New *Phaeoacremonium* species isolated from sandalwood trees in Western Australia

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**Abstract:** Thirty-eight *Phaeoacremonium* isolates collected from pruning wounds of tropical sandalwood in Western Australia were studied with morphological and cultural characteristics as well as phylogenetic analyses of combined DNA sequences of the actin and β-tubulin genes. Three known *Phaeoacremonium* species were found, namely *P. alvesii*, *P. parasiticum*, and *P. venezuelense*. *Phaeoacremonium venezuelense* represents a new record for Australia. Two new species are described: *P. luteum* sp. nov. can be identified by the ability to produce yellow pigment on MEA, PDA, and OA, the predominance of subcylindrical to subulate type II phialides, and the mycelium showing prominent exudate droplets observed as warts; and *P. santali* sp. nov. which can be separated from other species producing pink colonies on MEA by the predominance of type I and II phialides, the distinct brownish olive colonies in OA, and slow growth.

**Key words:** actin

β-tubulin

DNA phylogeny

*Santalum*

systematics

*Togninia*

**INTRODUCTION**

Tropical Sandalwood (*Santalum album*) is one of the world’s most valuable tropical tree species and demand has led to the overexploitation of natural sandalwood stands, in Timor from which it originates, and in India where it became naturalized over 2000 yr ago (Harbaugh & Baldwin 2007). To combat this destruction, plantations have been established in various countries including Australia, with commercial plantations established in 1999. The Ord River Irrigation Scheme near Kununurra has a Tropical sandalwood plantation estate presently occupying approximately 5 000 ha.

Sandalwood is a slow growing root hemi-parasite. The valuable sandalwood oil has been reported to start developing in the heartwood from 5 yr onwards. Plantation diseases usually only become a risk factor in plantation systems after two to three generations. However, disease risk in tropical plantation systems can become an issue in the first generation (Barry 2002, Barbour *et al.* 2010).

There have been few reports of fungal diseases infecting the Tropical sandalwood. Reports have been made of the presence of *Phytophthora cinnamomi* and in the early 1990s, an isolation of *Ganoderma steyartanum* was undertaken from host species, but not from Tropical sandalwood (Len Nelson pers. comm.). More recently Rural Industries Research and Development Corporation (RIRDC) supported an investigation by Barbour *et al.* (2010) into the identification of the heartwood rot in Tropical sandalwood and the impact on oil levels within the heartwood. Several rot fungi were isolated and sections suggested that the fungi were entering the branches and main stem via wounds made during pruning or when branches are damaged. The immediate response to this knowledge was to establish a pruning trial examining the effect of tree age and timing (season) of pruning on infection development. The pruned trees were destructively harvested after 6 and 12 mo, and over 70 endophytes, canker, and rot fungi recovered. Among these fungi were several *Phaeoacremonium* isolates.

The genus *Phaeoacremonium* was established by Crous *et al.* (1996), and 40 species have been described so far (Crous *et al.* 1996, Dupont *et al.* 2000, Groenewald *et al.* 2001, Mostert *et al.* 2005, 2006, Damm *et al.* 2008, Essakhli *et al.* 2008, Graham *et al.* 2009, Gramaje *et al.* 2009, 2012, Úrbez-Torres *et al.* 2014), including three species originally described as *Togninia* species with *Phaeoacremonium* asexual morphs: *T. africana* and *T. griseo-olivacea* (Damm *et al.* 2008), and *T. vibratilis* (Réblová & Mostert 2007). Several species of this genus have been studied intensively because of the involvement of these taxa in two complex fungal diseases of grapevine, namely Petri disease in young vines and esca disease in adult vines (Mostert *et al.* 2006), as well as with human infections, so-called phaeohyphomycoses (Mostert *et al.* 2005). However, numerous species of *Phaeoacremonium* have also been associated with disease...
Table 1. The described *Phaeoacremonium* (and *Togninia* sexual morphs) species known from soil and host plants other than *Vitis vinifera*, and their worldwide distribution. * = epithets not yet transferred to *Phaeoacremonium*.

