



A co-evolutionary relationship exists between *Endoraecium* (*Pucciniales*) and its *Acacia* hosts in Australia

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Key words

Atelocauda
endocyclic rusts
Mimosoideae
Racospermyces
Raveneliaceae
Uredinales

Abstract *Endoraecium* is a genus of rust fungi that infects several species of *Acacia* in Australia, South-East Asia and Hawaii. This study investigated the systematics of *Endoraecium* from 55 specimens in Australia based on a combined morphological and molecular approach. Phylogenetic analyses were conducted on partitioned datasets of loci from ribosomal and mitochondrial DNA. The recovered molecular phylogeny supported a recently published taxonomy based on morphology and host range that divided *Endoraecium digitatum* into five species. Spore morphology is synapomorphic and there is evidence *Endoraecium* co-evolved with its *Acacia* hosts. The broad host ranges of *E. digitatum*, *E. parvum*, *E. phyllodiorum* and *E. violae-faustiae* are revised in light of this study, and nine new species of *Endoraecium* are described from Australia based on host taxonomy, morphology and phylogenetic concordance.

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INTRODUCTION

Endoraecium (*Pucciniales*, *Raveneliaceae*) was established for two endocyclic species of rust fungi with pedicellate spores, *E. acaciae* (the type species) and *E. hawaiiense*, which were found on *Acacia koa* (*Fabaceae*, subfamily *Mimosoideae*) in Hawaii (Hodges & Gardner 1984). There are over 1 000 species of *Acacia* s.str. (hereafter referred to as *Acacia*) known in Australia (Murphy et al. 2010, Maslin 2013). The classification of *Acacia* has moved from a traditional morphological one based on sections, to a molecular phylogenetic one based on clades (Murphy et al. 2010, Miller et al. 2013). At the commencement of this study, 20 species of *Acacia* were recorded as hosts of seven species of *Endoraecium* in Australia (Berndt 2011). These 20 host species fall within three clades of the plurinerved, uninerved and Botrycephalae (p.u.b.) group of *Acacia* (Murphy et al. 2010), namely the i) Botrycephalae subclade (Murphy et al. 2010); ii) Juliflorae p.p. clade (Miller et al. 2013); and iii) Plurinerves p.p. clade (Miller et al. 2013), hereafter referred to as Botrycephalae, Juliflorae and Plurinerves, respectively.

Species of *Endoraecium* produce a range of symptoms on *Acacia* that include bullate swellings or pulvinate sori on phyllodes, or galls and witches' brooms on stems. These rust fungi produce spores of three types: i) spermogonia; ii) pedicellate spores, which have reticulate to foveolate ornamentations and were variously called aecial urediniospores (Walker 2001), uredo-like aeciospores (Berndt 2011) or teliospores in the

endocyclic species (Hodges & Gardner 1984); iii) teliospores, which are single celled, subhyaline and smooth walled. The teliospores are morphologically similar to those of *Uromyces* s.l. and several species of *Endoraecium* were at one time classified in that genus, including *U. digitatus* (Winter 1886), *U. bicinctus* (McAlpine 1906), *U. phyllodiorum* (McAlpine 1906), *U. hyalosporus* (Sawada 1913) and *U. koae* (Stevens 1925). On the basis of morphology, some species currently considered as *Endoraecium* were previously placed in other genera, including *Pileolaria* (Dietel 1921), *Maravalia* (Dietel 1924), *Poliotelium* (Mains 1939), *Atelocauda* (Cummins & Hiratsuka 1983, Ono 1984, Gardner 1991), and *Racospermyces* (Walker 2001).

Gardner (1991) proposed that *Endoraecium* may be closely related to *Uromycladium* (*Pileolariaceae*), another genus of rust that occurs on *Acacia* in Australia. However, in combined analyses of the Large Subunit (LSU) and Small Subunit (SSU) regions of ribosomal DNA (rDNA), the systematic position of *Endoraecium* was shown by Scholler & Aime (2006) and Aime (2006) to have an affinity with mimosoid rusts in the *Raveneliaceae* rather than with the *Pileolariaceae*.

In this study, the systematic relationships of *Endoraecium* on *Acacia* in Australia were investigated with freshly collected field material and herbarium specimens. Currently, *Endoraecium* contains 13 species, all on *Acacia*, with seven endemic to Australia, one to South-East Asia and five to Hawaii (McAlpine 1906, Sawada 1913, Hodges & Gardner 1984, Berndt 2011). The species are *E. acaciae* (type on *A. koa*, Hawaii), *E. angustiphylloidi* (type on *A. koa*, Hawaii), *E. bicinctum* (type on *A. fasciculifera*, Australia), *E. digitatum* (type on *A. notabilis*, Australia), *E. hawaiiense* (type on *A. koa*, Hawaii), *E. hyalosporum* (type on *A. confusa*, Taiwan), *E. kauaianum* (type on *A. koa*, Hawaii), *E. koae* (type on *A. koa*, Hawaii), *E. parvum* (type on *A. leiocalyx*, Australia), *E. phyllodiorum* (type on *Acacia* sp., Australia), *E. tierneyi* (type on *A. harpophylla*, Australia), *E. violae-faustiae* (type on *Acacia* sp., Australia) and *E. walkerianum* (type on *A. penninervis*, Australia). The SSU, Internal Transcribed Spacer (ITS) and LSU regions of rDNA, and cytochrome c oxidase subunit 3 (CO3) of mitochondrial DNA, were used for phylogenetic reconstruction to determine

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Table 1 Specimens of *Endoraecium* included in this study.

Taxon	Accession number	Subclade ¹ of <i>Acacia</i> s.str.	Host	GenBank accession			
				LSU	ITS	SSU	CO3
<i>E. acaciae</i>	BPI 871098	Plurinerves	<i>A. koa</i>	DQ323916 ²	N/A	DQ323917 ²	N/A
<i>E. auriculiforme</i>	BRIP 55609	Juliflorae	<i>A. auriculiformis</i>	KJ862296	KJ862353	N/A	KJ862430
	BRIP 56550	Juliflorae	<i>A. auriculiformis</i>	KJ862297	KJ862354	N/A	KJ862431
	BRIP 56548*	Juliflorae	<i>A. auriculiformis</i>	KJ862298	KJ862355	N/A	KJ862432
	BRIP 56549	Juliflorae	<i>A. auriculiformis</i>	KJ862299	KJ862356	N/A	KJ862433
<i>E. carnegiei</i>	BRIP 57926	Botrycephalae	<i>A. dealbata</i>	KJ862300	KJ862357	N/A	KJ862434
	BRIP 57924*	Botrycephalae	<i>A. dealbata</i>	KJ862301	N/A	N/A	KJ862435
	BRIP 59218	Botrycephalae	<i>A. dealbata</i>	KJ862302	N/A	N/A	N/A
<i>E. disparrimum</i>	BRIP 55659	Juliflorae	<i>A. disparrima</i>	KJ862303	N/A	KJ862402	KJ862436
	BRIP 55626*	Juliflorae	<i>A. disparrima</i>	KJ862304	KJ862358	KJ862403	KJ862437
	BRIP 55632	Juliflorae	<i>A. disparrima</i>	KJ862305	KJ862359	KJ862404	KJ862438
<i>E. falciforme</i>	BRIP 57583*	Botrycephalae	<i>A. falciformis</i>	KJ862306	KJ862360	KJ862405	KJ862439
	BRIP 57643	Botrycephalae	<i>A. falciformis</i>	KJ862307	N/A	N/A	N/A
<i>E. hawaiiense</i>	BPI 871064	Plurinerves	<i>A. koa</i>	DQ323920 ²	N/A	N/A	N/A
<i>E. irroratum</i>	BRIP 55671	Botrycephalae	<i>A. irrorata</i>	KJ862310	KJ862364	KJ862406	KJ862442
	BRIP 57279	Botrycephalae	<i>A. irrorata</i>	KJ862311	KJ862365	N/A	KJ862443
	BRIP 57286*	Botrycephalae	<i>A. irrorata</i>	KJ862312	KJ862366	KJ862407	N/A
	BRIP 58054	Botrycephalae	<i>A. irrorata</i>	KJ862313	N/A	N/A	N/A
<i>E. koae</i>	BPI 871071	Plurinerves	<i>A. koa</i>	DQ323918 ²	N/A	DQ323919 ²	N/A
<i>E. maslinii</i>	BRIP 57872*	Botrycephalae	<i>A. daphnifolia</i>	KJ862314	KJ862367	KJ862408	KJ862444
<i>E. parvum</i>	BRIP 57514	Juliflorae	<i>A. leiocalyx</i>	KJ862315	KJ862368	N/A	N/A
	BRIP 57524	Juliflorae	<i>A. leiocalyx</i>	KJ862316	KJ862369	KJ862409	KJ862445
	BRIP 57527	Juliflorae	<i>A. leiocalyx</i>	KJ862317	KJ862370	N/A	N/A
	BRIP 57534	Juliflorae	<i>A. leiocalyx</i>	KJ862318	KJ862371	N/A	N/A
	BRIP 57568	Juliflorae	<i>A. leiocalyx</i>	KJ862319	KJ862372	N/A	N/A
	BRIP 57631	Juliflorae	<i>A. leiocalyx</i>	KJ862320	KJ862373	KJ862410	KJ862446
	BRIP 57512	Juliflorae	<i>A. leiocalyx</i>	KJ862321	KJ862374	N/A	N/A
	BRIP 53616	Juliflorae	<i>A. leiocalyx</i>	KJ862322	KJ862375	N/A	N/A
<i>E. peggii</i>	BRIP 55602*	Juliflorae	<i>A. holoserica</i>	KJ862308	KJ862361	N/A	KJ862440
	BRIP 55631	Juliflorae	<i>A. holoserica</i>	KJ862309	KJ862362	N/A	KJ862441
	BRIP 58324	Juliflorae	<i>A. holoserica</i>	N/A	KJ862363	N/A	N/A
<i>E. phyllodiorum</i>	BRIP 57310	Juliflorae	<i>A. aulacocarpa</i>	KJ862323	KJ862377	N/A	N/A
	BRIP 57516	Juliflorae	<i>A. aulacocarpa</i>	KJ862324	KJ862378	KJ862411	KJ862447
	BRIP 57569	Juliflorae	<i>A. aulacocarpa</i>	KJ862325	KJ862379	N/A	N/A
	BRIP 57570	Juliflorae	<i>A. aulacocarpa</i>	KJ862326	KJ862380	N/A	N/A
	BRIP 57578	Juliflorae	<i>A. aulacocarpa</i>	KJ862327	KJ862381	KJ862412	KJ862448
	BRIP 57579	Juliflorae	<i>A. aulacocarpa</i>	KJ862328	N/A	KJ862413	N/A
	BRIP 57580	Juliflorae	<i>A. aulacocarpa</i>	KJ862329	KJ862382	N/A	N/A
	BRIP 57588	Juliflorae	<i>A. aulacocarpa</i>	KJ862330	KJ862383	N/A	N/A
	BRIP 57589	Juliflorae	<i>A. aulacocarpa</i>	KJ862331	KJ862384	N/A	N/A
	BRIP 57590	Juliflorae	<i>A. aulacocarpa</i>	KJ862332	KJ862385	N/A	N/A
	BRIP 57294	Botrycephalae	<i>A. podalyriifolia</i>	KJ862333	KJ862386	N/A	N/A
	BRIP 57576*	Botrycephalae	<i>A. podalyriifolia</i>	KJ862334	KJ862387	KJ862414	KJ862449
<i>E. tierneyi</i>	BRIP 27071*	Plurinerves	<i>A. harpophylla</i>	KJ862335	N/A	KJ862415	KJ862450
	BRIP 27880	Plurinerves	<i>A. harpophylla</i>	N/A	KJ862388	N/A	N/A
	BRIP 27887	Plurinerves	<i>A. harpophylla</i>	N/A	KJ862389	N/A	N/A
<i>E. tropicum</i>	BRIP 56555	Juliflorae	<i>A. tropica</i>	KJ862336	KJ862390	KJ862416	KJ862451
	BRIP 56557*	Juliflorae	<i>A. tropica</i>	KJ862337	KJ862391	KJ862417	KJ862452
<i>E. violae-faustiae</i>	BRIP 55601	Juliflorae	<i>A. aulacocarpa</i>	KJ862338	KJ862392	KJ862418	KJ862453
	BRIP 55629	Juliflorae	<i>A. aulacocarpa</i>	KJ862339	KJ862393	KJ862419	KJ862454
	BRIP 55660	Juliflorae	<i>A. aulacocarpa</i>	KJ862340	N/A	KJ862420	KJ862455
	BRIP 56544	Juliflorae	<i>A. difficilis</i>	KJ862341	KJ862395	KJ862421	N/A
	BRIP 55616	Juliflorae	<i>A. difficilis</i>	N/A	KJ862396	KJ862422	KJ862456
	BRIP 55611	Juliflorae	<i>A. difficilis</i>	N/A	KJ862397	KJ862423	N/A
	BRIP 56539	Juliflorae	<i>A. difficilis</i>	KJ862342	KJ862398	KJ862424	KJ862457
	BRIP 56540	Juliflorae	<i>A. difficilis</i>	KJ862343	KJ862399	KJ862425	N/A
	BRIP 56545	Juliflorae	<i>A. difficilis</i>	KJ862344	KJ862400	KJ862426	N/A
	BRIP 56547	Juliflorae	<i>A. difficilis</i>	KJ862345	KJ862401	KJ862427	KJ862458
	BRIP 56909	NA	<i>Breynia cernua</i> (Euphorbiaceae)	KJ862346	N/A	KJ862428	KJ862459
<i>Ravenelia neocaledoniensis</i>	BRIP 56907	NA	<i>Vachellia farnesiana</i>	KJ862347	N/A	N/A	N/A
	BRIP 56908	NA	<i>V. farnesiana</i>	KJ862348	N/A	N/A	KJ862460
<i>Ravenelia</i> sp.	BRIP 56904	NA	<i>Cassia</i> sp.	KJ862349	N/A	N/A	KJ862461
<i>Sphaerophragmium</i> sp.	BRIP 56910	NA	<i>Albizia</i> sp.	KJ862350	N/A	KJ862429	KJ862462
<i>Uromycladium simplex</i>	BRIP 57571	Botrycephalae	<i>A. pycnantha</i>	KJ632990 ³	N/A	N/A	N/A

