Luteocirrhus shearii gen. sp. nov. (Diaporthales, Cryphonectriaceae) pathogenic to Proteaceae in the South Western Australian Floristic Region

Colin Crane¹, and Treena I. Burgess²

¹Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia; corresponding author e-mail: colin.crane@dec.wa.gov.au
²Centre of Excellence for Climate Change, Woodland and Forest Health, School of Veterinary and Life, Murdoch University, Perth, 6150, Australia

Abstract: Morphological and DNA sequence characteristics of a pathogenic fungus isolated from branch cankers in Proteaceae of the South West Australian Floristic Region elucidated a new genus and species within Cryphonectriaceae (Diaporthales). The pathogen has been isolated from canker lesions in several Banksia species and Lambertia echinata subsp. citrina, and is associated with a serious decline of the rare B. verticillata. Lack of orange pigment in all observed structures except cirri, combined with pulvinate to globose black semi-immersed conidiomata with paraphyses, distinguishes the canker fungus from other genera of Cryphonectriaceae. This was confirmed by DNA sequence analysis of the ITS regions, ß-tubulin, and LSU genes. The fungus (sexual morph unknown) is described as Luteocirrhus shearii gen. sp. nov. Lesions in seedlings of Banksia spp. following wound inoculation and subsequent recovery confirm Koch’s postulates for pathogenicity. This pathogen of native Proteaceae is currently an emerging threat, particularly toward B. baxteri and B. verticillata.

Key words: Australia
Banksia
Cryphonectriaceae
Emerging pathogen
Fungal pathogen
Canker
Natural ecosystems
Phylogenetics
Proteaceae
Zythiostroma

INTRODUCTION

In a previous study of twig and branch cankers in Banksia coccinea, Shearer et al. (1995) isolated several pathogens including a purported Zythiostroma sp. (IMI 336153). The Zythiostroma sp. was shown to be a virulent pathogen of both B. baxteri and B. coccinea.

Recent studies on the causal agents of severe canker disease affecting Banksia communities and Lambertia spp. across the South West Australian Floristic Region (SWAFR) consistently returned Neofusicoccum australe, N. macrocallatum, and Cryptodiaporthe melanocraspeda, along with an undescribed species (Crane et al. 2012) which shared morphological and ITS sequences with the purported Zythiostroma sp. previously reported by Shearer et al. (1995). Based on GenBank searches this undescribed species grouped within Cryphonectriaceae, and thus its taxonomic status, needs to be revised as Zythiostroma resides in Nectriaceae and not Cryphonectriaceae.

Species of Cryphonectriaceae living within the bark and wood of trees have a worldwide distribution, include some of the world’s most important pathogens of trees, such as chestnut blight (Cryphonectria parasitica) and serious canker diseases of plantation eucalypts (Gryzenhout et al. 2009). Approximately one species in each of the recognised genera within the family are virulent pathogens, while the remainder are either facultative parasites or saprophytes (Gryzenhout et al. 2009).

Symptoms of the Zythiostroma sp. cankers on Proteaceae in the SWAFR include sunken lesions initially visible on one side of a twig or branch (Fig.1a), cracking and splitting of bark before girdling, and death of the branch. The fungus may kill only one branch before being contained by the host. However, infection can cause multiple branch deaths (Fig.1b), with complete crown dieback of individuals, and in the case of B. baxteri and B. verticillata infrequent collapse of entire communities. This occurs when pathogen growth within an individual continues unchecked until discrete twig cankers coalesce to girdle the main collar or basal stem, ensuing in death of the host.