| Phaeoacremonium species | Host/Substrate | Country and Reference |
|-------------------------|----------------|-----------------------|
| *P. aleophilum* (T. minima) | *Actinidia chinensis* | Italy (Crous & Gams 2000) |
| *Malus domestica* | | Iran (Arzanlou et al. 2014); South Africa (Cloete et al. 2011) |
| *Olea europea* | | Italy (Crous & Gams 2000); USA (Úrbez-Torres et al. 2013) |
| *Phoenix dactylifera* | | Iran (Mohammadi 2014) |
| *Prunus armeniaca* | | Iran (Arzanlou et al. 2014); South Africa (Damm et al. 2008) |
| *Prunus persica* | | South Africa (Damm et al. 2008) |
| *Prunus salicina* | | South Africa (Damm et al. 2008) |
| *Prunus peninsularis* | | USA (Hausner et al. 1992) |
| *Pyrus communis* | | South Africa (Cloete et al. 2011) |
| *Salix sp.* | | USA (Hausner et al. 1992) |
| *Soil* | | Spain (Agusti-Brisach et al. 2013); USA (Rooney et al. 2001) |
| *P. alvesii* | *Dodonaea viscosa* | Australia (Mostert et al. 2005) |
| *Olea europea* | | Italy (Nigro et al. 2013) |
| *P. amygdalinum* | *Prunus dulcis* | Spain (Gramaje et al. 2012) |
| *P. argentinense* (T. argentinensis) | *Soil* | Argentina (Crous & Gams, 2000) |
| *P. australiense* | *Prunus salicina* | South Africa (Damm et al. 2008) |
| *P. fuscum* | *Prunus salicina* | South Africa (Damm et al. 2008) |
| *P. griseorubrum* | *Prunus salicina* | South Africa (Damm et al. 2008) |
| *P. inflatipes* | *Hypoxylon truncatum* | USA (Mostert et al. 2005) |
| *Nectandra sp.* | | Costa Rica (Groenewald et al. 2001) |
| *Quercus virginiana* | | USA (Groenewald et al. 2001) |
| *Soil* | | USA (Rooney et al. 2001) |
| *P. iranianum* | *Actinidia chinensis* | Italy (Mostert et al. 2006) |
| *Malus domestica* | | Iran (Arzanlou et al. 2013) |
| *Prunus armeniaca* | | South Africa (Damm et al. 2008) |
| *Prunus dulcis* | | Spain (Gramaje et al. 2012) |
| *Pyrex communis* | | South Africa (Cloete et al. 2011) |
| *P. mortoniae* (T. fraxinopennisylvanica) | *Actinidia chinensis* | Italy (Prodi et al. 2008) |
| *Fraxinus excelsior* | | Sweden (Groenewald et al. 2001) |
| *Fraxinus latifolia* | | USA (Eskalen et al. 2005) |
| *Fraxinus pennsylvanica* | | USA (Hausner et al. 1992) |
| *Prunus salicina* | | South Africa (Damm et al. 2008) |
| *Pyrex communis* | | South Africa (Cloete et al. 2011) |
| *Quercus agrifolia* | | USA (Lynch et al. 2013) |
| *P. novae-zealandiae* (T. novae-zealandiae) | *Cupressus macrocarpa* | New Zealand (Hausner et al. 1992) |
| *Pinus radiata* | | New Zealand (Hausner et al. 1992) |
| *P. parasiticum* (T. parasitica) | *Actinidia chinensis* | Italy (Di Marco et al. 2004) |
| *Aquilaria agallocha* | | n.d. (Mostert et al. 2006) |
| *Cupressus sp.* | | n.d. (Mostert et al. 2006) |
| *Nectandra sp.* | | Costa Rica (Hawksworth et al. 1976) |
| *Olea europea* | | Italy (Nigro et al. 2013) |
| *Phoenix dactylifera* | | Iran (Mohammadi 2014); Iraq (Hawksworth et al. 1976) |
| *Prunus armeniaca* | | South Africa (Damm et al. 2008); Tunisia (Hawksworth et al. 1976) |
| *Prunus avium* | | Greece (Rumbos 1986) |
| *Quercus virginiana* | | USA (Halliwell 1966) |
| *Soil* | | Tahiti (Dupont et al. 2002); Spain (Agusti-Brisach et al. 2013) |
| *P. pallidum* | *Prunus armeniaca* | South Africa (Damm et al. 2008) |
| *P. pruniculum* | *Prunus salicina* | South Africa (Damm et al. 2008) |
| *P. rubriopenum* (T. rubrigena) | *Dactylis glomerata* | Spain (Sánchez-Márquez et al. 2007) |
Phaeoacremonium species from sandalwood