* Holotype

¹ Botrycephalae = Botrycephalae subclade (Murphy et al. 2010); Juliflorae = Juliflorae p.p. clade (Miller et al. 2013); Plurinerves = Plurinerves p.p. clade (Miller et al. 2013).² Scholler & Aime (2006).³ Doungsa-ard et al. (2014).

whether the current taxonomy of Australian species was supported by molecular data and if further diversity existed within *Endoraecium*.

MATERIALS AND METHODS

Taxon selection

Fresh specimens were collected from New South Wales, the Northern Territory, Queensland, Victoria and Western Australia, representing a broad diversity of *Acacia* (Table 1). Species from representative genera of *Raveneliaceae*, namely *Diorchidium*, *Kernkampella*, *Ravenelia* and *Sphaerophragmium*, were selected as outgroup taxa for the phylogenetic analyses.

Uromycladium simplex (*Pileolariaceae*) was included as an outgroup to the *Raveneliaceae*, as it represented another Australian endemic rust on *Acacia*.

Morphology

Spores were scraped from leaf material, mounted in lactic acid and gently heated to boiling. Preparations were examined with a Leica DMLB microscope and images were taken with a Leica DFC500 camera. Measurements were made digitally from photographed spores. All previous taxonomic studies of *Endoraecium* have adopted an ontogenic approach to spore classification (Hodges & Gardner 1984, Walker 2001, Scholler & Aime 2006, Berndt 2011). Aeciospores, urediniospores and

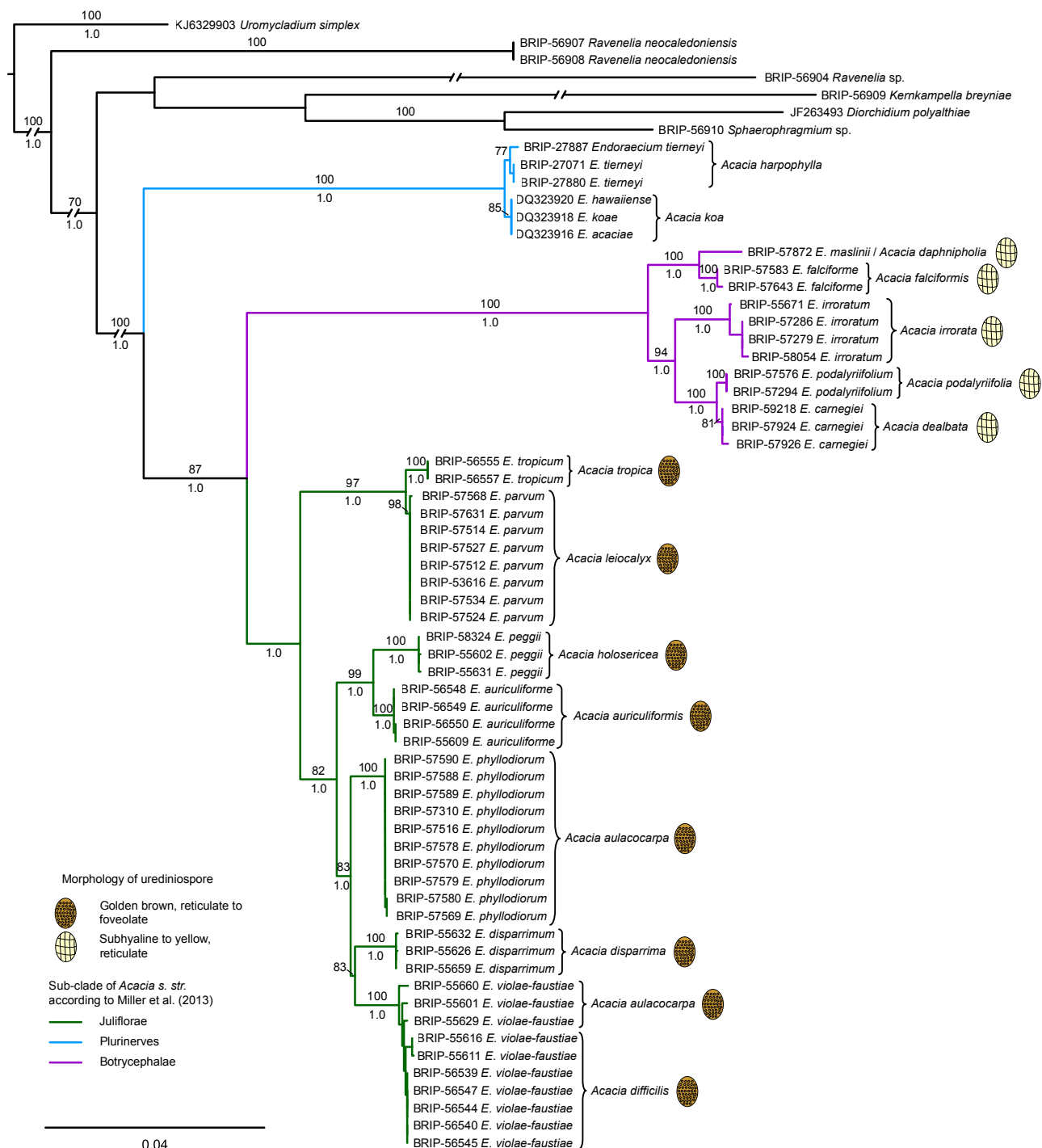


Fig. 1 Phylogram obtained in a maximum likelihood search in RAxML of the SSU, ITS, LSU and CO3 gene regions. Bootstrap support ($\geq 70\%$) values from 1 000 replicates above nodes. Posterior probabilities (≥ 0.95) from 4 200 trees in a Bayesian search are shown below nodes. Synapomorphic characters, namely host subclade and morphology of urediniospores, are mapped on to the topology.

in some cases teliospores, are often indistinguishable with this approach. For this reason, we used a morphological approach to classify spores (Laundon 1967), which recognises urediniospores of *Endoraecium* as spores with reticulate ornamentation, pedicels and germ pores.

DNA extraction, PCR and sequencing

DNA was extracted from fresh or archived material. Spores were obtained from leaf material according to the protocol in McTaggart et al. (2014), and then extracted with the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, CA, USA).

