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ARTICLE

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

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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 cirrhi, 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
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Cryphonectriaceae
Emerging pathogen
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Natural ecosystems
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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. macroclavatum*, and *Cryptodiaporthe melanocraspeda*, along with an undescribed species (Crane *et al.* 2012) which shared morphology 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, Wooller *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), ß-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

Twig samples 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 2011with 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 mm^2 spanning the lesion-healthy margin were 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 (19.5 g of Difco™ PDA and 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 mm^2 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 *Zythiostroma* sp. were determined in the dark on half-strength PDA medium for temperatures between 1–40 ºC at 5 ºC 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 (ITS1-5.8S-ITS2) 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 LR5 (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

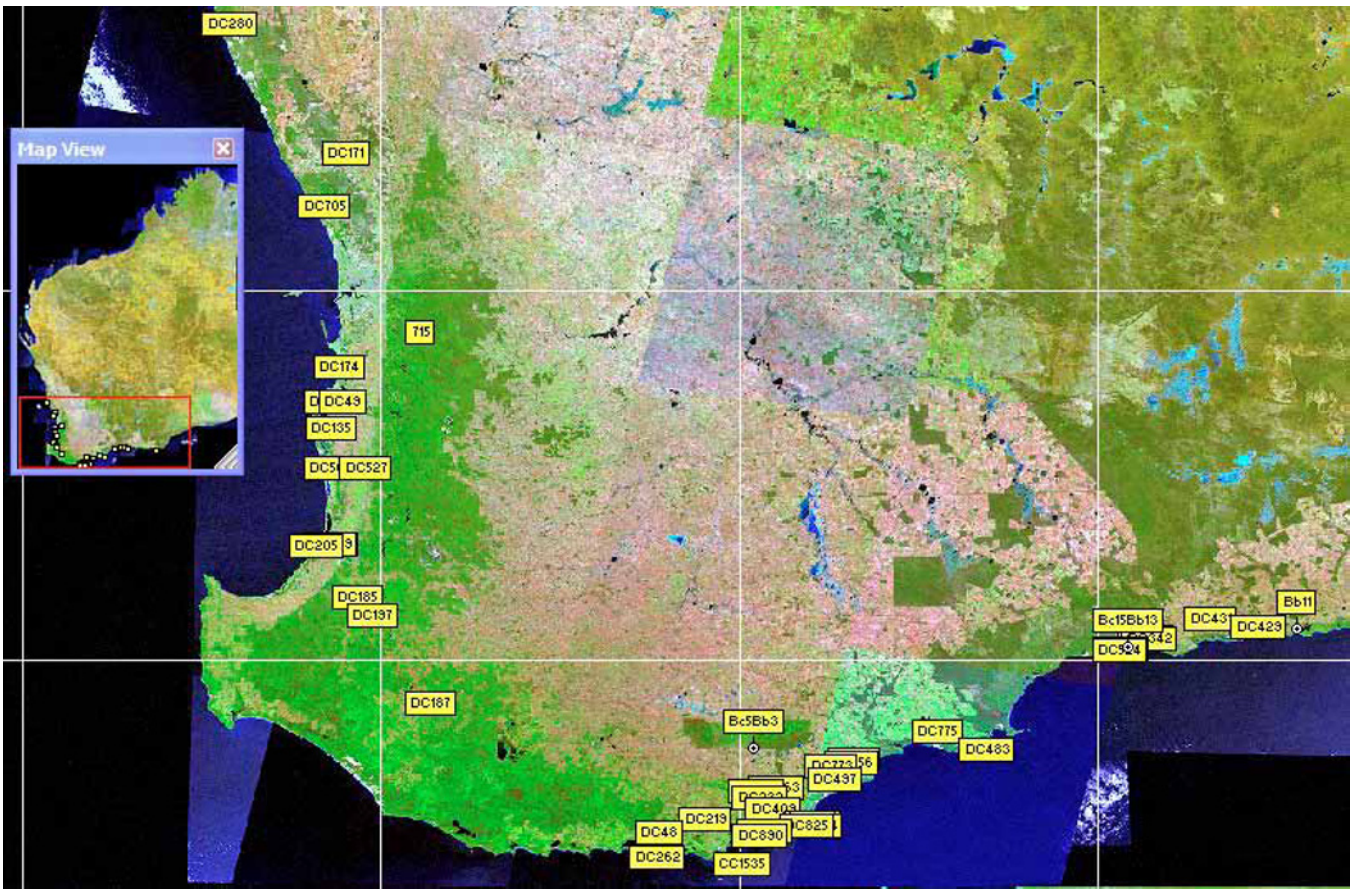

Fig. 2. Distribution of *Luteocirrhus shearii* from cankered branches in the South Western Australian Floristic Region.