The South West Australian Floristic Region is one of the worlds Biodiversity hotspots (Myers 2001) comprising at least 5710 described plant species, 79 % of which are endemic (Beard et al. 2000). The vegetation is predominantly shrubland or woodland, with Banksia species (Proteaceae) often being dominant larger perennials, together with other trees of low diversity and an understory of predominantly woody shrubs (Beard 1989, Shearer & Dillon 1996, Pate & Bell 1999). Several Banksia spp. are widespread throughout the region though some, such as B. verticillata, occupy narrow ecological niches resulting in restricted geographic distributions. Lambertia species (Proteaceae) occur as...
Fig. 1. A. Young canker of *Luteocirrhus shearii* in petiole scar of *Banksia baxteri*. B. Multiple branch death impact in *Banksia grandis*. 
shrubs or small trees, often within Banksia woodland and can also be major ecosystem components within the communities in which they occur. Proteaceous flowers provide nectar for birds and mammals (Hopper 1980, Wooler et al. 2000). The climate of the SWAFR is Mediterranean with long hot dry summers and the soils are infertile with little structure and low phosphorus. The impact of the introduced plant pathogen Phytophthora cinnamomi is a major threat to the Banksia woodland communities within the region (Shearer et al. 2007) and further threats could thus be more devastating. Since the mid-1970s, the rainfall in the SWAFR has decreased by 14 % (Bates et al. 2008). Forecast climate change scenarios may place 5–20 % of the endemic plant species of south-western Australia into range declines severe enough to threaten their persistence (Fitzpatrick et al. 2008). Concomitant shifts in corresponding pathogen impacts and distributions could reasonably be expected. Opportunistic sampling and observations suggest that an increase in canker incidence and severity across the region is possibly related to changing climate (Crane et al. 2012).

Comparisons of DNA sequence data from the rDNA internal transcribed spacer regions (ITS), β-tubulin and LSU gene regions placed the new species in the Cryphonectriaceae, and different to currently described genera (Gryzenhout et al. 2009, Vermeulen et al. 2011, Chen et al. 2012). In this study sequence data was used in combination with morphological characteristics of the asexual morph to describe this new pathogenic genus and species.

MATERIALS AND METHODS

Collection and isolation
Twigs from proteaceous plants exhibiting canker symptoms were collected across the SWAFR from Nambung National Park near Cervantes in the north to Cape Arid National Park near Esperance in the southeast (Fig. 2, Table 1). Opportunistic sampling of cankered plants began in 1990 (Shearer et al. 1995) and culminated in 2011 with an intensive survey of cankers in B. baxteri and B. coccinea across their respective geographic ranges (Crane et al. 2012).

Cankered branches were removed and transported to the laboratory, and samples containing mature conidiomata were examined under the microscope. Cankers with no visible conidiomata had the bark scraped away and diseased tissue pieces of approximately 3 mm² spanning the lesion-healthy conidiomata had the bark scraped away and diseased tissue was then incubated at 20 °C in the dark for 24 h then removed and surface sterilised in 70 % ethanol for 1 min, followed by washing in two changes of sterile distilled water then blotted dry and plated onto half-strength potato-dextrose agar (PDA) medium (Becton, Dickinson, Sparks, MD; 19.5 g of Difco™ PDA, 7.5 g Bacto agar in 1 L of distilled water). The plated tissue was then incubated at 20 °C in the dark for 24 h then under near-UV light at 20 °C for 2 wk. This treatment usually resulted in formation of mature conidiomata for microscopic examination. Isolates obtained were then subcultured from colony margins and stored using 5 mm² agar pieces containing conidiomata, placed under sterile distilled water (Boesewinkel 1976) in glass McCartney bottles and stored at room temperature.

Morphology
Conidiomata in bark from naturally infected cankers were used for morphological comparison and characterisation. Stems were initially examined at 250× under a Wild Heerbrugg stereo microscope and gross morphology of characteristic fruiting structures measured and described. Conidiomata were then hand sectioned and mounted in 3 % potassium hydroxide (KOH) and 85 % lactic acid for microscopic observation under a compound Olympus BH – 2 microscope. Detailed gross morphology was recorded for 15 representative cankers and 80 conidial measurements each from 30 conidiomata under oil immersion at 1000 ×.

Optimal growth conditions for two isolates (CBS 130776 and WAC13426) of the Zylostromata sp. were determined in the dark on half-strength PDA medium for temperatures between 1–40 °C at 5 °C intervals. Isolates were in a randomised design with four replicates. Growth was measured at 4, 6, and 11 d along two perpendicular lines intersecting at the centre of the agar inoculum plug. Plates showing no growth at 1 and 40 °C were returned to 20 °C to determine isolate viability.

