Ten new species of *Macalpinomyces* on *Eriachne* in northern Australia

Ying-Ming Li[a,b], Roger G. Shivas[c], Alistair R. McTaggart[d], Peng Zhao[a,b], and Lei Cai[a,b,*]

[a]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China;

[b]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, 100049, China;

[c]Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Queensland 4102, Australia;

[d]Department of Microbiology and Plant Pathology, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa

*Correspondence to: Lei Cai (cail@im.ac.cn)

ABSTRACT

Macalpinomyces was established in 1977, with the type species *M. eriachnes* described from a specimen collected in northern Australia on the grass *Eriachne* sp. in 1855. Subsequently, *M. eriachnes* has been reported on more than 21 species of *Eriachne* in northern Australia. In this study, a polyphasic approach was employed to determine whether *M. eriachnes* masked cryptic diversity. On the basis of morphology, multilocus phylogeny, and coalescent methods of generalized mixed Yule-coalescent (GMYC) and Poisson tree processes (PTP) models, 26 specimens of *Macalpinomyces* on 13 species of *Eriachne* held in Australian herbaria were studied. Consequently, 10 new species of *Macalpinomyces* that satisfied the phylogenetic species recognition criteria are described.

KEYWORDS: GMYC, phylogeny, PTP, smut fungi, taxonomy, *Ustilaginaceae*

Introduction

There are about 317 species of smut fungi in Australia (Shivas et al. 2014), including *Macalpinomyces eriachnes, Tilletia geeringii, T. mactaggartii*, and *T. marjaniae*, on *Eriachne* spp. (Li et al. 2014). The original collection of *M. eriachnes* was made from northern Australia in 1855 by Baron Ferdinand von Mueller. Two duplicate specimens were sent to European mycologists, Mordecai Cooke in England and Felix von Thümen in Germany (Langdon and Fullerton). Consequently, two new species were described in different genera based on this single collection, *Sorosporium eriachnes* (as '*eriachnis*') Thümen (1878) and *Ustilago australis* Cooke (1879). A monotypic genus, *Macalpinomyces*, was established by Langdon and Fullerton (1977) to accommodate the type specimen of *Sorosporium eriachnes*. It was characterized by sori without columellae, comparatively large, thick-walled, pale-colored sterile cells, and polyangular spores. The type of spore germination showed that *Macalpinomyces* belonged to the *Ustilaginaceae* (Langdon and Fullerton 1977). Subsequently, Vánky (1996, 1997) broadened the original concept of *Macalpinomyces* to include species with sori in the culms or spikelets, and also ovaricolous species with small sterile cells between the spores. This led to the transfer of several species from *Ustilago* and *Sporisorium* to *Macalpinomyces*, as well as the expansion of its host range to include genera of grasses other than *Eriachne*. Vánky (2011) listed 46 species of *Macalpinomyces* on more than 38 genera of grasses.

The application of molecular phylogenetic analyses showed that *Macalpinomyces* had become polyphyletic within the *Ustilaginaceae* (Stoll et al. 2005; McTaggart et al. 2012a). More recently, some species of *Macalpinomyces* have been transferred to other genera, including *Stollia* (McTaggart et al. 2012b) and *Mycosarcoma* (McTaggart et al. 2016).

Macalpinomyces eriachnes has been reported from 21 different species of *Eriachne*, namely, *E. agrostidea, E. aristidae, E. armittii, E. avenacea, E. capillaris, E. basedowii, E. ciliata, E. festucacea, E. glauca, E. glabrata, E. helmsii, E. melicacea, E. mucronata, E. obtusa, E. pallescens, E. pauciflora, E. pulchella, E. rara, E. scleranthoides, E. sulcata*, and *E. triseta* (Vánky and Shivas). Significant molecular distances were found between specimens of *M. eriachnes* on different *Eriachne* spp. (Stoll et al. 2005), which indicated that *M. eriachnes* might comprise a number of cryptic species. The main objectives of this study were to determine whether *M. eriachnes* was a complex of cryptic species on multiple hosts, as well as to delimit the species boundaries in *Macalpinomyces.*

Materials and methods

Specimen examination

Specimens held in herbarium BRIP (Department of Agriculture and Fisheries, Dutton Park, Queensland) were examined under a light microscope (TABLE 1). Spores were mounted in lactic acid (100% *v*/*v*) for examination. Spore measurements were expressed as ranges: (min–)mean − standard deviation–mean + standard deviation(–max) ($n \geq 20$). Images were captured by a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast (Wetzlar, Germany). Helicon Focus 4.46.1 (Helicon Soft Ltd., Kharkiv, Ukraine) was used to combine images in order to increase depth of field. For scanning electron microscopy (SEM), dried spores were dusted onto double-sided adhesive tape, fixed on specimen stubs, sputter-coated with gold, ca. 20 nm thick, and examined with a FEI Quanta 200 electron microscope (Hillsboro, Oregon, USA). Nomenclatural novelties and descriptions were registered in FungalName (http://fungalinfo.im.ac.cn/fungalname/fungalname.html).

DNA extraction, PCR amplification, and sequencing

Mature sori were carefully removed from herbarium specimens with a fine needle and deposited in cell lysis solution. Gentra Puregene kits (Qiagen, Valencia, California) were used to extract the total genomic DNA according to the manufacturer's protocol.

For fungi, fragments of nuclear rDNA ITS1-5.8S-ITS2 (internal transcribed spacer [ITS]) were amplified by polymerase chain reaction (PCR) with primers M-ITS 1/ITS 4 (White et al. 1990; Stoll et al. 2003) at 62 C; fragments of nuc 28S rRNA (28S) were amplified with the primers LR0R/LR7 (Vilgalys and Hester 1990) at 60 C; a fragment of nuc 18S rRNA (18S) was amplified with the primers NS1 and NS4 (White et al. 1990) at 60 C.

PCRs were performed in a 20 µL reaction containing 7 µL distilled water, 10 µL of 5× Phusion HF Buffer Pack (New England Biolabs, Ipswich, UK), 1 µL each primer (10 µM), and 1 µL DNA template. Amplification reactions were run as follows: initial denaturation of 98 C for 5 min, followed by 35 cycles of denaturing at 95 C for 30 s, annealing at related temperature for 30 s, and extension of 72 C for 1 min, followed by 10 min at 72 C for extension. PCR products were sent to Macrogen (Seoul, Korea) for sequencing with the forward and reverse primers mentioned above. DNA sequences were assembled and analyzed in Sequencher 5.0.

Table 1. List of specimens and their hosts examined in this study.

