



# Diversity of *Kordyana* species (*Brachybasidiaceae*) on *Commelinaceae* in Australia

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## Abstract

The identity and diversity of *Kordyana* species on three native species of *Commelinaceae* in Australia were studied following surveys in 2020–2022 for *Kordyana brasiliensis*, which had been deliberately released as a biocontrol agent for the environmental weed *Tradescantia fluminensis*. Three new species of *Kordyana* are described from Australia based on DNA sequence analysis of the ITS and LSU rDNA regions, morphology, host associations, and geographic distributions. Two new species, *Kordyana spectabilis* on *Aneilema acuminatum* and *Kordyana luteoalba* on *Pollia crispata*, occur in shaded rainforest habitats in eastern Australia. The third new species, *Kordyana occidentalis* on *Commelina ensifolia*, occurs in forests and woodlands of the Kimberley region of Western Australia. Morphological descriptions are provided for these three new species of *Kordyana* as well as for the conidial stage of *K. brasiliensis*.

**Keywords** Classical biological control · *Exobasidiales* · Taxonomy · 3 new species

## Introduction

*Kordyana* (*Brachybasidiaceae*, *Exobasidiomycetes*) accommodates dimorphic biotrophic leaf pathogens that cause leaf spots with non-pigmented spores on the abaxial side, mostly on host plants in the *Commelinaceae* (Piepenbring et al. 2020). *Kordyana* species have been reported from Africa, Asia, Australia, Russia, Central America (Panama, Costa Rica), and South America (Raciborski 1900; Gäumann 1922; Sawada 1929; Petrak 1950; Gruèzo 1990; Gómez and Kisimova-Horovitz 1997; Barreto and Evans 1988; Piepenbring et al. 2010; Macedo et al. 2016; Park et al. 2021; Dudka 2023). *Kordyana* is typified by the type species, *K. tradescantiae* (Pat.) Racib. (Raciborski 1900),

and accommodates seven additional valid species, *K. aneilemae* Sawada; *K. boswelliae* Thirum., Patel, G.W. Dhande & V.V. Bhatt; *K. brasiliensis* D.M. Macedo, O.L. Pereira & R.W. Barreto; *K. celebensis* Gäum.; *K. commelinae* Petch; *K. cyphelloidis* Viégas; and *K. polliae* Gäum. (Piepenbring et al. 2020). There are two additional species which lack a diagnosis, *K. indica* and *K. polliae* var. *microspora* (is considered a synonym of *K. polliae*) (Piepenbring et al. 2020; see Online Resource 1).

The classification of *Kordyana* species has traditionally been based on morphology and host range (Raciborski 1900; Gäumann 1922; Sawada 1929; Petrak 1950; Gruèzo 1990). However, the morphological approach to fungal classification is often unreliable due to the variability, or lack thereof, of phenotypic traits (including morphology and host range). In a critical revision of the *Brachybasidiaceae*, Piepenbring et al. (2020) used phylogenetic analysis of the ITS and LSU rDNA sequences to show that *K. brasiliensis*, *K. celebensis*, *K. tradescantiae*, and *Dicellomyces gloeosporus* formed a well-supported clade. This phylogenetic analysis was unable to resolve the relations between these taxa and contrasts the findings of Park et al. (2021), who placed *K. commelinae* sister to *Marantokordyana* species and not *D. gloeosporus* based on ITS rDNA. Unfortunately, the taxonomic resolution of *Kordyana* has been hampered as molecular barcodes are available for only four of the eight known species

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(Begerow et al. 2002; Macedo et al. 2016; Park et al. 2021; Dudka 2023).

*Kordyana brasiliensis* was deliberately released in New Zealand in 2018 and in Victoria, Australia, in 2019 as a biocontrol agent for the environmental weed *Tradescantia fluminensis* (Landcare Research 2020; Winston et al. 2021; Morin et al. 2022). *Kordyana brasiliensis* was discovered and described during exploratory surveys for potential biocontrol agents for *T. fluminensis* in Brazil (Macedo et al. 2016) and is reported as host-specific to *T. fluminensis*. Preliminary monitoring in Australia and New Zealand has shown that *K. brasiliensis* has become well-established in both countries since its release, with high levels of disease recorded at some locations (Morin et al. 2022; Landcare Research 2020). *Kordyana brasiliensis* is the only *Kordyana* species recorded in Australia according to the Fungi Name Index (FNI) (Australian National Species List 2024). Herbarium records show collections of unidentified specimens of *Kordyana* collected on *Commelina* sp., *C. ensifolia*, and *Aneilema acuminatum* in Australia. Seven genera within *Commelinaceae* occur naturally in Australia including *Aneilema*, *Cartonema*, *Commelina*, *Cyanotis*, *Floscopa*, *Murdannia*, and *Pollia* (PlantNET 2022; Western Australian Herbarium 2024; Queensland Government 2024). The diversity of *Kordyana* species on native *Commelinaceae* in Australia has not been previously studied.

From 2020 to 2022, surveys were made before and after the release of *K. brasiliensis* in New South Wales (NSW), Australia, to determine the success of the biological control release program. During these surveys, specimens of native Australian *Commelinaceae*, co-occurring with *T. fluminensis*, with white leaf lesions were found and collected. The identities of these specimens were studied using multigene phylogenetic analyses to determine whether *K. brasiliensis* had extended its known host range to include native Australian *Commelinaceae*, or whether they represented other *Kordyana* species. Among the specimens collected and including herbaria records, three new species of *Kordyana* were found, each restricted to a single host species, i.e., *Aneilema acuminatum*, *Commelina ensifolia*, and *Pollia crispata*.

## Materials and methods

### Specimens examined

Leaves of *Aneilema acuminatum*, *Commelina* spp., and *Pollia crispata* with white leaf lesions were collected before and after the release of *K. brasiliensis* in NSW in eastern Australia between 2020 and 2022 (CSIRO 2023). Additional surveys of native Australian *Commelinaceae*, including *A. acuminatum*, *Commelina* spp., and *P. crispata*, with white

leaf lesions, were conducted in Queensland (QLD) in 2021. Only native *Commelinaceae* species co-occurring with *T. fluminensis* at the study sites were examined. Plant hosts were identified by morphological descriptions and known geographical distributions in Flora of NSW (PlantNET 2022). Young leaf lesions were excised and attached with petroleum jelly to the inner lids of Petri dishes and inverted over potato dextrose agar (PDA) plates. The PDA plates were incubated in the dark at 21–22 °C for 1–2 days to capture discharged basidiospores on the agar surface. Single germinated basidiospores were transferred to fresh plates with a sterile needle and incubated in the dark at 21–22 °C for several weeks.

Selected specimens (dried leaves and fungal cultures) were deposited in the Queensland Plant Pathology Herbarium (BRIP) (Table 1). Fungal cultures were permanently preserved in a metabolically inactive state at –80 °C on agar pieces in 15% glycerol (v/v) in the Queensland Plant Pathology Herbarium (BRIP). Furthermore, three herbarium specimens of possible *Kordyana* on *Commelinaceae* collected in Australia (BRIP 53424, BRIP 65743, and PERTH 3203697) were borrowed and examined (Table 1). The identity of the fungus causing the white lesions in these previous collections had not been published.

### DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analysis

Genomic DNA was isolated directly from leaf lesions of herbarium specimens, as well as from pure fungal cultures grown on PDA. Samples were homogenised in a Fast-Prep®-24 Classic Tissue and Cell Homogeniser (MP Biomedicals LLC, USA), and genomic DNA was extracted with the ISOLATE II Plant DNA Kit (Meridian Bioscience, Australia) and the manufacturer's protocol. Genomic DNA was diluted 1:10 or 1:100 for PCR amplification by MyFi Mix (Meridian Bioscience, Australia) and manufacturer's instructions with a final reaction volume of 25 µl. The primers used were ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for ITS; LROR (Moncalvo et al. 1995) and LR5/LR6 (Vilgalys and Hester 1990) for LSU; and NS1 and NS4 (White et al. 1990) for SSU. PCR products were amplified with the following parameters: initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 53 °C for ITS, 62 °C for LSU and 52 °C for SSU for 15 s, and an extension at 72 °C for 30 s followed by a final extension at 72 °C for 7 min. PCR amplicons were cleaned with ISOLATE II PCR and Gel Kit (Meridian Bioscience, Australia) and then submitted to either Macrogen Inc (Seoul, Korea) or the Biomolecular Resource Facility (BRF) (Canberra, Australia) for DNA sequencing.