DNA sequence comparisons
Representative isolates (Table 1) were grown on half-strength PDA medium (Becton, Dickinson, Sparks, MD; 19.5 g PDA, 7.5 g of agar and 1 L of distilled water) at 20 °C for 2 wk and the mycelium was harvested by scraping from the agar surface with a sterile blade and placed in a 1.5 mL sterile Eppendorf® tube. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted according to Andjic et al. (2007).

For each isolate the region spanning the internal transcribed spacer (ITS 1-5.8S-ITS 2) region of the ribosomal DNA was amplified using the primers ITS-1 and ITS-4 (White et al. 1990). β-tubulin (BT) was amplified with primer pairs BT1a/BT1b and BT2a/BT2b respectively (Glass & Donaldson 1995). The large sub-unit (LSU) of the ribosomal DNA was amplified using the primers LR0R and LRS (Vilgalys & Hester 1990). The PCR reaction mixture and conditions were as described by Andjic et al. (2007). The clean-up of products and sequencing were as described by Sakalidis (2011) with the DNA fragments being sequenced with the same primer pairs used in the PCR amplification.

Sequence data were initially cleaned and subsequent manual adjustments made in Geneious v. R6 (Biomatters; http://www.geneious.com/). Sequences were aligned to those published for fungi in Cryphonectriaceae (Gryzenhout et al. 2009; Begoude et al. 2010, Chen et al. 2011, Vermeulen et al. 2011) in Geneious. The alignments were deposited in TreeBASE SN14068 (www.treebase.org).

Parsimony analysis was performed in PAUP (Swofford 2003). After the exclusion of the uninformative sites, the most parsimonious trees were obtained using heuristic searches with random stepwise addition in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-descent option off. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis 1992). Branch
and branch node support was determined using 1000 bootstrap replicates (Felsenstein 1985). Analyses were done for the ITS, BT, conserved BT exon data and LSU regions separately and for conserved BT exon data and ITS combined after a 1000 replicate partition homogeneity test was performed to test the null hypothesis that the data sets were homologous and could be combined. *Diaporthe ambiguа* was used as the out-group taxon for the combined ITS-BT data set and *D. eres* and *D. fibrosa* were used as the outgroup taxa for the LSU dataset.

Bayesian analysis was conducted on the same datasets as that used in the parsimony analysis. First, JModeltest v. 0.1.1 (Posada 2008) was used to determine the best nucleotide substitution model. Bayesian analyses were performed with MrBayes v. 3.1 (Ronquist & Heuelsenbeck 2003). Two independent runs of Markov Chain Monte Carlo (MCMC) using four chains were run over 1 000 000 generations. Trees were saved each 1 000 generations, resulting in 1 000 trees. Burn-in was set at 100 000 generations (i.e. 100 trees), well after the likelihood values converged to stationary, leaving 900 trees from which the consensus trees and posterior probabilities were calculated.

**Pathogenicity testing**

One-year-old potted seedlings of *Banksia attenuata*, *B. baxteri*, *B. coccinea* and *B. verticillata* were stem wound inoculated in a shadehouse using two isolates (CBS 130776 and WAC13426) with three single plant/pot replicates of each. Prior to inoculation, isolates were grown on half-strength PDA medium in the dark for 4 d. A 4 mm diam agar disk of each test fungus was inserted into a fresh cut made to the vascular cambium of each stem and bound with moist cotton wool and tape. A sterile agar disk was inserted in control inoculations. Stems were harvested, the outer bark shaved off and lesions measured 3 wk post inoculation after Shearer *et al.* (1995). Lesion lengths were compared by analysis of variance (ANOVA) with lesion length the random factor and host the fixed factor. The ANOVA assumptions of normality were checked by plotting residuals (Kirby 1993). Where appropriate means and standard errors of the mean (mean ± s.e.) were calculated.

**RESULTS**

**Collection and isolation**

Ninety-two isolates were collected from cankered branches of *Banksia baxteri*, *B. coccinea*, *B. grandis*, *B. ilicifolia*, *B. littoralis*, *B. pteridifolia*, *B. quercifolia*, *B. sessilis*, *B. speciosa*, *B. sphaerocarpa*, *B. verticillata*, and *Lambertia echinata* subsp. *citrina* across the SWAFR. Symptoms on all hosts with cankers were cracking of periderm with diffuse or contained lesions in twigs and stems. In *B. baxteri* there was also a 14 % (*n* = 21) recovery of the purported *Zythiostroma* sp. from analogue healthy branches.