The ITS region was amplified with ITS1F/ITS4B (Gardes & Bruns 1993). The ITS2-LSU region was amplified with Rust2inv (Aime 2006) / LR7 (Vilgalys & Hester 1990) and nested with LROR/LR6 (Vilgalys & Hester 1990). The SSU region was amplified with NS1 (White et al. 1990) / Rust 18S-R (Aime 2006) according to the protocol by Aime (2006). CO3 was amplified with the primers CO3_F1/CO3_R1 (Vialle et al. 2009). All PCRs were performed with high fidelity Phusion enzyme (New England Biolabs Inc.) according to the manufacturer's instructions. The PCRs were performed with the following annealing temperatures: SSU, ITS and nested LSU at 62 °C, the initial LSU at 60 °C, and CO3 at 55 °C. PCR products were sent to Macrogen Korea for direct sequencing. Sequences were uploaded to GenBank under the accession numbers listed in Table 1.

Phylogenetic analyses

The SSU (19), ITS (47), LSU (51) and CO3 (29) sequences were aligned separately in MAFFT (Katoh et al. 2009) (available: <http://www.ebi.ac.uk/Tools/msa/mafft/>). Non-homologous regions in the ITS alignment were removed using GBlocks (Castresana 2000). The sequences from each locus were concatenated in a supermatrix and run as partitioned datasets with maximum likelihood and Bayesian inference as phylogenetic criteria. CO3 was translated and included as amino acid coding sequence. JTT was selected as a protein coding model of evolution in MEGA5 (Tamura et al. 2011). Maximum likelihood was implemented as a search criterion in RAXML (Stamatakis 2014). GTRGAMMA was specified as the model of evolution for nucleotide sequence data. The RAXML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1 000 maximum likelihood bootstrap replicates. A Markov Chain Monte Carlo (MCMC) search in a Bayesian analysis was conducted with MrBayes (Ronquist & Huelsenbeck 2003). Four runs, each consisting of four chains, were implemented for 5 million generations. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every

500 generations and trees were saved every 1 000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander et al. 2008) (available at: ceb.csit.fsu.edu/awty/) and a burn-in of 18 000 generations was calculated. The ML and Bayesian analyses were run three times to test accuracy. Alignments and trees were uploaded to TreeBASE (<http://purl.org/phylo/treebase/phyloWS/study/TB2:S15410>).

RESULTS

The topologies recovered by Bayesian inference and maximum likelihood were identical (Fig. 1). No differences were recovered in the tree topologies between nucleotide and amino acid coding sequences of CO3. *Endoraecium* was recovered as a monophyletic group sister to other members of the *Raveneliaceae*.

Three major clades of *Endoraecium* corresponded to three clades in *Acacia*, namely Plurinerves, Juliflorae and Botrycephalae. The clade of *Endoraecium* that diversified on Plurinerves contained the Hawaiian species of *Endoraecium* and the Australian species, *E. tiermeyeri*. Rust taxa formed well-supported clades corresponding to host species. This is contrary to the current broad host ranges proposed for *E. digitatum*, *E. parvum*, *E. phyllidorum* and *E. violae-faustiae* (Berndt 2011).

Morphology of the urediniospores was a synapomorphic character for the two large clades on Juliflorae and Botrycephalae (Fig. 1). Urediniospores of species on Juliflorae were golden brown with reticulate to foveolate ornamentation. Urediniospores of those on Botrycephalae were subhyaline to pale yellow with reticulate ornamentation.

TAXONOMY

Endoraecium auriculiforme McTaggart & R.G. Shivas, *sp. nov.*
— MycoBank MB808980; Fig. 2

Etymology. Name refers to the host, *Acacia auriculiformis*, on which it was found.

Type. AUSTRALIA, Northern Territory, Darwin, Howard Springs (-12.4564, 131.0522), on *A. auriculiformis*, 15 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas (holotype BRIP 56548).

Uredinia on both phyllode surfaces, subepidermal, erumpent, on bullate swellings up to 1 cm long, or pulverulent on phyllode surface, linear to round, yellowish brown; paraphyses intrasoral, cylindrical, apex 6–9-digitate, subhyaline to yellow, 35–70 × 10–14 µm, thickened at apex, pedicel up to 40 µm. *Urediniospores* obovoid, oval to fusiform, apex acute, yellowish brown, 31–46 × 16–20 µm; wall 3–5 µm thick at sides and

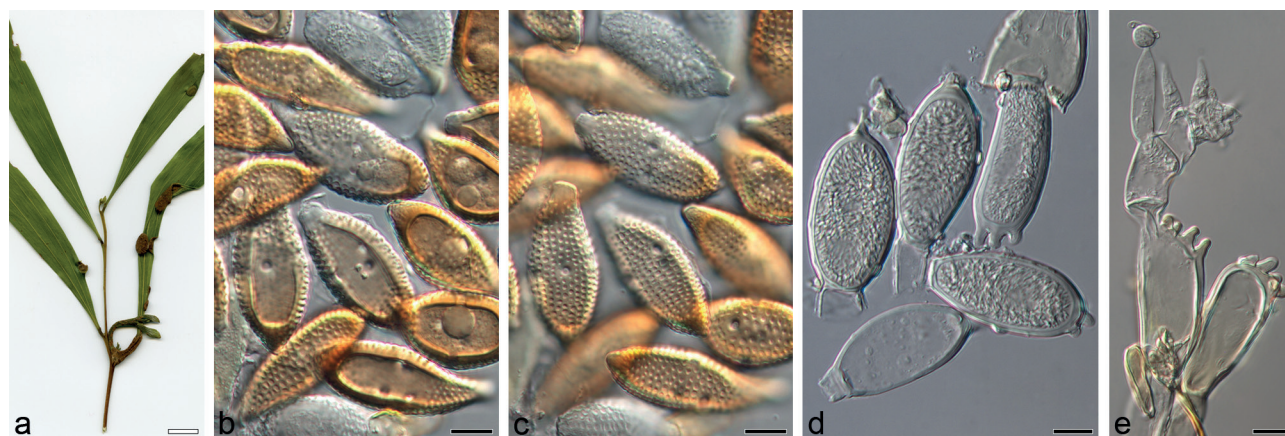


Fig. 2 *Endoraecium auriculiforme*. a. Bullate sori on leaf (BRIP 56550); b, c. urediniospores (BRIP 56609); d. teliospores (BRIP 56550); e. germinating teliospore (BRIP 56549). — Scale bars: a = 1 cm; b–e = 10 µm.

5–9 µm thick at apex, foveolate, with 3–4 equatorial germ pores; pedicel 3–5 µm, or absent. *Telia* formed from uredinia, erumpent, reddish brown. *Teliospores* cylindrical to fusiform, apex rounded, 2–4-digitate, hyaline to subhyaline, 36–54 × 13–25 µm; wall 1.5–2.5 µm thick at sides, 6–8 µm thick at apex; pedicel persistent, up to 38 µm. *Basidia* cylindrical, 3-septate, up to 35–40 × 8–12 µm. *Basidiospores* globose, hyaline, 4–6 µm, smooth-walled.

On phyllodes of *Acacia auriculiformis* (Juliflorae).

Additional specimens examined. AUSTRALIA, Northern Territory, Howard Springs, on *A. auriculiformis*, 9 May 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55609; 15 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, R.G. Shivas, BRIP 56550; Northern Territory, Darwin, Bronzewing Ave, Howard Springs, 15 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt & R.G. Shivas, BRIP 56549.

Notes — *Endoraecium auriculiforme* has foveolate urediniospores with an acute and thickened apex, and digitate paraphyses that resemble narrow teliospores. *Endoraecium violae-faustiae* and *E. peggii* also produce similar paraphyses. These three species all occur on hosts in the Juliflorae, with *E. auriculiforme* restricted to *A. auriculiformis*; *E. violae-faustiae* to *A. aulacocarpa* and *A. crassicarpa*, and *E. peggii* to *A. holosericea*. In the phylogenetic analyses, *E. auriculiforme* was recovered as sister to *E. peggii* (Fig. 1).

Endoraecium bicinctum (McAlpine) M. Scholler & Aime, Mycoscience 47: 163. 2006

Basionym. *Uromyces bicinctus* McAlpine, Rusts of Australia: 93. 1906.
 ≡ *Pileolaria bicincta* (McAlpine) Dietel, Ann. Mycol. 19: 302. 1921.
 ≡ *Atelocauda bicincta* (McAlpine) Cummins & Y. Hirats., Illustr. Gen. Rust Fungi, rev. edn (St. Paul): 147. 1983.
 ≡ *Racospermyces bicinctus* (McAlpine) J. Walker, Australas. Mycol. 20: 16. 2001.

Type. AUSTRALIA, Queensland, Rockhampton, on *A. fasciculifera*, 30 Nov. 1866, G.H. Robinson (holotype VPRI 5751).

Notes — *Endoraecium bicinctum* is known from four collections, which were examined by Walker (2001). It is only known to occur on *A. fasciculifera* in Botrycephalae (Murphy et al. 2010, Maslin 2013).

Endoraecium carnegiei McTaggart & R.G. Shivas, sp. nov.
 — MycoBank MB808981; Fig. 3

Etymology. Named after the Australian forest pathologist and fungal taxonomist, Dr Angus J. Carnegie, who generously assisted this study by collecting many specimens of rust on *Acacia*, including the type specimen on which this taxon is based.

Type. AUSTRALIA, New South Wales, Ando, Glen Allen State Forest, Poddy Hut Road (–36.7150, 149.3486), on *A. dealbata*, 6 Oct. 2012, A.J. Carnegie (holotype BRIP 57924).

Uredinia forming galls on stems, irregular, up to 3 cm, yellowish brown. *Urediniospores* ellipsoidal to ovoid, subhyaline to yellow, 34–40 × 21–25 µm; wall 4.0–4.5 µm thick, uniform or slightly thicker at sides, with 6–8 equatorial germ pores. *Telia* on leaves, pulverulent, erumpent, elliptical, less than 1 mm, yellowish brown. *Teliospores* cylindrical to fusiform, apex rounded, 1–5-digitate, hyaline to subhyaline, 41–76 × 10–17 µm; wall 1.0–1.5 µm thick at sides, 6–19 (–32) µm thick at apex; pedicel persistent, up to 51 µm.

On stems and leaves of *A. dealbata* (Botrycephalae).

