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New records of the Cryphonectriaceae from southern Africa including *Latruncellus aurorae* gen. sp. nov.

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Abstract: The Cryphonectriaceae accommodates some of the world's most important tree pathogens, including four genera known from native and introduced Myrtales in Africa. Surveys in the past 3 y in southern Africa have led to the discovery of cankers with fruiting structures resembling those of the Cryphonectriaceae on trees in the Myrtales in Namibia, South Africa, Swaziland and Zambia. These fungi were identified with morphological characteristics and DNA sequence data. For the first time we report *Chrysoporthe austroafricana* from Namibia and on *Syzygium guineense* and *Holocryphia eucalypti* in Swaziland on a *Eucalyptus grandis* clone. The host and geographic ranges of *Celoporthe dispersa* are expanded to include *S. legatti* in South Africa and *S. guineense* in Zambia. In addition a monotypic genus, *Latruncellus aurorae* gen. sp. nov., is described from *Galpinia transvaalica* (Lythraceae, Myrtales) in Swaziland. The present and other recent studies clearly emphasize the limited understanding of the diversity and distribution of fungi in the Cryphonectriaceae in Africa.

Key words: canker pathogens, *Celoporthe*, *Chrysoporthe*, *Galpinia transvaalica*, *Holocryphia*, Myrtales, tree diseases

INTRODUCTION

The Cryphonectriaceae includes fungi previously treated broadly in the *Cryphonectria-Endothia* complex (Gryzenhout et al. 2009) and currently accommodates 12 genera (Gryzenhout et al. 2009, 2010a; Begoude et al. 2010; Chungu et al. 2010). The family includes important tree pathogens, such as *Chrysoporthe austroafricana* Gryzenh. & M.J. Wingf., *Chr. cubensis* (Bruner) Gryzenh. & M.J. Wingf., *Cryphonectria parasitica* (Murrill) M.E. Barr and *Endothia gyrosa* (Schwein. : Fr.) Fr. (Gryzenhout et al. 2009). Of these the best known is *C. parasitica*, the causal agent of chestnut blight that devastated native populations of chestnut trees (*Castanea dentata* [Marsh.] Borkh. and *Castanea sativa* Mill. [Fagaceae]) in North America and Europe (Anagnostakis 1987, Heiniger and Rigling 1994). *Chrysoporthe austroafricana* and *Chr. cubensis* are important canker pathogens of plantation-grown *Eucalyptus* trees, resulting in considerable economic losses to forestry enterprises in Africa and South America (Hodges et al. 1979, Wingfield 2003). In Africa four genera of the Cryphonectriaceae are known, including *Aurifilum* (Begoude et al. 2010), *Celoporthe* (Nakabonge et al. 2006a), *Chrysoporthe* (Gryzenhout et al. 2004) and *Holocryphia* (Gryzenhout et al. 2006a), all of which include species that are tree pathogens.

Species of *Chrysoporthe* have a wide distribution in tropical and subtropical areas of the world (Gryzenhout et al. 2009). Five species are known in Africa, including *Chr. cubensis*, *Chr. austroafricana* (Gryzenhout et al. 2009), *Chr. deuterocubensis* Gryzenh. & M.J. Wingf. (Van der Merwe et al. 2011), *Chr. syzygiicola* Chungu, Gryzenh. & Jol. Roux and *Chr. zambiensis* Chungu, Gryzenh. & Jol. Roux (Chungu et al. 2010). *Chrysoporthe cubensis* is known from Cameroon (Gibson 1981), the Democratic Republic of Congo (Hodges et al. 1986), and Ghana (Roux and Apetorgbor 2009), while *Chr. deuterocubensis* is known from Kenya, Malawi, Mozambique (Nakabonge et al. 2006b) and Republic of Congo (Roux et al. 2003). *Chrysoporthe austroafricana* has been found only in eastern and southern African countries including Malawi, Mozambique, South Africa and Zambia (Nakabonge et al. 2006b), while *Chr. syzygiicola* and *Chr. zambiensis* were discribed recently as new species from Zambia (Chungu et al. 2010). Hosts of these species include native and introduced trees in the Myrtales, namely *Eucalyptus* spp. (Myrtaceae), *Syzygium* spp. (Myrtaceae) and *Tibouchina granulosa* Cogn. ex Britton (Melastomataceae) (Gryzenhout et al. 2009, Chungu et al. 2010).

Chr. cubensis and *Chr. deuterocubensis* are considered to have been introduced respectively into Africa from South America and southeastern Asia (Wingfield 2003; Nakabonge et al. 2006b, 2007; Van der Merwe 2011). In contrast the widespread presence of *Chr. austroafricana* on native hosts and their absence

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from other continents suggests that *Chr. austroafricana* is an African fungus (Heath et al. 2006, Nakabonge et al. 2006b).

Holocryphia eucalypti (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf. is a stress-associated pathogen of *Eucalyptus* spp. and is of relatively minor importance in South Africa (van der Westhuizen et al. 1993, Gryzenhout et al. 2003). In Australia, however, it has been reported as a pathogen of some concern, resulting in death of trees (Yuan and Mohammed 2000). Its only other known host is *Tibouchina urvilleana* (DC.) Cogn. (Melastomataceae) (Heath et al. 2007), and it evidently has undergone a host shift (Slippers et al. 2005) to this tree. Although previously known only from Australia and South Africa, it recently was reported from *E. grandis* Hill. ex Maid. in Uganda (Roux and Nakabonge 2009) and *Eucalyptus* spp. in New Zealand (Gryzenhout et al. 2010b). *Holocryphia eucalypti* is considered to have been introduced into South Africa because the South African population has little genetic diversity compared to that of Australian populations (Nakabonge et al. 2007).

Aurifilum marmelostoma Begoude, Gryzenh. & Jol. Roux (Begoude et al. 2010) and *Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf. (Nakabonge et al. 2006a) are two recently described monotypic genera in the Cryphonectriaceae that occur in Africa. *Celoporthe dispersa* has been reported only from South Africa, where it infects native *Heteropyxis canescens* Oliv. (Heteropyxidaceae, Myrtales) and *S. cordatum* trees and non-native *T. granulosa* trees (Nakabonge et al. 2006a). *Celoporthe dispersa* is thought to be native to southern Africa (Nakabonge et al. 2006a). *Aurifilum marmelostoma* was described from cankers on native *Terminalia ivorensis* (Combretaceae, Myrtales) A. Chev. and non-native *T. mantaly* H. Perrier in Cameroon (Begoude et al. 2010).

The economic importance of fungi in the Cryphonectriaceae, their ecological impact, seemingly wide host ranges in the Myrtales and their ability to cross infect trees (Gryzenhout et al. 2009) make these fungi important to plantation industries as well as to biodiversity and conservation programs in Africa. The aim of this study was to expand on surveys for the Cryphonectriaceae on Myrtales in southern Africa and to improve the base of knowledge regarding their species diversity and distribution in the subregion.

MATERIALS AND METHODS

Fungal isolates.—Native and non-native trees belonging to the Myrtales, especially trees from which members of the Cryphonectriaceae have been reported (Gryzenhout et al. 2009), were inspected for stem cankers and fruiting bodies resembling those of the Cryphonectriaceae. Surveys were conducted 2006–2008 in four southern African countries (Namibia, South Africa, Swaziland and Zambia) where scientific collaborations have been established to promote the study of plantation forestry diseases. Isolations were made from pieces of bark bearing fruiting structures resembling those of the Cryphonectriaceae with techniques described by Gryzenhout et al. (2009). Resulting cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (TABLE I) and duplicates of isolates representing a new genus were deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (TABLE I). Herbarium specimens of fruiting structures on bark representing the new genus were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa (TABLE I).

DNA sequence comparisons.—DNA was extracted from the mycelium of cultures grown on 2% malt extract agar (MEA). Mycelium was scraped from the surfaces of the MEA plates and freeze dried. Freeze-dried mycelium was ground to a fine powder with 2 mm diam metal beads in a Retsch cell disrupter (Retsch Gmbh, Germany) after which the protocol described by Möller et al. (1992) was followed for DNA extraction. DNA concentrations were determined on a NanoDrop 3.1.0 (ND-1000 uv/Vis spectrometer, NanoDrop Technologies, Wilmington, Delaware).