Species	Strain no./Herbarium no.	Host	GenBank accession no.		
			ITS	28S	18S
Anthracoidea karii	FO 46417 (TUB)	*Carex brunnescens*	—	DQ875358[1]	DQ875376[1]
Cintractia amazonica	MP 2008 (USJ)	*Rhynchospora barbata*	DQ875342[1]	AJ236142[1]	DQ363302[1]
Cintractia axicola	HUV 17460	*Fimbristylis tetragona.*	AY344967[1]	AF009847[1]	DQ875378[1]
Dermatosorus cyperi	HUV 15991	*Cyperus cellulloso-reticulatus*	DQ875343[1]	AJ236157[1]	—
Farysia chardoniana	MP 2062 (USJ)	*Carex polystachya*	AY344968[1]	AF009859[1]	—
Heterotolyposporium piluliforme	HUV 15732	*Juncus planifolius*	DQ875345[1]	AF009871[1]	—
Leucocintractia leucodermoides	MP 10431 (HAJB)	*Rhynchospora holoschoenoides*	DQ875346[1]	DQ875363[1]	—
Macalpinomyces australiensis	56574 (M)	*Eriachne helmsii*	AY740038[4]	AY740091[4]	—
M. australiensis	BRIP 43954	*Eriachne helmsii*	**KX686926**	**KX686969**	**KX686974**
M. australiensis	BRIP 27740	*Eriachne helmsii*	**KX686924**	**KX686968**	**KX686972**
M. cookei	BRIP 55386	*Eriachne pallescens*	**KX686942**	**KX686951**	**—**
M. eendrachtslandiae	BRIP 46732	*Eriachne ciliata*	**KX686928**	**KX686965**	**—**
M. eendrachtslandiae	BRIP 51816	*Eriachne ciliata*	**KX686937**	**KX686966**	**KX686980**
M. eriachnes	BRIP 49698	*Eriachne* sp.	**KX686932**	**KX686956**	**—**
M. eriachnes	BRIP 39636	*Eriachne obtusa*	**KX686925**	**KX686955**	**KX686973**
M. eriachnes	BRIP 49717	*Eriachne* sp.	**KX686934**	**KX686957**	**KX686958**
M. eriachnes	BRIP 51817	*Eriachne* sp.	**KX686938**	**KX686958**	**KX686981**
M. eriachnes	BRIP 54352	*Eriachne* sp.	**KX686940**	**KX686960**	**KX686982**
M. eriachnes	BRIP 55053	*Eriachne* sp.	**KX686941**	**KX686959**	**—**
M. eriachnes	BRIP 27683	*Eriachne obtusa*	**KX686923**	**KX686954**	**KX686971**
M. fullertonii	HUV 961	*Eriachne aristidea*	**JN367287**	**JN367312**	**JN367340**
M. fullertonii	56573 (M)	*Eriachne aristidea*	**AY740037**	**AY740090**	**—**
M. fullertonii	BRIP 27399	*Eriachne aristidea*	**KX686920**	**KX686944**	**—**
M. fullertonii	BRIP 27408	*Eriachne aristidea*	**KX686921**	**KX686945**	**—**
M. fullertonii	BRIP 27455	*Eriachne aristidea*	**KX686922**	**KX686947**	**—**
M. fullertonii	BRIP 43964	*Eriachne aristidea*	**KX686927**	**KX686948**	**—**
M. fullertonii	BRIP 46832	*Eriachne aristidea*	**KX686929**	**KX686946**	**KX686975**
M. langdonii	BRIP 57639	*Eriachne glauca*	**KX686943**	**KX686963**	**—**
M. langdonii	BRIP 49691	*Eriachne glauca*	**KX686931**	**KX686962**	**—**
M. langdonii	BRIP 51851	*Eriachne festucacea*	**KX686939**	**KX686964**	**—**
M. muelleri	BRIP 49638	*Eriachne* sp.	**KX686930**	**KX686949**	**KX686976**
M. novae-hollandiae	BRIP 49716	*Eriachne sulcata*	**KX686933**	**KX686967**	**KX686977**
M. terrae-australis	BRIP 49786	*Eriachne nodosa*	**KX686936**	**KX686953**	**KX686979**
M. terrae-australis	BRIP 26938	*Eriachne nodosa*	**KX686919**	**KX686952**	**—**
M. thuemenii	BRIP 49742	*Eriachne basedowii*	**KX686935**	**KX686961**	**KX686978**
M. vankyi	BRIP 26914	*Eriachne pulchella*	**KX686918**	**KX686950**	**KX686970**
Melanopsichium pennsylvanicum	HUV 17548	*Polygonum glabrum*	AY740040[1]	AY740093[1]	DQ363314[1]
Melanotaenium cingens	L.E. Kari 191 (M)	*Linaria vulgaris*	DQ875347[1]	DQ875364[1]	—
Melan. euphorbiae	HUV 17733	*Euphorbia geniculata*	JN367289[2]	JN367314[2]	DQ198789[2]
Moesziomyces bullatus	CBS 425.34	*Paspalum distichum*	DQ831013[3]	DQ831011[3]	DQ831012[3]
Mo. seriocauli	56580 (M)	*Eriocaulon cinereum*	AY740041[4]	AY740094[4]	—
Moreaua bulbostylidis	56581 (M)	*Bulbostylis capillaris*	DQ875349[1]	DQ875366[1]	—
Mor. fimbristylidis	56582 (M)	*Fimbristylis dichotoma*	DQ875350[1]	DQ875367[1]	—
Mundkurella kalopanacis	HUV 16732	*Kalopanax pictus*	DQ875351[1]	AF009869[1]	—
Mycosarcoma maydis	MUCL 30488	*Zea mays*	AY345004[8]	—	—
My. maydis	MS 115	*Zea mays*	—	AF453938[10]	—
Pericladium grewiae	HUV 18334	*Grewia retusifolia*	—	DQ875370[1]	—
Pseudozyma flocculosa	AFTOL-ID 864	—	DQ411535	AY745712	DQ092923
P. fusiformata	AP 6	—	FJ919774[5]	GQ281760[5]	—
P. rugulosa	JCM 10323	—	JN942670	JN940523	JN940458
P. tsukubaensis	1D 11	—	AB550283	AB550287	—
Restiosporium restionum	HUV 17980	*Restio nitens*	—	DQ875372[1]	—
Schizonella melanogramma	FO 37174	*Carex pilulifera*	DQ191252[6]	AF009870[6]	DQ363308[6]
Sporisorium erythraeense	Ust. Exs. 849 (M)	*Hackelochloa granularis*	AY740049[1]	AY740102[1]	—
S. reilianum	AFTOL-ID 490	—	DQ832230[2]	DQ832228[2]	DQ832229[2]
S. scitamineum	UMa697	*Saccharum officinarum*	JN367296[2]	JN367321[2]	JN367349[2]
S. sorghi	AFTOL-ID 867	*Sorghum bicolor*	DQ200931[7]	AY745726[7]	DQ234548[7]
Stegocintractia luzulae	MP2340 (M)	*Luzula pilosa*	DQ875353[1]	AJ236148[1]	—
Tolyposporium junci	HUV 17168	*Juncus bufonius*	AY3449948	AF009876[8]	—
Tranzscheliella hypodytes	RB3056 (TUB)	*Poa cita*	DQ191249[4]	DQ191255[4]	—
Trichocintractia utriculicola	MP2075 (USJ)	*Rhynchospora corymbosa*	DQ875354[1]	AF009877[1]	—
Urocystis colchici	CBS 283.28	*Colchicum autumnale*	DQ839596	DQ838576	DQ839595
Ustanciosporium taubertianum	MP 2276 (HAJB)	*Rhynchospora tenuis*	AY740024[8]	AJ236156[8]	—
Ustilago avenae	—	—	JN367306[2]	JN367333[2]	—
U. bullata	MP 2363	*Bromus diandrus*	AY344998[8]	AF453935[8]	—
U. cynodontis	MP 1838 (XAL)	*Cynodon dactylon*	AY345000[1]	AF009881[1]	—
U. davisii	HUV 19252	*Glyceria multiflora*	AY740169[1]	DQ875374[1]	—
U. hordei	UMa 699	*Hordeum vulgare*	—	JN367329[2]	JN367357[2]
U. hordei	Ust. Exs. 784	*Hordeum vulgare*	AY345003[8]	—	—
U. striiformis	HUV 18286	*Alopecurus pratensis*	AY740172[1]	DQ875375[1]	JN367359[1]
U. trichophora	MP 1898 (XAL)	*Echinochloa colona*	AY740023[1]	AJ236141[1]	—
U. tritici	AFTOL-ID 1398	—	DQ846894	DQ094784	DQ846895
Websdanea lyginiae	HUV 17900	*Lyginia barbata*	—	AJ236159[9]	—
W. lyginiae	56539 (M)	*Lyginia barbata*	DQ875357[1]	—	—

Note. The accession numbers marked in bold face refer to sequences new in this study.

[1]Begerow et al. (2006). [2]Kellner et al. (2011). [3]Diagne-Leyev et al. (2010). [4]Stoll et al. (2005). [5]Zhang et al. (2010). [6]Begerow et al. (2000). [7]Matheny et al. (2007). [8]Stoll et al. (2003). [9]Piepenbring et al. (1999). [10]Piepenbring et al. (2002).

Phylogenetic analyses

The sequences included in this study (TABLE 1) were aligned online with MAFFT (mafft.cbrc.jp/alignment/server/index.html) (Katoh and Toh 2008) using the L-INS-i method, and observed in MEGA 5 (Tamura et al. 2011). The nucleotide diversity (π) and its standard deviation were estimated for ITS in DNasp 5 (Rozas et al.).

Phylogenetic analyses were completed to resolve both the familial placement of *Macalpinomyces*, as well as the delimitation of species in *Macalpinomyces*. The relationships between *Macalpinomyces* and other genera in the *Ustilaginaceae* were inferred from a phylogenetic tree based on the ITS, 28S, and 18S data sets. The final data set comprised sequences from 18 specimens of *M. eriachnes* and 40 reference specimens (GenBank accessions). The final matrix contained 4484 characters and was deposited in TreeBASE (http://www.treebase.org) as S19698. The concatenated ITS and 28S data sets were utilized in the phylogenetic analyses of species of *Macalpinomyces*. The final matrix was deposited in TreeBASE as S19696.

Phylogenetic analyses were based on both maximum likelihood (ML) and Bayesian inference (BI). ML was implemented as a search criterion in RAxML (Stamatakis 2014) and PhyML 3.0 (Guindon et al. 2010). GTRGAMMA was specified as the model of evolution in both programs. The RAxML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1000 ML bootstrap replicates. The PhyML analyses were implemented with the ATGC bioinformatics platform (http://www.atgcmontpellier.fr/phyml/), with six substitution types and subtree pruning and regrafting (SPR) tree improvement, and support obtained from an approximate likelihood-ratio test (Anisimova et al. 2011).

BI was performed with MrBayes 3.1.2. (Huelsenbeck and Ronquist) with Markov chain Monte Carlo analyses that incorporated four runs, each consisting of four chains, until the standard deviation of split frequencies reached 0.01. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 50 generations, and trees were saved every 5000 generations; 26 779 trees were summarized for the final topology. A user-defined tree obtained from PhyML analyses was used as a starting point for all of the Bayesian analyses, which helped to improve convergence of the four runs.

Coalescent-based species delimitation

Generalized mixed Yule-coalescent (GMYC) model

Three data sets (i.e., ITS, 28S, and ITS+28S) were analyzed under the single-threshold model and multiple-threshold model. The GMYC uses ultrametric trees constructed by unique haplotypes, and duplicate haplotypes were deleted by Arlequin 3.1 (Excoffier et al. 2005). The ultrametric trees were generated by Beast 1.7.5 by the same methods indicated by Millanes et al. (2014). The selected topologies were used to optimize the single-threshold and multiple-threshold GMYC models online (http://species.h-its.org/gmyc/).

