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The PhyloCode applied to *Cintractiellales*, a new order of smut fungi with unresolved phylogenetic relationships in the *Ustilaginomycotina*

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Abstract: The PhyloCode is used to classify taxa based on their relation to a most recent common ancestor as recovered from a phylogenetic analysis. We examined the first specimen of *Cintractiella* (*Ustilaginomycotina*) collected from Australia and determined its systematic relationship to other *Fungi*. Three ribosomal DNA loci were analysed both with and without constraint to a phylogenomic hypothesis of the *Ustilaginomycotina*. *Cintractiella* did not share a most recent common ancestor with other orders of smut fungi. We used the PhyloCode to define the *Cintractiellales*, a monogeneric order with four species of *Cintractiella*, including *C. scirpodendri* sp. nov. on *Scirpodendron ghaeri*. The *Cintractiellales* may have shared a most recent common ancestor with the *Malasseziomycetes*, but are otherwise unresolved at the rank of class.

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INTRODUCTION

Higher taxonomic ranks of smut fungi and their asexual yeast-like morphs (*Ustilaginomycotina*, *Basidiomycota*) were first classified by patterns of teliospore germination (Tulasne & Tulasne 1847), later by ultrastructural characters (Bauer et al. 1997), and most recently by their evolutionary relationships based on genes (Begerow et al. 2006, Wang et al. 2015) and genomes (Kijpornyongpan et al. 2018). Confidence in the phylogenetic relationships and taxonomy of the *Ustilaginomycotina* has increased as genomes of more taxa are sequenced (Kijpornyongpan et al. 2018).

The smut fungi are evolutionary divergent and polyphyletic (Begerow et al. 2006, McTaggart et al. 2012). The sexual phenotype of smut fungi, when known, replaces the reproductive or other organs (leaves, roots) of their host plants with powdery masses of fungal spores. Phylogenetic studies have re-classified some traditional groups of smut fungi into higher taxonomic ranks outside of the *Ustilaginomycotina*, e.g. the *Entorrhizomycota* (*Entorrhizomycota*) (Aime et al. 2006, Bauer et al. 2015) and *Microbotryales* (*Pucciniomycotina*) (Aime et al. 2006). The unresolved relationships between some taxa in the *Ustilaginomycotina* can be explained by

(i) undiscovered biodiversity (missing data in phylogenetic analyses); (ii) few speciation events, and (iii) many extinctions between extant species and their most recent common ancestors. Several monogeneric classes and orders are known in the *Ustilaginomycotina*, namely, *Malasseziomycetes*, *Ceraceosorales*, *Golubeviales*, *Robbauerales*, *Uleiellales* and *Violaceomycetales* (Begerow et al. 2006, Wang et al. 2014, Albu et al. 2015, Wang et al. 2015, Riess et al. 2016).

Hibbett et al. (2018) used the PhyloCode to define higher taxonomic ranks of *Fungi* with known phylogenetic relationships. The PhyloCode transcends descriptive taxonomy in that it delimits taxa by their most recent common ancestor in a phylogenetic analysis, rather than by characters that may be homoplasious or difficult to recognise. Phylogenetic taxon definitions are suited to monotypic yeasts of the *Ustilaginomycotina*, as there are few apomorphies, whether morphological or biological, and their current taxonomies are based on phylogenetic hypotheses (Wang et al. 2014, Wang et al. 2015).

Species of *Cintractiella* (*Ustilaginomycotina*) form foliar pseudo-spikelets or modify the reproductive units (see Prychid & Bruhl 2013) of some tropical sedges (*Cyperaceae*, subfamily *Mapanioideae*). Three species of *Cintractiella* have been described, the type, *C. lamii* on an unidentified species of