Sequences for two gene regions, shown as appropriate to identify species in the Cryphonectriaceae (Gryzenhout et al. 2009), were amplified with the polymerase chain reaction (PCR). The internal transcribed spacer (ITS) regions (ITS1, ITS2) and the conserved 5.8S gene of the ribosomal RNA operon were amplified with the primer pair ITS1 and ITS4 (White et al. 1990). The β-tubulin 1 and β-tubulin 2 regions of the β-tubulin gene (BT) were amplified respectively with primer pairs BT1a, BT1b and BT2a, BT2b (Glass and Donaldson 1995). A third gene region, a part of the large subunit (LSU) nuclear ribosomal DNA shown to be appropriate to differentiate between genera in the Cryphonectriaceae, was also used. The LSU region was amplified with primer pairs LR0R and LR7 (Vilgalys and Hester 1990, Rehner and Samuels 1994). Reactions for ITS and BT were performed in a total volume of 25 µL composed of 40–60 ng DNA template, 0.25 µL (0.5 µM) each primer, 2.5 µL dNTPs (0.2 mM each dNTP), 0.25 µL (0.5 U) super-therm polymerase Taq, 2.5 µL (10×) dilution buffer, 2.5 µL $MgCl_2$ (Southern Cross Biotechnology, Cape Town, South Africa) and sterile distilled water (12.25 µL). LSU reactions were performed in a total volume of 50 µL as described by Castleburg et al. (2002). PCR reactions were carried out on a thermal cycler (Master Cycle®, Perkin Elmer Corp., Massachusetts). The program included an initial denaturation step at 94 C for 3 min, followed by 40 amplification cycles consisting of 30 s at 94 C, 45 s annealing at 55 C for ITS, BT 1 and LSU, 65 C for BT 2 and 1 min at 72 C, followed by a final step of 4 min at 72 C. PCR products were viewed with UV light on 1% agarose gels containing ethidium bromide. PCR products were cleaned with 0.06 g/mL Sephadex G-50 (Sigma-Aldrich, Amersham

TABLE I. Isolates for sequences generated in this study[1]

Identity	Isolate number CMW [a]	CBS [b]	PREM [c]	Host	Country and region	Collector	GenBank accession numbers [d]
Aurifilum marmelostoma	28285	124929	60257	*T. mantaly*	Cameroon, Centre	D. Begoude	HQ171215[4]
Aurifilum marmelostoma	28288	124930		*T. ivorensis*	Cameroon, Centre	D. Begoude	HQ171216[4]
Celoporthe dispersa	29378			*S. legatti*	South Africa, Limpopo	J Roux, M Vermeulen	GU726942[2], GU726954[3]
Celoporthe dispersa	29898			*S. legatti*	South Africa, Limpopo	J Roux, M Vermeulen	GU726943[2], GU726955[3]
Celoporthe dispersa	29900			*S. legatti*	South Africa, Limpopo	J Roux, M Vermeulen	GU726944[2], GU726956[3]
Celoporthe dispersa	29375			*S. guineense*	Zambia, Copperbelt	J Roux, M Vermeulen	GU726940[2], GU726952[3]
Celoporthe dispersa	29376			*S. guineense*	Zambia, Copperbelt	J Roux, M Vermeulen	GU726941[2], GU726953[3]
Celoporthe dispersa	29905			*S. cordatum*	South Africa, Limpopo	J Roux, M Vermeulen	GU726945[2], GU726957[3]
Chrysoporthe austroafricana	22760			*S. guineense*	Namibia, Caprivi	J Roux	GU726949[2], GU726961[3]
Chrysoporthe austroafricana	22751			*S. guineense*	Namibia, Caprivi	J Roux	GU726948[2], GU726960[3]
Chrysoporthe austroafricana	32954			*S. guineense*	South Africa, Limpopo	J Roux, M Vermeulen	GU726951[2], GU726963[3]
Chrysoporthe austroafricana	29904			*S. guineense*	South Africa, Limpopo	J Roux, M Vermeulen	GU726950[2], GU726962[3]
Cryptometrion aestuescens	18790	124008	60249		Indonesia	MJ Wingfield	HQ171211[4]
Cryptometrion aestuescens	18793	124007			Indonesia	MJ Wingfield	HQ171212[4]
Holocryphia eucalypti	11690			*E. grandis* clone	Swaziland	J Roux	GU726938[2], GU726936[3]
Holocryphia eucalypti	11689			*E. grandis* clone	Swaziland	J Roux	GU726939[2], GU726937[3]
Latruncellus aurorae	28274	124904	60349	*G. transvaalica*	Swaziland	J. Roux	GU726946[2], GU726958[3], HQ171213[4]
Latruncellus aurorae	28275			*G. transvaalica*	Swaziland	J. Roux	HQ171209[2], HQ171207[3], HQ171214[4]
Latruncellus aurorae	28276[1]	125526	60348	*G. transvaalica*	Swaziland	J. Roux	GU726947[2], GU726959[3]
Latruncellus aurorae	30633[1]	124905	60347	*G. transvaalica*	Swaziland	J. Roux	HQ171210[2], HQ171208[3]

[a] Culture collection of Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
[b] CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.
[c] PREM, Agricultural Research Council, Pretoria, South Africa.
[d] GeneBank accession numbers for sequence data of the ITS[2] (primers ITS1/2), BT[3] 1 and 2 (primers Bt1a/1b and Bt21/2b) and LSU[4] (primers LR0R and LR7).

Biosciences Ltd., Sweden) according to the manufacturer's instructions.

DNA fragments were sequenced with the same primer pairs used in the PCR amplification reactions. Sequencing reactions were performed as described by Begoude et al. (2010). The products were sequenced in both directions with the Big Dye Cycle Sequencing Kit (Applied Biosystems, Foster City, California) on an ABI Prism™ 3100 DNA sequencer (Applied Biosystems). Sequences were viewed and edited with Vector NTI Advanced 10 (Vector NTI® Software, 1600 Faraday Avenue, Carlsbad, California 92008). Sequences were aligned with those published for fungi in the Cryphonectriaceae (Gryzenhout et al., 2009, 2010a, b; Begoude et al. 2010) with the Web interface (http://timpani.genome.adjp/%7Emafft/server/) of the alignment program MAFFT 5.8 (Katoh et al. 2002). The alignments were deposited at TreeBase (www.treebase.org) (submission No. 10804).

Phylogenetic analyses were performed with the software package Phylogenetic Analysis Using Parsimony (PAUP) 4.01b10 (Swofford 2000). Phylogenetic analyses were done for each gene region separately with maximum parsimony (MP) (heuristic search with 100 random sequence additions). A 1000-replicate partition homogeneity test (PHT) was performed to examine the null hypotheses that the BT and ITS gene datasets were homologous and could be combined for further analyses (Farris et al. 1994). This was done after the exclusion of uninformative sites with a heuristic search with 100 random sequence additions, tree bisection-reconnection (TBR) branch swapping and MAXTREES set to 5000 to allow completion of analysis. A 1000-bootstrap replication was performed to determine the support of branches for the most parsimonious tree for datasets representing each gene region (Felsenstein 1985). *Diaporthe ambigua* Nitschke was used as outgroup taxon (Gryzenhout et al. 2009). The same analyses were repeated (PHT MAXTREES set to auto increase) for the conserved BT exon data supplemented with DNA sequences of the LSU (Gryzenhout et al. 2009) to ascertain placement of genera within the greater Cryphonectriaceae. The LSU and BT exon dataset was midpoint rooted.

Additional phylogenetic analyses were conducted based on maximum likelihood (ML) and Bayesian analyses for the combined data of the ITS and BT gene regions and for the LSU and BT exon gene regions respectively. The correct model for the datasets was identified with jModeltest 0.0.1 (Posada 2008). The TrN + I + G model (Tamura and Nei 1993) was shown to be appropriate for the ITS and BT dataset and the TIM2 + I + G model (Posada 2008) for the LSU and BT exon dataset. Maximum likelihood analyses were performed with PhyML 3 (Guindon and Gascuel 2003). A 1000 replicate bootstrap analysis was done to assess the confidence levels of the branch nodes in the phylogenetic trees. The Bayesian analyses were performed on the two datasets with the Markov chain Monte Carlo (MCMC) algorithm in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The number of generations was set to 3 000 000, and sample frequency set to 100. Burn-in was at 8000 for the ITS and BT dataset and 6000 for the LSU and BT exon dataset.