Poisson tree processes (PTP) model

A RAxML tree constructed from the best markers selected by GMYC analysis was used for the PTP analysis. The analysis was run on the Web server for PTP (http://species.h-its.org/ptp/) and 10 000 MCMC generations with a thinning of 100 and burn-in of 0.2 (Zhang et al.).

Results

Phylogenetic analyses of Macalpinomyces

The GenBank accession numbers of new sequences derived from this study, along with reference sequences, are showed in the TABLE 1. A summary of the polymorphism and diversity of the ITS, 28S, and 18S gene regions between species of *Macalpinomyces* on *Eriachne* is presented in TABLE 2. 18S had the lowest overall nucleotide diversity (π = 0.00029) and ITS the highest (π = 0.034). The number of polymorphic (segregating) sites of ITS, 28S, and 18S were 87, 19, and 2, respectively.

Table 2. Global polymorphism of the nucleotide alignments of *M. eriachnes* sequences for the three genomic regions analyzed.

Taxon	Locus	n	bp	S	h_d	π
All specimens	ITS	29	878	87	0.938	0.034
	28S	29	1802	19	0.91	0.0036
	18S	29	1478	2	0.275	0.00029

Note. n = sample size; bp = total number of sites; S = number of segregating sites; h_d = haplotypic (allelic) diversity; π = average number of differences per site.

The phylogenetic relationships of specimens of *Macalpinomyces* on *Eriachne* spp. had identical topologies from analyses in PhyML, RAxML, and MrBayes. The topology of the RAxML tree is shown in FIG. 1. All specimens of *Macalpinomyces* on *Eriachne* spp. formed a well-supported monophyletic clade in the *Ustilaginaceae* (Begerow et al. 2006; Wang et al. 2015).

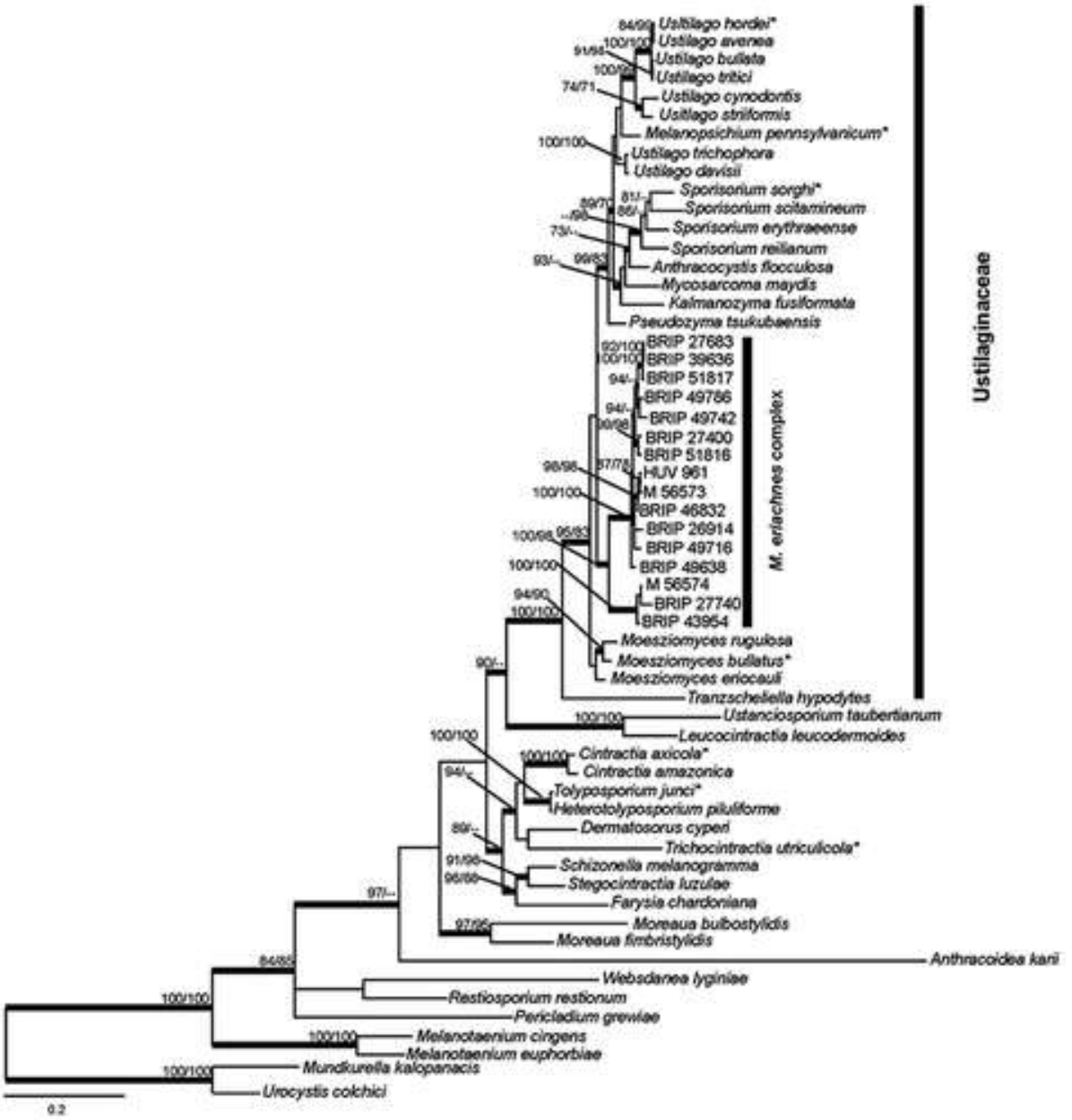

Figure 1. Phylogram obtained from a ML analysis based on the ITS, 28S, and 18S sequence alignment. Values above the branches represent ML bootstrap values (>75%) from RAxML and PhyML analyses, respectively. Thickened branches represent posterior probabilities (>0.95) from BI. The scale bar indicates 0.2 substitutions per site. The type specimens are indicated with an *.

Species delimitation for Macalpinomyces on Eriachne spp

The 18S region had low nucleotide diversity and provided less useful information for systematic analyses at the species level. The phylogenetic trees generated from the concatenated ITS and 28S data set with ML and BI were similar in topology (FIG. 2). The phylogenetic analyses recovered 11 well-supported clades (in FIG. 2).