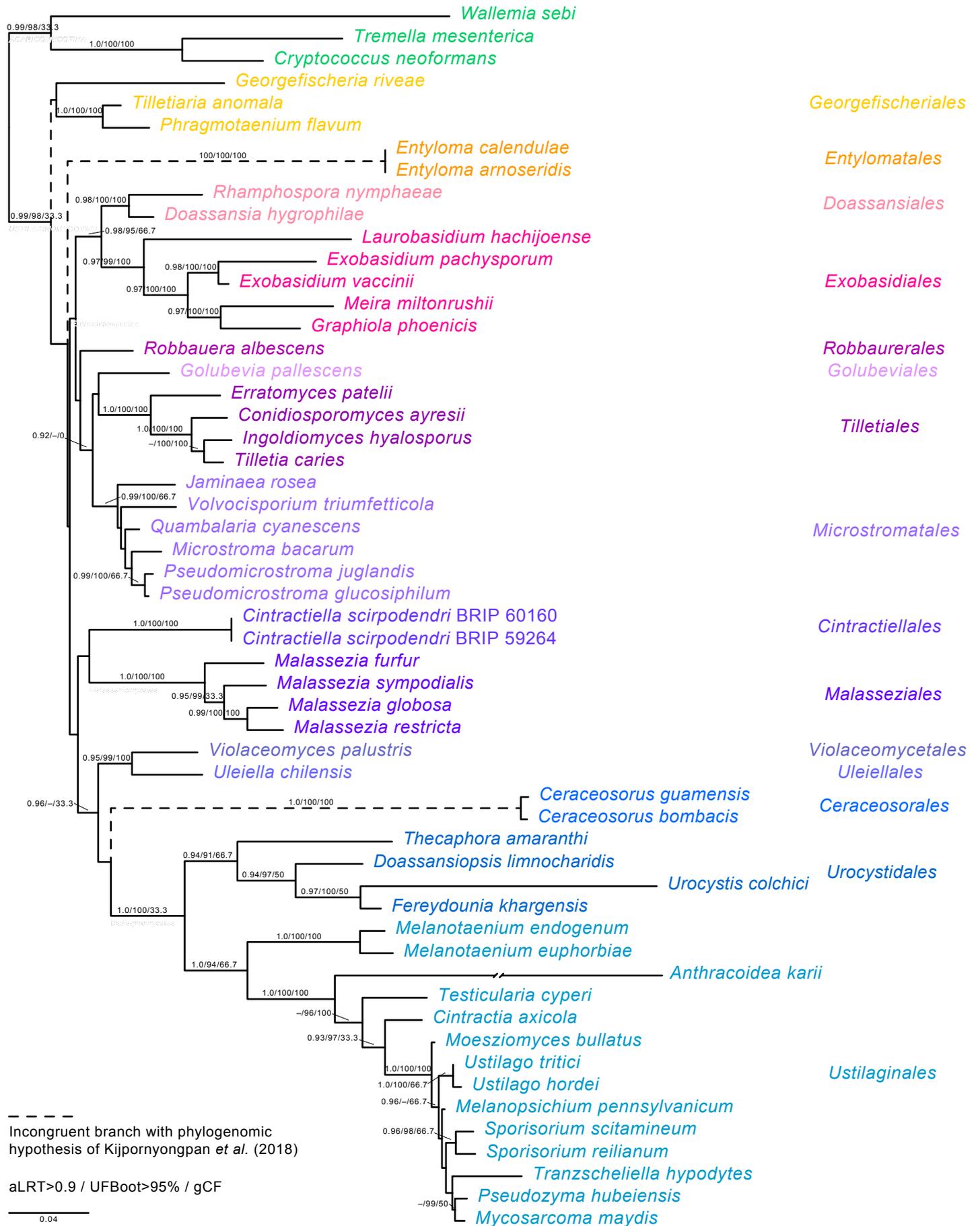


Fig. 1. Phylogram obtained from a maximum likelihood search in IQTree v. 1.7 beta, with a GTR gamma FreeRate heterogeneity model of evolution and a different rate for each partition of ribosomal DNA. aLRT values (≥ 0.9) and ultrafast bootstrap values ($\geq 95\%$) from 10 000 replicates, and genealogical concordance factors for three ribosomal DNA loci above nodes. Dashed lines indicate incongruence between the topology obtained from a phylogenomic dataset by Kijpornyongpan et al. (2018).

Hypolytrum in Irian Jaya, Indonesia (Boedijn 1937); *C. diplasiae* on *Diplasia karataefolia* in South America (Piepenbring 2001); and *C. kosraensis* on *Mapania pacifica* in the Caroline Islands, Federated States of Micronesia (Aime *et al.* 2018).

Boedijn (1937) did not propose a higher taxonomic rank for *Cintractiella*. Vánky (2003) established the *Cintractiellaceae* in the *Ustilaginales* for two species of *Cintractiella*. Begerow *et al.* (2018) were uncertain of the relationship of *Cintractiella* to other genera of the *Ustilaginomycotina* and treated the group with an unknown taxonomic affiliation. Our aim was to resolve the systematic placement of *Cintractiella* within *Fungi*. We used the PhyloCode to place and classify a new species of *Cintractiella* in relation to other taxa in the *Ustilaginomycotina*.

METHODS

Plant and fungal material

Specimens of *Scirpodendron ghaeri* and its parasitic smut fungus were collected using standard botanical methods. Associated material was dried on silica gel for DNA sequencing and material fixed in FAA (formaldehyde 10 mL, acetic acid 5 mL, 100 % ethanol 60 mL and distilled water 25 mL) and stored in 70 % ethanol. Plant and fungal specimens were lodged in the N.C.W. Beadle Herbarium and fungal specimens were sent to the Queensland Plant Pathology Herbarium (BRIP).

Microscopy

Spores were mounted in lactic acid (100 % v/v) for examination by light microscopy. Ranges were expressed as min–max with values rounded to 0.5 μ m. Images were captured with a Leica DFC 550 camera attached to a Leica DM2500 compound microscope with Nomarski differential interference contrast.

DNA extraction, PCR and sequencing

DNA was extracted from single sori (pseudo-spikelets) with a MoBio Microbial DNA extraction kit as specified by the manufacturer. Three ribosomal DNA (rDNA) loci, the internal transcribed spacer (ITS) and the large and small subunit regions (LSU and SSU), were amplified from BRIP 59246, and the ITS region was amplified from BRIP 60160. The ITS was amplified with primers ITS1/ITS4 (White *et al.* 1990), the LSU with LR0R/LR7 (Vilgalys & Hester 1990) and the SSU with NS1/NS4 (White *et al.* 1990). All reactions were amplified with high fidelity Phusion proof reading polymerase as specified by the manufacturer. The annealing temperatures in each PCR were 60 °C for ITS and SSU, and 62 °C for LSU. Amplified products were cleaned and sequenced with the primers from PCR by Macrogen (Korea).