The aim of the present study was to identify isolates of Phaeoacremonium collected from pruning wounds of sandalwood trees and to characterise those that appeared to be morphologically and genetically different from known species of the genus.

**MATERIAL AND METHODS**

**Sampling and fungal isolation**

Two sandalwood plantations were selected (1-yr-old and 5-yr-old). In each plantation 40 trees were selected for pruning, with the condition that more than two branches required pruning per tree. Twenty were used for the “post wet-season” pruning treatment completed on the 31 May 2011, and the remaining 20 trees were pruned for the “pre wet-season” treatment on the 4 November 2011. Branches were pruned using secateurs, and when required pruning saws, with cuts made as close to the stem as possible without causing damage to the stem bark. Trees were pruned to a maximum of one half of tree height.

Each wound sample was split longitudinally through the centre of the wound using a chisel cleaned with 70% ethanol between samples. For each pruning wound, shavings were taken using a sterilised scalpel from the margin between stained and healthy wood. These shavings were then transferred onto two media; (1) half strength Potato Dextrose Agar (PDA, Becton Dickinson, Sparks, MD; 19.5 g/L PDA, 7.5 g/L agar) containing 133 µg/mL streptomycin; and (2) Basidiomycete selective medium (5 g/L Bacto peptone (Difco, NSW, Australia), 20 g/L agar, 0.25 g/L MgSO\(_4\)•7H\(_2\)O, 0.5 g/L K\(_2\)HPO\(_4\), 0.016 g/L benomyl, 100 µg/L streptomycin, 2 ml/L 50% (v/v) lactic acid, and 20 ml/L 95% ethanol). After 2 wk, representative fungal colonies were transferred onto fresh ½ strength PDA. The cultures were examined regularly and any contaminated cultures were cleaned.

Once clean, all isolates from a single harvest time were subcultured on the same day onto ½ strength PDA. This was to enable comparison of culture morphology. After 2 wk, cultures were grouped based on morphology and representative isolates from each group were selected for molecular study.

**Morphological identification and characterisation**

Morphological characters used in this study to distinguish Phaeoacremonium species include conidiophore morphology, phialide type and shape, size of hyphal warts, and conidial size and shape. Colony characters and pigment production on MEA, PDA and oatmeal agar (OA; 60 g oatmeal; 12.5 g agar; Difco, Madrid, Spain) (Crous et al. 2009) incubated at 25°C were noted after 8 and 16 d.

Microscopic observations were made from aerial mycelium of colonies cultivated on MEA or by using the slide culture

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Table 1. (Continued).

| Phaeoacremonium species | Host/Substrate | Country and Reference |
|------------------------|----------------|-----------------------|
| *P. scolyti*           | Olea europea  | Italy (Nigro et al. 2013) |
|                        | Prunus armeniaca | South Africa (Damm et al. 2008) |
|                        | Prunus persica | South Africa (Damm et al. 2008) |
|                        | Prunus persica var. nucipersica | South Africa (Damm et al. 2008) |
|                        | Prunus salicina | South Africa (Damm et al. 2008) |
| *P. subulatum*         | Prunus armeniaca | South Africa (Damm et al. 2008) |
| *P. theobromatis*      | Theobroma gileri | Equador (Mostert et al. 2006) |
| *P. venezuelense*      | Prunus armeniaca | Spain (Olmo et al. 2014) |
| *P. viticola* (T. viticola) | Actinidia chinensis | France (Hennion et al. 2001) |
|                        | Prunus armeniaca | South Africa (Damm et al. 2008) |
|                        | Prunus salicina | South Africa (Damm et al. 2008) |
|                        | Pyrus communis | South Africa (Cloete et al. 2011) |
|                        | Sorbus intermedia | Germany (Mostert et al. 2006) |
| *T. africana* *         | Prunus armeniaca | South Africa (Damm et al. 2008) |
| *T. griseo-olivacea* * | Prunus armeniaca | South Africa (Damm et al. 2008) |
| *T. vibratilis* *      | Fagus sylvatica | France and Hungary (Réblová & Mostert 2007) |
|                        | Prunus padus | Sweden (Réblová & Mostert 2007) |
|                        | Sorbus sp. | Italy (Réblová & Mostert 2007) |