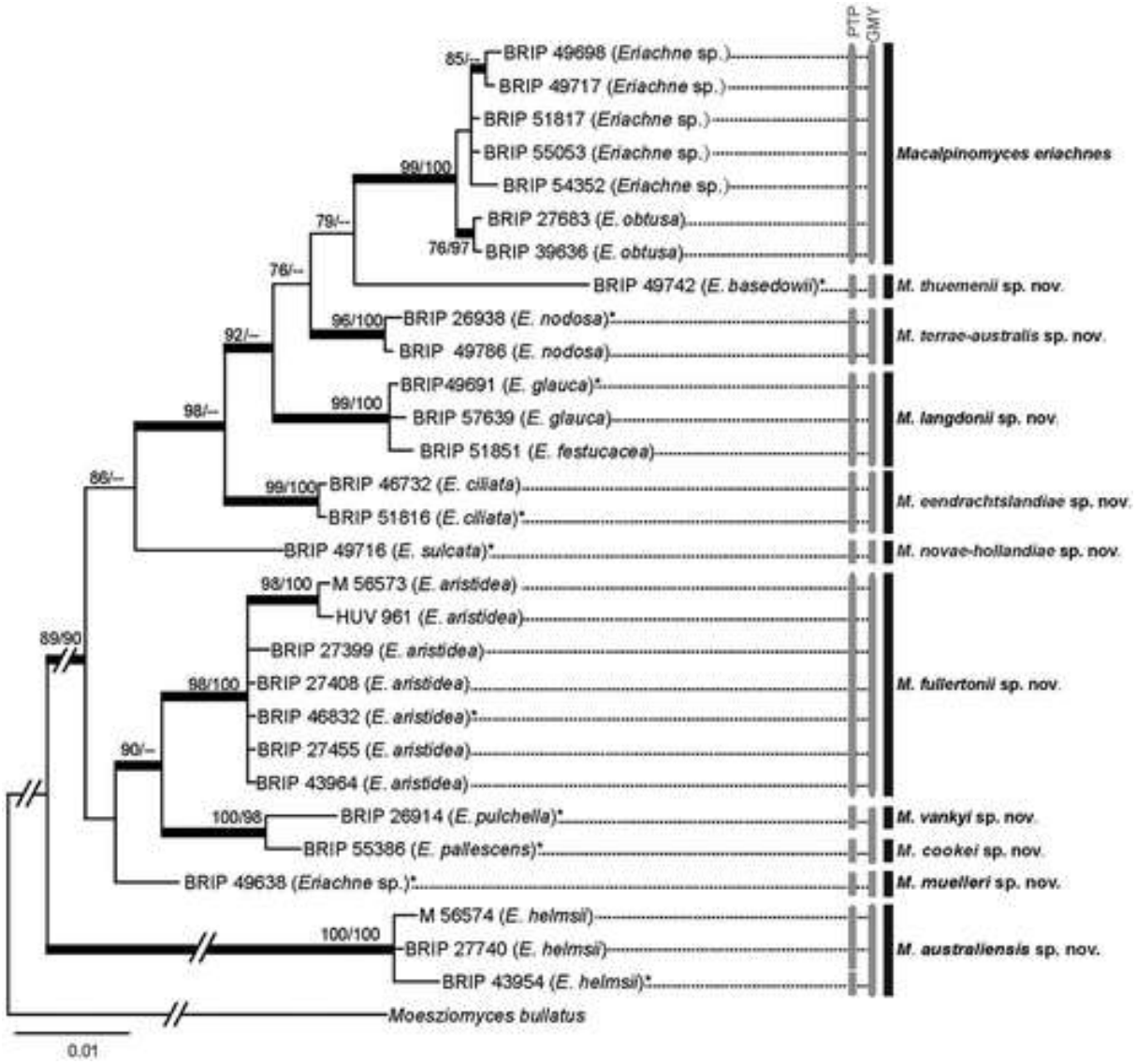

Figure 2. Phylogram obtained from a ML analysis based on the ITS+28S sequence alignment. Values above the branches represent ML bootstrap values (>75%) from RAxML and PhyML analyses, respectively. Thickened branches represent posterior probabilities (>0.95) from BI. The scale bar indicates 0.01 substitutions per site. The type specimens are indicated with an *. Results of the single-threshold and multiple-threshold GMYC analyses and PTP analysis by using combined ITS and 28S data are shown.

For the GMYC and PTP analyses, 29, 24, and 12 haplotypes of ITS, 28S, and 18S, respectively, were included. For the ITS or 28S data sets, both the single-threshold and multiple-threshold GMYC models of independent data sets accepted the null model (TABLE 4). For the ITS+28S data set, the single-threshold and multiple-threshold GMYC models provided a better fit to the ultrametric tree than a null model of uniform coalescent branching across the entire tree (single-threshold: likelihood ratio [LR] = 7.9, $P < 0.05$; multiple-threshold: LR = 8.9, $P < 0.05$), which supported the delimitation of taxa into 10 putative species (FIG. 2). The ITS+28S data set was used for the PTP analyses, and 12 putative species were inferred from specimens of *Macalpinomyces* on species of *Eriachne* (FIG. 2) on the basis of the best-fit ML tree and BI topology. The species delimitation based on PTP and GMYC methods were mostly congruent, with the exception of three putative species. The GMYC analyses did not support the specimens on *E. pallescens* (BRIP 55386) and *E. pulchella* (BRIP 26914) as independent entities. The morphological differences of spores and sterile cells between these two specimens (FIG. 2), together with their host ranges, supported their treatment as novel species. Three specimens on *E. helmsii* were treated as one species, although two (BRIP 27740 and BRIP

43954) split into two subclades in the PTP analysis (FIG. 2). However, there was no corresponding support in morphology, host affiliation, or GMYC analysis. In summary, all specimens of *Macalpinomyces* on *Eriachne* were resolved as 11 species by the phylogenetic analyses, GMYC and PTP, in conjunction with morphological characters and host affiliation. The pairwise identity of ITS sequences for each of these proposed new species is showed in the TABLE 3.

Table 3. Pairwise identity (%) of ITS sequences of type specimens.

	BRIP 26938	**BRIP 49691**	**BRIP 49742**	**BRIP 51816**	**BRIP 49716**	**BRIP 46832**	**BRIP 49638**	**BRIP 26914**	**BRIP 55386**	**BRIP 43954**
BRIP 51817	98	97	97	98	93	96	96	96	96	86
BRIP 26938		97	98	98	93	96	96	96	96	87
BRIP 49691			96	97	94	94	95	96	95	90
BRIP 49742				98	93	96	96	96	95	87
BRIP 51816					94	97	97	96	96	88
BRIP 49716						95	95	93	94	88
BRIP 46832							97	96	97	88
BRIP 49638								97	97	88
BRIP 26914									97	88
BRIP 55386										88

Table 4. Summary of the results of the GMYC analyses.

Analysis	**ITS+28S (24 haplotypes)**	**ITS (29 haplotypes)**	**28S (12 haplotypes)**
Single threshold			
Likelihood of null model	147.8573	190.3219	54.29365
Maximum likelihood of GMYC model	151.8272	191.6869	55.63607
Likelihood ratio	7.93985	2.730056	2.684842
Result of LR test	**0.01887484***	0.2553735	0.2612125
Number of ML clusters (confidence interval)	7 (3–7)	7 (1–9)	3 (1–4)
Number of ML entities (confidence interval)	**10 (4–15)**	12 (1–28)	3 (1–11)
Threshold time	−0.002614121	−0.002214422	−0.01806288
Multiple threshold			
Likelihood of null model	147.8573	190.3219	54.29365
Maximum likelihood of GMYC model	152.3128	192.2428	56.74379
Likelihood ratio	8.91101	3.841984	4.900275
Result of LR test	**0.01161445***	0.1464616	0.08628174
Number of ML clusters (confidence interval)	8 (3–8)	6 (1–7)	3 (3–3)
Number of ML entities (confidence interval)	**10 (4–14)**	10 (1–14)	4 (3–4)
Threshold time	−0.008496762	−0.01036862	−0.01806288
	−0.0001645725	−0.0003076432	−0.002297918

Taxonomy

Macalpinomyces Langdon & Fullerton, Trans Br Mycol Soc 68:30. 1977, **emend. Y.M. Li, McTaggart & R.G. Shivas**

Sori in all of the ovaries of species of *Eriachne*, covered by a peridium of host tissue permeated by hyphae, without a columella. Spores brown to opaque, subpolyhedrally to polyhedrally irregular, smooth or rugulose. **Sterile cells mostly larger than the spores and thick-walled**, often laminate, subhyaline to pale brown, smooth.

Type species: Macalpinomyces eriachnes (Thüm.) Langdon & Fullerton, Trans Br Mycol Soc 68:30. 1977.

Notes: Species of *Macalpinomyces* cause systemic infection, producing sori that occupy all of the ovaries in an inflorescence. This character distinguishes *Macalpinomyces* from three species of *Tilletia*, which replace some individual ovaries of infected inflorescences of *Eriachne* (Li et al. 2014). The floral parts (glumes, lemma, palea) of plants infected by *Macalpinomyces* remain mostly intact.

Macalpinomyces australiensis Y.M. Li, R.G. Shivas, McTaggart & L. Cai, sp. nov. FIG. 3a–d

FungalName FN570376

Typification: **AUSTRALIA**. QUEENSLAND: Cunnamulla, on *Eriachne helmsii*, 17 Mar 2004, D.R. Beasley, T.S. Marney & R.G. Shivas, BRIP 43954 (**holotype)**.

Etymology: From Australia, the name of country and continent from where the fungus was found.

Sori in all of the ovaries of ***Eriachne helmsii***. Spores 12–15(–18) × (8–)9.5–12(–13) µm; wall ca. 1 µm thick. Sterile cells (20–)21–34(–48) × (16–)17.5–29(–40) µm; wall 3–4 µm thick, laminate, contents granular.

Geographic distribution and host range: Known only from Australia on *Eriachne helmsii*.

Other specimens examined: **AUSTRALIA**. NORTHERN TERRITORY: Alice Springs, on *E. helmsii*, 27 Mar 2000, C. Vánky & K. Vánky, BRIP 27740.

Notes: Macalpinomyces australiensis is sister to all other known species of *Macalpinomyces* on *Eriachne* (FIG. 2). *Macalpinomyces australiensis* has significantly lager sterile cells than *M. eriachnes* (19–23 × 18.5–22 µm) (TABLE 5).

Macalpinomyces cookei Y.M. Li, R.G. Shivas, McTaggart & L. Cai, sp. nov. FIG. 3e–g

FungalName FN570374

Etymology: The name honors Mordecai Cubitt Cooke (1825–1914), an eminent English botanist and mycologist, who described *Ustilago australis* in 1879 from specimens sent to him by Ferdinand von Mueller.

Typification: **AUSTRALIA**. QUEENSLAND: Cooloola, on *E. pallescens*, 6 Mar 2012, *A.R. McTaggart*, BRIP 55386 (**holotype**).

Sori in all of the ovaries of ***Eriachne pallescens***. Spores (11–)12–14.5(–16) × (7–)8.5–11(–13) µm; wall ca. 1 µm thick. Sterile cells (16–)17–22(–26) × (10–)14.5–21(–25) µm; wall 3.5–5 µm thick, laminate.

Note: Macalpinomyces cookei is known from a single specimen, which differs molecularly from *M. eriachnes* (96% identity in ITS) and *M. australiensis* (88% identity in ITS).

Macalpinomyces eendrachtslandiae Y.M. Li, R.G. Shivas, McTaggart & L. Cai, sp. nov. FIG. 4i–l

FungalName FN570372

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: Between Wyndham and Kununurra, on *Eriachne ciliata*, 8 Apr 2008, A.R. McTaggart, V.L. Challinor, A.D.W Geering, M.D.E Shivas & R.G. Shivas, BRIP 51816 (**holotype**).