DNA sequence chromatograms were assembled and manually checked in Geneious Prime 2023.01.1

**Table 1** DNA sequences of reference taxa and novel taxa included in this study. DNA sequences of novel taxa generated in this study are indicated in bold. Collection details of novel taxa included

Species	Host/substrate	Fungarium Accession <sup>1</sup>	Culture no. <sup>1</sup>	Locality <sup>2</sup>	GenBank Accessions <sup>3</sup>			References			Collection details (this study)		
					ITS	LSU	SSU	Location	Collector	Date	Location	Collector	Date
<i>Acaromyces ingoldii</i>	Citrus rust mite	-	CBS 110050	Israel	NR_073342	NG_058540	NG_061199	Boekhout et al. (2003)	-	-	-	-	-
<i>Aricomyces warmingii</i>	<i>Frullania dilatata</i>	-	CBS 146033	UK	MT223780	MT223875	N/A	Crous et al. (2020)	-	-	-	-	-
<i>Clinocnidium bullatum</i>	<i>Aiouea neurophylla</i> Sydow, Fgi. exot. exs. 553 (M)	-	-	-	N/A	AF487383	N/A	Begerow et al. (2002)	-	-	-	-	-
<i>Cl. lauracearum</i>	-	-	NFCCI 4483	India	MN061349	MN061363	N/A	Singh et al. (2020)	-	-	-	-	-
<i>Coniodictyum chevalieri</i>	<i>Zizyphus mucronata</i>	PREM 59000-WM3450	-	South Africa	N/A	DQ334805	N/A	Maier et al. (2006)	-	-	-	-	-
<i>Dicellomyces gloeosporus</i>	<i>Pleioblastus argenteosriatus</i>	TMI 7283	-	Japan	AB427190	AB427190	LC575091	Tanaka et al. (2008)	-	-	-	-	-
<i>D. scirpi</i>	<i>Scirpus sylvaticus</i>	R.B. 1032	-	-	N/A	AF487385	DQ363304	Begerow et al. (2002)	-	-	-	-	-
<i>Drepanoconis larviformis</i>	<i>Nectandra cuspidata</i>	M.P. 4525	-	Panama	N/A	GU586973.1	N/A	Piepenbring et al. (2010)	-	-	-	-	-
<i>Exobasidium vaccinii</i>	-	TUB 019109	-	-	N/A	FJ644526	FJ641898	Kottke et al. (2010)	-	-	-	-	-
<i>E. vaccinii</i>	-	-	D.B. 160d	-	KP322983	N/A	N/A	Wang et al. (2015)	-	-	-	-	-
<i>Graphiola cylindrica</i>	Palm	JCM 8561	-	-	N/A	AF487400	N/A	Begerow et al. (2002)	-	-	-	-	-
<i>G. fimbriata</i>	-	-	IBRC-M 30158 <sup>T</sup>	Iran	KM403454	KM403453	KM403455	Nasr et al. (2019)	-	-	-	-	-
<i>G. geonomae</i>	<i>Geonoma interrupta</i>	M.P. 0141254	-	Panama	N/A	JN185909	N/A	Piepenbring et al. (2012)	-	-	-	-	-
<i>G. phoenicis</i>	<i>Phoenix canariensis</i>	HRUTA0025/2016	-	Chile	KX344499	KX344500	N/A	Sepúlveda et al. (2017)	-	-	-	-	-

Table 1 (continued)

Species	Host/substrate	Fungarium Accession <sup>1</sup>	Culture no. <sup>1</sup>	Locality <sup>2</sup>	GenBank Accessions <sup>3</sup>		References	Collection details (this study)		
					ITS	LSU		SSU	Location	Collector
<i>Kordyana brasiliensis</i>	<i>Tradescantia fluminensis</i>	VIC 3136 <sup>T</sup>	-	Brazil	N/A	HM105582	Macedo et al. (2016)	-	-	-
	<i>T. fluminensis</i>	-	BRIP 75726a	ACT	OR614366	OR764851	This study	CSIRO Black Mountain Laboratories, Canberra	Zeil-Rolfé, I.	12 Jan. 2022
	<i>T. fluminensis</i>	BRIP 70304a	-	Brazil	OR614356	OR764850	This study	Vicosa, Minas Gerais	Macedo, D.M.	10 Dec. 2009
	<i>T. fluminensis</i>	-	BRIP 75726b	ACT	OR614367	OR764852	This study	CSIRO Black Mountain Laboratories, Canberra	Zeil-Rolfé, I.	25 Jun. 2020
<i>K. celebensis</i>	<i>Commelina</i> sp.	HB 17	-	-	N/A	AF487401	Begerow et al. (2002)	-	-	-
<i>K. commeliniae</i>	<i>C. communis</i>	-	KACC 49694	Korea	MW267817	MW250223	Park et al. (2021)	-	-	-
<i>K. tradescantiae</i>	<i>Tradescantia</i> sp.	F.O. 47147	-	-	N/A	AF487402	Begerow et al. (2002)	-	-	-
<i>K. occidentalis</i>	<i>C. ensifolia</i>	BRIP 65743a <sup>T</sup>	-	WA	OR791408	OR764856	This study	Geikie Gorge	Lemana, B. Ványk, K., Ryley, M.J., Thompson, S.M., Shivas, M.D.E., Shivas, R.G.	20 Apr. 2017
<i>K. luteoalba</i>	<i>C. ensifolia</i>	PERTH 3203697	-	WA	OR791406	N/A	This study	Kununurra	Shivas, R.G.	11 Mar. 1993
	<i>Politia crispata</i>	BRIP 75709a	-	NSW	OR614364	OR764855	This study	Mt Kiera	Gooden, B.	20 Feb. 2021
	<i>P. crispata</i>	BRIP 75710a	-	NSW	N/A	OR764854	This study	Jerrara Dam	Gooden, B.	20 Feb. 2021
	<i>P. crispata</i>	BRIP 75711a	-	NSW	N/A	N/A	This study	Jerrara Dam	Zeil-Rolfé, I., Lester, J.	19 Jun. 2022

Table 1 (continued)

Species	Host/substrate	Fungarium Accession <sup>1</sup>	Culture no. <sup>1</sup>	Locality <sup>2</sup>	GenBank Accessions <sup>3</sup>			References	Collection details (this study)		
					ITS	LSU	SSU		Location	Collector	Date
<i>P. crispata</i>		BRIP 75712a	BRIP 75712a	NSW	N/A	N/A	N/A	This study	Jerrara Dam	Zeil-Rolfé, I., Lester, J	1 Sep. 2022
<i>P. crispata</i>		BRIP 75713a	-	NSW	N/A	N/A	N/A	This study	Ourimbah Creek	Patterson, B	6 May 2021
<i>P. crispata</i>		BRIP 75714a	-	QLD	N/A	N/A	N/A	This study	Main Range National Park	Gooden, B.	22 Jun. 2021
<i>P. crispata</i>		BRIP 75715a	-	QLD	N/A	N/A	N/A	This study	Lepidozania Break, Mount Glorious	Gooden, B.	27 Jun. 2021
<i>P. crispata</i>		BRIP 75716a	-	QLD	N/A	N/A	N/A	This study	Mary Cairncross Scenic Reserve	Gooden, B.	28 Jun. 2021
<i>P. crispata</i>		BRIP 75717a	BRIP 75717a	QLD	<b>OR614365/ OR614371</b>	<b>OR802997/ OR802998</b>	<b>OR616664/ OR616665</b>	This study	Maiala Recreation Area, Mount Glorious	Gooden, B.	25 Jun. 2021
<i>P. crispata</i>		BRIP 75718a	BRIP 75718a	NSW	<b>OR614361/ OR614369</b>	<b>OR802994/ OR802995</b>	<b>OR616658/ OR616659</b>	This study	Boorganna Nature Reserve	Zeil-Rolfé, I., Lester, J	8 Jun. 2022
<i>P. crispata</i>		BRIP 75719a	-	NSW	N/A	N/A	N/A	This study	Boorganna Nature Reserve	Zeil-Rolfé, I., Lester, J	22 Apr. 2021
<i>P. crispata</i>		BRIP 75720a	BRIP 75720a	NSW	<b>OR614362/ N/A</b>	N/A	<b>OR616660/ N/A</b>	This study	Boorganna Nature Reserve	Zeil-Rolfé, I., Lester, J	8 Jun. 2022
<i>P. crispata</i>		BRIP 75725a	BRIP 75725a <sub>T</sub>	NSW	<b>OR614363/ OR614370</b>	<b>OR802996/N/A</b>	<b>OR616661</b>	This study	Macquarie Pass National Park	Zeil-Rolfé, I., Lester, J	2 Sep. 2022
<i>K. spectabilis</i>	<i>Anellema acuminatum</i>	BRIP 53424a	-	QLD	<b>OR791407</b>	N/A	N/A	This study	Mount Glorious	McNeil, B.C., Shi- vas, R.G., Terhem, R.	09 Jun. 2010

Table 1 (continued)

