	83	92	98	133	158	172	176	188	189	190	192	219	228	229	424	427	458	462	466	468	484
Gondwanamyces serotectus	CMW36767	—	C	G	A	T	G	A	T	—	T	—	C	T	A	A	C	T	G	A	—	—	C	T	T	G	T	C	C
G. serotectus	CMW34100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
G. serotectus	CMW36768	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
G. serotectus	CMW34101	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gondwanamyces ubusi	CMW36769	C	G	A	T	A	A	G	C	T	C	A	T	A	G	C	T	A	A	C	A	A	T	A	G	T	C	G	A
G. ubusi	CMW36700	C	G	A	T	A	A	G	C	T	C	A	T	A	G	C	T	A	A	C	A	A	T	A	G	T	C	G	A

TABLE III. Number of fixed base pair differences between species of *Gondwanamyces*

	G. serotectus	*G. ubusi*	*G. capensis*	*G. proteae*	*G. scolytodis*	*G. cecropiae* (CCF3568)	*G. cecropiae* (CCF3565)
G. serotectus	0	19	53	56	85	92	78
G. ubusi	19	0	54	53	70	77	72
G. capensis	53	54	0	9	72	74	69
G. proteae	56	53	9	0	67	70	65
G. scolytodis	85	70	72	67	0	38	35
G. cecropiae (CCF3568)	96	77	74	70	38	0	2
G. cecropiae (CCF3565)	94	72	69	65	35	2	0

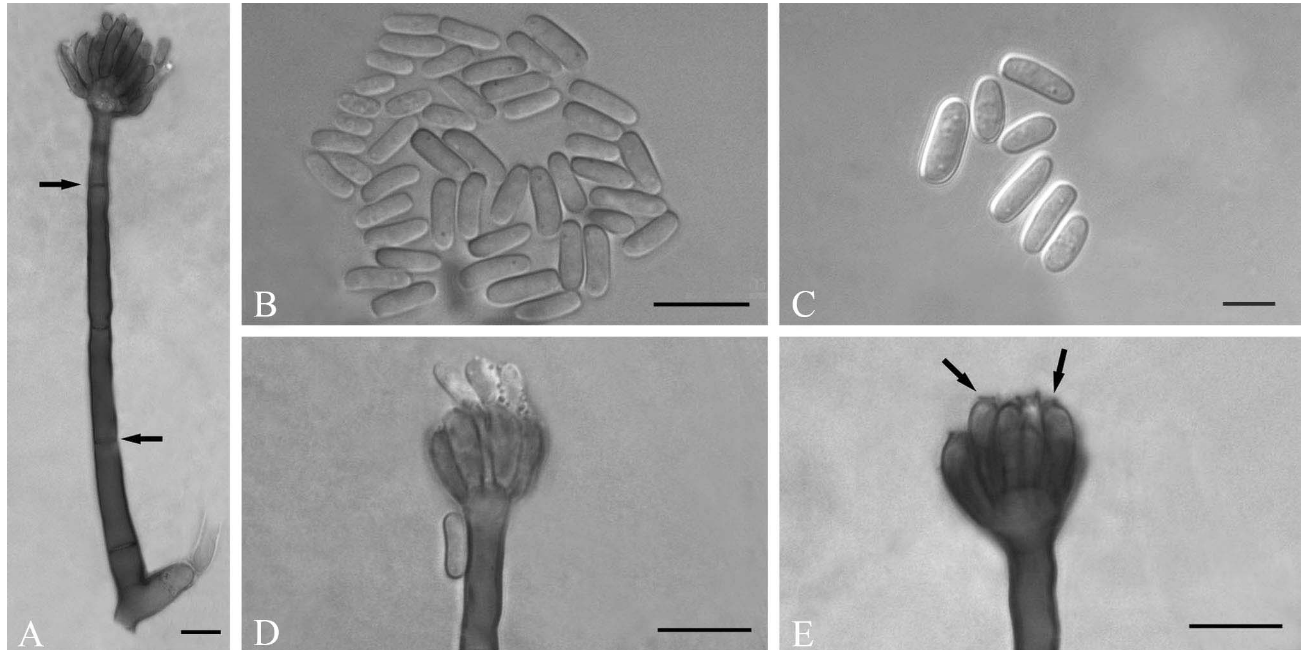

FIG. 3. *Gondwanamyces serotectus.* A. Conidiophore showing foot cell and sinuate stipe. B. Obovate conidia. C. Conidia of variable size. D. Conidiophore with phialidic conidiogenous cells and newly produced conidia. E. Conidiophore with conidiogenous cells showing phialides with distinct collarettes. Bars: A, B, D, E = 10 μm; C = 5 μm.