Additional specimens examined. AUSTRALIA, New South Wales, Mila, Bondi State Forest (–37.0847, 149.1078), on *A. dealbata*, 4 Oct. 2012, A.J. Carnegie, BRIP 57926; Victoria, Kergunyah, Murrumbidgee Range, Simpson Road (–36.3142, 146.9819), on *A. dealbata*, 13 May 2013, C. Doungsa-ard, W. Khem-muk & A.D.W. Geering, BRIP 59218.

Notes — *Endoraecium carnegiei* is one of three species that forms galls on the stems of its host. The other two species, *E. digitatum* and *E. irroratum*, also infect species of *Acacia* in the Botrycephalae. *Endoraecium carnegiei* is only known to occur on *A. dealbata* in south-eastern Australia.

Endoraecium digitatum (G. Winter) M. Scholler & Aime, Mycoscience 47: 163. 2006.

Basionym. *Uromyces digitatus* G. Winter, Rev. Mycol. (Toulouse) 8: 209. 1886.
 ≡ *Coeomurus digitatus* (G. Winter) Kuntze (as '*Caeomurus*'), Revis. Gen. Pl. 3: 450. 1898.
 ≡ *Atelocauda digitata* (G. Winter) Cummins & Y. Hirats., Illustr. Gen. Rust Fungi, rev. edn (St. Paul): 147. 1983.
 ≡ *Racospermyces digitatus* (G. Winter) J. Walker, Australas. Mycol. 20: 13. 2001.

Type. AUSTRALIA, South Australia, near Gawler, on *A. notabilis*, 1 July 1885, J.G.O. Tepper (holotype SF35352); South Australia, west of Gawler, along road to Mallala (–34.5665, 138.7184), on *A. notabilis*, 16 Oct. 2009, V. Faust-Berndt & R. Berndt (epitype SF35352).

Notes — Our study shows that the known host range of *E. digitatum* s.str. is restricted to *A. notabilis* in Botrycephalae. Walker (2001) adopted a broad host range for *E. digitatum*, but suspected it was a complex of closely related taxa, confined to one or a small group of hosts. Berndt (2011) divided *E. digitatum* into five species in Australia. He accepted *E. phyllodiorum* as distinct from *E. digitatum*, and further described three new taxa, *E. parvum*, *E. violae-faustiae* and *E. walkerianum*. Berndt (2011)

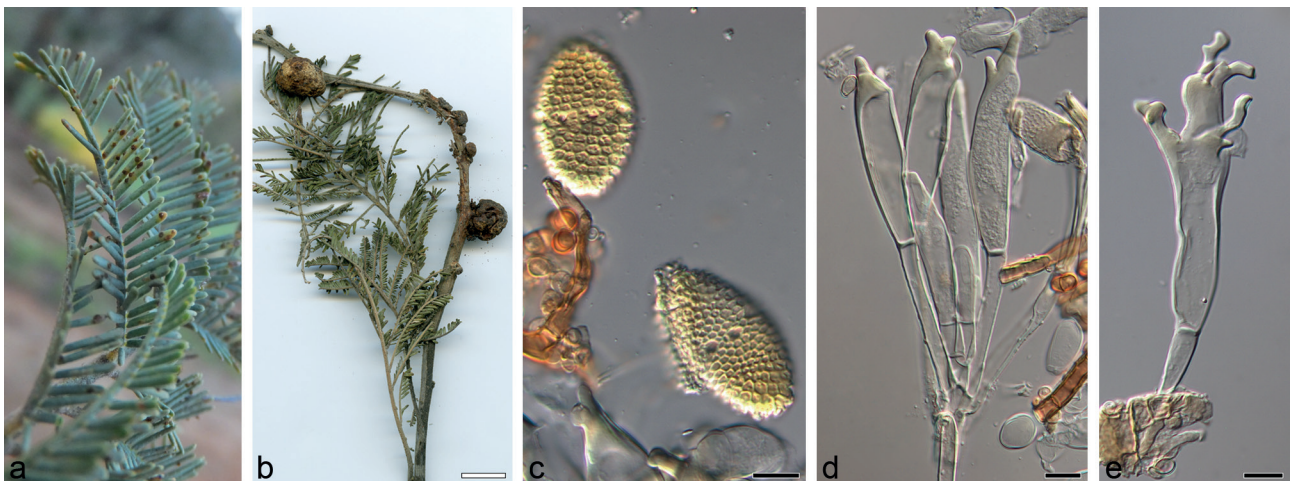


Fig. 3 *Endoraecium carnegiei*. a. Telia on leaves (BRIP 59218); b. gall (BRIP 57924); c. urediniospores (BRIP 57924); d, e. teliospores (BRIP 57924). — Scale bars: b = 1 cm; c–e = 10 µm.

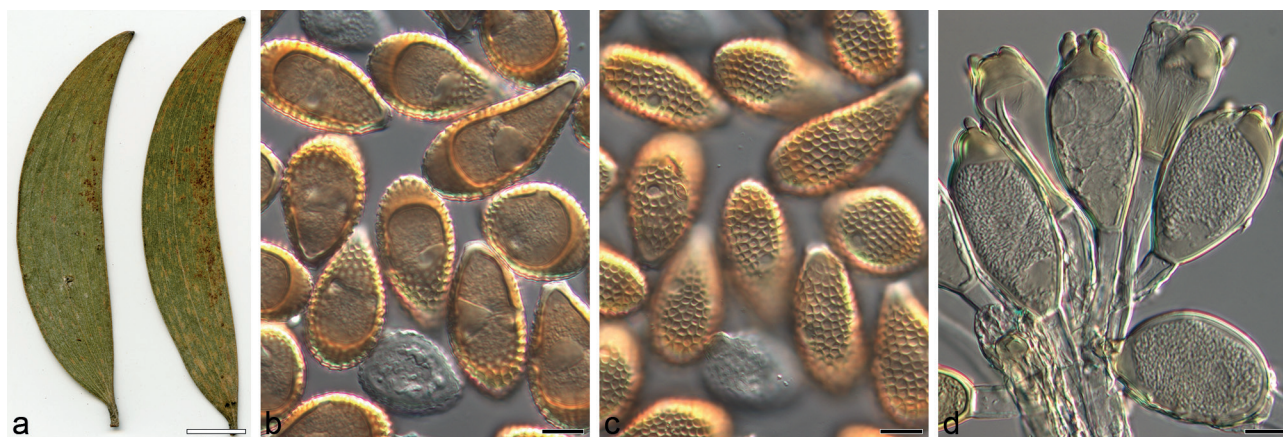


Fig. 4 *Endoraecium disparrimum*. a. Host (BRIP 55659); b, c. urediniospores (BRIP 55659); d. teliospores (BRIP 55626). — Scale bars: a = 1 cm; b–d = 10 µm.

designated an epitype for *E. digitatum* on *A. notabilis*, and listed six species of *Acacia* as hosts. We found four of these hosts, *A. dealbata*, *A. falciformis*, *A. irrorata* and *A. podalyriifolia*, were infected by novel species of *Endoraecium*. The remaining two hosts, *A. deanei* and *A. oshanesii*, most likely represent two new hosts of *Endoraecium*. The urediniospores of *E. digitatum* were not examined in this study, however, the description of the epitype (Berndt 2011) is similar to other species of *Endoraecium* with subhyaline to golden yellow urediniospores and reticulate ornamentation on species of *Acacia* in Botrycephalae.

Endoraecium disparrimum McTaggart & R.G. Shivas, *sp. nov.*
— MycoBank MB808982; Fig. 4

Etymology. Name refers to the host, *Acacia disparrima*, on which it was found.

Type. AUSTRALIA, Queensland, Babinda (−17.3397, 145.8675), on *A. disparrima*, 3 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas (holotype BRIP 55626).

Uredinia on both phyllode surfaces, forming bullate swellings up to 1 cm long, or pulverulent, linear to round, up to 1 mm diam, yellowish brown. *Urediniospores* subglobose to ovoid, apex obtuse, yellowish brown, 29–41 × 20–26 µm; wall 2–4 µm thick at sides and 5.5–9.0 µm thick at apex, reticulate,



Fig. 5 *Endoraecium falciforme* (BRIP 57583). a. Uredinia and telia surrounding spermatogonia; b. uredinium; c. teliospores; d, e. urediniospores. — Scale bars: b = 1 cm; c–e = 10 µm.

with 2–5 equatorial germ pores; pedicel 2 μm or absent. *Telia* formed from uredinia, yellowish brown. *Teliospores* cylindrical to fusiform, apex acuminate to rounded, hyaline to subhyaline, 31–50 \times 20–26 μm ; wall 1–2 μm thick at sides, 4–14 μm thick at apex; pedicel persistent, up to 33 μm .

On phyllodes of *A. disparrima* (Juliflorae).

Additional specimens examined. AUSTRALIA, Queensland, Herberton, Silver Valley Drive, on *A. disparrima*, 10 Apr. 2012, R. Berndt & V. Faust-Berndt, BRIP 55659; Queensland, near Yungaburra, Danbulla State Forest, at the Chimney's Rest Area, on *A. disparrima*, 9 Apr. 2012, R. Berndt & V. Faust-Berndt, BRIP 55632.

Notes — *Endoraecium disparrimum* occurs on *A. disparrima* in Juliflorae. It was recovered as sister to *E. violae-faustiae*, which occurs on closely related species of *Acacia*. It differs from *E. violae-faustiae* in that the urediniospores have an obtuse apex with reticulate rather than foveolate ornamentation, and paraphyses are absent.

Endoraecium falciforme McTaggart & R.G. Shivas, *sp. nov.*
— MycoBank MB808983; Fig. 5

Etymology. Name refers to the host, *Acacia falciformis*, on which it was found.

Type. AUSTRALIA, Queensland, Mt Tibrogargan (-26.9283, 152.9494), on *A. falciformis*, 15 Aug. 2012, C. Doungsa-ard & A.R. McTaggart (holotype BRIP 57583).

Spermogonia on fruit or phyllodes, amphigenous, subepidermal, erumpent, c. 100 μm . *Uredinia* on fruit or on both phyllode surfaces, causing distortion, surrounding spermogonia, subepidermal, erumpent, pulverulent, linear, round to ellipsoidal, greater than 10 mm, reddish brown. *Urediniospores* ellipsoidal to ovoid, subhyaline to yellow, 34–43 \times 21–25 μm ; wall unevenly thickened at equator, 3.0–6.5 μm and 3–5 μm thick at apex, reticulate, with 4–9 equatorial germ pores; pedicel 2 μm . *Telia* on both phyllode surfaces, formed separately from uredinia, erumpent, c. 1 mm, orange to yellowish brown. *Teliospores* cylindrical to clavate, apex rounded, 2–5-digitate, subhyaline, 47–63 \times (13–)15–23 μm ; wall 1.5–2.0 μm thick at sides, 12–24 μm thick at apex; pedicel persistent, up to 64 μm long \times 6–8 μm thick.