Phylogenetic hypotheses

We added the rDNA sequences of *Cintractiella* to the dataset of Kijpornyongpan *et al.* (2018), excluding *Cystobasidium minutum* and *Mixia osmundae* (Table 1; alignments publicly available at TreeBASE, accession S24092). The rDNA loci were aligned separately with MAFFT (Katoh & Standley 2013) and concatenated in Geneious Prime. Non-homologous parts were curated from the ITS alignment using Gblocks (Castresana 2000). The most likely tree was searched in IQTree v. 1.7 beta (Nguyen

et al. 2015) with a GTR gamma FreeRate heterogeneity model of evolution and a different rate for each partition (command -spp -m GTR+R), 10 000 ultrafast bootstraps (Hoang *et al.* 2018), an approximate likelihood ratio test with 10 000 replicates (Guindon *et al.* 2010) and genealogical concordance factors calculated from each locus (Minh *et al.* 2018).

We used two approaches to determine the phylogenetic relationships of *Cintractiella*. A maximum likelihood search on the concatenated alignment i) without constraint, and ii) constrained to the class rank topology recovered by Kijpornyongpan *et al.* (2018). The constrained tree had monophyletic *Exobasidiomycetes* and *Ustilaginomycetes*, but relationships below class rank were non-constrained. The topologies of the constrained and non-constrained trees were compared in IQTree v. 1.7 beta, using log-likelihoods, an approximate unbiased test (Shimodaira 2002) and the Kishino-Hasegawa test (Kishino & Hasegawa 1989).

RESULTS

Specimen identification

The eight specimens of *Cintractiella* on *Scirpodendron ghaeri* examined in this study differed in host specificity and morphology to the three known species of *Cintractiella*. A new species is proposed for this taxon. Smut fungi belonging to *Cintractiella* were also found on one herbarium specimen of *Hypolytrum nemorum* from Thailand and on ten specimens of *Diplasia karatifolia* from South America.

Phylogenetic hypotheses

Cintractiella was recovered as sister to the *Malasseziomycetes* in both the non-constrained (Fig. 1) and constrained (Fig. 2) trees. The *Exobasidiomycetes* was non-monophyletic in the non-constrained tree, which was congruent with the findings of Wang *et al.* (2015). The *Entylomatales*, *Georgefischeriales*, *Golubeviales*, *Microstromatales*, *Robbaurerales* and *Tilletiales* were paraphyletic with the *Exobasidiomycetes* in respect to the *Malasseziomycetes*, *Monilellomycetes* and *Ustilaginomycetes*, and the *Ceraceosorales* was sister to the *Ustilaginomycetes* in the non-constrained tree. The non-constrained tree also differed to the phylogenomic hypothesis of Kijpornyongpan *et al.* (2018), as the *Monilellomycetes* was sister to the *Ustilaginomycetes*. The orders of *Ustilaginomycotina* defined by Wang *et al.* (2015) were monophyletic in the non-constrained tree, although their relationships to each other were not well-supported or concordant between the three loci.

The constrained tree supported the sister relationship of *Cintractiella* with the *Malasseziomycetes*. However, the constrained tree had a lower log-likelihood than the non-constrained tree (-23712.10 compared to -23691.44), was less significant with the Kishino-Hasegawa test (p -KH 0.07 compared to 0.93, significant topology ≥ 0.05) and was less significant with the approximate unbiased test (p -AU 0.057 compared to 0.94, significant topology ≥ 0.05). Based on these tests, the non-constrained tree is a better hypothesis of evolution based on rDNA loci. We expected the topologies recovered from rDNA loci to differ to the phylogenomic dataset, as there is less phylogenetic signal in rDNA markers when compared to the 910 single copy orthologs used by Kijpornyongpan *et al.* (2018).

Table 1. Taxa and their corresponding GenBank numbers used in phylogenetic analyses based on Kijpornyongpan *et al.* (2018).