n.d. = no data.
Phaeoacremonium species from sandalwood

Molecular characterization: DNA isolation and amplification
Fungal mycelium and conidia from pure cultures grown on PDA for 2 wk at 25 ºC in the dark were scraped and mechanically disrupted by grinding to a fine powder under liquid nitrogen with a mortar and pestle. Total DNA was extracted with the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek, Norcross, GA) following the manufacturer’s instructions. DNA was viewed on 0.7 % agarose gels stained with ethidium bromide and stored at -20 ºC.

Approximately 600 bp of the 5’ end of the β-tubulin (BT) and approximately 300 bp of the 5’ end of the actin (ACT) genes were amplified as described by Mostert et al. (2006) using primer sets T1 (O’Donnell & Cigelnik 1997) and Bt2b, and ACT-512F and ACT-783R, (Carbone & Kohn 1999), respectively. PCR products were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany) and sequenced in both directions by Macrogen (Sequencing Center, Seoul). Sequences were edited using Sequencher software v. 4.7. (Gene Codes, Ann Arbor, MI).

Phylogenetic analyses
The new Phaeoacremonium sequences (BT and ACT), together with reference sequences (Mostert et al. 2006, Damm et al. 2008, Essakhi et al. 2008, Graham et al. 2009, Gramaje et al. 2009, 2012, Úrbez-Torres et al. 2014) and the outgroup, Pleurostomophora richardsiae (ACT = AY579271, BT = AY579334) obtained from GenBank, were aligned using MAFFT sequence alignment program v. 7 (Katoh & Standley 2013) followed by manual adjustments of the alignments in BioEdit Sequence Alignment Editor v. 7.2.3.

A partition homogeneity test of the BT and ACT alignments was conducted with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b (Swofford 2000) to test pairwise congruence between sequence data sets. Phylogenetic analyses of all aligned sequence data were performed with MEGA v. 5.05 software (Tamura et al. 2011). Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. All characters were unordered and of equal weight.

Maximum parsimony analysis was performed for the combined Phaeoacremonium dataset using the heuristic search option with 10 random simple taxon additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm with the option of saving no more than 10 trees with a score greater than or equal to 5 (Harrison & Langdale 2006). The robustness of the trees obtained was evaluated by 1 000 bootstrap replications (Hillis & Bull 1993). Tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC), were calculated.

Sequences derived in this study were lodged at GenBank, the alignments in TreeBASE (www.treebase.org/), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004). GenBank accession numbers of the strains collected during this study are listed in Table 2.

RESULTS
Fungal identification
The fungal isolates obtained in this study were characterised by having flat slow-growing cultures on MEA. Different types of phialides that were variable in size and shape were observed in the aerial mycelium, and either discrete or integrated in conidiophores. Sporulation was abundant and conidia hyaline and aseptate. All morphological characters corresponded to the genus Phaeoacremonium (Mostert et al. 2006). Based on their appearance in culture, the isolates could be assigned to five different clades (Table 2).