Morphology.—Fruiting structures representing an apparently undescribed species in the Cryphonectriaceae on original bark of *Galpinia transvaalica* N.E. Brown were cut from the bark under a dissection microscope and its morphology studied with methods described by Gryzenhout et al. (2009). Fifty measurements of asci, ascospores, conidia, conidiophores and conidiogenous cells were taken from the holotype specimen (PREM 60348) and 20 measurements from other specimens (PREM 60349, PREM 60347). Measurements are presented as (min–)average − SD–average + SD(–max) mm. Digital images were captured with a HRc Axiocam digital camera, and measurements were computed with Axiovision 3.1 software (Carl Zeiss Ltd., Germany). Characteristics of fruiting bodies were compared with those of other genera and species in the Cryphonectriaceae (Gryzenhout et al. 2009, 2010a; Begoude et al. 2010; Chungu et al. 2010).

Cultural characteristics of the apparently undescribed species were determined on two representative isolates (CMW28276, CMW30633). Disks were taken from the edges of actively growing cultures on 2% MEA and transferred to the centers of 90 mm Petri dishes containing 2% MEA. Five plates per isolate were placed in the dark in incubators set at 15–35 C at five degree intervals. Two measurements of diameter, perpendicular to each other, were taken of each culture until the fastest growing culture had covered the surface of the plate. Growth data were analyzed in Microsoft Excel to determine the optimum temperature for growth. Colony colors were described with the charts of Rayner (1970).

Pathogenicity tests.—Pathogenicity experiments were conducted with two isolates (CMW28276, CMW30633) from *G. transvaalica* on 10 *G. transvaalica* trees and 10 2 y old plants of the *Eucalyptus* (species) clone ZG14, which had been shown to be highly susceptible to infection by both *Cel. dispersa* and *Chr. austroafricana* (van Heerden and Wingfield 2001, Nakabonge et al. 2006a). Trees were maintained at 25 C in a greenhouse under natural day/night conditions for 2 wk to acclimatize, after which they were inoculated. For inoculations the bark was removed from trees with a 5 mm cork borer and a plug of mycelium was placed into the wound with the mycelium facing the cambium. Wounds were sealed with a strip of Parafilm to prevent desiccation and cross contamination.

Six weeks after inoculation the bark associated with the inoculation sites was removed and the lengths of lesions on the cambium were measured. Pieces of necrotic tissue were transferred to MEA to re-isolate the inoculated fungus. Variation in lesion lengths was assessed in Excel, using one-way analysis of variance (ANOVA) with a 95% level of significance.

RESULTS

Fungal isolates.—Fruiting structures resembling those of the Cryphonectriaceae were found on *G. transvaalica* (FIG. 1a–c) and an *E. grandis* clone in Swaziland, *S. guineense* and *S. legatti* in South Africa, *S. guineense* in the Caprivi region of Namibia (Katima

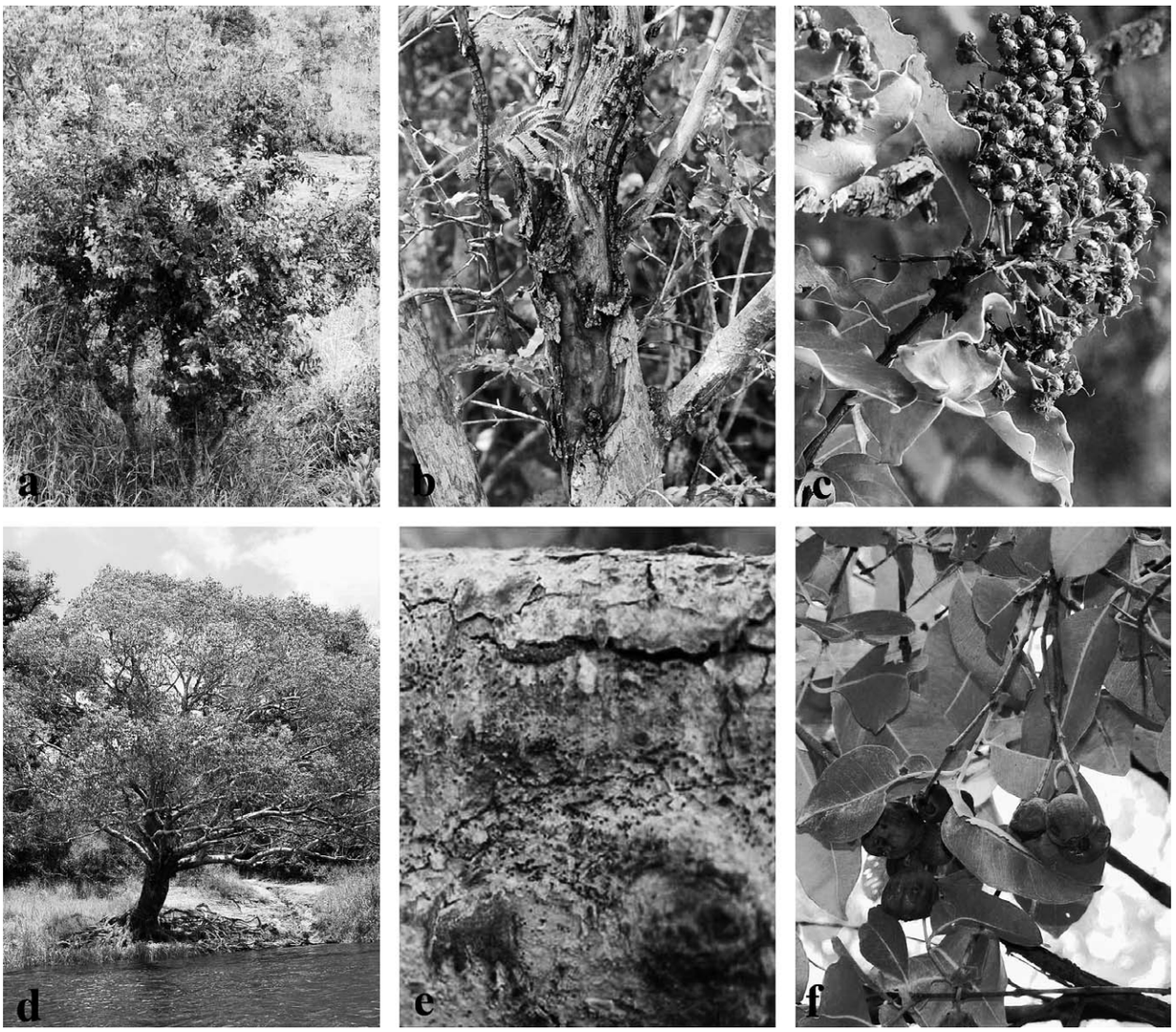

FIG. 1. Hosts and symptoms associated with infection by Cryphonectriaceae. a. *Galpinia transvaalica*. b. Canker on *G. transvaalica*. c. Berries of *G. transvaalica*. d. *Syzygium guineense*. e. Canker with fruiting structures of *Chrysoporthe austroafricana* on a root of *S. guineense* in Katima Mulilo. f. Berries of *S. guineense*.

Mulilo and Popa Falls) and *S. guineense* in Zambia (FIG. 1d–f). In Katima Mulilo infections were found on the roots (FIG. 1d–e) of *S. guineense* trees that are submerged by the Zambezi River at least 2 mo each year, as well as on branch stubs and stem cankers on these trees. Infections on *S. guineense* in Zambia and on *S. guineense* and *S. legatti* in South Africa were relatively inconspicuous and were found on branch cankers and branch stubs. Infections on *G. transvaalica* were associated with obvious branch and stem cankers (FIG. 1b). Fruiting structures were present both on the surface and beneath the bark of *G. transvaalica*, unlike those on the *Syzygium* and *Eucalyptus* trees that were found only on the surfaces of cankers or on necrotic areas. No fruiting structures were observed on the stems of native *Dissotis* sp. (Melastomataceae), a woody shrub related to *Tibouchina*, growing in riverine areas of South Africa and Zambia or on *E. camaldulensis* Dehnh. growing in woodlots in the Caprivi.

DNA sequence comparisons.—DNA for two Cryphonectriaceae isolates from Namibia, six from South Africa, six from Swaziland and two from Zambia were amplified and sequenced. The datasets for the ITS and BT gene regions consisted of 61 taxa each and for the LSU and BT exon gene region of 27 taxa each. Alignment lengths of different gene regions were 547–928 bp (TABLE II).