Taxon	GenBank numbers			References
	SSU	ITS	LSU	
<i>Anthracoidea kariii</i>	DQ875376	–	DQ875358	Begerow <i>et al.</i> (2006)
<i>Calocera viscosa</i>	DQ520102	DQ520102	DQ520102	Garnica & Weiss unpubl.
<i>Ceraceosorus bombacis</i>	DQ875377	KT984941	DQ875361	Begerow <i>et al.</i> (2006); Kijpornyongpan & Aime (2016)
<i>Ceraceosorus guamensis</i>	KT984925	KT984926	KT984924	Kijpornyongpan & Aime (2016)
<i>Cintractia axicola</i>	DQ875378	AY344967	AF009847	Stoll <i>et al.</i> (2003); Begerow <i>et al.</i> (2006)
<i>Cintractiella scirpodendri</i> (BRIP 59264)	MK584805	MK584806	MK584752	Present study
<i>Cintractiella scirpodendri</i> (BRIP 60160)	–	MK584807	–	Present study
<i>Conidiosporomyces ayresii</i>	DQ363303	–	AF009848	Begerow <i>et al.</i> (1997); Begerow <i>et al.</i> (2006)
<i>Cryptococcus neoformans</i>	AJ560316	HQ851403	AJ551296	Boekhout unpubl.; Kidd unpubl.
<i>Doassansia hygrophilae</i>	DQ198788	–	AF007524	Begerow <i>et al.</i> (1997); Oberwinkler <i>et al.</i> (2006)
<i>Doassansiopsis limnocharidis</i>	–	DQ875344	AF009850	Begerow <i>et al.</i> (1997); Begerow <i>et al.</i> (2006)
<i>Entyloma arnosseridis</i>	DQ645529	DQ911609	DQ645528	Matheny <i>et al.</i> (2006)
<i>Entyloma calendulae</i>	DQ663688	DQ663689	DQ663687	Matheny <i>et al.</i> (2006)
<i>Erratomyces patelii</i>	DQ663693	DQ663692	AY818966	Castlebury <i>et al.</i> (2005); Matheny <i>et al.</i> (2006)
<i>Exobasidium pachysporum</i>	DQ875379	AB180352	AF487392	Begerow <i>et al.</i> (2002); Begerow <i>et al.</i> (2006); Nagao <i>et al.</i> unpubl.
<i>Exobasidium vaccinii</i>	DQ198792	AB180362	AF009858	Begerow <i>et al.</i> (1997); Oberwinkler <i>et al.</i> (2006); Nagao <i>et al.</i> unpubl.
<i>Fereydounia khargensis</i>	KJ490646	KJ490645	KJ490644	Nasr <i>et al.</i> (2014)
<i>Georgefischeria riveae</i>	DQ363312	–	AF009861	Begerow <i>et al.</i> (1997); Begerow <i>et al.</i> (2006)
<i>Golubevia pallescens</i>	D83191	AY259059	AJ235291	Takashima & Nakase (2001); Boekhout <i>et al.</i> (2006)
<i>Graphiola phoenicis</i>	DQ363306	–	AF009862	Begerow <i>et al.</i> (1997); Begerow <i>et al.</i> (2006)
<i>Ingoldiomyces hyalosporus</i>	–	AF399891	AF133576	Begerow <i>et al.</i> (2000); Zhang <i>et al.</i> unpubl.
<i>Jaminaea rosea</i>	KR912076	KR912071	KR912073	Kijpornyongpan & Aime (2017)
<i>Laurobasidium hachijoense</i>	–	AB180359	AB177562	Nagao <i>et al.</i> unpubl.
<i>Malassezia euphorbiae</i>	JN367342	JN367289	JN367314	Kellner <i>et al.</i> (2011)
<i>Malassezia furfur</i>	KF706457	AY743634	AF063214	Guillot & Guého (1995); Cabañes <i>et al.</i> (2005); Wang <i>et al.</i> (2014)
<i>Malassezia globosa</i>	EU192364	AY743630	AY743604	Cabañes <i>et al.</i> (2005); Xu <i>et al.</i> (2007); Paulino & Blaser unpubl.
<i>Malassezia restricta</i>	EU192367	AY743636	AF064026	Guillot & Gueho (1995); Cabañes <i>et al.</i> (2005); Paulino & Blaser unpubl.
<i>Malassezia sympodialis</i>	KF706460	AY743632	AY387283	Cabañes <i>et al.</i> (2005); Wang <i>et al.</i> (2014)
<i>Meira miltonrushii</i>	JX432964	NR_120190	JQ906774	Rush & Aime (2013)
<i>Melanopsichium pennsylvanicum</i>	JN367341	JN367288	JN367313	Kellner <i>et al.</i> (2011)
<i>Melanotaenium endogenum</i>	DQ789980	DQ789981	DQ789979	Matheny <i>et al.</i> (2006)
<i>Microstroma bacarum</i>	AJ496257	DQ317629	AF190002	Fell <i>et al.</i> 2000; de Beer <i>et al.</i> (2006)
<i>Moesziomyces bullatus</i>	DQ831012	DQ831013	DQ831011	Matheny <i>et al.</i> (2006)

Table 1. (Continued).