Molecular identification and phylogenetic analyses
The partition homogeneity test of the BT and ACT alignments of Phaeoacremonium gave a P-value of 0.263 indicating that the datasets were congruent and could be combined. The combined sequence dataset consisted of 78 isolates including the outgroup and had 977 characters, of which 535 characters were parsimony-informative, 180 parsimony-uninformative and 262 constant. Sixty equally most parsimonious trees were retained (length = 2539 steps, CI = 0.471, RI = 0.837, RC = 0.399). A tree that closely resembled the strict consensus tree was chosen and is presented in Fig. 1. The isolates of the clade 1 grouped together in a polyphyletic clade with 100 % bootstrap support, with P. griseorubrum as closely related species. The isolates of the clade 2 grouped together in a polyphyletic clade with 100 % bootstrap support, with P. tardicrescens as closely related species. The isolates of clades 3 and 4, grouped inside the P. alvesii and P. parasiticum clades respectively, with 99 % bootstrap support.

The BT and ACT sequences of the first clade of Phaeoacremonium isolates were 98 % identical to those of P. griseorubrum CBS 111657 (GenBank AY579294, AY579227). Differences were found between the first clade of Phaeoacremonium isolates and P. griseorubrum CBS 111657 sequences with five nucleotides varying in the ACT region and nine nucleotides in the BT region. The BT and ACT sequences of the second clade of Phaeoacremonium...
isolates were 97% identical to those of *P. tardicrescens* CBS 110573 (GenBank AY579300, AY579233). Differences were found between the second clade of *Phaeoacremonium* isolates and *P. tardicrescens* CBS 110573 sequences with six nucleotides varying in the ACT region and 20 nucleotides in the BT region. The BT and ACT sequences of the third clade of isolates had 100% identity with *P. alvesii* isolates CBS 113590 (GenBank AY579304) and STE-U 6988 (GenBank JQ038925), respectively. A BLASTn search showed that the BT and ACT sequences of the fourth clade of isolates had 100% identity with isolates previously identified as *P. parasiticum* CBS 860.73 (GenBank AY579253). The BT and ACT sequences of the isolate corresponding to the fifth clade had 100% identity with *P. venezuelense* isolate CBS 651.85 (GenBank AY579320).

**Table 2. Phaeoacremonium species, accession numbers, and collection details of isolates studied.**

| Species                        | Isolate          | Accession No.¹ | GenBank Accessions |
|-------------------------------|------------------|----------------|-------------------|
|                               |                  |                | ACT               | BT               |
| *Phaeoacremonium santali* (clade 1) | E2.1B            | A2             | KF835395          | KF823789         |
|                               | E9.3A            | A3             | KF835396          | KF823790         |
|                               | E19.2C           | A4             | KF835397          | KF823791         |
|                               | -                | A5             | KF835398          | KF823792         |
|                               | E11.2A           | A6             | KF835399          | KF823793         |
|                               | E15.1A           | A7             | KF835400          | KF823794         |
|                               | F3.1             | A26            | KF835401          | KF823795         |
|                               | F6.7             | A27            | KF835402          | KF823796         |
|                               | F2.3             | A28, CBS 137498| KF835403          | KF823797         |
|                               | E7.7             | A29            | KF835304          | KF823798         |
|                               | F2.4             | A30            | KF835405          | KF823799         |
|                               | E9.3             | A35            | KJ533536          | KJ533532         |
|                               | E9.2             | A36            | KJ533537          | KJ533533         |
|                               | F32.2C           | A37            | KJ533538          | KJ533534         |
|                               | E38.3B           | A38            | KJ533539          | KJ533535         |
| *Phaeoacremonium luteum* (clade 2) | F3.7             | A16, CBS 137497| KF835406          | KF823800         |
|                               | F2.4             | A17            | KF835407          | KF823801         |
|                               | F3.3             | A18            | KF835408          | KF823802         |
|                               | F2.1             | A19            | KF835409          | KF823803         |
|                               | F2.5             | A20            | KF835410          | KF823804         |
|                               | E30.5B           | A33            | KJ533542          | KJ533540         |
|                               | F37.3B           | A34            | KJ533543          | KJ533541         |
| *Phaeoacremonium alvesii* (clade 3) | F1.2A            | A21            | KF790540          | KF790535         |
|                               | F11.5B           | A22            | KF790541          | KF790536         |
|                               | F15.3A           | A23            | KF790542          | KF790537         |
|                               | F15.5A           | A24            | KF790543          | KF790538         |
|                               | F5.4A            | A25            | KF790544          | KF790539         |
| *Phaeoacremonium parasiticum* (clade 4) | E15.3A           | A8             | KF790555          | KF790545         |
|                               | E9.3B            | A9             | KF790556          | KF790546         |
|                               | E8.2             | A10            | KF790557          | KF790547         |
|                               | F11.4E           | A11            | KF790558          | KF790548         |
|                               | E15.1B           | A12            | KF790559          | KF790549         |
|                               | E8.3             | A13            | KF790560          | KF790550         |
|                               | F15.5B           | A14            | KF790561          | KF790551         |
|                               | F11.2C           | A15            | KF790562          | KF790552         |
|                               | F11.5C           | A31            | KF790563          | KF790553         |
|                               | F5.5             | A32            | KF790564          | KF790554         |
| *Phaeoacremonium venezuelense* (clade 5) | F32.2A           | A39            | KJ496346          | KJ496345         |