**Fig. 2.** Distribution of *Luteocirrhus shearii* from cankered branches in the South Western Australian Floristic Region.
Luteocirrhus C. Crane & T. I. Burgess, gen. nov.

**Description:** Conidiomata pulvinate with or without neck, typically separate, fuscous black, subcortical semi-immersed or sometimes superficial erupting through bark, ostiolate, uni- to multiloculate, convoluted, paraphyses present and cylindrical or slightly allantoid, exuded as orange/yellow cirrhi, bright luteus on mass, exuded as cirrhi or tendrils. Ascotromata not seen.

**Type species:** Luteocirrhus shearii C. Crane & T. I. Burgess 2013

**Diagnosis:** Luteocirrhus shares entirely black conidiomata with mature Celoporthe and Crysoporthe in the Cryphonectriaceae as described previously (Gryzenhout et al. 2009), but differs in having some semi-immersed conidiomata, paraphyses within the locules and cylindrical conidia. Tissues stain purple in 3 % KOH and yellow in 85 % Lactic acid. This genus is separated from other genera in the Cryphonectriaceae primarily on ITS, BT and LSU DNA sequences.

**Etymology:** Latin, luteus, yellow; cirrus, a tendril like mass of forced out spores referring to the characteristic conidiophore mass extruded by the conidiomata.

**Hosts:** Banksia baxteri, B. coccinea, B. grandis, B. ilicifolia, B. littoralis, B. pteridifolia, B. quercifolia, B. sessilis, B. speciosa, B. sphaerocarpa, B. verticillata, and Lambertia echinata ssp. citrina (Proteaceae).

**Notes:** Morphologically, L. shearii shares entirely fuscous black conidiomata with Crysoporthe and mature conidiomata of Celoporthe, being distinct from other genera within the family which contain some orange colour. With Celoporthe, L. shearii shares conidiospore shape, presence of paraphyses, absence of periphyses, conidial shape and colour in mass. Luteocirrhus shearii differs from Celoporthe in having basal textura globulosa conidialstromat tissue. With Crysoporthe, L. shearii shares the absence of periphyses, conidial colour en mass, and differs by having semi-immersed conidiomata, paraphyses and cylindrical or slightly allantoid conidia (Table 2).

The LSU data aligned L. shearii most closely to Aurifilum marmelostoma and Latruncilla aurora, which differ morphologically in having orange pigment in most structures including conidiomata (Begoude et al. 2010, Vermeulen et al. 2011). ITS-BT sequences showed close alignment with Cryphonectria radicalis which shares pulvinate semi-immersed neckless conidiomata, paraphyses and differs in having orange conidiomata and cylindrical conidia.

Optimal temperature for both isolates was 25 ºC with no growth at 1 and 40 ºC (Fig. 4). Both isolates incubated at 1 ºC and WAC13426 incubated at 40 ºC resumed growth when returned to 20 ºC, though CBS 130776 failed to grow after 2 d at 40 ºC.

**Phylogenetic analysis**

The LSU data set (Fig. 5) consisted of 495 characters of which 44 were parsimony informative. Heuristic searches resulted in over 125 most parsimonious trees of 95 steps (CI = 0.58, RI = 0.84) (TreeBASE SN14068, Fig. 6). The topology of the Bayesian tree was very similar. Sequences of all Luteocirrhus shearii isolates were identical and reside in a highly supported terminal clade. Interestingly, many
genera within the Cryphonectriaceae such as Celoporthe, Crypnonecria, Holocryphia, Immersiporthe, and Microthia could not be separated based on LSU alone.

The intron data for BT is highly variable and difficult to align and thus only the exon data was considered in the phylogenetic analysis. The aligned datasets for ITS and BT exons (Fig. 5) consisted of 612 and 603 characters, respectively. Based on partition homogeneity tests in PAUP, the ITS and BT datasets were congruent ($P = 0.17$) and were concatenated resulting in a combined dataset of

Fig. 3. *Luteocirrhus shearii* (PERTH 08355274). A. Conidiomata with cirri. B. Vertical section of conidiomata. C. Horizontal cross section of conidiomata. D. Paraphyses protruding from hymenium. E. Conidiomatal tissue of textura globosa. F. Conidia. Bars A = 1 mm; B and D = 100 µm; C and E = 10 µm; and F = 5 µm.
Luteocirrhus shearii gen. sp. nov.