Taxon	GenBank numbers			References
	SSU	ITS	LSU	
<i>Mycosarcoma maydis</i>	KJ081758	KF278464	KF278480	Amed & Holmstrom unpubl.; Gujjari <i>et al.</i> unpubl.
<i>Phragmotenium flavum</i>	D82819	AB025708	AJ235285	Boekhout <i>et al.</i> (1995); Takashima & Nakase (2001); Tamura <i>et al.</i> unpubl.
<i>Pseudomicrostroma glucosiphilum</i>	KR912075	KR912070	KR912072	Kijpornyongpan & Aime (2017)
<i>Pseudomicrostroma juglandis</i>	DQ363313	DQ317634	AF009867	de Beer <i>et al.</i> (2006); Begerow <i>et al.</i> (2006)
<i>Pseudozyma hubeiensis</i>	XR001099829	DQ008954	AB566327	Wang <i>et al.</i> (2006); Konishi <i>et al.</i> (2013); Yarita <i>et al.</i> unpubl.
<i>Quambalaria cyanescens</i>	KF706440	DQ317623	DQ317616	de Beer <i>et al.</i> (2006); Wang <i>et al.</i> (2014)
<i>Rhamphospora nymphaeae</i>	DQ363311	DQ831034	AF007526	Begerow <i>et al.</i> (1997); Begerow <i>et al.</i> (2006); Matheny <i>et al.</i> (2006)
<i>Robbauera albescens</i>	KP322968	KP322986	AJ235289	Boekhout <i>et al.</i> (1995); Wang <i>et al.</i> (2015)
<i>Sporisorium reilianum</i>	KF706441	KF706438	KF706430	Wang <i>et al.</i> (2014)
<i>Sporisorium scitamaneum</i>	JN367349	JN367296	JN367321	Kellner <i>et al.</i> (2011)
<i>Testicularia cyperi</i>	KU147241	KU147240	KU147242	Kijpornyongpan & Aime unpubl.
<i>Thecaphora amaranthi</i>	DQ875383	EF200013	EF200038	Begerow <i>et al.</i> (2006); Vánky & Lutz (2007)
<i>Tilletia caries</i>	U00972	AF398447	AY819007	Berbee & Taylor (1993); Castlebury <i>et al.</i> (2005); Zhang <i>et al.</i> unpubl.
<i>Tilletiaria anomala</i>	AY803752	DQ234558	AY745715	Matheny <i>et al.</i> (2006)
<i>Tranzscheliella hypodytes</i>	JN367351	JN367298	JN367323	Kellner <i>et al.</i> (2011)
<i>Tremella mesenterica</i>	KF036705	AF042448	AF042266	Liu <i>et al.</i> unpubl.; Chen (1998)
<i>Uleiella chilensis</i>	KF061293	KF061293	KF061293	Riess <i>et al.</i> (2016)
<i>Urocystis colchici</i>	DQ839595	DQ839596	DQ838576	Matheny <i>et al.</i> (2006)
<i>Ustilago hordei</i>	JN367357	JN367303	JN367329	Kellner <i>et al.</i> (2011)
<i>Ustilago tritici</i>	DQ846895	DQ846894	DQ094784	Matheny <i>et al.</i> (2006)
<i>Violaceomyces palustris</i>	KM591583	KM591585	KM591584	Albu <i>et al.</i> (2015)
<i>Volvocisporium triumfetticola</i>	DQ317637	–	AF352053	Begerow <i>et al.</i> (2001); de Beer <i>et al.</i> (2006)
<i>Walleimia sebi</i>	FJ641905	AY328917	DQ847518	Matheny <i>et al.</i> (2006); Garnica <i>et al.</i> unpubl.

Cintractiella was not recovered in any of the described orders of the *Ustilaginomycotina*. We propose a new order for these taxa, based on the PhyloCode.

TAXONOMY

Cintractiellales McTaggart & R.G. Shivas, *ord. nov.* MycoBank MB830085.

Diagnosis: The smallest clade containing *Cintractiella scirpodendri* but not *Malassezia furfur*, *Ceraceosorus bombacis*, *Entyloma calendulae*, *Exobasidium vaccinii*, *Georgefischeria riveae*, *Golubevia pallescens*, *Robbauera albescens*, *Tilletia caries*, *Uleiella chilensis*, *Ustilago hordei*, *Violaceomyces palustris*.

Diagnostic apomorphies: Sexual morph on *Cyperaceae* subfamily *Mapanioideae*, forms hypertrophied, pseudo-spikelets filled with reticulate spores.

Notes: The *Cintractiellales* has an unresolved class affiliation in the *Ustilaginomycotina*, but does not belong to the *Exobasidiomycetes s. lat.* or the *Ustilaginomycetes*. The *Cintractiellales* is a monogeneric and monofamilial order of four species.

Included family: *Cintractiellaceae*.

Cintractiellaceae Vánky, *Fungal Diversity* **13**: 172. 2003.

Sori in adventitious spikelets on vegetative or generative organs of the host. **Spore mass** black, formed within a hyaline,