¹ All the isolates were collected in Kununurra (Australia) by TI Burgess.

² CBS: Culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.
Phaeoacremonium species from sandalwood

ARTICLE

TAXONOMY

Based on the DNA sequence analyses and morphological characters, two species of Phaeoacremonium proved distinct from known species, and are newly described below.

Phaeoacremonium luteum D. Gramaje, T.I. Burgess & J. Armengol, sp. nov.
MycoBank MB808419
(Fig. 2)

Etymology: Named after the yellow pigment produced that diffused into the agar ahead of the leading edge of the colony in all culture media.

Diagnosis: Phaeoacremonium luteum can be distinguished from the other species producing yellow pigment on MEA, PDA and OA, namely P. alvesii, P. subulatum, P. globosum, and the asexual morph of Toginia africana, by the predominance of type II phialides, the mycelium having prominent exudate droplets evident as warts, and slow growth.

Type: Australia: Western Australia: Kununurra, isolated from Santalum album trees, Dec. 2012, T. I. Burgess (CBS H-21622 – holotype; CBS 137497 – ex-type culture A16).

Description: Aerial structures in vitro on MEA: Mycelium consisting of branched, septate hyphae that occurs singly or in bundles of up to 8; hyphae tuberculate with warts to 2 µm diam, verruculose, medium to pale brown and 1.5–2.5 µm wide. Conidiophores mostly short, usually unbranched, arising from aerial or submerged hyphae, erect to flexuose, to 5-septate, sometimes bearing next to the terminal phialide 1–2 lateral ones, medium brown to pale brown, verrucose on the lower part, (15–)15.5–30–40(–59) µm long and 1.5–2.2–3 µm wide. Conidiogenous cells phialides, terminal or lateral, mostly monophialidic, smooth to verruculose, hyaline, collarettes 1.5–2.5 µm long, 1–1.5 µm wide; type I phialides mostly cylindrical, (3–)4–5–6(–6.5) × 1.5–2(–2.5) µm; type II phialides predominant, subcylindrical to subulate, (10–)10.5–13.5–17 × 2–3.5 µm; type III phialides cylindrical to subcylindrical, 20–24.5–30(–32) × 2–2.5–3 µm. Conidia hyaline, oblong or obovate, some reniform, 4–5–6(–7.5) × (1.5–)2–2.5–3 (av. = 5 × 2.5) µm, L/W ratio = 2.2. On surface or submerged in the agar: Phialides hyaline, mostly
cylindrical, 3–6–9(–10) × 1–1.5–2 μm. Conidia hyaline, mostly allantoid, few reniform, 4.5–6(–7) × 1–1.5–2, L/W ratio = 4.3.