TABLE II. Statistics resulting from maximum parsimony analyses

Statistic	ITS	BT	LSU	BT exon	Combined ITS and BT	Combined LSU and BT exon
Aligned characters	567	928	636	547	1495	1183
Constant characters	350	510	607	442	860	1032
Parsimony uninformative characters	20	11	7	16	31	26
Parsimony informative characters	197	407	22	89	604	125
Tree length	453	1041	45	177	1514	258
Consistency index (CI)	0.664	0.598	0.689	0.655	0.610	0.655
Retention index (RI)	0.894	0.882	0.837	0.765	0.881	0.757
Rescaled consistency index (RC)	0.594	0.527	0.577	0.502	0.537	0.496

Results of the PHT showed that datasets for the ITS and BT gene regions were homologous ($P = 0.045$) and thus could be combined (Cummings et al. 1995). Results of the PHT for the more conserved analysis based on LSU and BT exon data showed that these datasets also could be combined ($P = 0.02$) (Cummings et al. 1995). This was supported by the trees for each gene region that essentially had the same topology and support for the various genera.

Based on the ITS and BT datasets individually (data not shown), as well as the combined dataset, isolates collected in this study grouped in four genera of the Cryphonectriaceae, namely *Celoporthe*, *Chrysoporthe*, *Holocryphia* and a group closely related to *Aurifilum*. Results of the parsimony analyses correlated with results from maximum likelihood and Bayesian analyses (FIG. 2). These trees displayed the clades observed previously for *Chrysoporthe* spp. in Africa with *Chr. austroafricana* (ML, bootstrap confidence level (BS)/posterior probabilities (BPP), 100/90), *Chr. zambiensis* (94/100), *Chr. syzygiicola* (88/100), *Chr. cubensis* (93/98) and *Chr. deuterocubensis* (100/100) grouping separately (Gryzenhout et al. 2004, Nakabonge et al. 2006b). In both trees isolates from *S. guineense* in Namibia and Soutpansberg grouped with the *Chr. austroafricana* isolates and separately from other *Chrysoporthe* spp.

The three subclades (3–5) observed for *Cel. dispersa* isolates from different hosts (Nakabonge et al. 2006a) also were observed previously in this study (FIG. 2). These included isolates from *T. granulosa* from Durban in KwaZulu-Natal Province (96/97), *S. guineense* from Tzaneen in Limpopo Province (100/100) and *H. canescens* from Buffelskloof Nature Reserve in Mpumalanga Province (100/100). Isolates from *S. cordatum* and *S. legatti* in Soutpansberg (94/100) and Zambia (97/100) formed two additional, distinct subclades (1, 2) within *Celoporthe* (FIG. 2). Isolates from the *E. grandis* clone in Swaziland grouped with *H. eucalypti* (100/100).

Based on ITS and BT datasets, isolates from *G. transvaalica* grouped close to *A. marmelostoma*. The isolates from *G. transvaalica* however formed a distinct subclade (100/100), suggesting a second species in this recently described genus or on the other hand a distinct genus (FIG. 2). The same groupings for genera in the Cryphonectriaceae were observed in the more conserved dataset containing BT sequence data, excluding the introns, and combined with the LSU data suggesting a previously undescribed taxon among isolates collected in this study (FIG. 3). Although there was only 1 bp difference based on LSU data between *A. marmelostoma* and the fungus from *G. transvaalica*, BT exon data showed a clear and highly supported distinction (MP, BS = 97) with 12 fixed base pair differences between the two taxa.

Morphology.—Fruiting structures found on *G. transvaalica* resembled those of members of the Cryphonectriaceae, with distinct orange stromatic tissue (Gryzenhout et al. 2009) that turns purple in the presence of 3% KOH and yellow in lactic acid (Castlebury et al. 2002). Teleomorph structures were scarce, which is consistent with observations for other Cryphonectriaceae such as *Cel. dispersa* (Nakabonge et al. 2006a). Teleomorph structures were orange, semi-emerged with pseudoparenchymatous to prosenchymatous tissue, similar to genera in the Cryphonectriaceae (Gryzenhout et al. 2009). Ascospores resembled those of other Cryphonectriaceae ranging from fusoid to ellipsoid with a single septum (Gryzenhout et al. 2009). Conidiomatal structures were more abundant on specimens examined, occurring both on the surface and on the underside of bark peeling from the cambium. Conidiomata were orange, semi-immersed, with uni- to multilocular conidial locules, and conidia were cylindrical with sizes similar to those of species in the Cryphonectriaceae (Gryzenhout et al. 2009).

The fungus on *G. transvaalica* was similar to genera and species in the Cryphonectriaceae that have uniformly orange sexual and asexual states (TABLE III) in contrast to genera that have black fruiting

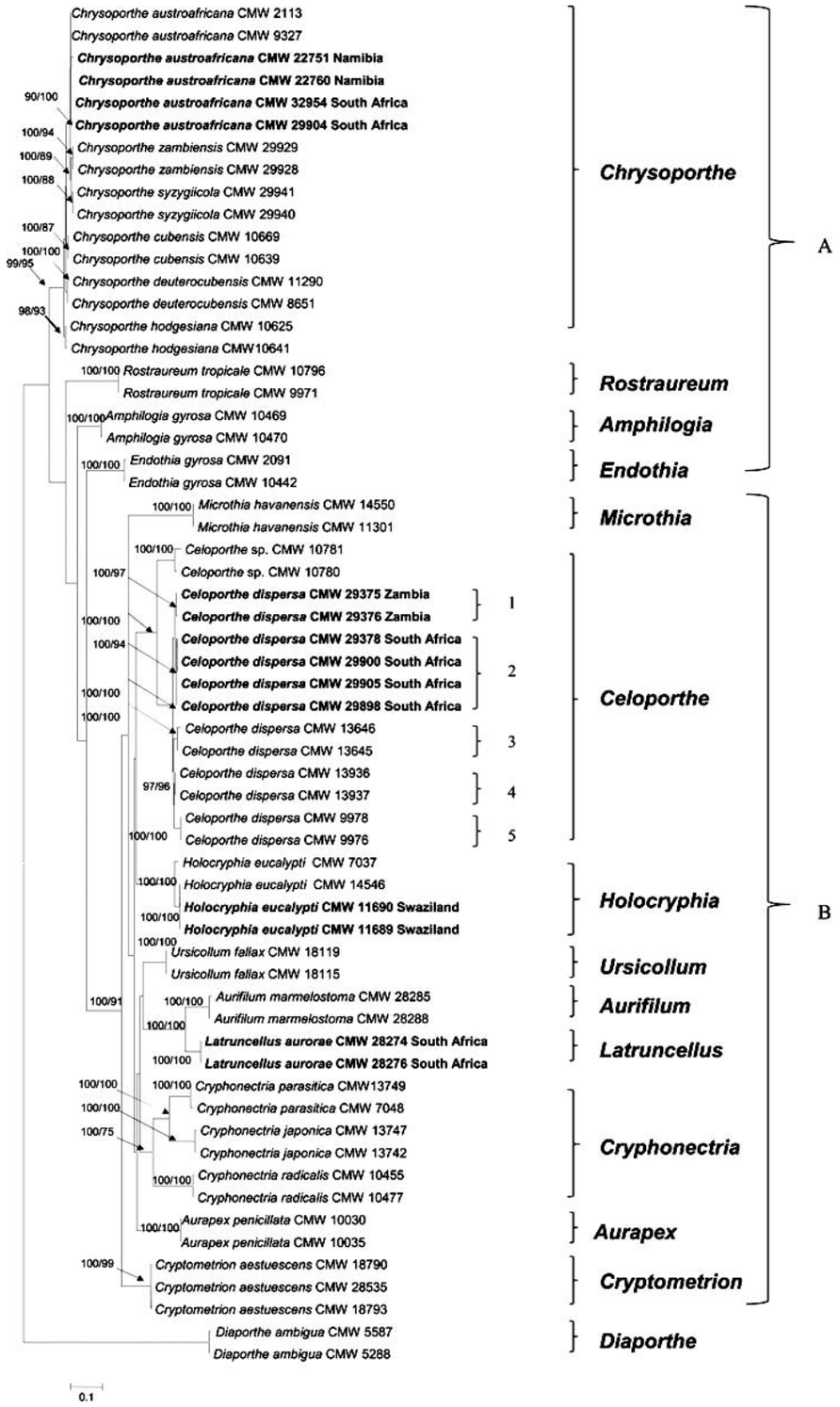

FIG. 2. Phylogram obtained from the combined datasets of the ITS and BT gene sequences. The phylogram was obtained with maximum likelihood analyses with the TrN + I + G parameter model. Confidence levels > 70% of the tree branch nodes, determined by posterior probabilities (BPP) and ML, 1000 replicate bootstrap analysis (BS) and are indicated on tree branches (BPP/BS). Isolates sequenced in this study are in boldface. Five clades of *Celoporthe dispersa* are marked 1–5. *Diaporthe ambigua* was defined as outgroup taxon.