On phyllodes and fruit of *A. falciformis* (Botrycephalae).

Additional specimen examined. AUSTRALIA, Queensland, Mt Tibrogargan, 4 Sept. 2012, K.M. Thomson, BRIP 57643.

Notes — *Endoraecium falciforme* is morphologically similar to *E. walkerianum*, which both occur on species of *Acacia* species in Botrycephalae. *Endoraecium falciforme* has distinctively thicker walls at the equator of the urediniospores (3.0–6.5 μm)

than *E. walkerianum* (3.0–4.5 μm). *Endoraecium falciforme* is recorded from south-east Queensland, whereas *E. walkerianum* is known from southern Australia. Urediniospores from a para-type specimen of *E. walkerianum* (BRIP 14205) were found to have thickened apices, whereas those of *E. falciforme* were thickened at the equator, or the wall was of uniform thickness.

Endoraecium irroratum McTaggart & R.G. Shivas, *sp. nov.*
— MycoBank MB808984; Fig. 6

Etymology. Name refers to the host, *Acacia irrorata*, on which it was found.

Type. AUSTRALIA, Queensland, Main Range National Park, on *A. irrorata*, 11 June 2012, A.D.W. Geering (holotype BRIP 57286).

Uredinia forming galls on stems, up to 2 cm long, yellowish brown. *Urediniospores* ovoid, apex obtuse, subhyaline, yellow to yellowish brown, 32–42 \times 17–24 μm ; wall 2.0–3.5 μm thick at sides, apex sometimes thickened (up to 4 μm), reticulate, with 4–10 equatorial germ pores. *Telia* formed from uredinia, yellowish brown. *Teliospores* cylindrical to subfusiform, apex acuminate to rounded, 2–7-digitate, subhyaline to yellow, 47–73 \times 15–22 μm ; wall 1.0–1.5 μm thick at sides, 11–21 μm thick at apex; pedicel persistent, up to 46 μm .

On stems and phyllodes of *A. irrorata* (Botrycephalae).

Additional specimens examined. AUSTRALIA, New South Wales, Warrumbungle National Park, on *A. irrorata*, 20 Mar. 2012, R. Berndt & V. Faust-Berndt, BRIP 55671; Queensland, Mt Mee, Mt Mee State Forest, on *A. irrorata*, 3 June 2012, C. Doungsa-ard & R.G. Shivas, BRIP 57279; Queensland, Mt Glorious, Wivenhoe Dam lookout area (-27.3077, 152.7136), *A. irrorata*, 30 Nov. 2012, A.D.W. Geering, BRIP 58054.

Notes — *Endoraecium irroratum* occurs on *A. irrorata* in Botrycephalae. Berndt (2011) considered that the rust on *A. irrorata* was *E. digitatum* based on morphology. There are no clear morphological differences that separate *E. irroratum* from the epitype of *E. digitatum* (Berndt 2011). However, the results from the molecular phylogenetic analysis in this study indicate the rusts on Botrycephalae are each restricted to a single host species.

Endoraecium maslinii McTaggart & R.G. Shivas, *sp. nov.* — MycoBank MB808985; Fig. 7

Etymology. Named after the Australian botanist Bruce R. Maslin, an expert on *Acacia* taxonomy, who has published approximately 250 *Acacia* taxa and kindly identified many specimens in this study.

Type. AUSTRALIA, Western Australia, Midlands Rd, 10 km SE of Mingenew (-29.2847, 115.5319), on *A. daphnifolia*, 28 Sept. 2012, C. Doungsa-ard & A.R. McTaggart (holotype BRIP 57872).

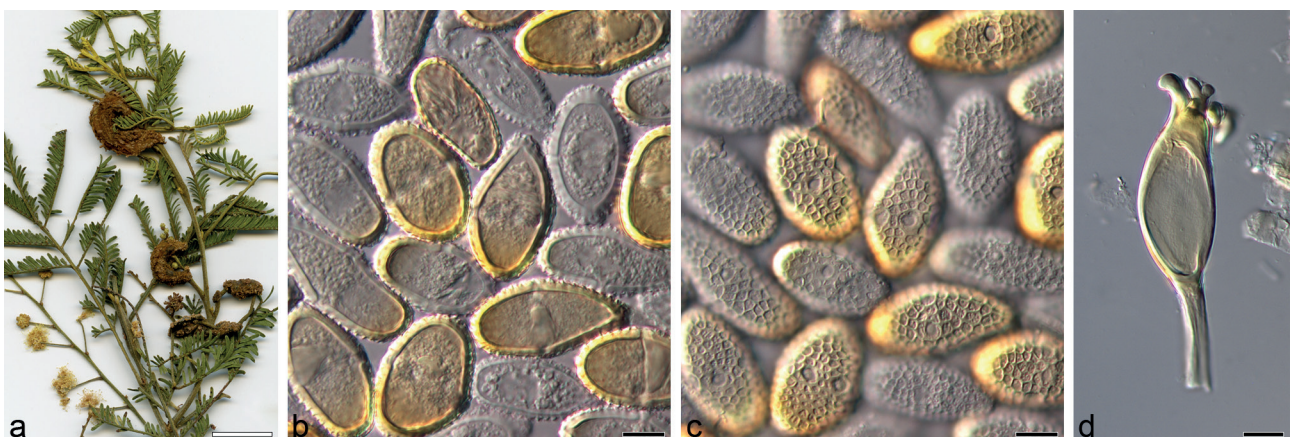


Fig. 6 *Endoraecium irroratum*. a. Stem gall (BRIP 55671); b, c. urediniospores (BRIP 55671); d. teliospore (BRIP 57286). — Scale bars: a = 1 cm; b–d = 10 μm .



Fig. 7 *Endoraecium maslinii* (BRIP 57872). a–c. Bullate swellings on leaves; d, e. urediniospores. — Scale bars: c = 1 cm; d, e = 10 μ m.

Uredinia forming bullate swellings on fruit and both phyllode surfaces, round, up to 1 cm, yellowish brown. *Urediniospores* ellipsoidal, ovoid to fusiform, apex obtuse, subhyaline to yellowish brown, 37–46 \times 20–26 μ m; wall 2.5–5.0 μ m thick at side, apex slightly thickened, 3.5–6.0 μ m, reticulate, with 6–8 equatorial germ pores; pedicel absent or up to 2.5 μ m.

On phyllodes of *A. daphnifolia* (Botrycephalae).

Notes — *Endoraecium maslinii* occurs on *A. daphnifolia* in Botrycephalae and is the only species of *Endoraecium* known from Western Australia. Only urediniospores have been found, which are yellowish brown and reticulate, and morphologically similar to other species on *Acacia* in Botrycephalae.

Endoraecium parvum Berndt, Mycol. Progr. 10: 510. 2011

Type. AUSTRALIA, Queensland, Caloundra, on *A. leiocalyx*, 25 Aug. 1932, S.T. Blake (holotype BRIP 75431).

Uredinia on both phyllode surfaces, subepidermal, erumpent, pulverulent, linear to round, yellowish brown. *Urediniospores* globose, subglobose to obovoid, apex obtuse, yellowish brown to reddish brown, 25–38 \times 16–22 μ m; wall 3–4 μ m thick at equator, hardly thickened at apex, reticulate, with 2–4 equatorial germ pores, pedicel 1.5–3.0 μ m. *Telia* formed from uredinia, erumpent, linear to dome-shaped, reddish brown. *Teliospores* oval, apex rounded, 1–3-digitate, hyaline to subhyaline, 31–60 \times 13–27 μ m; wall 1–2 μ m thick, mostly thickened at apex (5–14 μ m); pedicel persistent, up to 66 μ m.

Additional specimens examined. AUSTRALIA, Queensland, Caloundra, on *A. leiocalyx*, 25 Aug. 1932, S.T. Blake, BRIP 7543; Queensland, Mt Coolum (-26.5656, 153.0969), on *A. leiocalyx*, 28 July 2012, C. Doungsa-ard & A.R. McTaggart, BRIP 57512; Queensland, Mt Coolum (-26.5622, 153.0942), on *A. leiocalyx*, 28 July 2012, C. Doungsa-ard & A.R. McTaggart, BRIP 57514; Queensland, Cunningham Highway (-28.0317, 152.4697), on *A. leiocalyx*, 1 Aug. 2012, C. Doungsa-ard, A.R. McTaggart, A.D.W. Geering & R.G. Shivas, BRIP 57524; Queensland, Lake Moogerah Road (-28.0247, 152.5189), on *A. leiocalyx*, 1 Aug. 2012, C. Doungsa-ard, A.R. McTaggart, A.D.W. Geering & R.G. Shivas, BRIP 57527; Queensland, West Haldon (-27.7722, 152.0903), on *A. leiocalyx*, 1 Aug. 2012, C. Doungsa-ard, A.R. McTaggart, A.D.W. Geering & R.G. Shivas, BRIP 57534; Queensland, Brisbane, Geebung (-27.3375, 153.0511), on *A. leiocalyx*, 2 Sept. 2012, R.G. & M.D.E. Shivas, BRIP 57631; New South Wales, Shark Creek (-29.5664, 153.2003), on *A. leiocalyx*, 13 Aug. 2012, C. Doungsa-ard & A.R. McTaggart, BRIP 57568.

Notes — *Endoraecium parvum* is distinguished from other species by the comparatively short (25–38 μ m) urediniospores (Berndt 2011). Berndt (2011) described *E. parvum* from *A. leiocalyx* (as *A. concurrens*) and *A. mangium*. Specimens of rust on *A. mangium* were not included in this study and these warrant further study.

Endoraecium peggii McTaggart & R.G. Shivas, sp. nov. — MycoBank MB808986; Fig. 8

Etymology. Named after the Australian forest pathologist and rust specialist, Dr Geoff S. Pegg, who has discovered several new plant pathogens in Australia.