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 ambigua* 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.

Morphology and taxonomy

Luteocirrhus C. Crane & T. I. Burgess, **gen. nov.**
MycoBank MB563390

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

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.

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 base cell tissue of *textura globulosa*. *Conidiophores* phialidic, enteroblastic, hyaline, channel and collarette minute. *Conidia* hyaline, aseptate, cylindrical or slightly allantoid, exuded as orange/yellow cirrhi, bright luteus on mass, exuded as cirrhi or tendrils. *Ascotromata* not seen.

Luteocirrhus shearii C. Crane & T. I. Burgess. **sp. nov.**
MycoBank MB563472
(Fig. 3)

Etymology: *shearii* – taken from Bryan Shearer, who discovered the fungus on *Banksia baxteri,* and shortened for phonetic simplicity.

Type: **Australia**: *Western Australia*: Mettler Lake Nature Reserve, -34.55962, 118.62395 (lat/long), isolated from pycnidia in branch canker on *Banksia baxteri*, 17 Nov. 2009, *C. Crane*, (PERTH 08439362 – **holotype;** cultures ex-holotype, CBS 130776 = WAC 13425).

Description: Conidiomata pulvinate with or without neck, typically 200–600 µm high, 200–690 µm diam, separate, fuscous black, subcortical semi-immersed or sometimes superficial erupting through bark, ostiolate, uni- to multiloculate, convoluted, paraphyses present, 20–40 µm long and base cells tissue of *textura globulosa*. *Conidiophores* 8–18 × 2–3 µm, phialidic, enteroblastic, hyaline, channel and collarette minute. *Conidia* 3–4 × 1 µm, hyaline, aseptate, cylindrical or slightly allantoid, exuded as orange cirrhi, bright luteus on mass, exuded as cirrhi or tendrils. *Ascotromata* not seen.

Culture characteristics: Mycelium in culture (half-strength PDA), immersed, septate, initially hyaline turning pale brown (Mu 7.5YR4/4 “brown”; Munsell 1994) to olive green (Mu 5Y4/4 “olive”), squiggly appearance, producing copious conidiomata on older growth topped with orange yellow cirrhi, optimum 25 ºC no growth at 1 and 40 ºC (Fig. 4).

Additional specimens examined: **Australia:** *Western Australia*: Mt Groper, -34.51084, 118.79974 (lat/long), isolated from canker on *B. baxteri*, 19 Apr. 2010, *C. Crane* (PERTH 08355347, WAC 13426); Cape Riche, -34.567100, 118.707881, isolated from canker on *B. baxteri*, 24 May 2010, *C. Crane* (PERTH 08355339); Waychinicup National Park, -34.882433, 118.412117, isolated from canker on *B. baxteri,* 7 Nov. 2009, *C. Crane* (PERTH 08355282); Mt Groper, -34.510000, 118.800867, isolated from canker on *B. baxteri,* 19 Nov. 2009, *C. Crane (*PERTH 08355312); Cape Riche, -34.883417, 118.399850, isolated from canker on *B. baxteri*, 17 Nov. 2009, *C. Crane (*CBS 130775); South Sister Nature Reserve, -34.801100, 118.192400, isolated from cankers on *B. grandis*, 17 Nov. 2009, *C. Crane* (PERTH 08355266); Bremer Bay, -34.473717, 119.373683, isolated from cankers on *B. pteridifolia*, 18 Nov. 2009, *C. Crane (*PERTH 08355304); Hassell National Park, -34.576050, 118.515450, isolated from cankers on *Lambertia echinata* ssp. *citrina*, 19 Nov. 2009, *C. Crane* (PERTH 08355320).