The support for individual genera (terminal clades) is radicalis Microthia, Cryphonectriaceae other members of the shares entirely black conidiomata with Immersioporthe volume 4 · no. 1 Proteaceae.

This study describes a novel and serious pathogen of Luteocirrhus shearii is separated supported terminal clade. of Luteocirrhus shearii is separated from the phylogenetically closest genera Immersioporthe and Microthia by 120 and 115 steps respectively. All other genera in the Cryphonectriaceae, with the exception of Cryphonectria also form coherent highly supported groups. Cryphonectria radialis does not group with the other Cryphonectria species. While the support for individual genera (terminal clades) is high there is little support for higher level clustering.

Pathogenicity testing

All stems (mean 5 mm diam) except controls and one of Banksia baxteri were girdled by brown-black lesions within 21 d. Shade house mean daily maximum and minimum temperatures were 24 °C and 14 °C respectively with an average of 74 % humidity for the duration of the trial. Relative susceptibility of the hosts to the disease is indicated by lesion extension rates that were significantly (P ≤ 0.5) greater in B. verticillata and B. baxteri, than B. attenuata and B. coccinea (Fig. 7). Wounds healed over in control inoculations with no accompanying lesion. Where lesions had produced conidiomata (Fig. 3) their identity was confirmed morphologically or subsequently by culturing from the lesion margin and producing conidiomata as previously described. Recovery of L. shearii from these lesions confirmed Koch’s postulates for pathogenicity.

DISCUSSION

This study describes a novel and serious pathogen of Proteaceae in the SWAFR of Western Australia. Phylogenetic analysis and morphological features place Luteocirrhus as a new monotypic genus in the Cryphonectriaceae. Luteocirrhus shearii shares entirely black conidiomata with other members of the Cryphonectriaceae, Celoporthe and Chrysoporthe, but differs by being semi-immersed. The occurrence of paraphyses also separates L. shearii from Chrysoporthe. Aurapex, which also has black conidiomata, could be confused with these genera should its characteristic orange neck break off, therefore, multiple conidiomata should be examined.

Luteocirrhus shearii was first reported in 1991 as Zythiostroma sp. causing canker disease in Proteaceae (Shearer & Faiman1991). Concurrent studies of cankers in the region document the increasing incidence and severity of the pathogen in stressed environments, and the role the pathogen may play in a drying climate is of great concern (Crane et al. 2012). The family Cryphonectriaceae has a global distribution with a rapidly growing number of genera and species recognized (Lumbsch & Huhndorf 2007, Gryzenhout et al. 2009, Vermeulen et al. 2011, Chen et al. 2012, Crous et al. 2012) and contains many virulent pathogens affecting some 100 tree species in over 14 families (Gryzenhout et al. 2009). Apart from Cryphonectria parasitica in non-endemic chestnuts and oak of Victoria, the Australian members of the family have to date been recorded only from myrtaceous hosts. With a few exceptions, the fungi occurring on Myrtaceae have been largely host family specific (Cheewangkoon et al. 2009). Luteocirrhus shearii appears to be host family specific to Australian native Proteaceae (19 species to date) while absent from concurrent samples of myrtaceous species within the SWAFR.

Cryphonectriaceae affecting the Australian Myrtaceae, Aurantiosacculus spp. (Crous et al. 2012a), and Foliochyphra eucalypti (Cheekwangkoon et al. 2009), are found on the eastern side of the continent and in Tasmania to the southeast, Chrysorchyptra corymbiae in the Northern Territory (Crous et al. 2012) and the stem canker pathogen Holocyphra eucalypti across continental Australia including the SWAFR (Nakabonge et al. 2008). Population studies of H. eucalypti have shown it to be native to Australia (Nakabonge et al. 2008), though whether it is native to Western Australia is not known. While little is known of the continental distribution of L. shearii, which could reflect low sampling effort within the Proteaceae, a single Zythiostroma sp. has been reported causing canker disease in eucalypts in Tasmania (Yuan & Mohammed 1997). There is regional widespread distribution of L. shearii within the geographically isolated SWAFR on a diverse range of native Proteaceous hosts. Absence in the literature to date and being found only within the SWAFR suggests L. shearii may be endemic and host family specific to the Proteaceae within the region. Historical records of the incidence in B. coccinea also indicate this fungus is a long established endemic or at least well adapted ecologically prior to first isolation by Shearer in 1985.