Etymology: Taken from the Dutch word Eendrachtsland, which was one of the earliest names for Australia given in 1616 by the Dutch explorer Dirk Hartog, who was the first European to sight Western Australia.

Sori in all of the ovaries of ***Eriachne ciliata***. Spores (8–)8.5–10.5(–11) × (6–)6.5–8 µm; wall ca. 1 µm thick. Sterile cells 20–25(–30) × (15–)17–23(–28) µm; wall 3–3.5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne ciliata*.

Other specimens examined: AUSTRALIA. WESTERN AUSTRALIA: Wyndham, Five Rivers Lookout, on *Eriachne ciliata*, 6 May 2005, D.R. Beasley, T.S. Marney & R.G. Shivas, BRIP 46732.

Notes: Macalpinomyces eendrachtslandiae is closely related to *M. eriachnes* and to *M. thuemenii* (98% identity in ITS), which is also described in this study. However, *M. eendrachtslandiae* has smaller spores than both *M. eriachnes* and *M. thuemenii* (TABLE 5).

Macalpinomyces eriachnes (Thüm.) Langdon & Fullerton, Trans Br Mycol Soc 68:30. 1977, **emend. Y.M. Li, McTaggart & R.G. Shivas**

≡ *Sorosporium eriachnes* Thümen (as '*eriachnis*'), Flora 61:443. 1878.

≡ *Ustilago australis* Cooke, Grevillea 8:34. 1879.

Sori in **all of the ovaries of *Eriachne obtusa***, covered by a peridium of host tissue permeated by hyphae, without a columella. Spores brown to opaque, subpolyhedrally to polyhedrally irregular, (11–)13–15.5(–16) × (7–)8.5–11 µm, smooth in profile. Sterile cells globose, subglobose or ellipsoidal, (18–)19–23(–26) × (17–)18.5–22(–26) µm; wall 3–7 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne obtusa* and unidentified species of *Eriachne*.

Specimens examined: **AUSTRALIA**. NORTHERN TERRITORY: Fitzmaurice River, on *Eriachne* sp. (= *E. obtusa* det. B.K. Simon from image of holotype), Oct 1855, coll. F.J. Müller, K(M) 146202/3 (**holotype** of *U. australis*), VPRI 2957 (**isotype** of *U. australis*); Katherine, on *E. obtusa*, 14 Mar 2000, R.G. Shivas, I.T. Riley, C. Vánky & K. Vánky, BRIP 27683; Katherine, on *E. obtusa*, 14 Mar 2000, R.G. Shivas, I.T. Riley, C. Vánky & K. Vánky, BRIP 39636. WESTERN AUSTRALIA: Between Fitzroy Crossing and Halls Creek, on *Eriachne* sp., 10 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J.

Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49698; Derby, on *Eriachne* sp., 9 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J. Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49717; between Wyndham and Kununurra, on *Eriachne* sp., 8 Apr 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. Shivas & R.G. Shivas, BRIP 51817; Kununurra, Weaber Plain Road, on *Eriachne* sp., 20 Apr 2011, S.M. Thompson & M.J. Ryley, BRIP 55053; 30.5 km south-southwest of new Theda Homestead, 22 Aug 2010, M.D. Barrett & R.L. Barrett, on *Eriachne* sp., BRIP 54352.

Notes: Cooke (1879) described *Ustilago australis*, unaware that *Sorosporium eriachnes* had been described the previous year by Thümen (McAlpine 1910). This was a consequence of Ferdinand von Mueller sending duplicate specimens to both Thümen and Cooke (Langdon and Fullerton 1977). According to Dr. Kálmán Vánky (pers. comm.), Thümen's specimen in BUC is rather meager, whereas Cooke's specimen in K is rich.

The identity of the host for the type of *Ustilago australis* (K(M) 146202/3) was given as *Eriachne* sp. by Langdon and Fullerton (1977). However, Vánky (2011) identified the host as *E. festucacea* on the basis that a healthy specimen (MEL 92576) collected by Müller at the same time and location represented the host species. However, the late Dr. Bryan Simon identified K(M) 146202/3 as *E. obtusa* from a high resolution image made available by K. This is not surprising because several species of *Eriachne* occur in the region of northwestern Australia, where these specimens were collected.

Macalpinomyces fullertonii Y.M. Li, R.G. Shivas, McTaggart & L. Cai, **sp. nov.** FIG. 4a–d

FungalName FN570373

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: Onslow, on *Eriachne aristidea*, 10 Aug 2005, M.J. Ryley, T.S. Marney & R.G. Shivas, BRIP 46832 (**holotype**).

Etymology: The name honours Dr. Robert (Bob) Alexander Fullerton, an Australian–New Zealand plant pathologist and mycologist, whose PhD studies led to the establishment of *Macalpinomyces* with R.F.N. Langdon.

Sori in all of the ovaries of ***Eriachne aristidea***. Spores (10–)10.5–13(–14) × (8–)9–10 µm; wall ca. 1 µm thick. Sterile cells (26–)28–38(–40) × (20–)24–34(–37) µm; wall 4.5–6 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne aristidea*.

Other specimens examined: AUSTRALIA. NORTHERN TERRITORY: Alice Springs, on *E. aristidea*, 26 Mar 2000, C. Vánky & K. Vánky, BRIP 27399. WESTERN AUSTRALIA: Halls Creek, on *E. aristidea*, 28 Jun 1998, A.A. Mitchell, BRIP 27408. QUEENSLAND: Cunnamulla, on *E. aristidea*, 12 Mar 1998, C. Vánky & K. Vánky, BRIP 27455; Cunnamulla, on *E. aristidea*, 16 Mar 2004, D.R. Beasley, T.S. Marney & R.G. Shivas, BRIP 43964.

Notes: Seven specimens of *M. fullertonii* were resolved in a well-supported clade. The ITS identity between the specimens of *M. fullertonii* (FIG. 2) was 99–100%. *Macalpinomyces fullertonii* is the only smut fungus reported on *E. aristidea*.

Macalpinomyces langdonii Y.M. Li, R.G. Shivas, McTaggart & L. Cai, sp. nov. FIG. 5a–d

FungalName FN570368

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: 90 km southwest of Kununurra, on *Eriachne glauca*, 11 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J. Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49691 (**holotype**).

Etymology: The name honors the Australian mycologist and plant pathologist Raymond Forbes Langdon (1916–2014), who established *Macalpinomyces* with R.A. Fullerton.

Sori in all of the ovaries of ***Eriachne glauca*** and ***E. festucacea***. Spores (9–)9.5–11(–12) × (7–)7.5–9.5(–11) µm; wall ca. 1 µm thick. Sterile cells (19–)20–26(–33) × (16–)19–24(–30) µm; wall 3–4 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne glauca* and *E. festucacea.*

Other specimens examined: AUSTRALIA. WESTERN AUSTRALIA: Roadside creek between Lake Argyle and Kununurra, on *E. festucacea*, 9 Apr 2008, C. Vánky & K. Vánky, BRIP 51851. NORTHERN TERRITORY: Baines, Victoria Highway, on *E. glauca*, 19 Apr 2012, A.R. McTaggart & R.G. Shivas, BRIP 57639.

Notes: Macalpinomyces langdonii is the only smut fungus known to infect *E. glauca*. Together with *Tilletia geeringii*, it is the second smut fungus found on *E. festucacea* (Li et al. 2014).

Macalpinomyces muelleri Y.M. Li, R.G. Shivas, McTaggart & L. Cai, **sp. nov**. FIG. 3k–n

FungalName FN570366

Typification: **AUSTRALIA**. NORTHERN TERRITORY: Tennant Creek, on *Eriachne* sp., 25 Apr 2007, A.R. McTaggart, R.G. Shivas & J.R. Liberato, BRIP 49638 (**holotype**).

Etymology: The name honors Baron Ferdinand von Mueller (1825–1896), a German-Australian botanist, who first collected specimens of smut fungus on *Eriachne*.

Sori in all of the ovaries of ***Eriachne* sp**. Spores (10–)10. 5–12.5(–13) × (8–)8.5–10 µm; wall ca. 1 µm thick. Sterile cells (23–)25–40(–50) × (20–)21–32(–42) µm; wall 3–5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne* sp.

Notes: The pairwise ITS identity of *M. muelleri* and *M. vankyi* is 97%. However, *M. muelleri* has larger sterile cells than *M. vankyi* (20–27 × 19.5–25 µm).

Macalpinomyces novae-hollandiae Y.M. Li, R.G. Shivas, McTaggart & L. Cai, **sp. nov.** FIG. 4e–f

FungalName FN570367

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: Fitzroy Crossing from Derby, on *Eriachne sulcata*, 9 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J. Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49716 (**holotype**).

Etymology: Taken from New Holland, which was the first European name applied to Australia in 1644 by the Dutch explorer Abel Tasman.