Type. AUSTRALIA, Northern Territory, Darwin, 8 km from airport, on *A. holosericea*, 7 May 2012, R.G. Shivas (holotype BRIP 55602).

Uredinia on both phyllode surfaces, pulverulent, subepidermal, erumpent, linear to round, up to 2 mm, yellowish brown; paraphyses intrasoral, cylindrical, yellow, with pedicel, 37–84 \times 9–10 μ m, thickened at apex, digitate. *Urediniospores* ovoid, apex obtuse, yellowish brown to reddish brown, 31–45 \times 20–25 μ m; wall 3–5 μ m thick at sides, apex 4–10 μ m thick, foveolate to reticulate, with 2–3 equatorial germ pores; pedicel 3–6 μ m. *Telia* rare, on both surfaces of phyllode, darker than uredinia, erumpent, linear, brown to black. *Teliospores* oval, apex rounded, no digitations, hyaline to subhyaline, 45–60 \times (15–)19–27 μ m; wall 1.5–2.0 μ m thick at sides, sometimes thickened at apex, 7–17 μ m; pedicel persistent, up to 52 μ m.

On phyllodes of *A. holosericea* (Juliflorae).

Additional specimens examined. AUSTRALIA, Queensland, Dimbulah, Bourke Developmental Road, on *A. holosericea*, 6 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55631; Queensland, Brisbane, Mt Coot-tha Botanic Gardens (-27.477655, 152.972270), on *A. holosericea*, Nov. 2012, C. Doungsa-ard & A.R. McTaggart, BRIP 58324.

Notes — *Endoraecium peggii* is known from *A. holosericea* in Juliflorae. This species of *Acacia* is native to northern Australia (Maslin 2013). The rust was also collected in south-east Queensland on a plant in the Mt Coot-tha Botanic Gardens. The teliospores of *E. peggii* lack the apical digitations found in all other species of *Endoraecium*, although this ornamentation is present on the paraphyses.

Endoraecium phyllodiorum (Berk. & Broome) Berndt, Mycol. Progr. 10: 503. 2011

Basionym. *Melampsora phyllodiorum* Berk. & Broome, Trans. Linn. Soc. London 2, ser. 2: 67. 1883.

\equiv *Uromyces phyllodiorum* (Berk. & Broome) McAlpine, The Rusts of Australia: 95. 1906. (McAlpine (1906) described teliospores from the type specimen, which were overlooked by Berkeley & Broome (1883). Under previous rules of nomenclature, *U. phyllodiorum* was considered the name of a new species with a teleomorphic type (Berndt 2011). Under the current system of nomenclature, the name is cited as originally published, and is typified by the type of the basionym.)

\equiv *Pileolaria phyllodiorum* (Berk. & Broome) Dietel, Ann. Mycol. 19: 302. 1921.

= *Uromyces phyllodii* Cooke & Massee (as '*phyllodiae*'), in Cooke, Grevillea 17: 70. 1889.

≡ *Coeomurus phyllodii* (Cooke & Massee) Kuntze (as '*Caeomurus*'), Revis. Gen. Pl. 3: 450. 1898.

Type. AUSTRALIA, Queensland, Brisbane, on *Acacia* sp., *F.M. Bailey* no. 269, K(M) 146703.

Uredinia on both phyllode surfaces, subepidermal, erumpent, pulverulent or on bullate swellings, linear, round to irregular, yellowish brown. **Urediniospores** subglobose to oval, apex obtuse, yellowish brown, (28–)30–52 × 17–26 µm; wall 2.5–4.0 µm thick at sides, 5–10 µm thick at apex, reticulate, with 3–7 equatorial germ pores; pedicel absent or 1.5–3.5 µm. **Telia** formed from uredinia, erumpent, linear, reddish brown. **Teliospores** cylindrical to oval, apex rounded, 1–4-digitate, hyaline to subhyaline, 38–58 × 17–28 µm; wall 1–2 µm thick at sides, 6–17 µm thick at apex; pedicel persistent, up to 30 µm.

Additional specimens examined. AUSTRALIA, Queensland, Mount Coolum (-26.5611, 153.0839), on *A. aulacocarpa*, 28 July 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57310; Queensland, Mt Coolum (-26.5611, 153.0839), on *A. aulacocarpa*, 28 July 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57516; New South Wales, Woombah, at the beginning of Iluka Road (-29.3544, 153.2492), on *A. aulacocarpa*, 11 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57578; New South Wales, Woombah, at the beginning of Iluka Road (-29.3544, 153.2492), on *A. aulacocarpa*, 11 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57580; New South Wales, Woombah, Iluka Bluff (-29.3967, 153.3722), on *A. aulacocarpa*, 11 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57579; New South Wales, Shark Creek (-29.5664, 153.2003), on *A. aulacocarpa*, 13 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57569; New South Wales, Shark Creek (-29.5664, 153.2003), on *A. aulacocarpa*, 13 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57570; Queensland, Mt Tibrogargan (-26.9264, 152.9417), on *A. aulacocarpa*, 15 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57588; Queens-

land, Mt Tibrogargan (-26.9258, 152.9508), on *A. aulacocarpa*, 15 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57589; Queensland, Mt Tibrogargan (-26.9278, 152.9378), on *A. aulacocarpa*, 15 Aug. 2012, *C. Doungsa-ard & A.R. McTaggart*, BRIP 57590.

Notes — Berndt (2011) discussed the taxonomy of *E. phyllodiorum*, and listed the hosts as *A. aulacocarpa*, *A. crassicaarpa*, *A. holosericea*, *A. mangium* and tentatively *A. auriculiformis*. The type specimens of *Melampsora phyllodiorum* and *Uromyces phyllodii* were collected on unidentified species of *Acacia* in Brisbane, Australia. *Acacia aulacocarpa* is the only host species listed by Berndt (2011) that occurs naturally in or near to Brisbane. The other four species of *Acacia* are restricted to northern Queensland and the Northern Territory (Maslin 2013). Herbarium records and field observations by the authors show that *Endoraecium* spp. occur on four species, *A. aulacocarpa*, *A. irrorata*, *A. leiocalyx* and *A. podalyriifolia*, in the Brisbane region. The rust on *A. aulacocarpa* forms bullate swellings on phyllodes, very similar to those seen in the type specimen of *E. phyllodiorum* and illustrated by Berndt (2011). Consequently, we suggest that *A. aulacocarpa* is the host of the type of *E. phyllodiorum*. Berndt (2011) proposed that *E. phyllodiorum* had a wide host range, including *A. aulacocarpa*, *A. auriculiformis*, *A. holosericea* and *A. mangium*. The rusts on *A. auriculiformis* and *A. holosericea* are now identified as *E. auriculiforme* and *E. peggii*, respectively. The rust on *A. mangium* is unknown. *Acacia aulacocarpa* is the only species of *Acacia* known to host two species of *Endoraecium*, *E. phyllodiorum* and *E. violae-faustiae*.

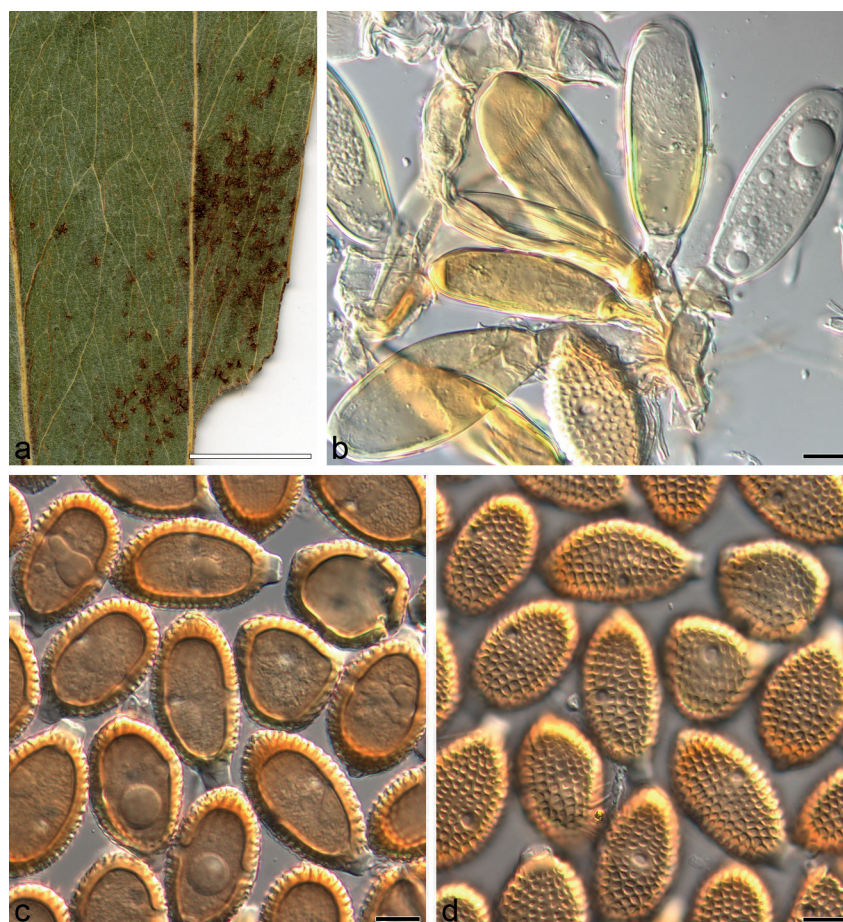


Fig. 8 *Endoraecium peggii*. a. Uredinia (BRIP 55631); b. teliospores (BRIP 55602); c, d. urediniospores (BRIP 55631). — Scale bars: a = 1 cm; b–d = 10 µm.