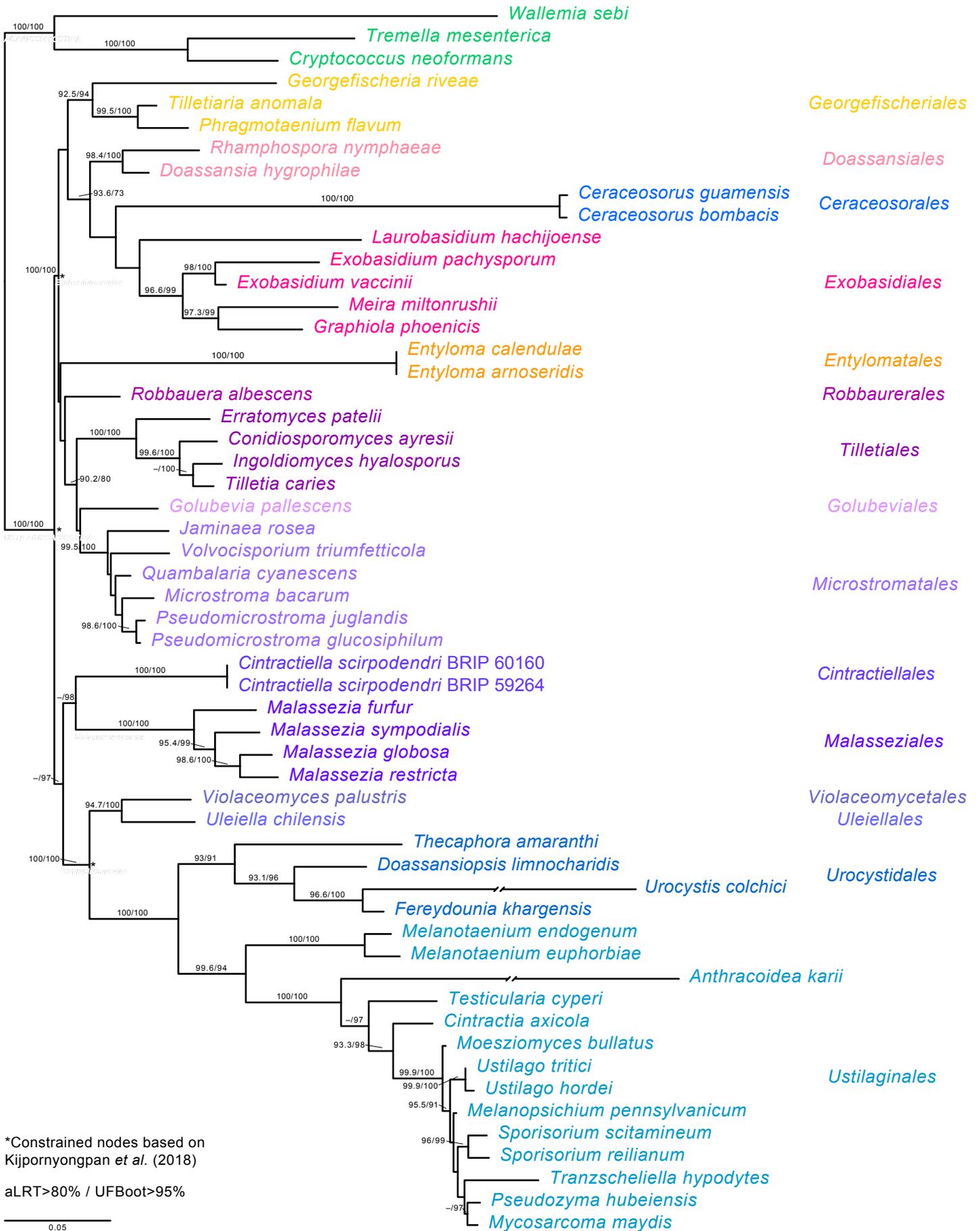


Fig. 2. Phylogram obtained from a maximum likelihood search in IQTree v. 1.7 beta constrained to the class rank topology of Kijpornyongpan et al. (2018). aLRT values (≥ 0.9) and ultrafast bootstrap values ($\geq 95\%$) from 10 000 replicates above nodes.

sporogenous fungal matrix, enclosed in the distal part of sterile spikelets. Spores single, relatively large, darkly pigmented, ornamented. Host-parasite interaction by intracellular haustoria. On *Cyperaceae*, subfamily *Mapanioideae*.

Included genus: *Cintractiella*.

Cintractiella Boedijn, *Bull. Jard. bot. Buitenz*, 3 Sér. **14**: 368. 1937.

Description: See Vánky (2011).

Sori in adventitious spikelets on vegetative or generative organs of host plants in *Cyperaceae*, subfamily *Mapanioideae*, in groups, forming witches' brooms or galls. Spore mass black, initially agglutinated, enclosed in the distal part of sterile spikelets, at maturity exposed at the opened tip of the spikelet. Spores develop embedded in hyaline, sporogenous fungal matrix, when mature solitary, relatively large and thick-walled, reddish brown, without tint of orange-red, ornamented. Host-parasite interaction by intracellular haustoria.

Included species: *C. diplasiae*, *C. kosraensis*, *C. lamii* and *C. scirpodendri*.

Cintractiella diplasiae (Henn.) M. Piepenbr., *Perspect. Pl. Ecol. Evol. Syst.* **4**: 120. 2001. Fig. 3J.

Basionym: *Ustilago diplasiae* Henn., *Hedwigia* **43**: 155. 1904.

Material examined: **Brazil**, *Diplasia karatifolia*, 24 Oct. 1977, Keel, BRIP 59673; 11 Feb. 1992, M. Nee, BRIP 66022. **Guyana**, *D. karatifolia*, 18 Jun. 1921, H.A. Gleason, BRIP 59670; Amatuk, Potaro River, 16 Aug. 1959, BRIP 59676; 18 Apr. 1993, T.W. Henkel & R. Williams, BRIP 59671. **Peru**, *D. karatifolia*, 22 Aug. 1980, Vasquez, BRIP 59677. **Suriname**, *D. karatifolia*, 10 Sep. 1963, H.S. Irwin, BRIP 59672. **Venezuela**, *D. karatifolia*, 25 Sep. 1928, G.H.H. Tate, BRIP 59669; 23 Mar. 1976, J.R.A. Lister, BRIP 59674; 10 May 1982, M. Morillo & R. Liesner, BRIP 59675.