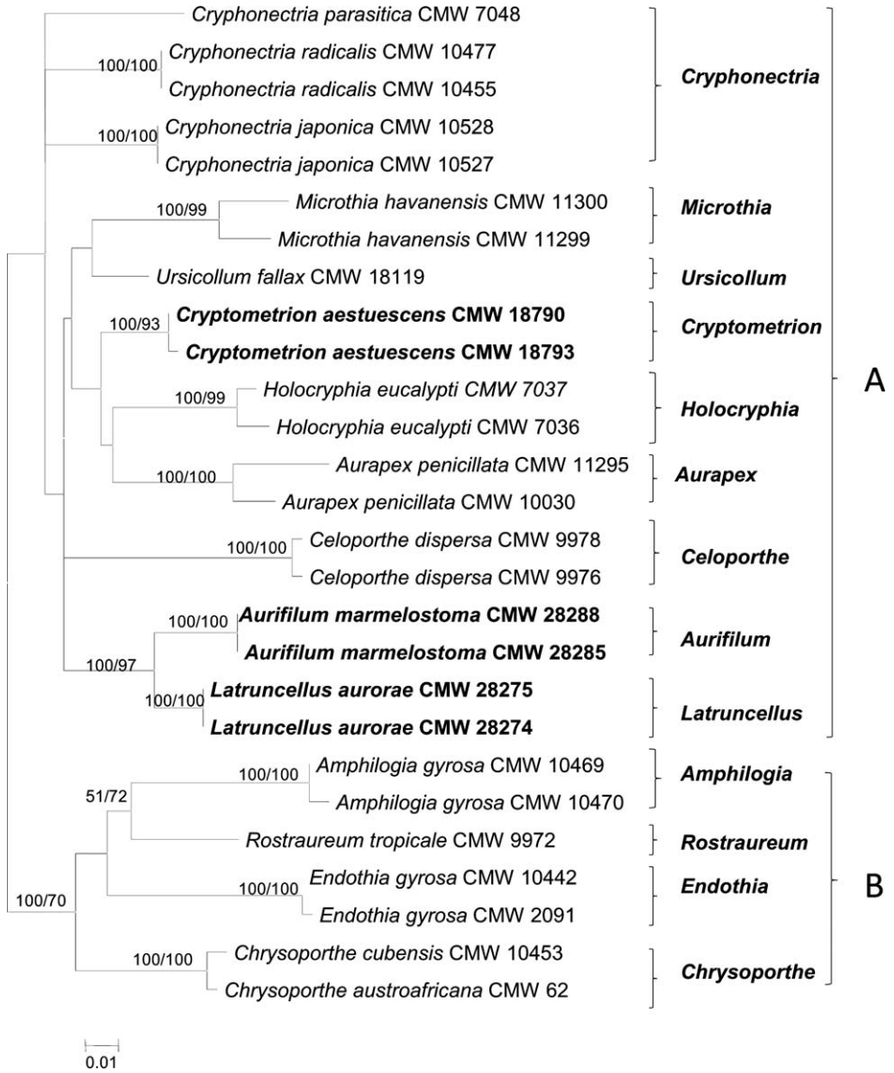

FIG. 3. Phylogram obtained from the combined datasets of the LSU and BT exon gene sequences. The phylogram was obtained with maximum likelihood using the TIM2 + I + G parameter model. Confidence levels > 70% of the tree branch nodes determined by posterior probabilities (BPP) and ML, 1000 replicate bootstrap analysis (BS) are indicated (BPP/BS). Isolates sequenced in this study are in boldface. The tree was midpoint rooted.

bodies or those that are partially black (Gryzenhout et al. 2009). It nonetheless could be distinguished from the taxa with uniformly orange sexual and asexual states (TABLE III) based on a number of characteristics. The fungus has long paraphyses (< 90 µm) between conidiophores (FIG. 4i), similar to those in conidiomata of *Aurifilum* (Begoude et al. 2010), *Holocryphia* and *Microthia* (Gryzenhout et al. 2009). The anamorph structures of this fungus are conical, thus similar to those of *Aurifilum*, *Amphilogia* and *Rostraureum*. However it could be distinguished from all these genera due to the presence of distinct conidiomatal necks (FIG. 4e) and more important the unique, constricted shape of the neck, which makes fruiting structures similar to the shape of pawns in chess (FIG. 4e).

Sequence data showed that the unknown fungus from *G. transvaalica* is most closely related to the monotypic genus *Aurifilum*. Several important differences however separate the unknown fungus from *A. marmelostoma*. The most important of these is the morphology of the conidiomata. Conidiomata of *A. marmelostoma* are broadly convex without necks, while those of the unidentified fungus are conical with distinctly constricted, fattened necks (FIG. 4e). The ostioles in the unidentified fungus are not obviously

darkened as they are in those of conidiomata of *A. marmelostoma* (TABLE III). The unidentified fungus has fusoid to oval ascospores that are constricted at median to off-median septa (FIG. 4d), whereas those of *A. marmelostoma* are fusoid to ellipsoid with a medium septum without constrictions. The conidiophores of *A. marmelostoma* are cylindrical with inflated bases, while those of the unidentified fungus are subulate. In addition conidia of *A. marmelostoma* are cylindrical to allantoid while those of the undescribed fungus (FIG. 4h) are only cylindrical (Begoude et al. 2010). Last, the unidentified fungus grows optimally at 25 C in contrast to *A. marmelostoma* that has an optimum at 30 C.

Taxonomy.—Comparisons of DNA sequence data of the unknown fungus from *G. transvaalica* with genera residing in the Cryphonectriaceae showed that it is related most closely to *A. marmelostoma*, possibly representing a second species in this genus (FIGS. 2, 3). However, anamorph structures of the unknown fungus are significantly different from those of *A. marmelostoma* or from any other genus in the Cryphonectriaceae. For example, anamorph structures of the unknown fungus are uniformly orange with constricted, fattened necks with a shape similar to that of a chess pawn while those of *A. marmelostoma* do not have necks and have blackened ostiolar openings. These differences are consistent with the magnitude of morphological differences used in the past to described new genera in the Cryphonectriaceae (Gryzenhout et al. 2009). Other differences probably represent those at the level of species, such as those in optimal growth temperature and shape of ascospores, conidiophores and conidia. We thus propose that the fungus from *G. transvaalica* most appropriately belongs in a new genus described as follows:

Latruncellus M. Verm., Gryzenh. & Jol. Roux. gen. nov.

MycoBank MB518285

Etymology. The name refers to the shape of the conidiomata, which have inflated heads that resemble chess pawns. The Romans did not know chess but played a similar battle game. The smallest and least valuable piece in the game was a latrunculus, meaning ''robber, brigand, bandit'', and this piece was apparently the analog of a chess pawn; -cellus refers to the small fruiting structures.

Ascostromata subimmersa pulvinata aurantiaca. *Asci* fusoidei vel ellipsoidei. *Ascosporae* uniseptatae hyalinae. *Conidiomata* conica collis distinctis complanatis, latrunculiformia subimmersa unilocularia vel multilocularia, marginibus pseudoparenchymatis intus prosenchymata, aurantiaca, collo textura globulosa. *Paraphyses* adsunt. *Conidia* minuta hyalina non septata, pro guttulis cirrhisque aurantiacis exsudatis.