Species	Host/substrate	Fungarium Accession <sup>1</sup>	Culture no. <sup>1</sup>	Locality <sup>2</sup>	GenBank Accessions <sup>3</sup>			References	Collection details (this study)		
					ITS	LSU	SSU		Location	Collector	Date
<i>A. acuminatum</i>		BRIP 75723a	-	NSW	<b>OR614357</b>	<b>OR802991</b>	N/A	This study	Macquarie Pass National Park	Gooden, B., Zeil-Rolf, I.	19 Aug. 2020
<i>A. acuminatum</i>		BRIP 75708a	-	NSW	<b>OR614358</b>	<b>OR764853</b>	<b>OR616654</b>	This study	Minnamurra Rainforest National Park	Gooden, B., Zeil-Rolf, I.	18 Feb. 2021
<i>A. acuminatum</i>		BRIP 75721a	-	NSW	<b>OR614359</b>	<b>OR802992</b>	<b>OR616655</b>	This study	Boorganna Nature Reserve	Zeil-Rolf, I., Lester, J	8 Jun. 2022
<i>A. acuminatum</i>		BRIP 75722a	-	NSW	N/A	N/A	N/A	This study	Boorganna Nature Reserve	Zeil-Rolf, I., Lester, J	8 Jun. 2022
<i>A. acuminatum</i>		BRIP 75723a	-	NSW	N/A	N/A	N/A	This study	Boorganna Nature Reserve	Zeil-Rolf, I., Lester, J	8 Jun. 2022
<i>A. acuminatum</i>		BRIP 75724a	BRIP 75724a <sup>T</sup>	NSW	<b>OR614360/</b> <b>OR614368</b>	N/A/ <b>OR802993</b>	<b>OR616656/</b> <b>OR616657</b>	This study	Darkwood	Zeil-Rolf, I., Lester, J	5 Jun. 2022
<i>Laurobasidium lauri</i>	<i>Laurus azorica</i>	M.P. 2371	-	-	N/A	AF487403.1	N/A	Begerow et al. (2002)	-	-	-
<i>Manan-tokordyana boliviana</i>	<i>Goepertia propinqua</i>	USZ, Curso de Hongos CH88 <sup>T</sup>	-	Bolivia	NR_173868	MN275901	N/A	Piepenbring et al. (2020)	-	-	-
<i>M. oberwinkleriana</i>	<i>G. panamensis</i>	PMA 0123802 <sup>T</sup>	-	Panama	NR_168427	MN275900	N/A	Piepenbring et al. (2020)	-	-	-
<i>Meira argovae</i>	Spider mite on leaf of <i>Ricinus communis</i>	-	CBS 110053 <sup>T</sup>	Israel	NR_073344	NG_042395	KP322953	Boekhout et al. (2003)	-	-	-
<i>Me. geulakonigae</i>	Citrus rust mite	-	CBS 110052 <sup>T</sup>	Israel	NR_073343	NG_042394	NG_065630	Boekhout et al. (2003)	-	-	-
<i>Me. miltorushii</i>	Leaf surface of <i>Magnolia grandiflora</i>	-	CBS 12591	USA	NR_120190	NG_059474	NG_065598	Rush and Aime (2013)	-	-	-

Table 1 (continued)

Species	Host/substrate	Fungarium Accession <sup>1</sup>	Culture no. <sup>1</sup>	Locality <sup>2</sup>	GenBank Accessions <sup>3</sup>		References	Collection details (this study)		
					ITS	LSU		SSU	Location	Collector
<i>Me. nashicola</i>	Fruit of <i>Pyrus pyrifolia</i>	-	CBS 117161 <sub>T</sub>	Japan	NR_119391	NG_060234	Yasuda et al. (2006)	-	-	-
<i>Me. nicotiana</i>	Rhizosphere of tobacco	-	CCCTC M2015704 <sub>T</sub>	China	MH188916	MH188917	Cao et al. (2018)	-	-	-
<i>Muriba-sidiospora indica</i>	Leaves of <i>Rhus myso-rensis</i>	CBS 6588	-	South Africa	N/A	AY204506	Crous et al. (2003)	-	-	-
<i>Rhamos-phospora nymphaea</i>	<i>Nymphaea tuberosa</i>	-	CBS 172.38	USA	NR_119615	NG_057757	Matheny et al. (2006)	-	-	-

<sup>1</sup>BRIP, Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; CBS, Westerdijk Fungal Biodiversity Institute, The Netherlands; CCTCC, China Centre for Type Culture Collection, China; D.B., Herbarium D. Begerow, Ruhr-Universität Bochum, Germany; F.O., Herbarium F. Oberwinkler, Tübingen, Germany; HB, Institut für angewandte Mikrobiologie, Wien, Austria; HRUTA, Universidad de Tarapacá (UTA), Arica, Chile; IBRC-M, Iranian Biological Resources Centre, Iran; JCM, Japan Collection of Microorganisms, Japan; KACC, Korean Agricultural Culture Collection; M, Botanische Staatssammlung, München, Germany; M.P., Herbarium M. Piepenbring, Frankfurt, Germany; NFCCI, National Fungal Culture Collection of India, New Delhi, India; PMA, Universidad de Panama; PREM, National Mycological Herbarium, Pretoria, South Africa; R.B., Herbarium R. Bauer, Tübingen, Germany; TMI, Tottori Mycological Institute, Japan; TUB, Herbarium Tübingense, University of Tübingen, Germany; USZ, Herbario del Oriente Boliviano, Bolivia; VIC, Universidade Federal de Viçosa, Brazil

<sup>2</sup>ACT, Australian Capital Territory, Australia; NSW, New South Wales, Australia; QLD, Queensland, Australia; VIC, Victoria, Australia; WA, Western Australia

<sup>3</sup>ITS, internal transcribed spacer region and intervening 5.8S nrRNA gene; LSU, nuclear ribosomal large subunit; SSU, nuclear ribosomal small subunit; N/A, not available. Species with two GenBank numbers = holotype (dried leaf lesions)/ex-type culture sequences

<sup>T</sup>Type

(Geneious Prime 2023.01.1, <https://www.geneious.com>). Basic Local Alignment Tool (BLAST) searches in GenBank using consensus sequences were performed to compare their identity to sequences of ex-type specimens in the *Exobasidiales*, and an alignment dataset for phylogenetic analysis was constructed. DNA sequences obtained in this study were deposited in GenBank (Table 1).

Sequence alignments were constructed in MEGA11 v. 11.0.13 (Tamura et al. 2021) with Muscle (Edgar 2004). DNA sequence alignments were edited manually to ensure correct alignment before the removal of poorly aligned regions with GBlocks v. 0.91.1 (Castresana 2000) through the NGPhylogeny.fr website (Lemoine et al. 2019). The parameters used for the ITS, LSU, and SSU were 18/29/11/5/with half, 22/35/8/5/with half, and 13/20/8/5/with half, respectively. The alignments were concatenated to provide a total alignment with 1983 positions (58% of the original 3442 positions).

Phylogenetic relationships were inferred by Bayesian inference (BI) and maximum likelihood (ML) based on the concatenated alignment (Online Resource 2). The BI analysis was run with MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) for two runs over four parallel chains of 1.2 million generations, with trees sampled every 400 generations after a 10% burn-in. Likelihood profiles were examined to verify that the burn-in was appropriate. Run convergence was confirmed when the average standard deviation was < 0.01 with effective sample sizes > 200. Model Finder (Kalyanamoothy et al. 2017) in IQ-TREE2 (Minh et al. 2020) was used to select the best-fit substitution model based on the Bayesian information criterion (BIC) (Nguyen et al. 2014): GTR + F + I + R3. Nodal support was assessed with Bayesian posterior probabilities (BPP). The ML analysis was run in RAxML (Stamatakis et al. 2008) with 1000 bootstrap replicates. Phylogenetic trees were visualised in Figtree v1.4.4, with the outgroup *Rhamphospora nymphaeae* (*Dossansiales*) as used by Piepenbring et al. (2020).

## Morphology

Fresh and dried leaf specimens were examined with a LEICA DLMB microscope (Leica Microsystems, Germany) for basidia, basidiospores, conidia, and hyphae. Colony characteristics of the yeast-like cultures on PDA were recorded. Conidial measurements were taken from PDA cultures (if available) and leaf surfaces. The range of dimensions of morphological features was determined ( $n = 50$  unless indicated otherwise). The colours of cultures were described with the colour chart of Rayner (1970).

## Results

### Sample collection and isolates

Forty sites in eastern Australia were surveyed for *Aneilema acuminatum*, *Commelina* spp., and *Pollia crispata* with white leaf lesions. *Pollia crispata* was the only *Pollia* species recorded during the surveys and was observed growing at 22 sites. At these sites, white leaf spots with white abaxial sides were found on *P. crispata* at 11 sites (17 detections) and remained undetected at 11 sites. *Aneilema acuminatum* was the only *Aneilema* species recorded during surveys and was observed growing at 15 sites. At these sites, white leaf spots with white abaxial sides were found on *A. acuminatum* at 7 sites (9 detections) and were undetected at 8 sites (Fig. 1; Table 1). *Kordyana* spp. were not found on *Commelina* spp. in eastern Australia. Cultures of *Kordyana* were successfully obtained from five fresh leaf specimens of *P. crispata* (BRIP 75725, BRIP 75719, BRIP 75718, BRIP 75717, and BRIP 75712) and one from a leaf of *A. acuminatum* (BRIP 75724).

### Phylogenetic analyses

The BI and ML trees produced similar topologies. The phylogenetic analysis based on the concatenated ITS, LSU, and SSU alignment revealed three well-supported clades that we consider to represent novel species of *Kordyana* (Fig. 2). Each of these three clades contained only Australian specimens, and members of each clade were restricted to *A. acuminatum*, *C. ensifolia*, or *P. crispata*, respectively. Sequences of *Kordyana* spp. formed a well-supported clade sister to *Marantokordyana*.