Sori in all of the ovaries of ***Eriachne sulcata***. Spores (8–)9–10.5(–11) × (6–)7–8.5(–10) µm; wall ca. 1 µm thick. Sterile cells (16–)16.5–21(–24) × (14–)14.5–19(–21) µm; wall 3–4.5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne sulcata*.

Notes: Macalpinomyces novae-hollandiae is most closely related to *M. fullertonii* and *M. muelleri* (97% identity in ITS). *Macalpinomyces novae-hollandiae* is the only smut fungus known on *Eriachne sulcata*.

Macalpinomyces terrae-australis Y.M. Li, R.G. Shivas, McTaggart & L. Cai, **sp. nov.** FIG. 5e–h

FungalName FN570364

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: 57 km west of Kununurra, on *Eriachne nodosa*, 31 Mar 2000, C. Vánky & K. Vánky, BRIP 26938 (**holotype**).

Etymology: Taken from *Terra Australis*, which is an early Latin name for a hypothetical continent in the Southern Hemisphere and the name from which Australia was coined by British explorer Matthew Flinders in the early 1800s.

Sori in all of the ovaries of ***Eriachne nodosa***. Spores (8–)9–11(–12) × (7–)7.5–9(–10) µm; wall ca. 1 µm thick. Sterile cells (15–)18–23(–25) × (16–)17–21(–22) µm; wall 3–3.5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne nodosa.*

Specimens examined: AUSTRALIA. WESTERN AUSTRALIA: Between Wyndham and Kununurra, on *E. nodosa*, 13 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J. Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49786.

Notes: Macalpinomyces terrae-australis was resolved as sister to *M. thuemenii* (98% identity in ITS) and *M. eriachnes* (98% identity in ITS). *Macalpinomyces terrae-australis* has smaller spores than both *M. eriachnes* and *M. thuemenii* (TABLE 5).

Macalpinomyces thuemenii Y.M. Li, R. Shivas, McTaggart & L. Cai, **sp. nov.** FIG. 4m–p

FungalName FN570365

Typification: **AUSTRALIA**. WESTERN AUSTRALIA: 10 km east of Kununurra, on *Eriachne basedowii*, 12 Apr 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J. Shivas, G.F. Shivas, M.D.E. Shivas & R.G. Shivas, BRIP 49742 (**holotype**).

Etymology: The name honours Felix von Thümen (1839–1892), a German botanist and mycologist, who described *Sorosporium eriachnes* (as '*eriachnis*') in 1878 from specimens sent to him by Ferdinand von Mueller.

Sori in all of the ovaries of ***Eriachne basedowii****. Spores* (12–)13.5–16.5(–18) × 10–11.5(–12) µm; wall ca. 1 µm thick. Sterile cells (17–)20–27(–30) × (14–)16.5–24(–26) µm; wall 3–4.5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne basedowii*.

Note: Macalpinomyces thuemenii has the largest spores of all species of *Macalpinomyces* on *Eriachne* (TABLE 5).

Macalpinomyces vankyi Y.M. Li, R.G. Shivas, McTaggart & L. Cai, **sp. nov.** FIG. 3h–j

FungalName FN570363

Typification: **AUSTRALIA**. NORTHERN TERR-ITORY: Alice Springs, on *Eriachne pulchella* subsp. *dominii*, 14 Mar 2000, R.G. Shivas, I.T. Riley, C. Vánky & K. Vánky, BRIP 26914 (**holotype**).

Etymology: The name honors the Hungarian mycologist Dr. Kálmán Vánky, whose taxonomic studies over decades underpin most contemporary work on smut fungi.

Sori in all of the ovaries of ***Eriachne pulchella*** subsp. ***dominii***. Spores (10–)11–14(–15) × (8–)8.5–10(–11) µm; wall ca. 1 µm thick. Sterile cells (20–)20–27(–30) × (18–)19.5–25(–30) µm; wall 3.5–5 µm thick, laminate.

Geographic distribution and host range: Known only from Australia on *Eriachne pulchella* subsp. *dominii.*

Notes: Two species of smut fungi, *M. vankyi* and *Tilletia marjaniae*, are known to infect *E. pulchella* (Li et al. 2014). Phylogenetic analysis shows that *M. vankyi* is closely related to *M. cookei* (97% identity in ITS). However, *M. vankyi* has larger sterile cells than *M. cookei* (17–22 × 14.5–21 µm).

Table 5. Morphological characteristics of species of *Macalpinomyces* on *Eriachne*.

Species	Spores (µm)	Sterile cells (µm)	Wall thickness of sterile cells (µm)	Host
Macalpinomyces australiensis	12–15 × 9.5–12	21–34 × 17.5–29	3–4	*Eriachne helmsii*
M. cookei	12–14.5 × 8.5–11	17–22 × 14.5–21	3.5–5	*E. pallescens*
M. eendrachtslandiae	8.5–10.5 × 6.5–8	20–25 × 17–23	3–3.5	*E. ciliata*
M. eriachnes	13–15.5 × 8.5–11	19–23 × 18.5–22	3–5	*E. obtusa*, *Eriachne* sp.
M. fullertonii	10.5–13 × 9–10	28–38 × 24–34	4.5–6	*E. aristidea*
M. langdonii	9.5–11 × 7.5–9.5	20–26 × 19–24	3–4	*E. glauca*, *E. festucacea*
M. muelleri	10.5–12.5 × 8.5–10	25–40 × 21–33	3–5	*Eriachne* sp.
M. novae-hollandiae	9–10.5 × 7–8.5	16.5–21 × 14.5–19	3–4.5	*E. sulcata*
M. terrae-australis	9–11 × 7.5–9	18–23 × 17–21	3–3.5	*E. nodosa*
M. thuemenii	13.5–16.5 × 10–11.5	20–27 × 16.5–24	3–4.5	*E. basedowii*
M. vankyi	11–14 × 8.5–10	20–27 × 19.5–25	3.5–5	*E. pulchella* subsp. *dominii*

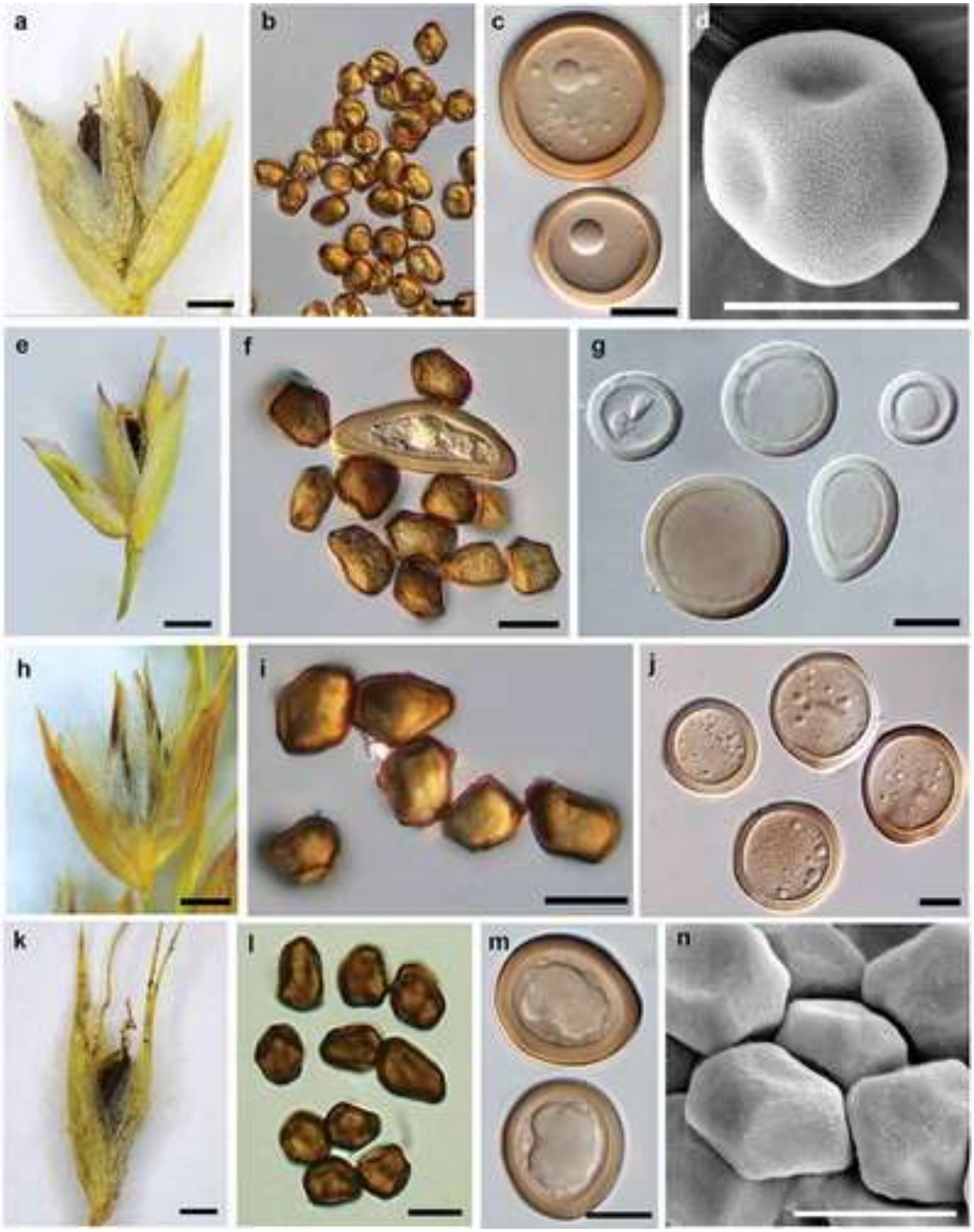

Figure 3. *Macalpinomyces australiensis* (BRIP 43954) (a–d), *M. cookei* (BRIP 55386) (e–g), *M. vankyi* (BRIP 26914) (h–j), and *M. muelleri* (BRIP 49638) (k–n). a, e, h, k. Sori. b, g, f, i, l. Spores under microscope. c, g, j, m. Sterile cells under microscope. d, n. Spores under the SEM. Bars: a, e, h, k = 1 mm; b, f, i, l, d, n = 10 µm.