Alternatively, the absence of the sexual morph on native hosts in the SWAFR suggests that the center of diversity for L. shearii is elsewhere. This behaviour is similar to H. eucalypti, where only the asexual morph has been found in Western Australia though the fungus is native to the Australian continent (Nakabonge et al. 2008).

Shearer et al. (1995) previously demonstrated the pathogenicity of L. shearii (as a Zythiostroma sp.) by girdling and killing B. baxteri and B. coccinea inoculated stems followed by 100 % recovery of the pathogen. Luteocirrhus shearii was
Fig. 5. One of 125 most parsimonious trees of 92 steps based on analysis of LSU gene region. Bootstrap values are given above the line. Trees are rooted to Diaporthe eres and D. fibrosa.
Fig. 6. One of 172 most parsimonious trees of 1041 steps based on analysis of combined DNA sequence data set of gene regions of the partial exon 4, exon 5, exon 6 and exon 7 of the BT genes, and the ITS gene region. Bootstrap values are given above the line. The trees are rooted to Diaporthe ambigua.
not considered a major cause of death in *B. coccinea* due to infrequent isolation. Pathogenicity has now been demonstrated in a further seven *Banksia* spp. (Shearer & Crane, unpubl. data) and the fungus has been recorded as occurring naturally across a wide geographic area within the range of *Proteaceae* in the SWAFR. The isolation of *L. shearii* from 14% of healthy *B. baxteri* stems suggests that the fungus is capable of a latent phase or has some type of endophytic stage in the disease epidemiology, which warrants further investigation.

Worldwide, the incidence of canker diseases caused by or associated with these types of fungi and other endophytes has been steadily increasing. Climate change is seen as the driving force in the apparent emerging pathogenicity of these normally minor diseases (Desprez-Loustau et al. 2006, Jurc & Ogris 2006, Daikin et al. 2010). Concurrent studies of the influence of climate on canker disease in *Proteaceae* in the SWAFR has shown that *L. shearii* is one of the causal organisms frequently isolated from aggressive cankers. *Neofusicoccum australe*,

---

### Table 1. Isolates and reference specimens of *Luteocirrhus shearii* used in the phylogenetic, morphological analysis.

| Isolate | Western Australian Herbarium specimen | Host | Location | ITS       | BT1     | BT2     | LSU       |
|---------|----------------------------------------|------|----------|-----------|---------|---------|-----------|
| Bb7.2   |                                        | *Banksia baxteri* | Waychinicup National Park WA | KC197020  | KC197011 | KC197005 |
| CBS1 130774 |                                    |      |          |           |         |         |           |
| Bb8.2   | PERTH 08355347                         | *B. baxteri* | Mt Groper WA | KC197025  | KC197016 | KC197010 |
| WAC13426 |                                    |      |          |           |         |         |           |
| Bb11.4  |                                        | *B. baxteri* | Stokes National Park WA | KC197022  | KC197013 | KC197007 | KC197017 |
| Bb16.7  | PERTH 08355339                         | *B. baxteri* | Cape Riche WA | KC197023  | KC197014 | KC197008 |
| Bb16H   | PERTH 08355290                         | *B. baxteri* | Cape Riche WA | KC197024  | KC197015 | KC197009 | KC197018 |
| CBS 130775 |                                    |      |          |           |         |         |           |
| Bb17.5  | PERTH 08439362                         | *B. baxteri* | Mettler Lake Nature Reserve WA | KC197021  | KC197012 | KC197006 | KC197019 |
| CBS 130776 WAC 134251 |                                    |      |          |           |         |         |           |
| CC1572  | PERTH 08355274                         | *B. grandis* | Palmdale rd Albany WA | KC197020  | KC197011 | KC197005 |
| CC1577  | PERTH 8355266                          | *B. grandis* | South Sister Nature reserve WA | KC197020  | KC197011 | KC197005 |
| CC1579  | PERTH 08355282                         | *B. baxteri* | Waychinicup National Park WA | KC197020  | KC197011 | KC197005 |
| CC1587  | PERTH 08355304                         | *B. pteridifolia* | Bremer Bay WA | KC197020  | KC197011 | KC197005 |
| CC1589  | PERTH 08355312                         | *B. baxteri* | Mt Groper WA | KC197020  | KC197011 | KC197005 |
| CC1590  | PERTH 08355320                         | *Lambertia echinata* subsp. *citrina* | Hassel National Park WA | KC197020  | KC197011 | KC197005 |