### Taxonomy

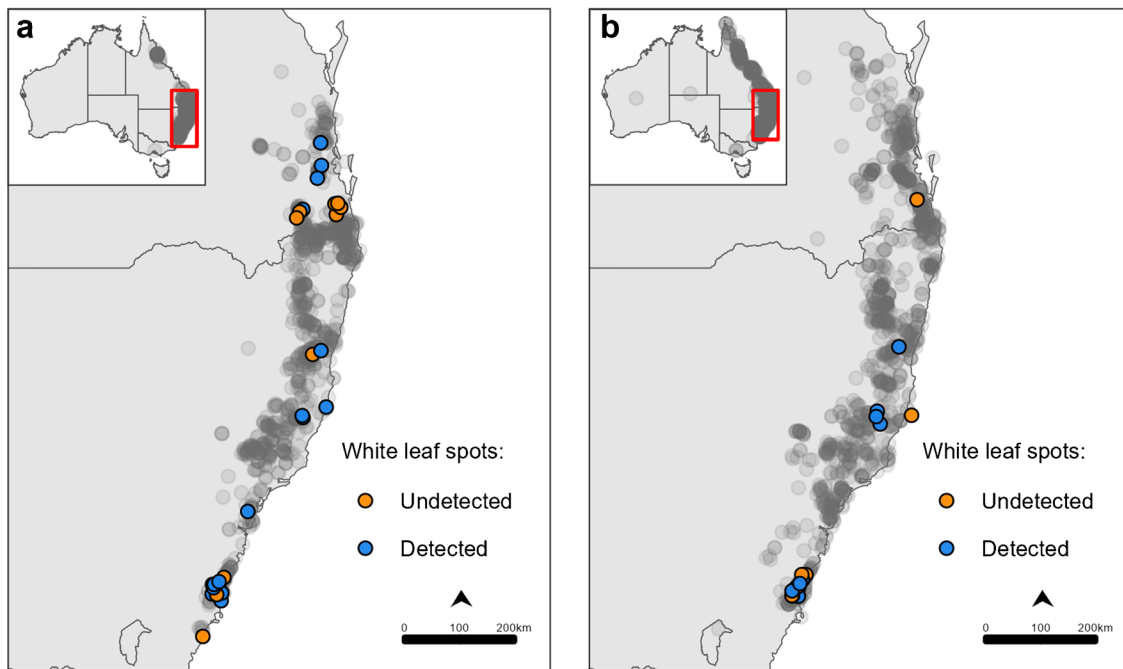
Three new species of *Kordyana* from Australia are described based on the phylogenetic analysis, morphology, and host species. Molecular barcodes and morphological descriptions, together with information about their known host range and distribution, are provided. The asexual life stage of *K. brasiliensis* is described for the first time.

***Kordyana luteoalba*** Zeil-Rolfe, Gooden, G.C. Hunter, C.C. Linde & R.G. Shivas, sp. nov., Fig. 3.

Mycobank: MB 852430.

**Holotype:** AUSTRALIA. New South Wales, Macquarie Pass National Park, 34°34'10" S 150°40'19" E, rainforest, on leaves of *Pollia crispata*, 2 Aug. 2022, I. Zeil-Rolfe & J. Lester (BRIP 75725a includes a culture permanently preserved in a metabolically inactive state); ITS, LSU, and





**Fig. 1** Distribution of white leaf spot symptoms detected on two *Comelinaceae* species native in eastern Australia (NSW, QLD). **a** *Pollia crispata* observed growing at 22 sites (coloured points), white leaf spots detected  $n=17$  at 11 different sites, undetected  $n=11$ ; **b**

*Aneilema acuminatum* observed growing at 15 sites (coloured points), white leaf spots detected  $n=9$  at 7 different sites, undetected  $n=8$ . Grey points represent records of the host plants downloaded from Atlas of Living Australia (ALA) (<https://www.ala.org.au/>)

SSU rDNA sequences GenBank OR614363, OR802996, and OR616661, respectively. For data on additional specimens examined, see Table 1.

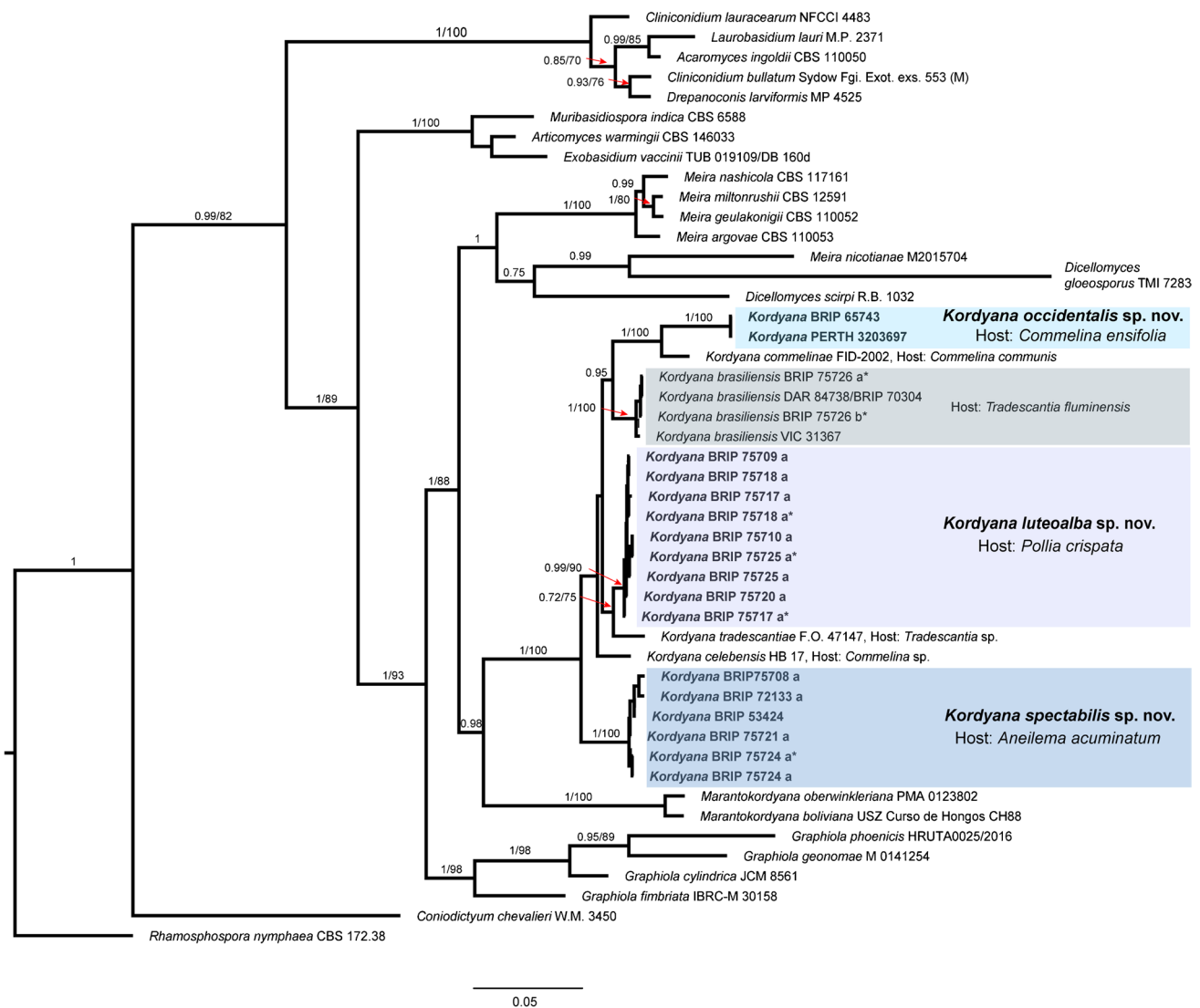
**Etymology:** Refers to the yellow leaf lesions surrounded by a distinctive white halo.

**Leaf lesions** amphigenous, circular to irregular, solitary or coalescent. Sporulation hypophyllous, leaf lesions at first pale yellow to orange with a white to cream halo around the lesion, the centre becoming brown with age, with white balls of basidia covering the lesion. *Substomatal chambers* in the lesions filled with dense fungal cells, predominantly thick hyphae of (2–) 3–4 (–4.5)  $\mu\text{m}$  width ( $n=32$ ). *Basidia* emerge through the stomatal openings from substomatal stromata, densely packed, pyriform to clavate to cylindrical, occasionally with probasidial swellings, (16–) 21–36.5 (–60)  $\times$  (2.5–) 3.5–5 (–6)  $\mu\text{m}$ , bisterigmatic. *Sterigmata* straight or curved, splayed slightly outwards with a slight bulge, (3–) 4.5–7 (–9.5)  $\times$  (1.5–) 2–3 (–3.5)  $\mu\text{m}$ , carrying a single basidiospore each. *Basidiospores* two-celled with a central septum at maturity or one-celled prior to germination, liberated in pairs or singly, hyaline, oblong to reniform, apex rounded, slightly narrowed towards the hilum, (11–) 12–14 (–16)  $\times$  (3.5–) 4–5 (–6)  $\mu\text{m}$ , germinating from one or both poles. Germ tubes 1–2  $\mu\text{m}$  wide ( $n=7$ ), often coiled, branched, hyphae forming conidia on sterigmata-like outgrowths. *Conidia* one-celled, fusiform or acerose, (2.5–) 3.5–6.5 (–9)  $\times$  0.5–1.5  $\mu\text{m}$ , hyaline. Colonies on PDA after

14 days of growth at 22 °C in the dark reach 7 mm diam., flat, gelatinous, corrugated, central colony Sienna (8) in colour and progresses outward to Luteous (12) and a Pale Luteous (11) border, comprised of conidia and hyphae, conidia forming on sterigmata-like outgrowths from hyphae or via budding.