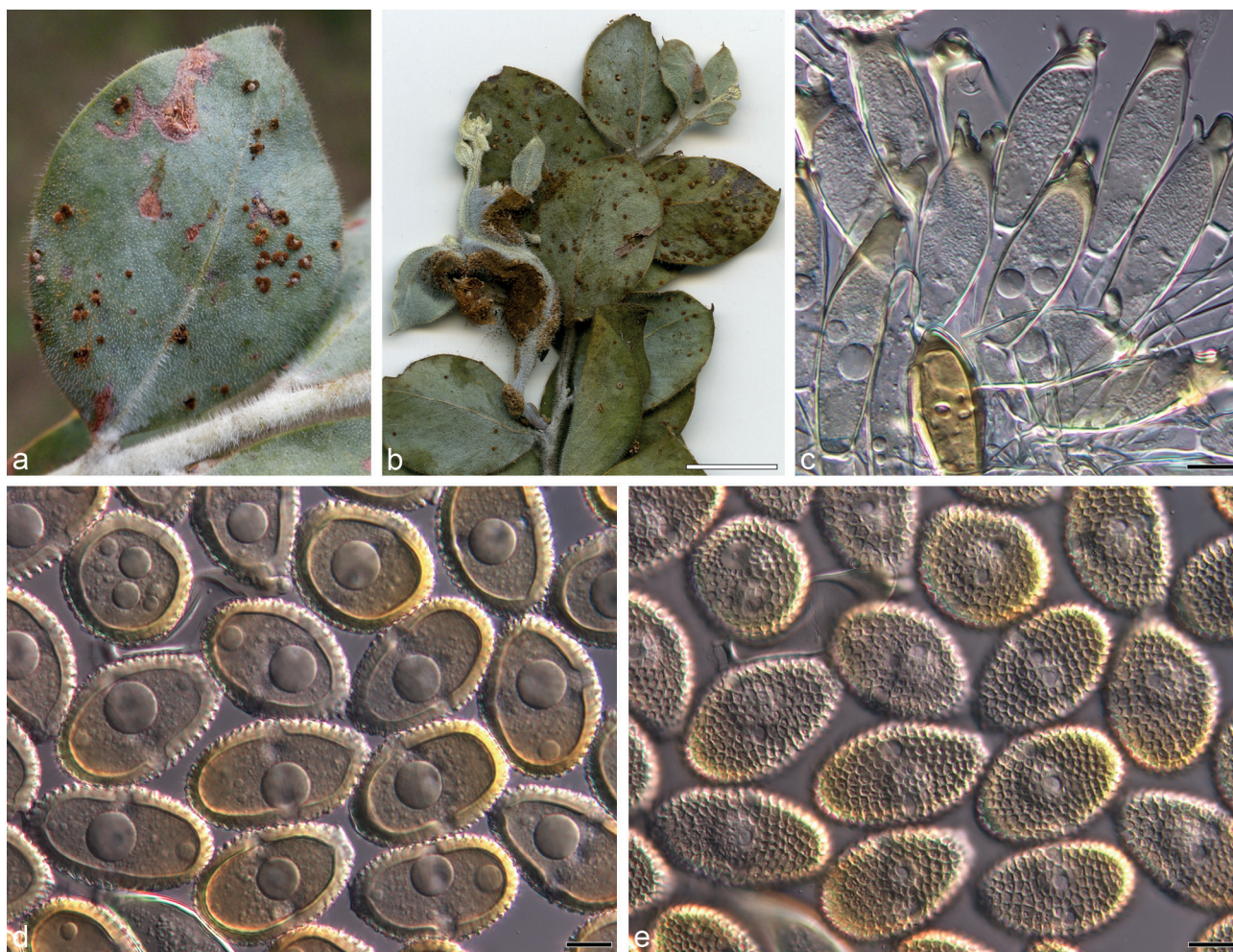


Fig. 9 *Endoraecium podalyriifolium* (BRIP 57576). a, b. Pulverulent sori on leaves and stems; c. teliospores; d, e. urediniospores. — Scale bars: b = 1 cm; c–e = 10 µm.

Endoraecium podalyriifolium McTaggart & R.G. Shivas, sp. nov. — MycoBank MB808987; Fig. 9

Etymology. Name refers to the host, *Acacia podalyriifolia*, on which it was found.

Type. AUSTRALIA, New South Wales, Maclean, Wharf Street (-29.4589, 153.2111), on *A. podalyriifolia*, 12 Aug. 2012, C. Doungsa-ard & A.R. McTaggart (holotype BRIP 57576).

Uredinia on both phyllode surfaces, subepidermal, erumpent, pulverulent, round, 0.5–10.0 mm, yellow to yellowish brown. *Urediniospores* cylindrical to ovoid, apex obtuse, subhyaline to yellow, (30–)33–44 × 23–27 µm; wall 3–4 µm thick, reticulate, with 4–6 germ pores. *Telia* formed from uredinia, subepidermal, erumpent, yellow. *Teliospores* cylindrical to fusiform, apex rounded, 2–5-digitate, subhyaline to yellow, 44–60 × 12–19 µm; wall 1 µm thick at sides, 6–12 µm thick at apex; pedicel persistent, over 60 µm.

On phyllodes of *A. podalyriifolia* (Botrycephalae).

Additional specimen examined. AUSTRALIA, Queensland, South Ripley, Ripley Road, on *A. podalyriifolia*, 17 July 2012, A.D.W. Geering, BRIP 57294.

Notes — Berndt (2011) considered the rust on *A. podalyriifolia* was *E. digitatum* based on morphology and the close relationships of host species within this group. However, the results from the molecular phylogenetic analysis in this study indicate the rusts on Botrycephalae are each restricted to a single host species.

Endoraecium tierneyi (J. Walker & R.G. Shivas) M. Scholler & Aime, Mycoscience 47: 163. 2006

Basionym. *Racospermyces tierneyi* J. Walker & R.G. Shivas, in Walker, Australas. Mycol. 20: 23. 2001.

Type. AUSTRALIA, Queensland, Tambo, near Castlevale, on *A. harpophylla*, 6 June 2000, G.S. Pegg (holotype BRIP 27071!).

Uredinia on both phyllode surfaces, subepidermal, erumpent, linear, up to 500 µm, yellowish brown. *Urediniospores* cylindrical, oval to broadly fusiform, apex obtuse, reddish brown, 26–36 × 13–18 µm; wall 2.0–2.5 µm thick at sides, 4.0–4.5 µm thick at apex, reticulate, with 4–8 equatorial germ pores. *Telia* on both phyllode surfaces, erumpent. *Teliospores* subglobose, ovoid to obovoid, apex rounded, reddish brown, darker at the apex, 27–41 × 20–26 µm; wall 1.0 µm thick at sides, 7–13 µm thick at apex, smooth; pedicel persistent, 50–55 µm long.

Additional specimens examined. AUSTRALIA, Queensland, Springsure, on *A. harpophylla*, 27 Feb. 2001, G.S. Pegg, BRIP 27887; Queensland, Caldervale, on *A. harpophylla*, 1 Mar. 2001, G.S. Pegg, BRIP 27880.

Notes — *Endoraecium tierneyi* occurs on *A. harpophylla* in Plurinerves. It was recovered in the phylogenetic analyses as sister to three species of *Endoraecium* in Hawaii that occur on *A. koa*, also in Plurinerves. The teliospores are subglobose to obovoid, while other species of *Endoraecium* in Australia have clavate teliospores.

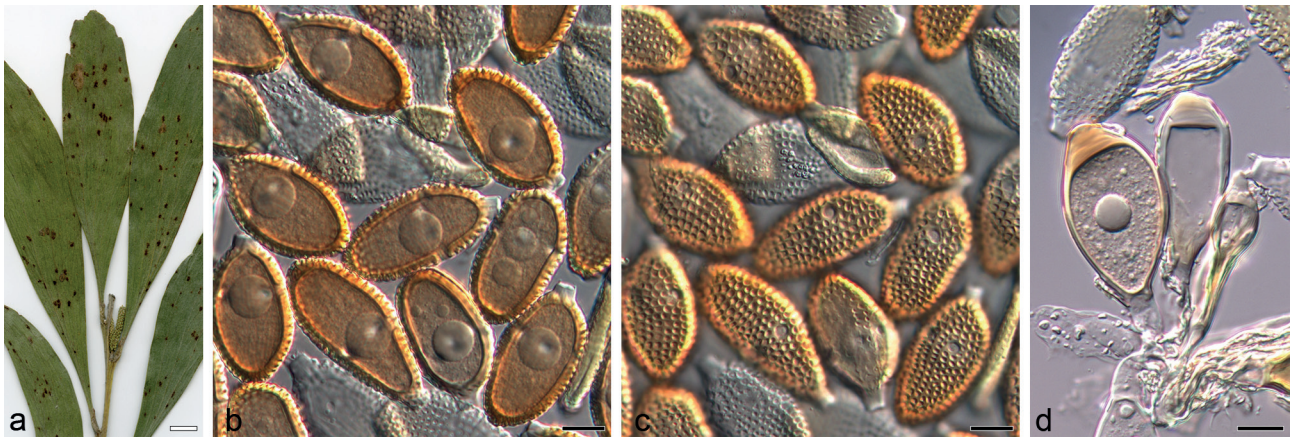


Fig. 10 *Endoraecium tropicum*. a. Pulverulent sori (BRIP 56557); b, c. urediniospores (BRIP 56557); d. teliospores (BRIP 56555). — Scale bars: a = 1 cm; b–d = 10 µm.

***Endoraecium tropicum* McTaggart & R.G. Shivas, sp. nov. —**
 MycoBank MB808988; Fig. 10

Etymology. Name refers to the host, *Acacia tropica*, on which it was found.

Type. AUSTRALIA, Northern Territory, Gregory, Victoria Highway (-15.6003, 131.2136), on *A. tropica*, 20 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas (holotype BRIP 56557).

Uredinia on both phyllode surfaces, subepidermal, pulverulent, up to 2 mm, brown. *Urediniospores* ovoid to fusiform, apex obtuse, yellowish brown, 30–40 × 17–22 µm; wall 3–4 µm thick at sides, apex usually 3–4 µm thick, sometimes slightly thickened (4–5 µm), reticulate, with 3–4, equatorial germ pores; pedicel 3–4 µm. *Telia* formed from uredinia, erumpent. *Teliospores* clavate, apex rounded, papillate rather than digitate, 40–53 × 16–23 µm; wall 1.5–2.0 µm thick at sides, 8–11 µm thick at apex; pedicel persistent, 5 µm.

On phyllodes of *A. tropica* (Juliiflorae).

Additional specimen examined. AUSTRALIA, Northern Territory, Gregory, Victoria Highway (-15.6014, 131.2136), on *A. tropica*, 20 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 56555.

Notes — *Endoraecium tropicum* was closely related to *E. parvum* in the phylogenetic analysis. Both species have urediniospores with uniformly thickened spore walls. The distribution of *A. tropica* is restricted to the Northern Territory and north-west Queensland. *Acacia leiocalyx*, the host of *E. parvum*, occurs in eastern Australia (Maslin 2013).