Culture characteristics: Colonies reaching a radius of 4.5–5 mm after 8 d at 25 ºC. Minimum temperature for growth 15 ºC, optimum 30–35 ºC, maximum 37 ºC. Colonies on MEA flat, feltly to powdery, with crenate margin; after 8 d and 16 d, brownish olive towards the edge above, buffy brown to buff-yellow in reverse. Colonies on PDA flat, feltly or woolly textured, with crenate margins; after 8 d and 16 d, buff-yellow and dark greyish brown above, buff-yellow to buffy brown in reverse. Colonies on OA flat, with woolly tufts, with entire margin; after 8 d and 16 d olive-grey and brownish vinaceous above, buffy brown to greyish brown in reverse. Yellow pigment produced on MEA, PDA and OA.

Additional cultures examined: Australia: Western Australia: Kununurra, isolated from Santalum album trees, Dec. 2012, T.I. Burgess A17, A18, A19, and A20.

Phaeoacremonium santali D. Gramaje, T.I. Burgess, J. Armengol, sp. nov.
MycoBank MB808420 (Fig. 3)

Etymology: Named after the host it was isolated from, Tropical sandalwood (Santalum album).

Diagnosis: Phaeoacremonium santali can be distinguished from the other species producing pink colonies on MEA, namely P. alvesii, P. armeniacum, P. griseorubrum, P. rubrigenum, P. scolyti, and P. viticola, by the predominance of type I and II phialides, the brownish olive colour in OA, and slow growth. Colonies reached a radius of only 6.6–7.5 mm in 8 d at 25 ºC on MEA. Phaeoacremonium griseorubrum overlaps with P. santali in growth rate, but has a temperature maximum for growth of 40 ºC, compared with 37 ºC in the latter species.

Type: Australia: Western Australia: Kununurra, isolated from Santalum album trees, Dec. 2012, T.I. Burgess (CBS H-21621 – holotype; CBS 137498 – ex-type culture A20).

Description: Aerial structures in vitro on MEA: Mycelium consisting of branched, septate hyphae that occurs singly or in bundles of up to six; smooth or rarely with warts, verruculose, yellow-brown to hyaline, 2–3.5 μm wide. Conidiophores mostly short, usually unbranched, arising from aerial or submerged hyphae, erect to flexuous, to 3-septate, often bearing besides the terminal phialide 1–2 lateral ones, pale brown, smooth to verruculose, (10–)10.5–15–27(–31) μm long and 1.5–2(–2.5) μm wide. Conidigenous cells phialides, terminal or lateral, mostly polyphialidic, smooth to verruculose, hyaline, collarettes 1.5–2.5 μm long, 1–1.5 μm wide; type I and II phialides predominant, type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, (2–)2.5–6–7.5(–8) × 1–1.5–2 μm; type II phialides elongate-ampulliform and attenuated at the base, or navicular, tapering towards the apex, (5.5–)6–7.9 × 2–3–3 μm; type III phialides subcylindrical to navicular, 12–14–19(–20) × 1.5–2–2.5 μm. Conidia hyaline, oblong ellipsoidal, some obovoid or reniform, (3–)4–4.5–5(–6) × 1.5–2–3 μm, L/W ratio = 2.1. On surface or submerged in the agar: Phialides hyaline, mostly cylindrical, 5–7.5–10(–12) × 1–1.5–2 μm. Conidia hyaline, mostly allantoid, 5–6–7(–11) × 1–1.5–2, L/W ratio = 4.1.

Culture characteristics: Colonies reaching a radius of 6.6–7.5 mm after 8 d at 25 ºC. Minimum temperature for growth 15 ºC, optimum 30 ºC, maximum 37 ºC. Colonies on MEA flat, erose or dentate; after 8 d pale rose to pinkish vinaceous towards the edge above, pale rose towards the edge in reverse, after 16 d rosy vinaceous to pinkish buff towards the edge above, vinaceous pink near the centre and pinkish buff towards the edge in reverse. Colonies on PDA flat, felt-like with few woolly tufts near the centre, with entire margin; after 8 d pale pinkish buff towards the edge above and in reverse, after 16 d isabelline to oliveaceous towards the edge above, violet-brown towards the centre and pale brown to orange-grey towards the edge in reverse. Colonies on OA flat, feltly to powdery; with entire margin; after 8 d and 16 d dull green to olive green above, brownish olive to dark vinaceous-brown towards the edge in reverse.