**Notes:** *Kordyana luteoalba* infects leaves of *P. crispata* in shaded rainforest habitats extending from south-east Queensland south to the Shoalhaven-Illawarra region in New South Wales. *Kordyana luteoalba* is phylogenetically distinct from all other sequenced *Kordyana* species and is restricted to *P. crispata*. *Kordyana luteoalba* is most closely related to *K. tradescantiae* (only 2% sequence divergence in LSU). Morphologically, *K. tradescantiae* differs from *K. luteoalba* by the germination of basidiospores from the sides of spores in addition to the poles (Begerow et al. 2002), presence of paraphyses (Raciborski 1900), shorter basidia (10–15  $\mu\text{m}$ ), shorter sterigmata (3.5–5  $\mu\text{m}$ ) and shorter conidia (3–5  $\mu\text{m}$ ). Piepenbring et al. (2020) note that probasidial swellings have been observed in *K. tradescantiae*; however, they are not reported or described in descriptions provided by Petrak (1950), Raciborski (1900), Gómez and Kisimova-Horovitz (1997), or Piepenbring et al. (2010) (see Online Resource 3).

*Kordyana polliae* on leaves of *P. secundiflora* in Indonesia (Gäumann 1922) is the only other species recorded on *Pollia*. *Kordyana polliae* differs from *K. luteoalba* by the presence of paraphyses, absence of conidia and probasidial



**Fig. 2** Phylogenetic tree based on BI analysis of the alignment of the combined ITS, LSU, and SSU rDNA sequences from selected species of *Exobasidiales*. *Rhamsphospora nymphaea* (CBS 172.38) was used as an outgroup. Numbers on the branches indicate Bayesian posterior

probabilities (>0.70) and ML bootstrap support values (>70%). The scale bar represents the number of substitutions per site. Novel taxa are shown in bold. References to sequences obtained from ex-type cultures are marked by an asterisk (\*)

swellings, presence of one-celled basidiospores only, and slightly longer and wider basidiospores (15–21  $\mu\text{m} \times 5\text{--}8\ \mu\text{m}$ ) (Gäumann 1922). Although *K. polliae* lacks DNA sequence data, we propose that the Australian specimens represent a novel species as they can be differentiated from *K. polliae* by morphological features, host species, and geographical distribution.

***Kordyana occidentalis*** Zeil-Rolfe, Gooden, G.C. Hunter C.C. Linde & R.G. Shivas, sp. nov., Fig. 4.

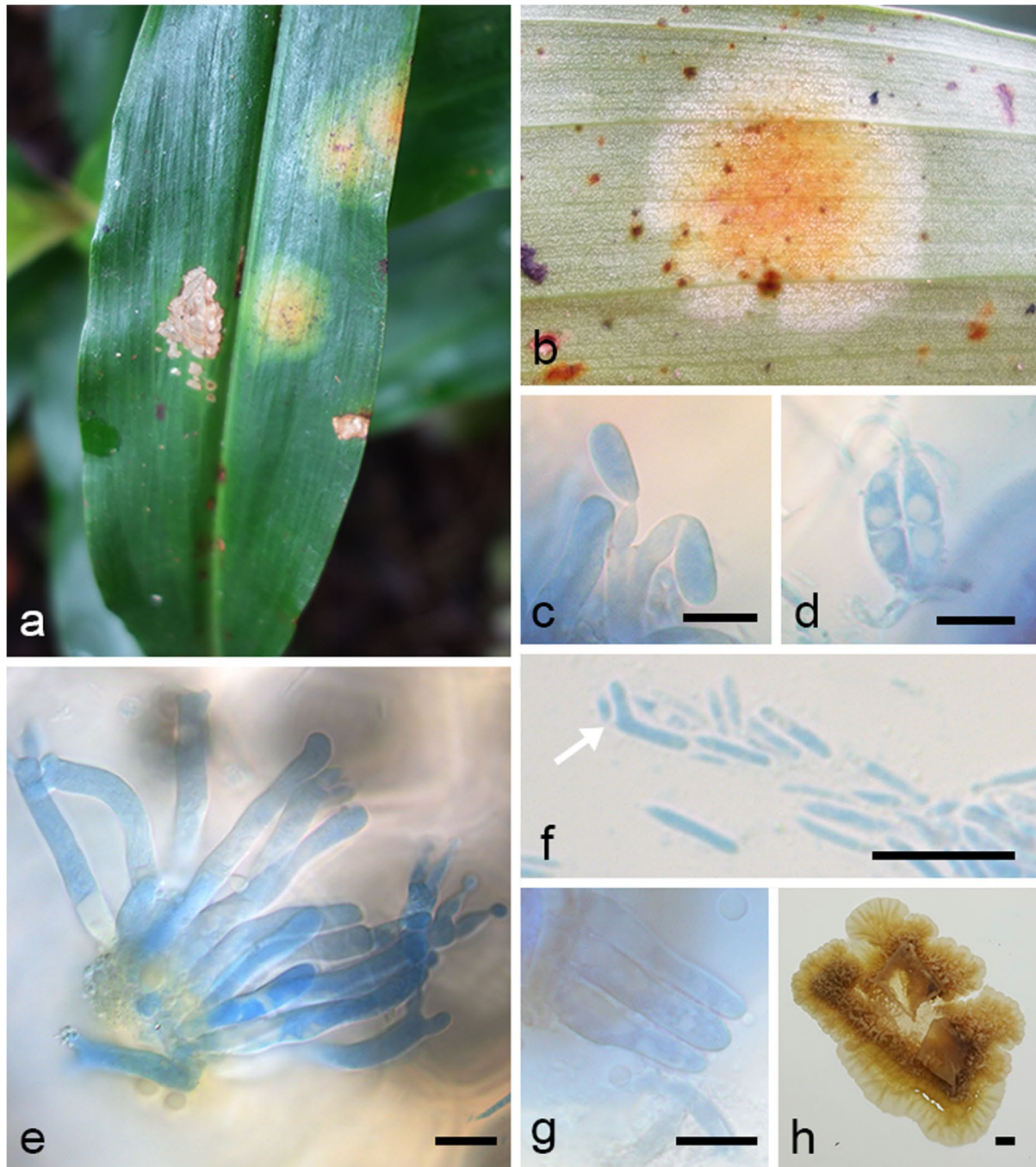
Mycobank: MB 852914.

**Holotype:** AUSTRALIA, Western Australia, Geikie Gorge, on leaves of *Commelina ensifolia*, 20 Apr. 2017, B. Lemana, K. Vánky, M.J. Ryley, S.M. Thompson, M.D.E. Shivas &

R.G. Shivas (BRIP 65743); ITS and LSU rDNA sequences GenBank OR791408 and OR764856, respectively. Additional specimens examined are shown in Table 1.

**Etymology:** Refers to the known distribution of this species in Western Australia.

**Leaf lesions** amphigenous, usually circular, coalescing into larger, irregular lesions, sporulation hypophyllous. **Balls of basidia** suprastomatal, densely packed with basidia of various ages, (42–) 53–84 (–95)  $\times$  (33–) 44–72 (–90)  $\mu\text{m}$  ( $n = 19$ ). **Basidia** holobasidia, single celled and hyaline, cylindrical with some probasidial swellings, bisterigmatic, (10.5–) 14.5–29 (–48)  $\times$  (2.5–) 3.5–5.5 (–6.5)  $\mu\text{m}$  ( $n = 33$ ). **Sterigmata** conical with a slight bulge in the middle, straight or slightly curved, (2.5–) 3–4.5 (–5.5)  $\times$  (1–) 2–2.5 (–3)  $\mu\text{m}$