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 *Chrysoporthe* and mature conidiomata of *Celoporthe*, being distinct from other genera within the family which contain some orange colour. With *Celoporthe*, *L. shearii* shares conidiomatal shape, presence of paraphyses, absence of periphyses, conidial shape and colour in mass. *Luteocirrhus shearii* differs from *Celoporthe* in having basal *textura globulosa* conidiomatal stromatic tissue. With *Chrysoporthe, 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 *Latrunclla aurorae,* 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

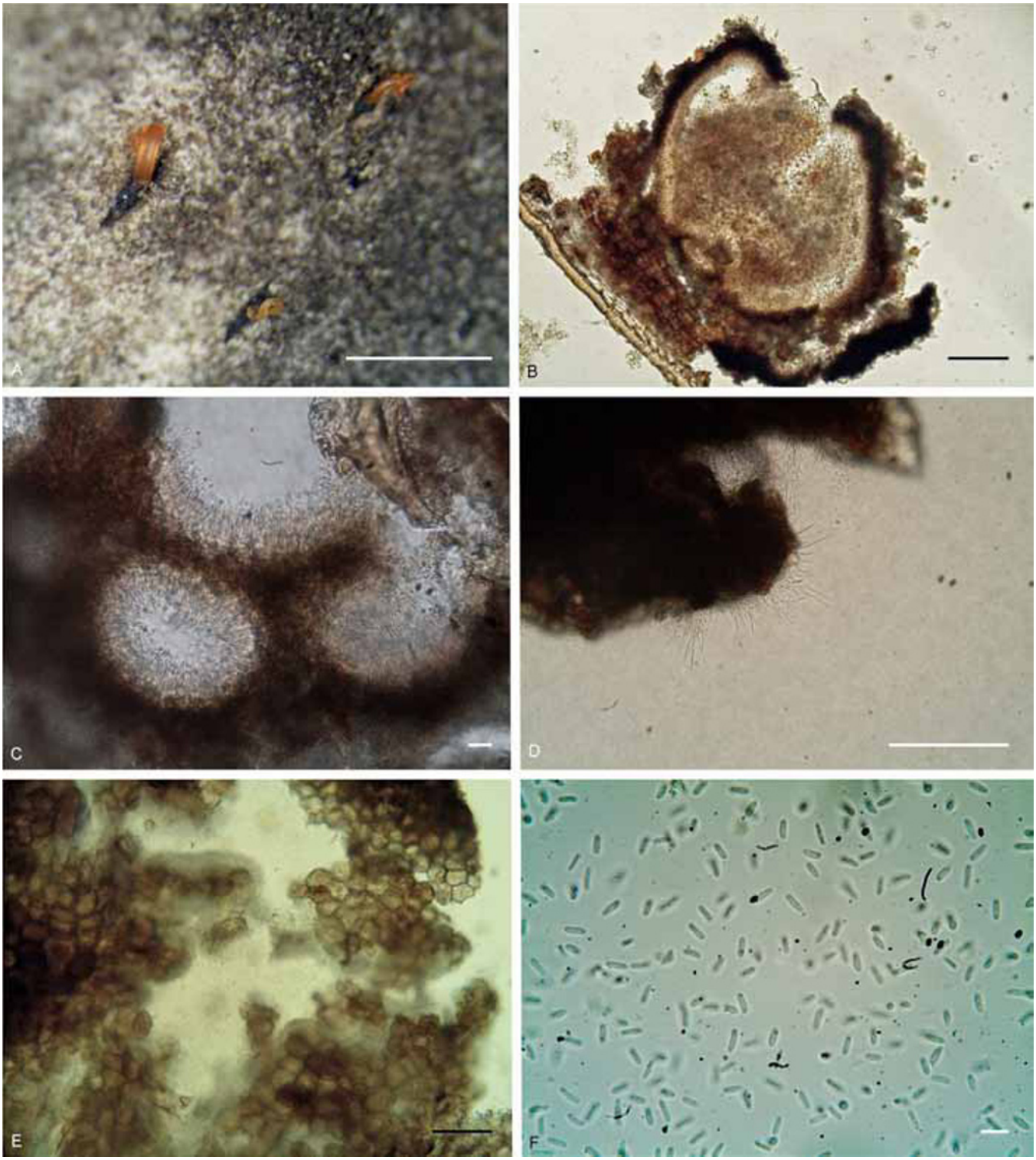

Fig. 3. *Luteocirrhus shearii* (PERTH 08355274). **A.** Conidiomata with cirrhi. **B.** Vertical section of conidiomata. **C.** Horizontal cross section of conidiomata. **D.** Paraphyses protruding from hymenium. **E.** Conidiomatal tissue of textura globosa. **F.** Conidia. Bars A = 1 mm; B and D =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

genera within the *Cryphonectriaceae* such as *Celoporthe, Cryponectria, 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

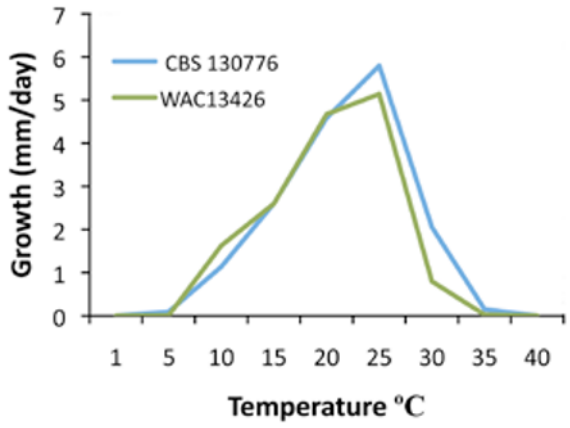

Fig. 4. Radial growth rates of two isolates of *Luteocirrhus shearii* on half-strength potato dextrose medium.