surface of agar, one to three rhizoids, (10.3–)13.7–28.1(–38.6) × (4.0–)5.2–8.2(–11.0) μm (av. 50 rhizoids 20.9 × 6.7 μm, l/w 3.1) at base and terminating in 3–7 conidiogenous cells, (11.0–)69.8–146.0 (–181.0) × (4.5–)5.6–8.1(–9.5) μm (av. 50 conidiophores 108.0 × 6.8 μm, l/w 15.9) (FIG. 4A). Conidiogenous cells fawn (13‴), terminating in distinct collarettes, occasionally with secondary conidiogenous

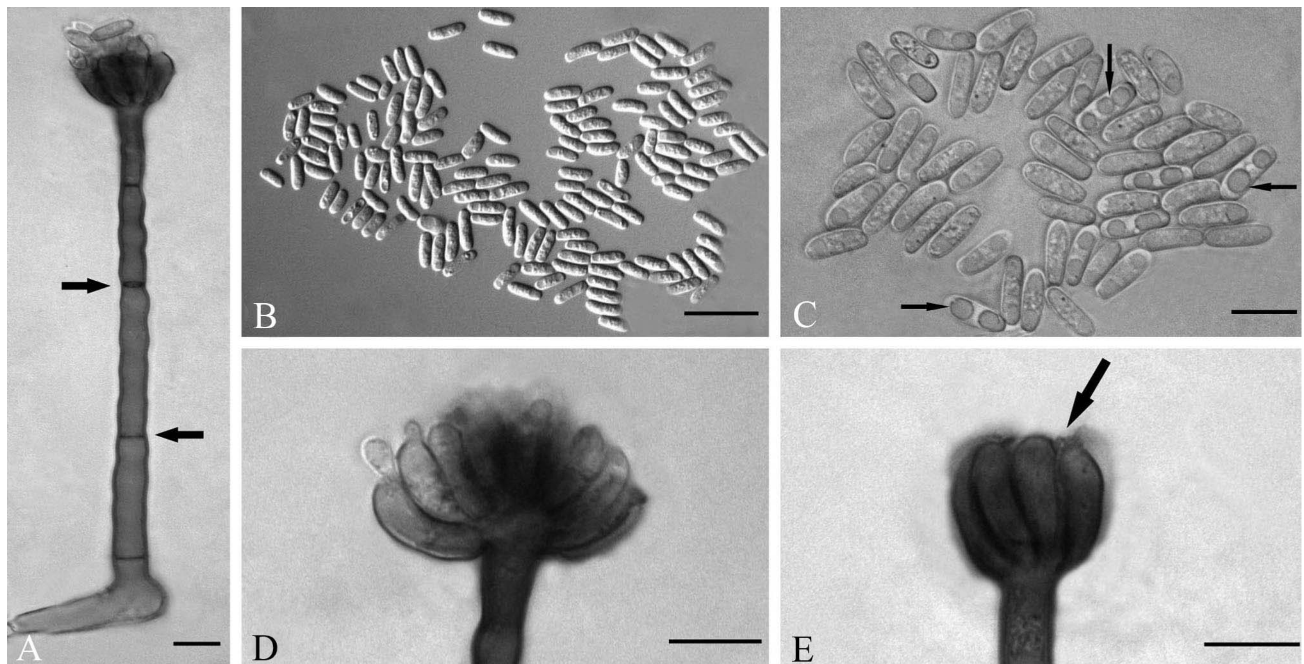

FIG. 4. *Gondwanamyces ubusi.* A. Conidiophore showing foot cell and sinuate stipe. B. Obovate conidia. C. Conidia with distinct guttules. D. Conidiophore with conidiogenous cells and newly produced conidia. E. Conidiophore with phialidic conidiogenous cells. Bars: A, C, D, E = 10 μm; B = 20 μm.