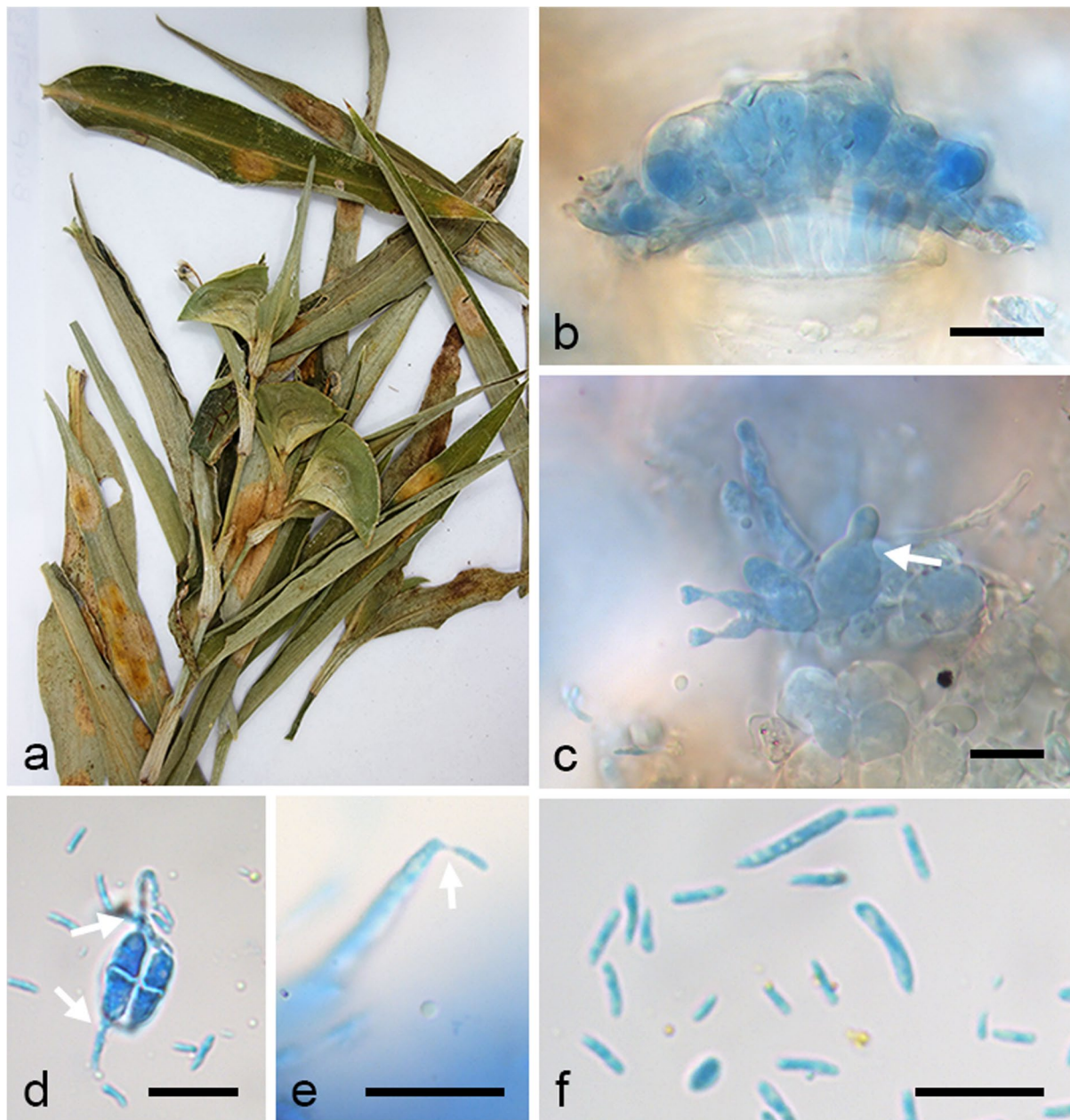
**Fig. 3** *Kordyana luteoalba* (BRIP 75725a) on *Pollia crispata*. **a** Adaxial leaf surface with lesions; **b** abaxial leaf surface with lesion; **c** two sterigmata with basidiospores; **d** bipolar germination of two

two-celled basidiospores; **e** ball of basidia; **f** conidia, one budding (arrow); **g** basidia, one with probasidial swelling; **h** colony on PDA after 8 wks. Scale bars: c–g = 10  $\mu$ m; h = 1 mm

( $n=47$ ), with a single basidiospore. *Basidiospores* liberated singly or in pairs, two-celled with a central septum at maturity or one-celled prior to germination, oblong to reniform, apex round, base narrower, (7–) 9–11.5 (–13)  $\times$  (2–) 3–4 (–6)  $\mu$ m, germinating with thin hyphae from one or both poles ca. 1  $\mu$ m wide, forming conidia on sterigmata-like outgrowths. *Conidia* abundant on the surface of leaf lesions, single

celled, rod-shaped to fusiform, apex curved, base acute, (2.5–) 3–5 (–6.5)  $\times$  0.5–1.5  $\mu$ m. Conidial budding was not observed. No cultures were obtained (isolations were not attempted from dried leaf samples).

*Notes:* *Kordyana occidentalis* infects the leaves of *C. ensifolia* in the Kimberley region of northern Western Australia (Table 1). Three other *Kordyana* species have been



**Fig. 4** *Kordyana occidentalis* (BRIP 65743) on *Commelina ensifolia*. **a** Dried leaves with lesions; **b** basidia emerging from a stomatal chamber; **c** basidia, one with sterigmata forming basidiospores, one

with probasidial swelling (arrow); **d** bipolar germination of basidiospores (arrows); **e** conidium on a sterigmata-like outgrowth (arrow); **f** conidia. Scale bars: b–f = 10  $\mu\text{m}$

described from *Commelina*, namely *K. celebensis* on *C. benghalensis* in Indonesia (Gäumann 1922, see Begerow et al. 2002 for reference sequence data); *K. commelinae* on *C. nudiflora* in Sri Lanka (Petch 1922, see Park et al. 2021 for reference sequence data); and *K. polliae* var. *microspora* on *C. maculata* in India (Narendra and Rao 1977, without sequence data).

*Kordyana commelinae* is phylogenetically most closely related to *K. occidentalis* but differs morphologically from *K. occidentalis* by the occasional formation of three

sterigmata per basidium (observed by Dudka 2023), an absence of probasidial swellings, conidial shape (described as globose by Petch 1922 and linear and acicular by Park et al. 2021), slightly narrower basidiospores (on av. 2.3  $\mu\text{m}$ ), and slightly shorter conidia (on av. 3.2  $\mu\text{m}$ ) (see Online Resource 3). *Kordyana celebensis* can be distinguished from *K. occidentalis* by phylogenetic analysis and differs by the absence of probasidial swellings and conidia, formation of one-celled basidiospores only, longer basidia (max. 60  $\mu\text{m}$ ), and slightly longer basidiospores (9–14  $\mu\text{m}$ ) (Gäumann

1922). *Kordyana polliae* var. *microspora* lacks sequence data, however, can be distinguished from *K. occidentalis* by host species, geographic distribution, and morphological characteristics by the presence of paraphyses, presence of one-celled basidiospores only, absence of probasidial swellings, and shorter basidiospores (6.7–7.6 µm) (Narendra and Rao 1977). Therefore, we propose that the Australian specimens on *C. ensifolia* represent a novel species as supported by morphological differences, unique host species, and distinct geographic range.

***Kordyana spectabilis*** Zeil-Rolfe, Gooden, G.C. Hunter C.C. Linde & R.G. Shivas, sp. nov., Fig. 5.

Mycobank: MB 852915.

**Holotype:** AUSTRALIA. New South Wales, Darkwood Road, rainforest, on leaves of *Aneilema acuminatum*, 30°27'01" S 152°36'39" E, 5 Jun. 2022, I. Zeil-Rolfe & J. Lester (BRIP 75724a includes a culture permanently preserved in a metabolically inactive state); ITS, LSU, and SSU rDNA sequences GenBank OR614368, OR802993, and OR616657, respectively. Additional specimens examined are shown in Table 1.

**Etymology:** Refers to the brightly coloured leaf lesions on the host.

**Leaf lesions** amphigenous, circular to irregular, solitary or coalescent. Sporulation hypophyllous, leaf lesions at first yellowish orange with a cream halo, later brown or orange with a pale yellowish green halo, covered by not pigmented balls of basidia. **Basidia** emerge through stomata from substomatal stroma, densely packed, clavate to cylindrical, (10–) 12–21 (–26) × (1–) 2–3 (–4) µm ( $n=28$ ), lacking probasidial swellings, bisterigmatic. **Sterigmata** straight or curved, slightly swollen, (2–) 3.5–5 (–5.5) × (0.7–) 1–1.5 (–2) µm ( $n=46$ ), with a single basidiospore each. **Basidiospores** one-celled or occasionally two-celled with a central septum, liberated in pairs or singly, hyaline, oblong to reniform or slightly allantoid, apex rounded, slightly narrowed towards hilum, (10–) 11–13 (–16) × (2–) 3–4 (–5) µm, germinating from one or both poles. **Germ tubes** 0.5–1.5 µm wide, branched, forming conidia on sterigmata-like outgrowths. **Conidia** one-celled, fusiform or slightly curved, (2.5–) 4–6.5 (–9) × 0.5–1.5 µm, hyaline. **Colonies** on PDA after 14 days growth at 22 °C in the dark reaching 7 mm diam., flat, gelatinous, colony centre Luteous (12) becoming paler towards the colony border presenting as Pale Luteous (11), comprised of conidia and hyphae, conidia forming on sterigmata-like outgrowths from hyphae or via budding.

**Notes:** *Kordyana spectabilis* infects leaves of *A. acuminatum* in shaded rainforest habitats in eastern Australia from south-east QLD to the Shoalhaven-Illawarra region, NSW. *Kordyana spectabilis* is phylogenetically distinct from all *Kordyana* species for which molecular barcodes are available. There are only two other reports of *Kordyana*

on *Aneilema*. One is *K. aneilematis* with the holotype collected on *A. angustifolium* in Taiwan (Sawada 1929 as *K. aneilemae*). The second is *Kordyana* sp. collected on *A. umbrosum* var. *ovato-oblongum* in Ecuador (collected by H. Sydow 1937 as *K. tradescantiae* according to Petrak 1950), which Petrak (1950) re-examined and noted that the lack of described morphological features meant species identification was not possible.