1215 characters of which 359 were informative. Heuristic searches resulted in 172 most parsimonious trees of 1041 steps (CI=0.53, RI=0.82) (TreeBASE SN14068, Fig. 6). The topology of the Bayesian tree was very similar. All isolates of *Luteocirrhus shearii* were identical and reside in a highly supported terminal clade. *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 radicalis* 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 ($P \leq 0.5$) 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 & Fairman1991). 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 *Foliocryphia eucalypti* (Cheekwangkoon *et al.* 2009), are found on the eastern side of the continent and in Tasmania to the south-east, *Chrysochrypta corymbiae* in the Northern Territory (Crous *et al.* 2012) and the stem canker pathogen *Holocryphia 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.*

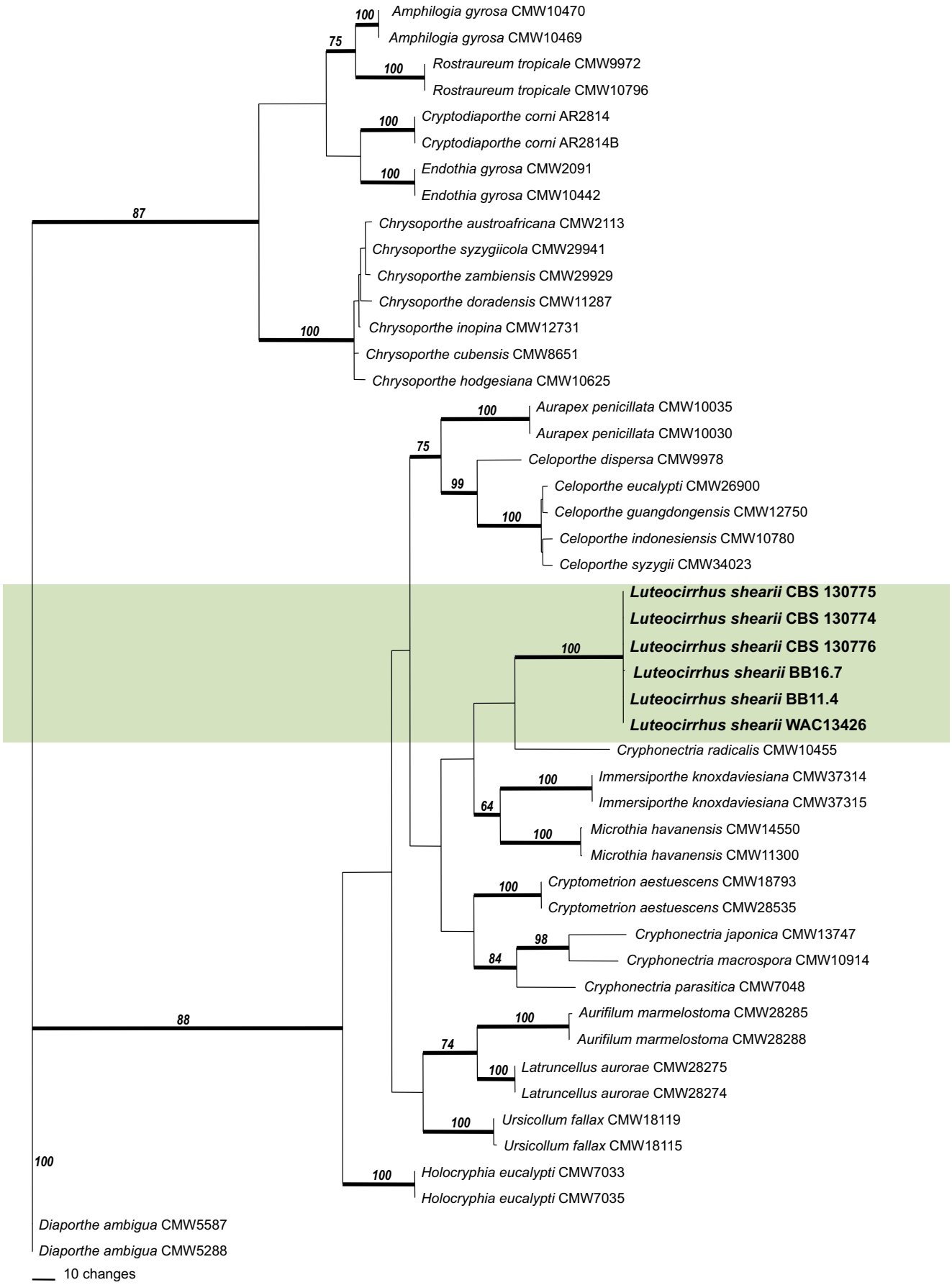

Fig. 6. One of 172 most parsimonious trees of 1041 steps based on analysis of combined DNA sequence data set of gene regions of the partial exon 4, exon 5, exon 6 and exon 7 of the BT genes, and the ITS gene region. Bootstrap values are given above the line. The trees are rooted to *Diaporthe ambigua*.

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 CBS[2] 130774		*Banksia baxteri*	Waychinicup National Park WA	KC197020	KC197011	KC197005	
Bb8.2 WAC[3]13426	PERTH 08355347	*B. baxteri*	Mt Groper WA	KC197025	KC197016	KC197010	
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 CBS 130775	PERTH 08355290	*B. baxteri*	Cape Riche WA	KC197024	KC197015	KC197009	KC197018