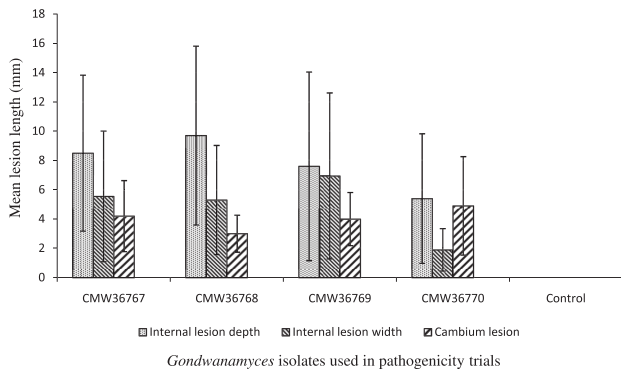

FIG. 5. Histogram of mean lesion lengths (mm) resulting from inoculations with two isolates of *G. serotectus* (CMW36767, CMW36768) and *G. ubusi* (CMW36769, CMW36770) used in the *E. ingens* pathogenicity trails. Bars indicate 95% confidence limits for each isolate.

cells, obovoid, (8.4–)10.1–12.5(–13.4) × (2.3–)3.2–4.5(–5.1) μm (av. 50 conidiogenous cells 11.3 × 3.9 μm, l/w 2.9) (FIG. 4D, E). Conidia vinaceous buff (17‴d), rounded at the base and apex, oblong, hyaline and aspetate, (5.6–)8.5–11.5(–13.6) × (1.7–)2.9–4.0(–4.7) μm (av. 50 conidia 10.0 × 3.5 μm, l/w 2.9) (FIG. 4B, C). Optimum for growth 30 C, growing at 9.5 mm/d, minimum growth at 10 C and maximum growth at 35 C.

HOLOTYPE. SOUTH AFRICA, EASTERN CAPE PROVINCE: Great Fish River Nature Reserve (S33 1.764 E26 48.702), isolated from insect tunnels on diseased *Euphorbia tetragona*, Mar 2010, Roux J, holotype (PREM 60568), a dry culture on MEA CMW 36769 = CBS 129742; ex-type culture CMW 36770 = CBS 129743 (PREM 60569). Teleomorph not observed.

G. serotectus and *G. ubusi* were identified based on a combination of DNA sequence data and morphology. Previous studies of *Gondwanamyces* using the ITS region of the nuclear DNA and this, together with differences in morphology (TABLE IV), provided robust evidence for the unique nature of the fungi from *Euphorbia* species. *G. ubusi* and *G. serotectus* are quite different morphologically and relatively easy to distinguish. The latter species has longer conidiophores with a greater number of rhizoids, which are shorter than those of *G. ubusi*. *G. serotectus*, also produces shorter conidia with a greater number of conidiogenous cells than those of *G. ubusi*. *G. serotectus* has conidiophores that proliferate, giving rise to new conidiophores at a higher level, a characteristic never seen in *G. ubusi*.

Nomenclatural changes.—Based on DNA sequence data from previous studies and the results of this study, most species of *Custingophora* and the previously described species of *Knoxdavesia* represent anamorph states of *Gondwanamyces* in the order Microascales. A single nomenclature for this group of fungi is presented, giving precedence to the teleomorph name. For that reason *Custingophora cecropiae* is transferred herein to *Gondwanamyces*. Even though *Custingophora olivaceae* shares similar morphological characters with *Gondwanamyces* anamorphs, this fungus appears to represent a distinct genus based on DNA sequence phylogeny and it is not placed in the *Gondwanamyces*.

Gondwanamyces Marais & M.J. Wingf., Mycologia 90, 1998

Globose to subglobose black ascomatal bases with long necks that tapers toward the apexes, ending in ostiolar hyphae. Asci evanescent, ascospores hyaline, aseptate, with or without sheaths (Réblová et al. 2011).

Gondwanamyces cecropiae (M. Kolařík) van der Linde, Jol. Roux & M.J. Wingf. comb. nov. PREM 858087. Basionym: *Custingophora cecropiae* M. Kolařík, Fungal Biol 1:113. 2009.