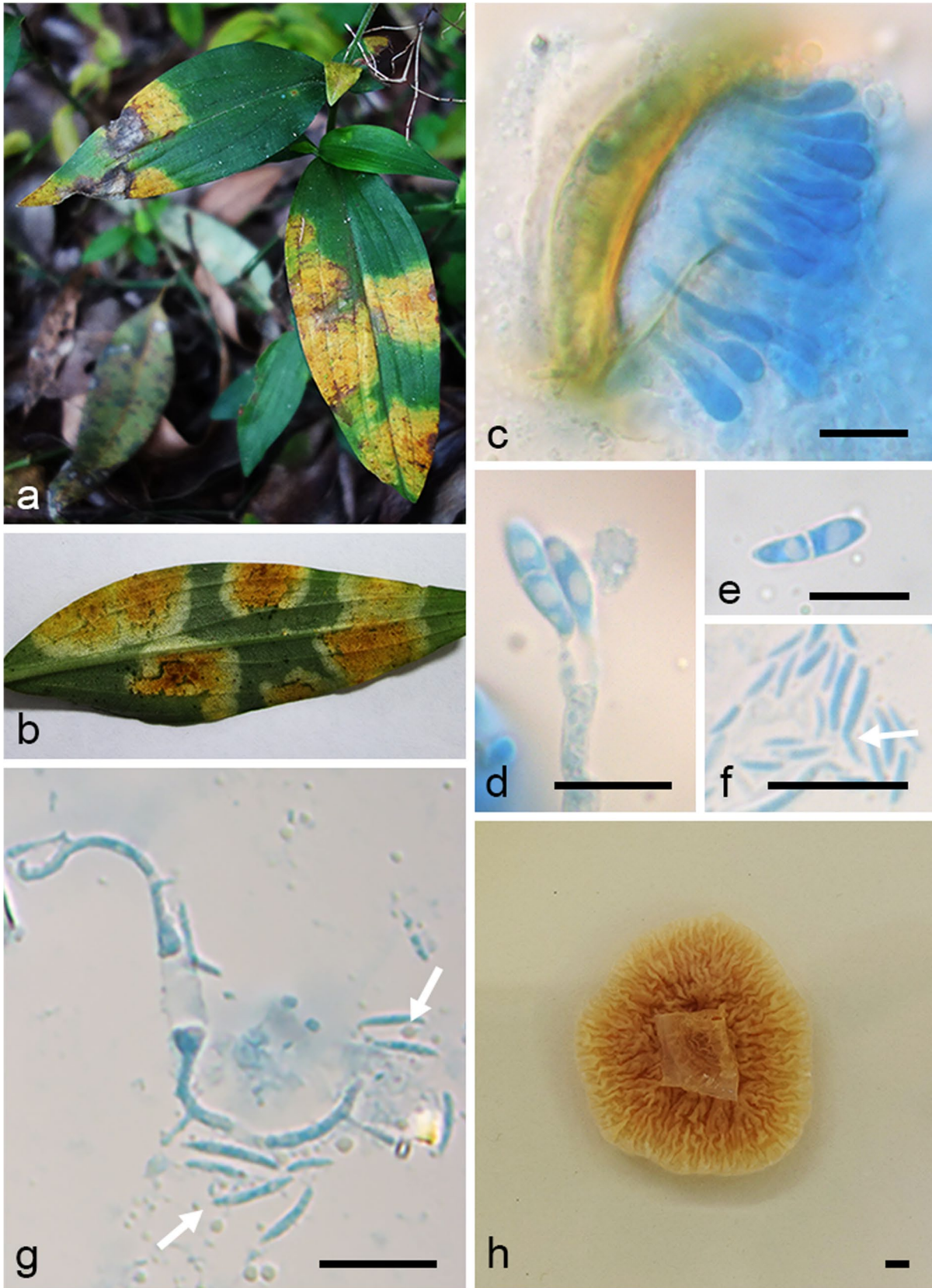
Morphologically, *K. spectabilis* is most similar to *K. aneilematis* and *K. commelinae*, lacking both paraphyses and probasidial swellings (see Online Resource 3). *Kordyana aneilematis* differs from *K. spectabilis* by longer and wider basidia (34–51 µm × 4–5 µm), slightly longer and wider sterigmata (5–7 µm × 2.5 µm), shorter basidiospores (10–14 µm), and the absence of conidia (Sawada 1929). *Kordyana commelinae* can be distinguished from *K. spectabilis* phylogenetically and morphologically by longer basidia (on av. 20 µm, Petch 1922; 25.9 µm, Park et al. 2021; 20–37 µm, Dudka 2023), narrower basidiospores (on av. 2.3 µm), and shorter conidia (on av. 3.2 µm) (Park et al. 2021) (see Online Resource 3). Despite the absence of sequence data from *K. aneilematis* for comparison, we have proposed the Australian specimens represent a novel species based on distinct morphological features, host species, and geographic distributions.

***Kordyana brasiliensis*** D.M. Macedo, O.L. Pereira & R.W. Barreto, Australas. Pl. Pathol. 45 (1): 51 (2016), Fig. 6.

Mycobank: MB 518070.

**Holotype:** BRAZIL. Minas Gerais, Viços, on living leaves of *Tradescantia fluminensis*, 10 Dec. 2009, D. M. Macedo (VIC 3136).

**Leaf lesions** amphigenous, circular to irregular, solitary or coalescent. Sporulation hypophyllous, leaf lesions pale white lesions becoming yellow to orange covered in white balls of basidia. **Balls of basidia** suprastomatal, spherical, densely packed with basidia of various developmental stages, germinated basidiospores and hyphae, (66–) 84–151 (–201) × (68–) 87–142 (–194) µm ( $n=40$ ). **Basidia** holobasidia, hyaline, cylindrical, some with probasidial swellings, bisterigmatic, (10–) 18–29 (–39) × (3–) 4–5 (–6) µm. **Sterigmata** conical in shape, straight or slightly curved, splayed slightly outwards, generally with a slight bulge, (3–) 3.5–5 (–6.5) × (1.5–) 2–3 (–3.5) µm, with a single basidiospore each. **Basidiospores** liberated singly or in pairs, one-celled or occasionally two-celled with a central septum after germination, oblong to reniform, somewhat allantoid, apex round, base narrowed, (12–) 14–18 (–19.5) × (3–) 3.5–3.5 (–5.5) µm, germinating by thin hyphae from one or both poles 1–2 µm wide forming conidia on sterigmata-like outgrowths. **Conidia** occasionally observed on the surface of leaf lesions, one-celled, fusiform or rod-shaped, often falcate, (4–) 5.5–12 (–17.5) × (0.5–) 1–1.5 (–2) µm, germinating to



**Fig. 5** *Kordyana spectabilis* (BRIP 75724a) on *Aneilema acuminatum*. **a** Lesions on the adaxial leaf surfaces of several leaves of *A. acuminatum* plant; **b** symptoms on the abaxial leaf surface; **c** basidia; **d** two basidiospores; **e** single two-celled basidiospore; **f** conidia, one budding (arrow); **g** basidiospore germinating from poles to form conidia (arrows) on small sterigmata-like outgrowths; **h** colony on PDA after 8 weeks. Scale bars: c–g = 10 µm; h = 1 mm

form hyphae. Colonies on PDA after 14 d growth at 22 °C reaching 7.5 mm diam., flat, Pale Luteous (11) in the centre of the colony and transitioning to Straw (46) at the colony border, comprised of conidia and hyphae, conidia forming on sterigmata-like outgrowths from hyphae or via budding.

**Specimen examined:** On leaves of *Tradescantia fluminensis*. AUSTRALIA. Australian Capital Territory, 35°16'301" S 149°06'49" E, cultured from material imported from Universidade Federal de Viçosa, MG, Brazil, 12 Jan. 2022, I. Zeil-Rolfe (BRIP 75726a).

**Notes:** *Kordyana brasiliensis* was introduced from its native range in Brazil into an Australian quarantine facility at CSIRO Black Mountain Science and Innovation Park, Australian Capital Territory (Morin et al. 2022). Since its field release in Australia in 2019, *K. brasiliensis* has spread across riparian habitats in rainforest and wet-sclerophyllous forests from north-eastern NSW southwards to the Dandenong Ranges in Victoria (ALA 2023; GBIF 2024; CSIRO 2023).

## Discussion

The taxonomic resolution of *Kordyana* is hampered as most species lack molecular barcodes. Further specimens and DNA sequence data are needed to clarify the taxonomy of this genus (Piepenbring et al. 2020). In this study, we provide molecular barcodes for three newly recognised Australian species, *K. luteoalba*, *K. spectabilis*, and *K. occidentalis*, together with phenotypic data, host associations, and geographic distributions. The three *Kordyana* species described in this study fit within the accepted concept of *Kordyana*, forming bisterigmatic basidia in suprastomatal balls, each basidium bearing two basidiospores which germinate generally becoming two-celled with hyphae that form conidia.