Note: Spores of *C. diplasiae* differ in the ornamentation on the spore walls to other species of *Cintractiella* (Piepenbring 2001).

Cintractiella scirpodendri Prychid & J.J. Bruhl, *sp. nov.* MycoBank MB830084. Fig. 3A–E.

Etymology: Named after the host genus, *Scirpodendron*.

Type in sterile foliar spikelet-like growths on *Scirpodendron ghaeri*.

Sori (Fig. 1A–B) form in adventitious shoots that form in clusters of 1–15 on mature leaves, naked around the apex of all adventitious shoots, develop from around the shoot apex as a cylindrical column, ca. 5 × 1 mm, hidden by the narrow bracts of the adventitious shoots, semi-agglutinated to granular mass of spores visible at shoot apex. Spores (Fig. 3C–D) globose to subglobose, 14–22 µm, hazel to pale brown; wall 1–2 µm thick, minutely reticulate with up to 30 meshes per spore diam, circumference smooth to minutely dentate in profile. Spore germination (Fig. 1E) on PDA after 48 h produce three-celled phragmobasidia, fusiform or cylindrical, 13–20 × 7.5–10 µm, with lateral and terminal basidiospores. Basidiospores fusiform, 7.5–15 × 1.5–3.5 µm, hyaline, easily separate and continue to produce basidiospores.

Typus: **Australia**, Queensland, Cape Tribulation Road, 12 May 2013, C.J. Prychid & J.J. Bruhl, NE 99776 (BRIP 59264 **holotype**), SSU: MK584805, ITS: MK584806, LSU: MK584752.

On *Cyperaceae* (subfamily *Mapanioideae*): *Scirpodendron ghaeri*. Known only from the type locality in northern Australia.

Additional materials examined: **Australia**, Queensland, Cape Tribulation Road, *Scirpodendron ghaeri*, 7 Dec. 2012, C.J. Prychid & J.J. Bruhl, NE 99315; Croquette Point Road, C.J. Prychid & J.J. Bruhl, NE 99319; Cape Tribulation, Dubuji Boardwalk, 12 May 2013, C.J. Prychid 67 & J.J. Bruhl, NE 99777; Croquette Point Road, 14 May 2013, C.J. Prychid 71 & J.J. Bruhl, NE 99781; Cape Tribulation, Dubuji Boardwalk, 4 Jan. 2014, R.G. & M.D.E. Shivas & J. & L. Marsh, BRIP 60160, ITS: MK584807; Cape Tribulation Road, 18 Jun. 2014, C.J. Prychid 79, J.J. Bruhl & I.R. Telford, NE 101800; Croquette Point Road, 18 Jun. 2014, C.J. Prychid, J.J. Bruhl & I.R. Telford, NE 101804.

Notes: *Cintractiella scirpodendri* differs from *C. kosraensis* and *C. lamii*, which have much larger spores 29 × 36 µm diam. Sori of *C. scirpodendri* develop similarly to *C. lamii* as described and illustrated by Boedijn (1937), although *C. scirpodendri* lacks an enveloping fungal membrane.

Cintractiella kosraensis Aime *et al.*, *MycoKeys* **42**: 3. 2018.

Cintractiella lamii Boedijn, *Bull. Jard. bot. Buitenz*, 3 Sér. **14**: 368. 1937. Fig. 3F–I.

Material examined: **Thailand**, *Hypolytrum nemorum*, 15 Sep. 1985, C. Niyondham, BRIP 59678.

Notes: The specimen examined morphologically resembled the type description of *C. lamii* (Boedijn 1937) and occurred on the same host, *Hypolytrum*. We have not designated a neotype because there is the possibility of cryptic speciation. Furthermore, the specimen that we examined was collected in Thailand and the type location is in Indonesia.

DISCUSSION

The *Cintractiellales* (*Ustilaginomycotina*) is a monofamilial and monogeneric order with four species of *Cintractiella*. We defined the *Cintractiellales* with the PhyloCode as this accounts for phylogenetic uncertainty within the *Ustilaginomycotina* (Hibbett *et al.* 2018), as well as provides flexibility for future descriptions of taxa, such as yeasts, that may belong to this order. Our study is the first to show the systematic placement of *Cintractiella* in the *Fungi*.

An advantage of the PhyloCode is that a taxon can be defined by exclusion of taxa that do not share a most recent common ancestor. The phylogenetic relationships of *Cintractiella* to other orders of smut fungi were unresolved in our phylogenetic analyses. However, all orders in the *Ustilaginomycotina* had phylogenetic support, and were excluded from the definition of *Cintractiellales* as they did not share a most recent common ancestor at the rank of order.