DISCUSSION

This study provides new insights into the distribution, species diversity and host range of the unusual and ecologically intriguing (Roets et al. 2009a, b) genus *Gondwanamyces*. Previously only two species of *Gondwanamyces* were known from South Africa, *G. proteae* and *G. capensis* (Wingfield et al. 1988, Wingfield and van Wyk 1993), both from Western Cape Province. These species were restricted to insect-colonized infructescences of *Proteae* species (Wingfield et al. 1988, Wingfield and van Wyk 1993, Roets et al. 2009a). The discovery of *G. cecropiae* (previously *C. cecropiae*) and *G. scolytodis* associated with *C. angustifolia* in Costa Rica (Kolařík and Hulcr 2009) showed for the first time that these fungi are not restricted to South Africa although they remain restricted to the Gondwana region. In this study two additional *Gondwanamyces* species were found on *E. ingens* and *E. tetragona* in South Africa. These results suggest that *Gondwanamyces* is more widespread, at least in the Gondwana region, than originally thought.

G. serotectus was isolated from discolored plant material as well as from the bodies of the weevil *Cossonus*. This insect has not been reported previously from *E. ingens* and was described in 1798 by the Swiss entomologist Joseph Philippe de Clairville. There is no clear understanding of its origin or global distribution (www.itis.gov). The association of a *Gondwanamyces* sp. with insects is not unexpected given that *G. proteae* and

TABLE IV. Morphological differences between *G. serotectus*, *G. ubusi* and their closest neighbours *G. capensis* and *G. proteae*

	Conidiophore	Conidiogenous cells	No. of conidiogenous cells	Conidia	Rhizoids	Reference
G. serotectus	141.0 × 7.8 µm, l/w 18.0	8.9 × 3.5 µm, l/w 2.6	4–10	8.4 × 4.2 µm, l/w 2.0	17.4 × 6.6 µm, l/w 2.6	This study
G. ubusi	108.0 × 6.8 µm, l/w 15.9	11.3 × 3.9 µm, l/w 2.9	3–7	10.0 × 3.5 µm, l/w 2.9	20.9 × 6.7 µm, l/w 3.1	This study
G. capensis	65.6 × 3.9 µm, l/w 16.8	9.5 × 5.3 µm, l/w 1.8	5–12	5.0 × 3.8 µm, l/w 1.3	no information available	Wingfield & Van Wyk 1993
G. proteae	132.0 × 8.0 µm, l/w 16.5	9.0 × 5.0 µm, l/w 1.8	7–12	5.0 × 3.0 µm, l/w 1.7	13.0 × 5.0 µm, l/w 2.6	Wingfield et al. 1988

G. capensis occur in insect-infested *Protea* infructescences and initially were thought to be vectored by one or more of the insects in this niche (Wingfield et al. 1988). Likewise *G. cecropiae* was isolated from the body of *S. unipunctatus* and *G. scolytodis* from galleries in the sapwood of *C. angustifolia* (Kolařík and Hulcr 2009).

Various ophiostomatoid fungi have been shown to be closely associated with mites, phoretic on the beetles that originally were thought to be the primary vectors of the fungi (Moser 1985, Klepzig et al. 2001). Intriguingly the *Ophiostoma* species that are found in *Protea* infructecenses (Wingfield and van Wyk 1993, Marais et al. 2001, Roets et al. 2009a) have been shown to be primarily vectored by mites (Roets et al. 2007, 2009b) and recently the same has been shown for *G. proteae* (Roets et al. 2011). Thus, it is likely that the *Gondwanamyces* species described in this study are vectored by mites that are associated with insects that infest *Euphorbia* species. Further studies will be required to resolve this interesting question.

This study presents the first evaluation of the pathogenicity of *Gondwanamyces* species on any plant. The *E. ingens* trees inoculated were growing in North West Province where these trees seem to be more healthy compared to those in Limpopo Province. Both newly described *Gondwanamyces* species produced lesions on healthy succulent branches in contrast to the control inoculation in which internal lesion development and discoloration was absent. This suggests that these fungi and the insects that carry them could play a role in the decline of *E. ingens* in Limpopo Province. Great variation in lesion length was found for the two species described in this study, which could be attributed to genotypic differences among individual *E. ingens* trees. Additional inoculations on both *E. tetragona* and *E. ingens* as well as other *Euphorbia* spp. in other regions of the country should be carried out to understand this relationship more clearly. Furthermore it appears that this fungus-insect-host interaction might be linked to an environmental and/or an anthropogenic trigger that has initiated the sudden and severe decline of these trees.

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