Ascostromata semi-immersed, pulvinate, upper region eustromatic, lower region pseudostromatic, edges pseudoparenchymatous with prosenchymatous tissue inside, orange. *Perithecia* embedded in host tissue at base of stroma, textura porrecta, perithecial bases hyaline when young. Asci fusoid to ellipsoid. *Ascospores* fusoid to oval, single septate, septum median to off-median, hyaline.

Conidiomata conical with distinct, constricted necks, pawn-shaped, semi-immersed, uni- to multilocular, convoluted, part of ascomata as conidial locules or as solitary structures, edges of conidiomata pseudoparenchymatous and inside prosenchymatous, orange, necks with textura globulosa. Conidiophores subulate to flask-shaped, aseptate or septate with attenuated apex, branched or unbranched, hyaline. Paraphyses present. Conidia minute, hyaline, cylindrical, aseptate, exuded as orange droplets and tendrils.

Latruncellus aurorae M. Verm., Gryzenh. & Jol. Roux. sp. nov. FIG. 4

Mycobank MB518286

Etymology. aurorae (Latin) ''of the dawn'' referring to the orange fruiting structures.

Ascostromata subimmersa globosa aurantiaca 131–343 µm supra corticem crescentia, 337–827 µm diam. Perithecia in contextu hospitis in basi stromatis immersa, e textura porrecta composita, bases peritheciorum paucae (1–3), juventute hyalinae, collis 45–119 µm longis 58–134 µm latis, solum perjuvenia visa. Asci fusoidei vel ellipsoidei (31.5–)34.5–46.5(–49.5) × (5–)6.5–10(–12) µm. Ascosporae fusoideae vel ovales, uniseptatae, septo medio vel prope medium, sporae saepe in septo constrictae, apice obtusae, hyalinae, (8.5–)9.5–11(–12.5) × (2.5–)3–3.5(–4.5) µm.

Conidiomata conica latrunculiformes subimmersa uniloculares vel multiloculares, solitaria vel loculi conidiales pro partes ascomatum, margines conidiomatum pseudoparenchymatae, intus prosenchymata, aurantiaca, supra corticem 51–199 µm alta, 63–129 µm lata, collis distinctiscomplanatis e textura globulosa formatis, 34–84 µm longis, maxime 52–98 µm latis. Conidiophorae subulatae vel ampulliformes, septatae vel non, apice attenuato, ramosae vel non, hyalinae, (7–)8.5–15.5(–22) µm × (1.5–)1.5–2(–2.5) µm. Paraphyses cylindricae apice obtusae, non septatae (22–)24.5–56.5(–76.5) × (1.5–)1.5–2.5(–3) µm. Conidia hyalina cylindrica non septata, 4(–4.5) × 1–1.5(–2) µm, pro guttulis cirrhisque aurantiacis exsudatis.

Ascostromata semi-immersed, pulvinate, upper region eustromatic, lower region pseudostromatic, edges pseudoparenchymatous with prosenchyma inside, orange, extended 131–343 µm above the bark, 337–827 µm diam. Perithecia embedded in host tissue at base of stroma, textura porrecta, perithecial bases

TABLE III. Morphological characteristics of genera in the Cryphonectriaceae having uniformly orange fruiting bodies compared with those of *Latruncellus aurorae* (adapted from Begoude et al. 2010)

Morphological characteristics	*Amphilogia*	*Cryphonectria*	*Endothia*	*Holocryphia*
Teleomorph				
Structure of ascostroma	pulvinate, erumpent, slightly immersed to superficial	Large, pulvinate, erumpent, semi-immersed	Large, pulvinate to clavate, erumpent, superficial	pulvinate, semi-immersed
Ascospore shape	Hyaline, ellipsoidal to fusoid	Hyaline, ellipsoidal to fusoid	Hyaline, cylindrical	Hyaline, cylindrical
Ascospore septation	1–3-septate	1-septate	Aseptate	Aseptate
Anamorph				
Structure of conidiomata	Conical to pyriform superficial	Pulvinate, semi-immersed, erumpent	Pulvinate, superficial, erumpent	pulvinate, erumpent, semi-immersed
Conidiomatal neck	Absent	Absent	Absent	Absent
Conidiomatal stromatic tissue	Prosenchyma and pseudoparenchyma	Prosenchyma and pseudoparenchyma	Prosenchyma and pseudoparenchyma	Prosenchyma and pseudoparenchyma
Paraphyses	Absent	Absent	Absent	Present
Conidia	Variable size, hyaline, oblong to slightly curved, aseptate	Minute, hyaline, cylindrical, aseptate	Minute, hyaline, cylindrical, aseptate	Hyaline, cylindrical, aseptate

Microthia	*Rostraureum*	*Ursicollum*	*Aurifilum*	*Latruncellus*
Large, pulvinate, erumpent, semi-immersed	pulvinate, erumpent, immersed to semi-immersed	Not known	Large, pulvinate to pyriform, semi-immersed	Pulvinate, semi-immersed
Hyaline, ellipsoidal to fusoid	Hyaline, ellipsoidal to fusoid	Not known	Hyaline, ellipsoidal to fusoid	Hyaline, fusoid to oval
1-septate	1-septate	Not known	One septate	One septate
Pulvinate, semi-immersed	Clavate to rostrate	Pyriform or rostrate, superficial	Broadly convex	Conical with inflated neck
Absent	Present	Present	Absent, ostiolar opening darkened	Present
Prosenchyma and pseudoparenchyma	Of different textura type	Prosenchyma and pseudoparenchyma	Prosenchyma and pseudoparenchyma	Prosenchyma and pseudoparenchyma
Present	Absent	Absent	Present	Present
Hyaline, cylindrical, aseptate	Uniform capitalshyaline, cylindrical, aseptate	Hyaline, cylindrical, aseptate	Minute, hyaline, cylindrical to allantoid, aseptate,	Minute, hyaline, cylindrical, aseptate,