---

1 Ex-type culture.
2 CBS, Centaalbureau voor Schimmelcultures, Utrecht, the Netherlands.
3 WAC, Department Agriculture Plant Pathogen Collection, Department of Agriculture Western Australia.

### Table 2. Morphological characteristics of *Luteocirrhus* compared with other genera of *Cryphonectriaceae* having entirely black conidiomata.

| Morphological characteristics | *Celoporthe* | *Chrysoporthe* | *Luteocirrus* |
|-------------------------------|-------------|---------------|--------------|
| Conidiomatal colour           | Entirely fuscous black when mature | Entirely fuscous black | Entirely fuscous black |
| Conidiomatal position in bark | Superficial | Superficial   | Semi immersed |
| Conidiomatal shape            | Pulvinate to conical/globose, ± neck | Pyriform to pulvinate, one to four attenuated necks | Pulvinate to globose, ± neck |
| Conidiomatal stromatic tissue | Prosenchyma and pseudoparenchyma | Textura globulosa and textura corpecta | Basal textura globulosa |
| Paraphyses                    | Present     | Absent        | Present      |
| Periphyses                    | Absent      | Absent        | Absent       |
| Conidial shape                | Cylindrical | Oblong        | Cylindrical or slightly allantoid |
| Conidial colour on mass       | Luteous     | Luteous       | Luteous      |
Luteocirrhus shearii gen. sp. nov.

ACKNOWLEDGEMENTS

We are grateful to Bryan Shearer, Sarah Barrett, Chris Dunne, Richard Fairman, Malcolm Grant, Eddie Lim, Peter Scott, and Meridith Spencer for assistance in isolate collection, Diane White for sequencing, Louise Ratcliff for inoculation and harvesting assistance and Jane Crane for reviewing an earlier version of the manuscript.

REFERENCES

Andjic V, Hardy GESU, Cortinas MN, Wingfield MJ, Burgess TI (2007) Multiple gene genealogies reveal important relationships between species of Phaeophloeospora infecting Eucalyptus leaves. FEMS Microbiology Letters 268: 22–33.

Bates BC, Hope P, Ryan B, Smith I, Charles S (2008) Key findings from the Indian Ocean Climate Initiative and their impact on policy development in Australia. Climatic Change 89: 339–354.

Beard JS (1989) Definition and location of Banksia woodlands. Journal of the Royal Society of Western Australia 71: 85–86.

Beard JS, Chapman AR, Gioia P (2000) Species richness and endemism in the Western Australian flora. Journal of Biogeography 27: 1257–1268.

Begoude BAD, Slippers B, Wingfield MJ, Roux J (2010) Botryosphaeriaceae associated with Terminalia catappa in Cameroon, South Africa and Madagascar. Mycological Progress 9: 101–123.

Boesewinkel HJ (1976) Storage of fungal cultures in water. Transactions of the British Mycological Society 66: 183–185.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-anun C, Crous PW (2009) Myrtaceae, a cache of fungal biodiversity. Persoonia 23: 55–85.

Chen SF, Gryzenhout M, Roux J, Xie YJ, Wingfield, MJ, Zhou XD (2011) Novel species of Celolophorte from Eucalyptus and Syzygium trees in China and Indonesia. Mycologia 103: 1384–1410.