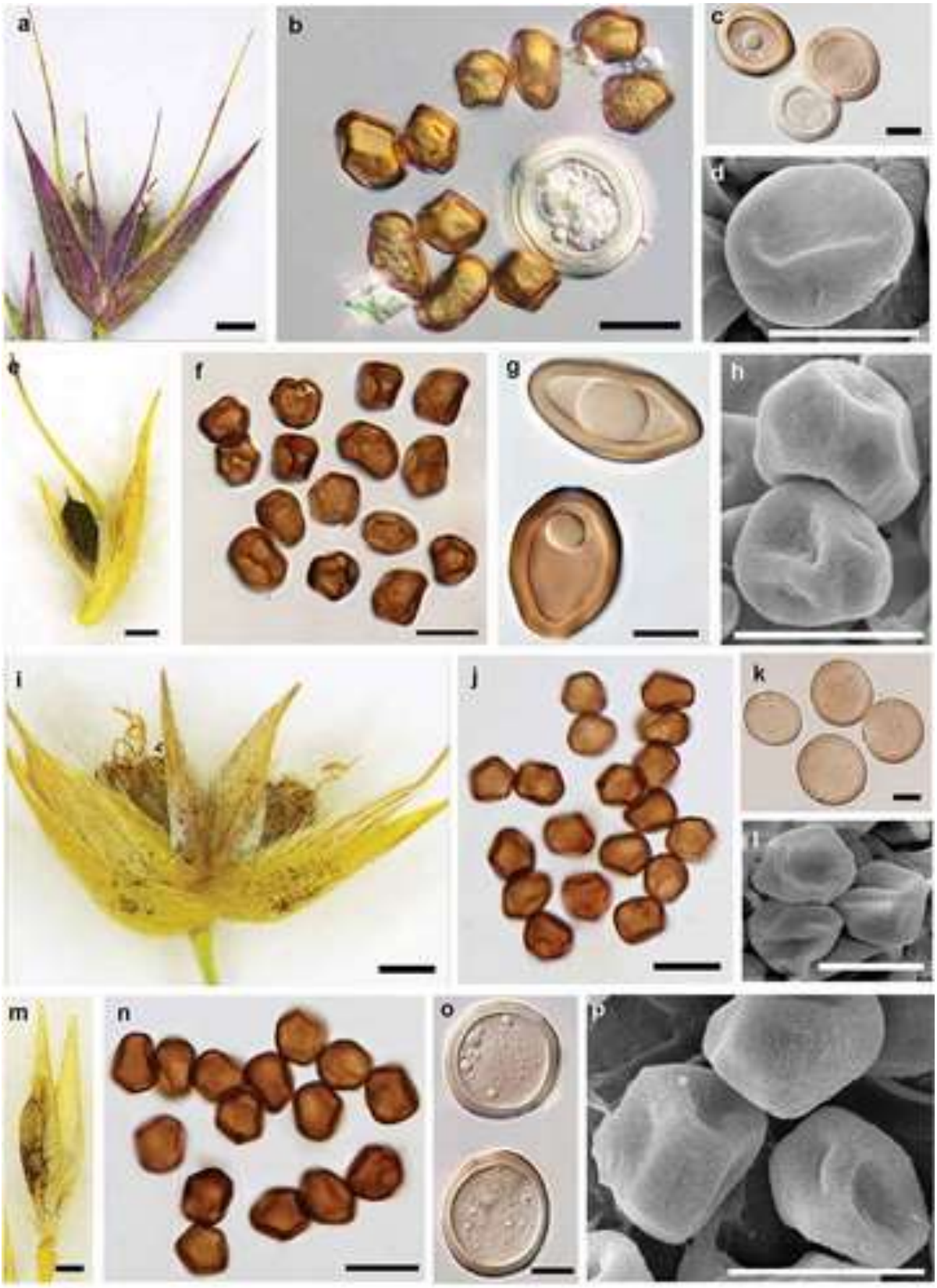

Figure 4. *Macalpinomyces fullertonii* (BRIP 46832) (a–d), *M. novae-hollandiae* (BRIP 49716) (e–h), *M. eendrachtslandiae* (BRIP 51816) (i–l), and *M. thuemenii* (BRIP 49742) (m–p). a, e, i, m. Sori. b, f, j, n. Spores under microscope. c, g, k, o. Sterile cells under microscope. d, h, l, p. Spores under the SEM. Bars: a, e, i, m = 1 mm; b, f, j, n, c, g, k, o, d, h, l, p = 10 µm.

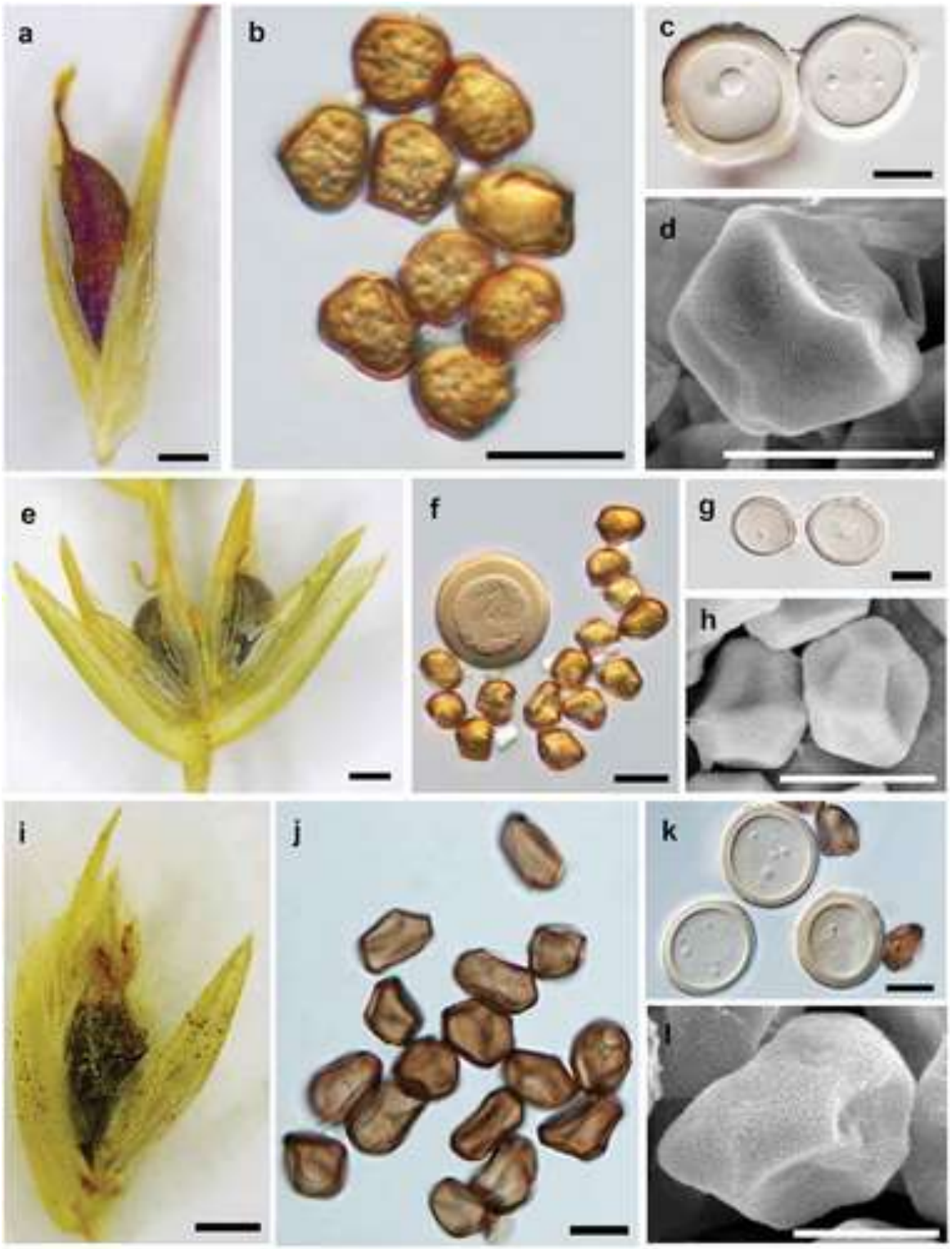

Figure 5. *Macalpinomyces langdonii* (BRIP 51851) (a–d) and *M. terrae-australis* (BRIP 26938) (e–f). a, e. Sori. b, f. Spores under microscope. c, g. Sterile cells under microscope. d, h. Spores under the SEM. Bars: a, e = 1 mm; b, f, c, g, d, h = 10 µm.