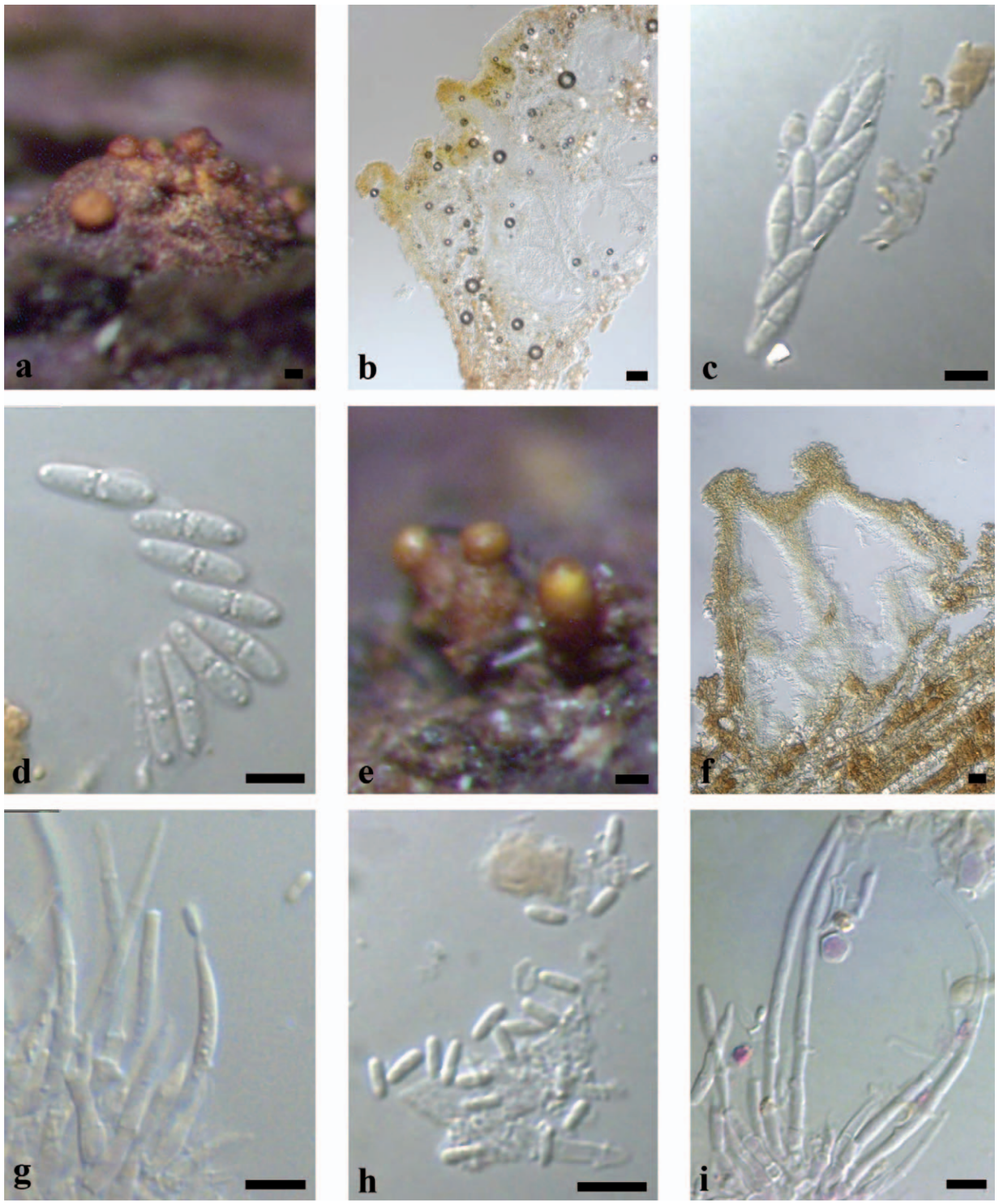

FIG. 4. Fruiting structures of *Latruncellus aurorae*. a. Ascostroma on bark. b. Longitudinal section through ascostroma. c. Ascus. d. Ascospores. e. Conidiomata on bark. f. Longitudinal section through conidioma. g. Conidiogenous cells. h. Conidia. e. Paraphyses. Bar: a, b, e, f = 50μm; c, d, g, h, i = 5 μm.

few (1–3), hyaline when immature, necks extending above ascostroma, 45–119 µm long and 58–134 µm wide. Asci fusoid to ellipsoid, (31.5–)34.5–46.5(–49.5) × (5–)6.5–10(–12) µm. Ascospores fusoid to oval, single septate, septum median to off-median, often constricted at septum, apex obtuse, hyaline, (8.5–)9.5–11(–12.5) × (2.5–)3–3.5(–4.5) µm.

Conidiomata conical, pawn-shaped, semi-immersed, uni- to multilocular, convoluted, part of ascomata as conidial locules or as solitary structures, edges of conidiomata pseudoparenchymatous and inside prosenchymatous, orange, 51–199 µm high above the bark and 63–129 µm wide. Necks distinct, constricted, of textura globulosa, 34–84 µm long, 52–98 µm wide at widest point. Conidiophores subulate to flask-shaped, aseptate to septate, with attenuated apex, branched or unbranched, hyaline, (7–)8.5–15.5(–22) µm × (1.5–)1.5–2(–2.5)µm. Paraphyses cylindrical with obtuse apex, aseptate, (22–)24.5–56.5(–76.5) × (1.5–)1.5–2.5(–3) µm. Conidia hyaline, cylindrical, aseptate, exuded as orange droplets and tendrils, 3–4(–4.5) × 1–1.5(–2) µm.

Culture characteristics.—On MEA luteous to pale luteous, covering a 90 mm plate in 7 d at an optimum temperature of 25 C. Minimal growth was observed at 15 C and no growth was observed at 35 C.

Specimens examined. SWAZILAND, LUBOMBO: close to the South Africa and Mozambique border. Isolated from bark of *G. transvaalica*, 2008, J. Roux, (HOLOTYPE, PREM 60348, ex-type culture CMW28276/CBS 125526, PARATYPES, PREM 60349, PREM 60347, living cultures CMW28274/CBS124904, CMW30633/CBS124905).

KEY, ADAPTED FROM BEGOUDE ET AL. (2010) AND BASED ON ANAMORPH AND TELEOMORPH CHARACTERISTICS, TO AID IN MORPHOLOGICAL IDENTIFICATION

1a. Orange conidiomata 2
1b. Conidiomata uniformly black to black with orange necks 11
2a. Conidiomata pulvinate to globose, ascospores septate or aseptate 3
2b. Conidiomata conical or rostrate or pyriform or convex, with or without a neck, ascospores septate 7
3a. Ascospores septate 4
3b. Ascospores aseptate 6
4a. Semi-immersed, usually no paraphyses 5
4b. Semi-immersed to superficial, paraphyses present . . . . . ***Microthia***
5a. Conidiomata usually larger than 350 µm, uni- to multilocular, ascospores with median septum . . . . . ***Cryphonectria***
5b. Conidiomata usually smaller than 350 µm diam, unilocular, ascospores with median to submedian septum . . . . . ***Cryptometrion***
6a. Stromata strongly developed, large, erumpent, mostly superficial, numerous conidial locules, no paraphyses . . . . . ***Endothia***
6b. Stromata small to medium, semi-immersed, few conidial locules or one convoluted locule, paraphyses present . . . . . ***Holocryphia***
7a. Conidiomata with necks, ascospores single septate 8
7b. Conidiomata without necks, ascospores single to multiple septate 10
8a. Conidiomata with prominent, delimited neck . . . 9
8b. Conidiomata with neck continuous with base, rostrate, white sheath of tissue surrounding perithecial necks when sectioned longitudinally . . . . . ***Rostraureum***
9a. Conidiomata rostrate to pyriform with large base, neck attenuated or not, teleomorph still unknown . . . . . ***Ursicollum***
9b. Conidiomata conical with constricted, fattened neck, shape like a chess pawn . . . . . ***Latruncellus***
10a. Conidiomata conical, uniformly orange, ascospores 1- to 3-septate . . . . . ***Amphilogia***
10b. Conidiomata convex, with blackened ostiolar openings, ascospores 1-septate . . . . . ***Aurifilum***
11a. Conidiomata uniformly black when mature 11
11b. Conidiomata black with orange neck, teleomorph still unknown . . . . . ***Aurapex***
12a. Conidiomata pulvinate to pyriform with attenuated neck, base tissue of *textura globulosa* when sectioned longitudinally, perithecial necks long and covered with dark tissue, emerging from orange stroma . . . . . ***Chrysoporthe***
12b. Conidiomata pulvinate or conical, occasionally with short necks, base tissue prosenchymatous, apices of conidiomata can be orange to scarlet when young, perithecial necks short and of same color (orange to umber) as stroma . . . . ***Celoporthe***

Pathogenicity tests.—Six weeks after inoculation with isolates of *L. aurorae* lesions were visible on both *G. transvaalica* (min = 11.96, max = 59.26, st. dev. = 12.84) and the *Eucalyptus* clone ZG14 (min = 12.62, max = 34.70, st. dev.= 6.23). No lesions were observed on trees inoculated with sterile MEA. Statistical analyses showed a significant difference in lesion lengths on trees inoculated with the test fungi and the controls for both *G. transvaalica* and *Eucalyptus* clone ZG14 ($P < 0.001$). No statistical differences ($P > 0.05$) were found between lesions on *G. transvaalica* and those on *Eucalyptus* clone ZG14. Average lesion lengths on *G. transvaalica* was 28 mm and average lesion length on *Eucalyptus* was 23 mm. *Latruncellus aurorae* was re-isolated from lesions on both *G. transvaalica* and *Eucalyptus*.

DISCUSSION

Results of this study considerably expand available knowledge regarding the distribution and host range

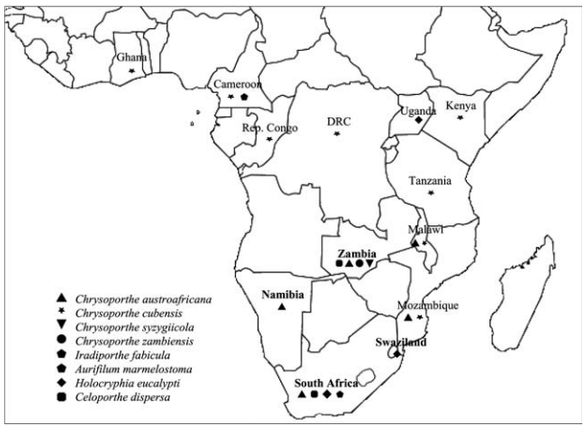

FIG. 5. Current distribution of Cryphonectriaceae in Africa (markers are only an indication of countries where Cryphonectriaceae occur and not specific locations). Countries in boldface represent new reports.

of the Cryphonectriaceae in southern Africa (FIG. 5). Previously unknown hosts and areas of occurrence have been discovered for these fungi. For example *Chr. austroafricana* was found in the Soutpansberg area, outside its previously known range in South Africa, and other members of the Cryphonectriaceae were recorded for the first time from Namibia and Swaziland (FIG. 5). Furthermore, a previously unknown genus in the Cryphonectriaceae was discovered on a member of the Lythraceae in Swaziland. This is the first report of Cryphonectriaceae infecting a member of the Lythraceae in Africa.