Chen SF, Wingfield MJ, Roets F, Roux J (2012) A serious canker disease caused by Immersiophorte knoxdaviesiana gen. et sp. nov. (Cryptonectriaceae) on native Rapanea melanophloeos in South Africa. Plant Pathology Doi: 10.1111/j.1365-3059.2012.02671. 62: 667–678.

Crane CE, Shearer BL, Barrett S, Dunne CP (2012) Influence of climate on canker disease in the Proteaceae of the Southwest Australian Floristic Region. In Proceedings of the Australasian Systematic Botany Society Conference: 26.

Crous PW, Summerell BA, Shivas RG, Burgess TI, Decock CA et al. (2012) Fungal Planet description sheets: 164–165. Persoonia 28: 138–182.

Crous PW, Summerell BA, Alfenas AC, Edwards J, Pascoe IG et al. (2012a) Coelomycetous genera associated with leaf spots of tree hosts. Persoonia 28: 66–75.

Daikin N, White D, Hardy G, Burgess T (2010) The opportunistic pathogen, Neofusicoccum australe, is responsible for crown dieback of peppermint (Agonis flexuosa) in Western Australia. Australasian Plant Pathology 39: 202–206.

Desprez-Laustau ML, Marciais B, Nageleisen LM, Piou D, Vannini A (2006) Interactive effects of drought and pathogens in forest trees. Annals of Forest Science 63: 597–612.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thieer T, Wilson A (2011) Geneious. Version 5.4. <http://www.geneious.com/>.

Felsenstein J (1985) Confidence intervals on phylogenetics: an approach using bootstrap. Evolution. 39: 783–791.

Fitzpatrick MC, Gove AD, Sanders NJ, Dunn RR (2008) Climate change, plant migration, and range collapse in a global biodiversity hotspot: the Banksia (Proteaceae) of Western Australia. Global Change Biology 14: 1337–1352.

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Gryzenhout M, Wingfield BD, Wingfield MJ (2009) Taxonomy, phylogeny, and ecology of bark-inhabiting and tree-pathogenic fungi in the Cryptonectriaceae. St Paul, MN: American Phytopathological Society Press.

Hillis DM, Huelsenbeck JP (1992) Signal, noise and reliability in molecular phylogenetic analysis. Journal of Heredity 83: 189–195.

Hopper SD (1980) Bird and mammal pollen vectors in Banksia communities at Cheyne Beach, Western Australia. Australian Journal of Botany 28: 61–75.

Jurc D, Ogris N (2006) First reported outbreak of charcoal disease caused by Biscogniauxia mediterranea on Turkey oak in Slovenia. Plant Pathology 55. 299.

Kirby KN (1993). Advanced data analysis with SYSTAT. Van Nostrand Reinhold, New York.

Lumbsch HT, Huhndorf SM (2007) Notes on ascomycete systematics. Nos 4408–4750. Mycologia 13: 59–99.
Shearer BR, Fairman RG (1991) Aerial canker fungi threaten Banksia coccinea. In: Proceedings of the Conservation Biology in Australia and Oceania Conference, University of Queensland: Abstract 85/C16.

Shearer BR, Fairman RG, Bathgate JA (1995) Cryptodiaporthe melanocraspeda canker as a threat to Banksia coccinea on the South Coast of Western Australia. *Plant Disease* **79**: 637–641.

Swofford DL (2003) *PAUP*: phylogenetic Analyses using parsimony (* and other methods). Version 4.0b10. Sunderland, MA: Sinauer Associates.

Vermeulen M, Gryzenhout M, Wingfield MJ, Roux J (2011) New records of the *Cryphonectriaceae* from southern Africa including *Latruncellus aurorae* gen. sp. nov. *Mycologia* **103**: 554–569.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4239–4246.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds.: 315–322. San Diego: Academic Press.

Woolier RD, Richardson KC, Garavanta CAM, Saffer VM, Bryant KA (2000) Opportunistic breeding in the polyandrous honey possum, *Tarsipes rostratus*. *Australian Journal of Zoology* **48**: 669–680.

Yuan, Zi-Qing, Mohammed C (1997) Investigation of fungi associated with stem cankers of eucalypts in Tasmania Australia. *Australasian Plant Pathology* **26**: 78–84.