Discussion

The present study found that species of *Eriachne* in northern Australia harbored a diversity of species of *Macalpinomyces*. Until this study, all species of *Macalpinomyces* on *Eriachne* had been identified and reported in the literature as *M. eriachnes* (Vánky and Shivas 2008; Vánky 2011). Although the sizes of spores for most species of *Macalpinomyces* on *Eriachne* overlapped, some species were distinguishable, for example, *M. eendrachtslandiae* has the smallest spores of known species and *M. thuemenii* has the largest spores. Generally, the size of sterile cells was not diagnostic. Similarly, spore ornamentation did not distinguish species, either under light microscopy or SEM (FIGS. 3, 4, and 5).

Host affiliation has been used for the delimitation of species of smut fungi (Begerow et al. 2006, 2014), especially when supported by phylogenetic and biological studies (Cai et al. 2011; McTaggart et al. 2012a, 2012b). In this study, 11 host specific species of *Macalpinomyces* were identified on *Eriachne*. Further, *M. eriachnes* s. str. was only found on *E. obtusa* and unidentified *Eriachne* spp. It is highly likely that additional species of *Macalpinomyces* remain to be discovered on *Eriachne*, as only 13 of the 22 species of *Eriachne* that are known hosts of *Macalpinomyces* were included in this study. Our results also showed that ITS sequences provided good resolution of species of *Macalpinomyces* on *Eriachne. Macalpinomyces australiensis* was sister to all other species of *Macalpinomyces* on *Eriachne* (FIG. 2), with a large molecular distance (ITS sequence identity 87–90%) (TABLE 3) that may indicate undiscovered intermediate species.

Eleven of the species of *Macalpinomyces* on *Eriachne* included in this study had overlapping geographic ranges in northern Australia (FIG. 2). We found that specimens from the same host species, but in different geographic regions, were genetically closer than specimens from the same geographic region, but on different host species. This highlighted the importance of host adaptation in the evolutionary process of this host-pathogen association. Begerow et al. (2004) suggested that host shift was the likely explanation for the present distribution of the smut fungi on their hosts. A cophylogenetic analysis of the *Macalpinomyces-Eriachne* relationships will depend on further specimens.

Acknowledgments

The authors are grateful to Dr. Kálmán Vánky (HUV) for lending specimens and Dr. Begoña Aguirre-Hudson (K) for providing high-resolution scanned images of the type of *Ustilago australis* (K(M) 146202/3). Dr. Andrew Geering and Marjan Shivas are thanked for assistance in specimen collection. Dr. Yuan-Ying Su and Ms. Fang Liu are thanked for technical assistance.

Funding

This study was financially supported by the National Natural Science Foundation of China (NSFC 31670017), China. Y.M. Li acknowledges the external Cooperation Program of the Chinese Academy of Sciences (GJHZ1310) for supporting her visit to Australia.

References

Anisimova M, Gil M, Dufayard JF, Dessimoz C, Gascuel O. 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Systematic Biology 60:685–699.

Begerow D, Bauer R, Boekhout T. 2000. Phylogenetic placements of Ustilaginomycetes anamorphs as deduced from nuclear LSU rDNA sequences. Mycological Research 104:53–60.

Begerow D, Göker M, Lutz M, Stoll M. 2004. On the evolution of smut fungi on their hosts. In: Agerer R, Piepenbring M, Blanz P, eds. Fronteirs in Basidiomycote mycology. Eching, Germany: IHW-Verlag. p. 81–98.

Begerow D, Schafer AM, Kellner R, Yurkov A, Kemler M, Oberwinkler F, Bauer R. 2014 Ustilaginomycotina. In: McLaughlin DJ, Spatafora JW, eds. The mycota VII: systematics and evolution part A. 2nd ed. Berlin: Springer-Verlag. p. 295–329.

Begerow D, Stoll M, Bauer R. 2006. A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. Mycologia 98:906–916.

Cai L, Giraud T, Zhang N, Begerow D, Cai G, Shivas RG. 2011. The evolution of species concepts and species recognition criteria in plant pathogenic fungi. Fungal Diversity 50:121–133.

Cooke MC. 1879. Undescribed fungi in the Kew Herbarium. Grevillea 8:34–35.

Diagne-Leye G, Almaraz-Lopez T, Mascarell G, Ba AT, Roux C. 2010. Systematic position of *Moesziomyces penicillariae* among Ustilaginaceae. African Journal of Microbiology Research 4:740–747.

Excoffier L, Guillaume L, Schneider S. 2005. Arlequin, version 3.1: an integrated software package for population genetics data analysis. Berne, Switzerland: University of Berne.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59:307–321.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9:286–298.

Kellner R, Vollmeister E, Feldbrügge M, Begerow D. 2011. Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. PLoS Genetics 7:e1002436.

Langdon RFN, Fullerton RA. 1977. *Macalpinomyces*, a new genus of smut fungi. Transactions of the British Mycological Society 68:27–30.

Li YM, Shivas RG, Cai L. 2014. Three new species of *Tilletia* on *Eriachne* from north-western Australia. Mycoscience 55:361–366.

Matheny PB, Wanga Z, Bindera M, Curtisa JM, Limb YW, Nilssonc RH, Hughesd KW, Hofstettere V, Ammiratif JF, Schochg CL, Langerh E, Langerh G, McLaughlini DJ, Wilsona AW, Frøslevj T, Gek ZW, Kerriganl RW, Slota JC, Yang ZL, Baronim TJ, Fischern M, Hosakao K, Matsuurap K, Seidlq MT, Vaurasr

J, Hibbetta DS. 2007. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution 43:430–451.

McAlpine D. 1910. The smuts of Australia. Their structure, life history, treatment, and classification. Melbourne, Australia: Government Printer. 285 p.

McTaggart AR, Shivas RG, Boekhout T, Oberwinkler F, Vánky K, Pennycook SR, Begerow D. 2016. *Mycosarcoma* (*Ustilaginaceae*), a resurrected generic name for corn smut (*Ustilago maydis*) and its close relatives with hypertrophied, tubular sori. IMA fungus, 7:309–315.

McTaggart AR, Shivas RG, Geering AD, Callaghan B, Vánky K, Scharaschkin T. 2012a. Soral synapomorphies are significant for the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae). Persoonia 29:63–77.

McTaggart AR, Shivas RG, Geering AD, Vánky K, Scharaschkin T. 2012b. Taxonomic revision of *Ustilago, Sporisorium* and *Macalpinomyces*. Persoonia 29:116–132.

Millanes AM, Truong C, Westberg M, Diederich P, Wedin M. 2014. Host switching promotes diversity in host-specialized mycoparasitic fungi: uncoupled evolution in the *Biatoropsis-Usnea* system. Evolution 8:1576–1593.

Piepenbring M, Dominik B, Franz O. 1999. Molecular sequence data assess the value of morphological characteristics for a phylogenetic classification of species of *Cintractia*. Mycologia 91:485–498.

Piepenbring M, Stoll M, Oberwinkler F. 2002. The generic position of *Ustilago maydis, Ustilago scitaminea*, and *Ustilago esculenta* (Ustilaginales). Mycological Progress 1:71–80.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55:595–609.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

Shivas RG, Beasley DR, McTaggart AR. 2014. Online identification guides for Australian smut fungi (Ustilaginomycotina) and rust fungi (Pucciniales). IMA Fungus 5:195–202.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Stoll M, Begerow D, Oberwinkler F. 2005. Molecular phylogeny of *Ustilago, Sporisorium*, and related taxa based on combined analyses of rDNA sequences. Mycological Research 109:342–356.

Stoll M, Piepenbring M, Begerow D, Oberwinkler F. 2003. Molecular phylogeny of *Ustilago* and *Sporisorium* species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences. Canadian Journal of Botany 81:976–984.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731–2739.

Vánky K. 1996. The genus *Macalpinomyces* (Ustilaginales). Mycotaxon 59:115–126.

Vánky K. 1997. Taxonomical studies on Ustilaginales. XV. Mycotaxon 62:127–150.

Vánky K. 2011 ['2012']. Smut fungi of the world. St. Paul, Minnesota: APS Press. p. 302–303.

Vánky K, Shivas RG. 2008. Fungi of Australia: the smut fungi. Australian Biological Resources Study. Melbourne, Australia: Canberra & CSIRO Publishing. p. 54–55.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172:4238–4246.

Wang QM, Begerow D, Groenewald M, Liu XZ, Theelen B, Bai FY, Boekhout T. 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Studies in Mycology 81:55–83.

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

Zhang D, Spadaro D, Garibaldi A, Gullino ML. 2010. Selection and evaluation of new antagonists for their efficacy against postharvest brown rot of peaches. Postharvest Biology and Technology 55:174–181.

Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29:2869–2876.