***Endoraecium violae-faustiae* Berndt, (as ‘*violae-faustae*’)**
 Mycol. Progr. 10: 513. 2011

Type. AUSTRALIA, Queensland, Cairns, Barron Gorge, MacDonald’s trail, on *A. crassicaarpa* (as *Acacia* sp.), 18 Aug. 2006, V. Faust-Berndt & R. Berndt (holotype BRIP 53388!).

Uredinia on both phyllode surfaces, subepidermal, erumpent, pulverulent, linear to round, yellowish brown, not forming bullate sori; paraphyses cylindrical with digitate heads, subhyaline to yellow, 35–88 × 6–15 µm thick at sides, thickened at apex. *Urediniospores* globose, subglobose, obovoid to oval, apex acute, yellowish brown, 28–53 × 17–26 µm; wall 2.5–4.5 µm thick at sides, apex mostly thickened, 5.0–10.5 µm thick, foveolate, with 3–5 equatorial germ pores; pedicel 3–5 µm. *Telia* formed from uredinia, erumpent, reddish brown. *Teliospores* obovoid to oval, apex rounded, 1–4-digitate, hyaline to subhyaline, 41–53 × 20–28 µm; wall 1.5–3.0 µm thick at sides, 5–14 µm thick at apex; pedicel persistent, up to 43 µm.

Additional specimens examined. AUSTRALIA, Queensland, Coen, Peach No. 7, on *A. crassicaarpa* 18 July 1999, R.G. Shivas & M. Gunther, paratype BRIP 25816; Queensland, Julatten, Abattoir Swamp (-16.6074, 145.3428), on *A. aulacocarpa*, 5 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55601; Queensland, Mareeba (-16.9414, 145.5411), on *A. difficilis*, 6 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55611; Queensland, Innisfail (-17.5003, 146.0756), on *A. difficilis*, 7 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55616; Queensland, Dimbulah (-17.2967, 144.9736), on *A. aulacocarpa*, 6 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt, V. Faust-Berndt, M.D.E. & R.G. Shivas, BRIP 55629; Northern Territory, Humpty Doo (-12.5964, 131.2083), on *A. difficilis*, 22 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt & R.G. Shivas, BRIP 56539; Northern Territory, Humpty Doo (-12.5964, 131.2083), on *A. difficilis*, 22 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt & R.G. Shivas, BRIP 56540; Northern Territory, Humpty Doo (-12.5964, 131.2083), on *A. difficilis*, 22 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt & R.G. Shivas, BRIP 56545; Northern Territory, Humpty Doo (-12.5964, 131.2083), on *A. difficilis*, 22 Apr. 2012, C. Doungsa-ard, A.R. McTaggart, R. Berndt & R.G. Shivas, BRIP 56547.

Notes — Berndt (2011) described the urediniospores of *E. violae-faustiae* as foveolate, with a thickened and acute apex. The most distinctive character was the variable paraphyses, which resembled thin teliospores, similar to those found in *E. auriculiforme* and *E. peggii*. *Endoraecium violae-faustiae* was reported on *A. aulacocarpa* and *A. crassicaarpa*, also in Juliiflorae, as well as *A. flavescens* in Plurinerves (Berndt 2011). The host of the holotype (BRIP 53388), was re-identified as *A. crassicaarpa*, which is closely related to *A. aulacocarpa* (Maslin 2013). The host plant of the specimen on *A. flavescens* (BRIP 25816) was re-identified as *A. crassicaarpa*. The distribution of *E. violae-faustiae* is restricted to the northern parts of Australia on *A. aulacocarpa*, *A. crassicaarpa* and *A. difficilis*.

***Endoraecium walkerianum* Berndt, Mycol. Progr. 10: 509.**
 2011

Type. AUSTRALIA, New South Wales, Cavan Gap near Yass, on *A. penninervis*, 20 Nov. 1951, E. Gauba (holotype SF35354).

Additional specimen examined. AUSTRALIA, New South Wales, Bald Rock, on *A. penninervis*, 26 Feb. 1984, J.W. Tierney, paratype BRIP 14205.

Notes — *Endoraecium walkerianum* was reported from *A. penninervis* and *A. obliquinervis* (Berndt 2011) in Botrycephalae (Maslin 2013).

A KEY TO THE KNOWN SPECIES OF ENDORAECIUM IN AUSTRALIA

1. Urediniospores hyaline to pale yellow (on Botrycephalae) 2
1. Urediniospores golden or reddish brown 7
2. Urediniospore wall up to 5 µm or thicker at sides 3
2. Urediniospore wall 2.0–4.5 µm thick at sides 4
3. Forming galls on stems of *A. notabilis* *E. digitatum*
3. Forming hypertrophied lesions on *A. falciformis* *E. falciforme*
3. Forming hypertrophied lesions on *A. daphnifolia* *E. maslinii*
4. Forming hypertrophied lesions on phyllodes 5
4. Forming galls on stem 6
5. On *A. fasciculifera* *E. bicinctum*
5. On *A. podalyriifolia* *E. podalyriifolium*
5. On *A. penninervis* *E. walkerianum*
6. On *A. irrorata* *E. irroratum*
6. On *A. dealbata* *E. carnegiei*
7. Teliospores globose to ellipsoid on *A. harpophylla* *E. tierneyi*
7. Teliospores clavate to obovoid or absent (on Juliflorae) 8
8. Urediniospore wall uniform 9
8. Urediniospore with thickened apex 10
9. Teliospores digitate, on *A. leiocalyx* *E. parvum*
9. Teliospores not digitate, on *A. tropica* *E. tropicum*
10. Urediniospores foveolate, paraphyses present 11
10. Urediniospores reticulate, paraphyses absent 13
11. Urediniospores with an acute apex 12
11. Urediniospores not acute, on *A. holosericea* .. *E. peggii*
12. On *A. auriculiformis* *E. auriculiforme*
12. On *A. aulacocarpa*, *A. crassocarpa* or *A. difficilis* *E. violae-faustiae*
13. On *A. disparrima* *E. disparrimum*
13. On *A. aulacocarpa* *E. phyllodiorum*

DISCUSSION

Phylogenetic analyses of loci from nuclear rDNA and mitochondrial DNA showed that species of *Endoraecium* in Australia have narrow host ranges. Savile (1971) hypothesised that rusts speciated either by divergence with their hosts or by host jumps. The close relationship between *Endoraecium* and species/subclades of *Acacia* observed in this study indicates that *Endoraecium* diversified by co-evolution with its hosts. For example, the five species of *Endoraecium* on Botrycephalae, namely, *E. carnegiei*, *E. falciforme*, *E. irroratum*, *E. maslinii* and *E. podalyriifolium*, were closely related with few differences in the studied genes and short branch lengths between species in the recovered phylogenetic trees. Species of *Acacia* in Botrycephalae diversified c. 3.4–3.8 million years ago (Miller et al. 2013), which would represent the maximum age of the corresponding *Endoraecium* species.

Endoraecium tierneyi on *A. harpophylla* in Australia was recovered as sister to the Hawaiian species, *E. acaciae*, *E. hawaiiense* and *E. koae*, on *A. koa*. These Australian and Hawaiian rusts all occur on species of *Acacia* in Plurinerves. Hodges & Gardner (1984) and Walker (2001) proposed that the Australian species of *Endoraecium* were the ancestor of the Hawaiian rusts. Our study supports this view by showing the rusts on Plurinerves had an Australian ancestor (plesiomorphic state). Further, the three rusts on *Acacia* in Hawaii have differences in life cycle and morphology, but are identical in the LSU region of

rDNA. These rusts have diversified on *A. koae* after its relatively recent split from Australian species of *Acacia* (Brown et al. 2012). Endocyclic species occur in Hawaii but not in Australia, which shows that a reduced life cycle is a derived character in *Endoraecium*.

Endoraecium hyalosporum from South-East Asia infects *A. confusa*, which is the only example of a species of *Endoraecium* on a host outside of the p.u.b. clade of *Acacia* (Brown et al. 2012). This may represent an example of a host jump. However the systematic position of *E. hyalosporum* is unknown, and it is possible that it does not have a close phylogenetic relationship to other species of *Endoraecium*. It is noteworthy that *E. hyalosporum* has been assigned to other genera at various times, namely, *Maravalia* (Dietel 1924), *Poliotelium* (Mains 1939) and *Atelocauda* (Ono 1984).

The morphology of urediniospores may be synapomorphic for the three clades of *Endoraecium*. For example, the rusts on Juliflorae have reticulate to foveolate ornamentation on golden-brown urediniospores. The rusts on Botrycephalae have a raised reticulate ornamentation on subhyaline to pale yellow urediniospores. *Endoraecium tierneyi* was the only rust examined on Plurinerves, and it had reticulate, reddish brown urediniospores. Berndt (2011) noted the urediniospores of species of *Endoraecium* on *A. koa* have similar morphology to the rusts on Juliflorae, such as *E. phyllodiorum*. We cannot propose any apomorphies for the rusts on Plurinerves at this stage. The rusts on Juliflorae, namely *E. auriculiforme*, *E. disparrimum*, *E. peggii*, *E. phyllodiorum* and *E. violae-faustiae*, have urediniospores with thickened apices, whereas their sister rusts *E. parvum* and *E. tropicum*, also in Juliflorae, have uniformly thickened spore walls.

Other morphological characters were less useful for species delimitation in *Endoraecium*. In general, the teliospores of rusts on species of *Acacia* in Botrycephalae had numerous (up to 15) apical digitations and were clavate. The teliospores of rusts on species of *Acacia* in Juliflorae had fewer (up to four) apical digitations and were obovoid. The presence of a gall or bullate swelling is not a useful character for species delimitation. For example, the sori of *E. auriculiforme* and *E. phyllodiorum* may occur on bullate swellings or on undistorted phyllodes (Berndt 2011).

Endoraecium is likely to show further diversity. Only 14 species of *Acacia* were sampled in this study. Other species such as *A. glaucocarpa* (Botrycephalae), *A. juliflorae* (Juliflorae) and *A. viscidula* (Plurinerves) were observed by the authors as hosts for potentially new species of *Endoraecium*. New taxa will need to be classified by a combined morphological, ecological (host range) and molecular approach. We predict that many new species of *Endoraecium*, which have diversified by coevolution with their *Acacia* hosts, remain to be found in Australia.

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