The class rank of the *Cintractiellales* was not defined in the present study, as the *Exobasidiomycetes* were paraphyletic with regard to the *Ustilaginomycetes*, similar to the phylogenetic hypothesis recovered by Wang *et al.* (2015). The boundaries

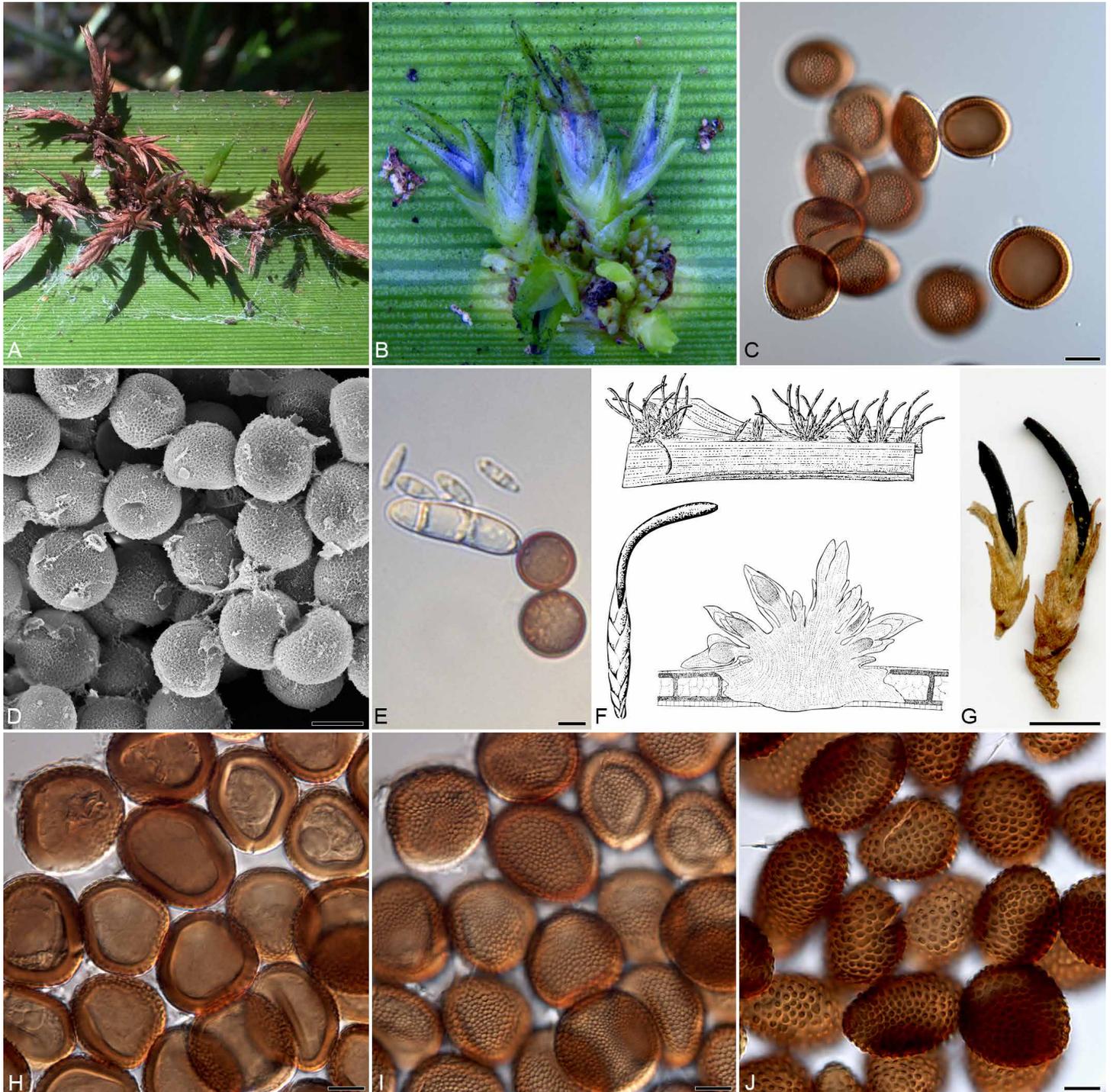


Fig. 3. A–E. *Cintractiella scirpodendri* on *Scirpodendron ghaeri*. A–B. Spikelet-like growths (BRIP 60160). C–D. Spores (BRIP 59264). E. Spore germination (BRIP 60160). F–I. *Cintractiella lamii* on *Hypolytrum nemorum*. F. Illustration by Boedijn (1937). G. Sori (BRIP 59678), H–I. Spores (BRIP 59678). J. *Cintractiella diplasiae* on *Diplasia karatifolia*, spores (BRIP 59671). Scale bars: G = 2 mm, all others = 10 μ m.

of known classes recovered with rDNA loci were not congruent with the phylogenomic dataset of Kijpornyongpan *et al.* (2018), which may be a consequence of less data in our study. Based on the trees recovered from rDNA loci, it is possible the *Cintractiellales* belong to another (monogeneric) class in the *Ustilaginomycotina*.

We examined two of the four known species of *Cintractiella*, and a specimen that closely resembled the type species, *C. lamii*. Based on morphology, *C. diplasiae* may not be congeneric with *Cintractiella*. *Cintractiella diplasiae* forms sori in the inflorescence rather than in pseudo-spikelets on leaves, and its spores have a tuberculate rather than reticulate ornamentation.

Our research is the first report of a species of *Cintractiella* from Australia. Images and a morphological description are provided on the Smut Fungi of Australia Lucid Key (available at <http://collections.daff.qld.gov.au/web/key/smutfungi/>) (Shivas *et al.* 2014).

Conflict of interest: The authors declare that there is no conflict of interest.

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