The presence and abundance of *Chr. austroafricana* on a native African host in Namibia and the fact that *S. guineense* appeared to be relatively tolerant to infection provides added support for the view (Heath et al. 2006) that *Chr. austroafricana* has an African origin. This is illustrated by the fact that *Chr. austroafricana* was relatively common on native *S. guineense* trees in the Caprivi region. In addition to being found on stems and branches of *S. guineense* at Popa Falls, the fungus also was common on the roots and branches of *S. guineense* at Katima Mulilo. Disease symptoms, such as stem girdling and tree death known to be associated with *Chrysoporthe* infections on *Eucalyptus* spp., were not observed on *S. guineense*.

An intriguing aspect of the *Chr. austroafricana* infections on *S. guineense* in Katima Mulilo was that they were on roots partially submerged in water at the time of sampling. This could have interesting implications regarding our understanding of the epidemiology of this fungus. Roots of *S. guineense* growing on the banks of the Zambezi River are known to be submerged during the rainy season. This raises questions as to when and how infection took place. For example, it is not known whether infection takes place after exposure of the roots to water or whether the fungus survives or even sporulates on the roots while they are submerged. Although it is known that both *Chr. austroafricana* (Wingfield et al. 1989) and *Chr. deuterocubensis* (Davison and Coates 1991) can infect roots, the only report of an association of a *Chrysoporthe* sp. with water is the fact that spores of the asexual state are typically rain-splash dispersed. The presence of *Chr. austroafricana* on the roots of *S. guineense* also could be related to the fact that submerged roots are stressed.

Chrysoporthe austroafricana occurs on non-native *Eucalyptus* spp. in Malawi, Mozambique, South Africa and Zambia (Wingfield et al. 1989, Nakabonge et al. 2006b, Chungu et al. 2010). This fungus however was not found on *Eucalyptus* spp. in Namibia and was present only on native *Syzygium* trees in that country. This could be due to the fact that only *E. camaldulensis* trees are planted in the Caprivi region while *E. grandis* is planted in most other African countries. The latter species is much more susceptible to infection by *Chr. austroafricana* than is *E. camaldulensis* (Van Heerden et al. 2005).

Celoporthe dispersa first was described from native *S. cordatum*, *H. canescens* and non-native *T. granulosa* in South Africa (Nakabonge et al. 2006a). Collections of the fungus in the present study greatly expand its geographic and host range. Nakabonge et al. (2006a) observed three distinct phylogenetic subclades for *Cel. dispersa*, corresponding to the three hosts (*Heteropyxis*, *Syzygium* and *Tibouchina*) and localities (Lydenburg, Tzaneen and Durban) where isolates were collected. The isolates from Soutpansberg in South Africa and Zambia collected in this study belong in two additional subclades within *Cel. dispersa*. It is possible that these five groups represent cryptic species. However, herbarium specimens found previously and linked to the groups from *Heteropyxis* and *Tibouchina* were limited and had only anamorph structures (Nakabonge et al. 2006a). Similarly infected plant tissue from the current study had few fruiting structures, making comprehensive comparisons among the collections difficult. Additional collections must be made to resolve the taxonomy of these isolates.

Holocryphia eucalypti was known previously only from South Africa and Australia and most recently has been found in Uganda (Roux and Nakabonge 2009) and New Zealand (Gryzenhout et al. 2010b). The presence of *H. eucalypti* in Swaziland is not unexpected because Swaziland shares most of its border with South Africa and it has similar climatic conditions. There is also a lack of quarantine and control of

movement of raw material between South Africa and Swaziland. The fungus is not a particularly important pathogen in South Africa (van der Westhuizen et al. 1993, Gryzenhout et al. 2003), and the report from Uganda (Roux and Nakabonge 2009) also indicates minimal effect on trees. *Holocryphia eucalypti*, however, can cause severe damage under stress (Old et al. 1986, Wardlaw 1999, Gryzenhout et al. 2003), and this could have implications for its role as a plantation pathogen in Swaziland and other African countries, especially under climate change (van Staden et al. 2004, Desprez-Loustau et al. 2007).

Based on phylogeny, the newly described genus *Latruncellus* might more appropriately represent a new species of *Aurifilum*. This, however, is not supported by morphology. Within the Cryphonectriaceae shape and color of anamorph structures represent the most important distinctive characteristic for the various described genera, and these are supported with 100% bootstrap and posterior probability values in phylogenetic studies (Gryzenhout et al. 2009, 2010a; Begoude et al. 2010). A number of genera thus have been described in the Cryphonectriaceae following the approach of supplementing phylogenetic groupings with anamorph morphology. Many of these genera are monotypic, but genera such as *Chrysoporthe* and *Cryphonectria* consist of species strongly supported by morphological characteristics and phylogenetic data.

The alternative to having the various phylogenetic groupings in the Cryphonectriaceae represent separate genera is for all species in the Cryphonectriaceae to belong to either a single genus or in two genera representing the two larger groupings observed in the Cryphonectriaceae (FIGS. 2, 3; clades A, B). Describing the phylogenetic clades in one genus is unfeasible because species shown to be closely related based on DNA sequence data are often morphologically vastly different. For instance, *Rostraureum* and *Chrysoporthe* and *Microthia* and *Aurapex* group closely based on DNA sequence data (FIGS. 2, 3) but *Rostraureum* and *Microthia* have orange conidiomata while *Chrysoporthe* and *Aurapex* have black conidiomata. Similarly, accepting only two genera in the Cryphonectriaceae, based on the two larger phylogenetic clades, includes inconsistent morphological differences between species in the two phylogenetic groups. For instance, fungi with orange and dark conidiomata and septate as well as aseptate ascospores are found in both groups. Based on currently available data describing the various phylogenetic groups united by a defined suite of morphological features as distinct genera appears to be the most logical means to treat the taxonomy of the Cryphonectriaceae (Gryzenhout et al. 2009). Isolates from *G. transvaalica* were described therefore in the new genus *Latruncellus* as opposed to being treated as a new species in the genus *Aurifilum*.

Little is known regarding the distribution and host range of *A. marmelostoma* and *L. aurorae*. *Aurifilum marmelostoma* is known only from Cameroon where it is associated with stem cankers on *T. ivorensis* and *T. mantaly* (Combretaceae). In contrast *L. aurorae* is known only from Swaziland on *G. transvaalica* (*Lythraceae*). All the tree genera on which these fungi are found belong in the Myrtales, and it seems likely that *Aurifilum* and *Latruncellus* have wider host and geographical ranges than those currently known for them.

While this study has greatly increased the base of knowledge regarding the distribution and taxonomy of the Cryphonectriaceae in Africa, it also has highlighted the fact that the host and geographic range data for these fungi in Africa remains incomplete. To control the movement of pathogens between countries in Africa and to anticipate potentially detrimental host jumps (Slippers et al. 2005) to other tree species it will be important to have an expanded knowledge regarding the host range and distribution of pathogens. This is especially true for pathogens of native trees for which limited information is available.

Tree pathogens are typically not highly destructive on their native hosts in their areas of origin, but they can cause significant damage when they are moved to new areas and exposed to related trees that have evolved in their absence (Anagnostakis 1987, Wingfield et al. 2008). In the Cryphonectriaceae the devastation caused by *C. parasitica* in Europe and North America provides an apt example (Anagnostakis 1987, Gryzenhout et al. 2009). Thus, pathogens occurring on seemingly unimportant hosts, such as *L. aurorae* on the native tree *G. transvaalica*, have the potential to cause serious disease if they are moved to new areas. Inoculations in this study showed that *L. aurorae* is pathogenic to both *G. transvaalica* and the *Eucalyptus* clone tested. Although *L. aurorae* has not been found on *Eucalyptus* spp., the results of this study show that it has the potential to cause disease on these trees. This and similar pathogens thus also should be considered when formulating quarantine standards.

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