Additional cultures examined: Australia: Western Australia: Kununurra, isolated from Santalum album trees, Dec. 2012, T.I. Burgess A2, A3, A4, A5, A6, A7, A26, A27, A29, and A30.

DISCUSSION

In this study, the integration of morphology, cultural characters, and DNA sequence data revealed the presence of five Phaeoacremonium species within pruning wounds of Santalum album in tropical Western Australia. Phaeoacremonium species were commonly isolated from both 1- and 5-year-old trees and in both harvests, approximately 12 and 18 mo after pruning. Two novel species of Phaeoacremonium, P. luteum and P. santali, were obtained from sandalwood, bringing the total number of known species of the genus to 42.

Micromorphological traits, such as conidiophore morphology, phialide type and shape, size of hyphal warts, and cultural characters are useful in distinguishing Phaeoacremonium species (Mostert et al. 2005). In addition, molecular analyses of part of the β-tubulin and actin gene regions have been shown to give high phylogenetic resolution within Phaeoacremonium in previous studies (Mostert et al. 2005, 2006, Rébélová & Mostert 2007, Essakhi et al. 2008, Graham et al. 2009, Gramaje et al. 2012). Distinct features of P. luteum include its ability to produce yellow pigment on MEA, PDA and OA, the predominance of subcylindrical to subulate type II phialides and the mycelium showing prominent exudate droplets observed as warts. Yellow pigment production on PDA and OA is a common culture characteristic of some Phaeoacremonium species, and is considered useful in distinguishing species in the genus, essentially on OA, which is an excellent medium to test pigment production (Mostert et al. 2006). It is also important to note the slow growth of this species on malt extract agar, with colonies reaching a radius of only 4.5–5 mm after 8 d. Phaeoacremonium santali
could be distinguished from the other species producing pink colonies on MEA by the predominance of type I and II phialides, its distinct brownish olive colonies in oatmeal agar, and slow growth.

Growth temperature studies showed that all the isolates of *P. luteum* and *P. santali* had a maximum growth temperature of 37 ºC, suggesting that they have the potential to survive at human body temperature. Several thermotolerant *Phaeoacremonium* species, such as *P. alvesii*, *P. griseorubrum*, *P. krajdenii*, *P. parasiticum*, and *P. venezuelense*, are associated with phaeohyphomycosis in humans and also have been isolated from woody hosts (Mostert et al. 2005, Essakhi et al. 2008).

In addition to the two new taxa, three previously known species were also found on sandalwood. *Phaeoacremonium alvesii* and *P. parasiticum* had been previously reported from *Dodonaea viscosa* (Mostert et al. 2005) and *Vitis vinifera* (Pascoe & Cottral 2000) in Australia, respectively. *Phaeoacremonium venezuelense* represents a new record for Australia and has previously been reported from humans in Brazil (Guarro et al. 2006), Canada (Mostert et al. 2005), France (Mostofi et al. 2012), and Venezuela (de Albornoz 1974), from grapevine in Algeria (Berraf-Tebbal et al. 2011) and South Africa (Mostert et al. 2005), and more recently from wood decay of apricot trees in Spain (Olmo et al. 2014). Species of *Phaeoacremonium* obtained in this study were all collected from pruning wounds. Some *Phaeoacremonium* spp., such as *P. aleophilum* and *P. mortoniae*, produce perithecia (i.e. a *Togninia* sexual morph) in old, rotted, vascular tissue of pruning wounds and in deep cracks in cordons, trunks, and spurs of grapevine (Eskalen et al. 2005, Rooney-Latham et al. 2005, Baloyi et al. 2013). Ascospores are released from these overwintering structures by rain and can infect the grapevine through fresh pruning wounds, which are recognized as the main point of entry for *Phaeoacremonium* species into grapevines (Eskalen et al. 2005). Of the species found in this study, only *P. parasiticum* has a known sexual morph and could possibly be present as perithecia on sandalwood trees. Aerial inoculum could be released by these ascocarps on infected tress, thus becoming a major source of fungal infection. Insect transmission of sexual spores or conidia may also occur (Moyo et al. 2014).

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