Bb17.5 CBS 130776 WAC 13425[1]	PERTH 08439362	*B. baxteri*	Mettler Lake Nature Reserve WA	KC197021	KC197012	KC197006	KC197019
CC1572	PERTH 08355274	*B. grandis*	Palmdale rd Albany WA				
CC1577	PERTH 8355266	*B. grandis*	South Sister Nature reserve WA				
CC1579	PERTH 08355282	*B. baxteri*	Waychinicup National Park WA				
CC1587	PERTH 08355304	*B. pteridifolia*	Bremer Bay WA				
CC1589	PERTH 08355312	*B. baxteri*	Mt Groper WA				
CC1590	PERTH 08355320	*Lambertia echinata* subsp. *citrina*	Hassel National Park WA				

[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***	***Luteocirrhus***
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 porrecta	Basal textura globulosa
Paraphyses	Present	Absent	Present
Periphyses	Absent	Absent	Absent
Conidial shape	Cylindrical	Oblong	Cylindrical or slightly allantoid
Condial colour on mass	Luteous	Luteous	Luteous

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*,

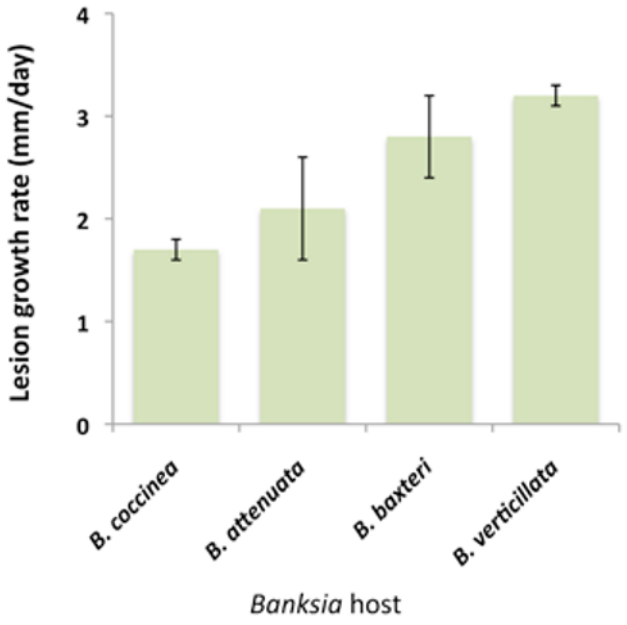

Fig. 7. Mean (± standard error) visible lesion growth rates of *Luteocirrhus shearii* following stem wound inoculation of four *Banksia* hosts. Wounds healed over in control inoculations.

N. macroclavatum, and *Cryptodiaporthe melanocraspeda,* along with *L. shearii,* are forming a disease complex that is having an increasing impact across many proteaceous species in the region (Crane *et al.* 2012). This increasing impact has so far been positively correlated with minimum temperatures (Crane *et al.* 2012) and the complex appears to be an emerging disease issue in a changing environment.

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