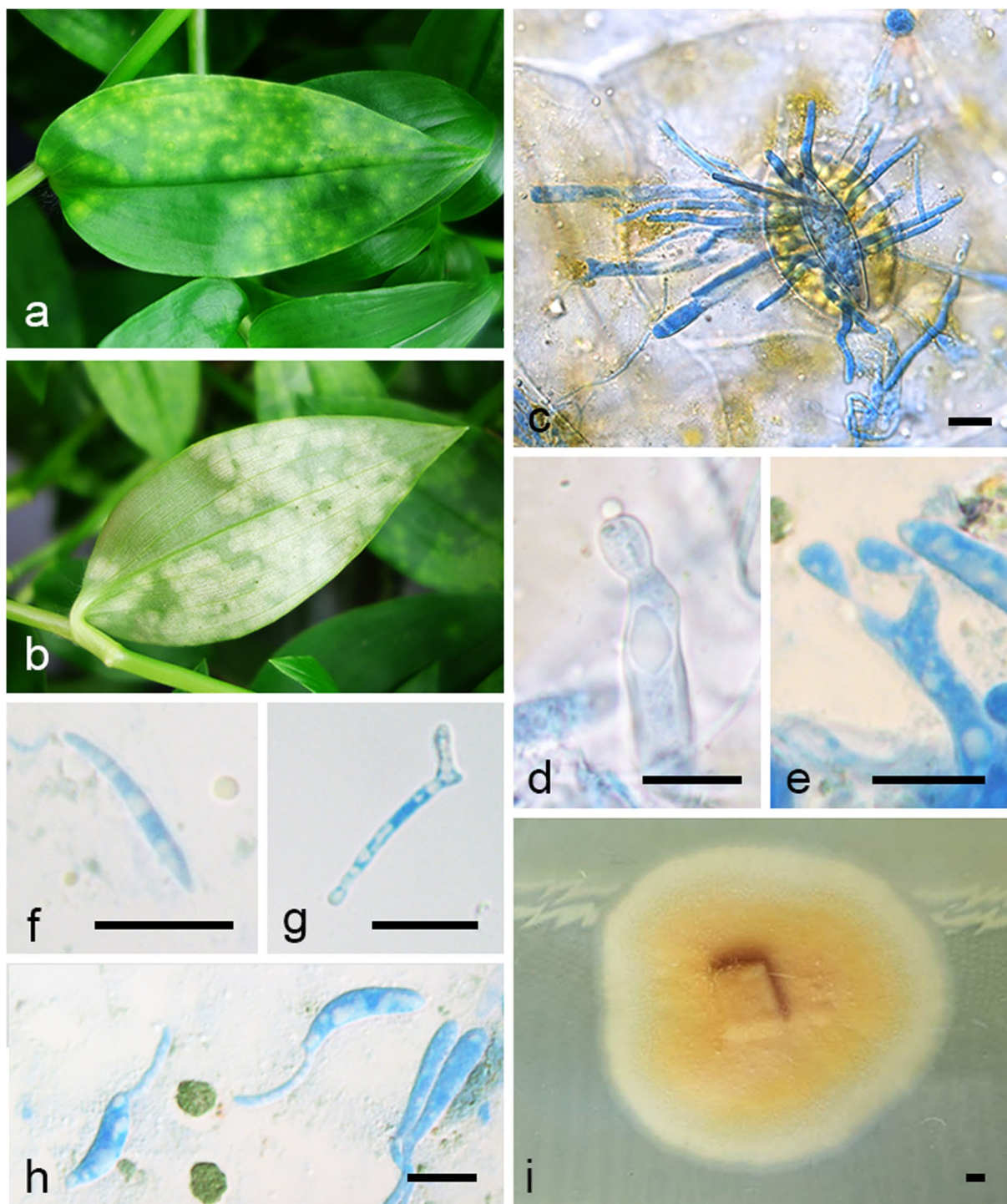
*Kordyana luteoalba*, *K. occidentalis*, and *K. spectabilis* are morphologically similar and can be differentiated by the absence of probasidial swellings and shorter basidia in *K. spectabilis*, the rod-shaped conidia in *K. occidentalis* and the longer basidia, slightly longer and wider sterigmata, and longer and wider basidiospores in *K. luteoalba*. The species can be differentiated from all other described *Kordyana* species from *Commelinaceae* hosts by phylogenetic data (if available), morphology, unique host species, and geographical distributions. Notably, all three Australian species lack paraphyses which are described in several *Kordyana*

species including *K. tradescantiae*, *K. polliae*, *K. polliae* var. *microspora*, and *K. commelinae* (Raciborski 1900; Gäumann 1922; Narendra and Rao 1977; Dudka 2023).

Distinguishing *Kordyana* species, particularly those lacking DNA sequence data, is complicated by the lack of complete morphological descriptions for several species (Piepenbring et al. 2020) and variability in the intraspecific morphological descriptions of collections (e.g. *K. tradescantiae* and *K. commelinae*). Descriptions of morphological details of *K. commelinae* and *K. tradescantiae* differ depending on the specimen studied as well as the author. For example, the description of *K. commelinae* from Russia includes the presence of paraphyses and occasionally basidia with three sterigmata (Dudka 2023), which differs to descriptions of *K. commelinae* from Sri Lanka and Korea (Park et al. 2021; Petch 1922). Specimens of *K. tradescantiae* are described as lacking paraphyses (e.g. Raciborski 1900, Gómez and Kisimova-Horovitz 1997; Piepenbring et al. 2010) and probasidial swellings (e.g. Raciborski 1900, Petrak 1950; Gómez and Kisimova-Horovitz 1997) except for Petrak (1950) who observed paraphyses and drawings by F. Oberwinkler (Piepenbring et al. 2020) and Piepenbring et al. (2010) who illustrated probasidial swellings. There are issues, however, with the use of probasidial swellings to differentiate taxa as they are commonly overlooked and common among *Brachybasidiaceae* species (Piepenbring et al. 2020).

Measurements of morphological features have been traditionally used to differentiate *Kordyana* species (e.g. Macedo et al. 2016; Park et al. 2021); however, comparisons of collections demonstrate that these also vary within a species. For example, Piepenbring et al. (2010) record significantly longer and wider basidiospores of *K. tradescantiae* compared to Raciborski (1900), Petrak (1950), and Gómez and Kisimova-Horovitz (1997). Basidia and basidiospore lengths vary between different descriptions of collections of *K. commelinae* (Petch 1922; Park et al. 2021; Dudka 2023). These issues highlight that the morphological approach to fungal classification of *Kordyana* species is unreliable and requires DNA barcodes to reliably distinguish species.

The discovery of *K. luteoalba*, *K. occidentalis*, and *K. spectabilis*, each apparently restricted to one of three native Australian species of *Commelinaceae* (*P. crispata*, *C. ensifolia*, and *A. acuminatum*, respectively), was a consequence of surveys before and after the release of *K. brasiliensis*. *Kordyana spectabilis* and *K. luteoalba* were found to be broadly distributed across eastern Australia, and *K. occidentalis* was only found in the Kimberly region in Western Australia. In 2019, *K. brasiliensis* was introduced into New South Wales and Victoria in eastern Australia as a classical biocontrol agent for *T. fluminensis*. The co-occurrence of white leaf lesions on *T. fluminensis* as well as on *A. acuminatum* and *P. crispata* in eastern Australia raised the concern that *K.*



**Fig. 6** *Kordyana brasiliensis* (BRIP 75726a) on *Tradescantia fluminensis*. **a** Adaxial leaf surface with lesions; **b** abaxial leaf surface with lesions; **c** basidia emerging from a stoma to form a suprastomatal ball; **d** basidium with probasidial swelling; **e** basidium with ster-

igmata and basidiospores; **f** conidia; **g** budding conidium; **h** germinating basidiospores; **i** colony on PDA after 12 weeks. Scale bars: c–h = 10  $\mu\text{m}$ ; i = 1 mm

*brasiliensis* may have extended its host range to native Australian *Commelinaceae*. This study provides strong evidence that *K. brasiliensis* remains highly host-specific to *T. fluminensis*.

The inclusion of additional sequence data for *K. brasiliensis*, *K. spectabilis*, *K. luteoalba*, and *K. occidentalis* has clarified the taxonomic relationship between *Kordyana* and *Dicellomyces*. We found that *Kordyana* formed



a well-supported clade sister to *Marantokordyana* and not a sister to *D. gloesporus*. Further, *M. nicotianae* was sister to *D. gloesporus* in our analysis rendering *Meira* polyphyletic.

Dimorphic life cycles that include an asexual yeast-like stage (conidia) are known for several taxa in the *Brachybasidiaceae*, including *K. spectabilis*, *K. luteoalba*, and *K. occidentalis* (in our study), as well as *Exobasidium*, *Marantokordyana*, and *Meira* (Ingram et al. 2019; Limtong et al. 2017; Park et al. 2021; Piepenbring et al. 2020; Tanaka et al. 2008). *Meira* spp. and one unknown species of *Kordyana* have been isolated from leaf phylloplanes (Albu 2012; Limtong et al. 2017; Tanaka et al. 2008). In a study of the diversity of phylloplane basidiomycetous yeasts on fern leaves in the USA, Albu (2012) used the spore-fall method and isolated *Kordyana* sp. from a senescent fertile frond of *Pelazoneuron kunthii* (*Polypodiales*). This may indicate that *Kordyana* spp. can survive epiphytically on the leaf phylloplane, either as basidiospores or conidia. Ungerminated conidia were often seen on the surface of leaf lesions around the suprastomatal balls of *K. spectabilis*, *K. luteoalba*, and *K. occidentalis*, in contrast to *K. brasiliensis*, which produced few conidia. The growth of these pathogens in a yeast-like state is thought to be advantageous for survival, dispersal, and multiplication (Bauer et al. 2001; Ingram et al. 2019).

This study demonstrates that knowledge of local fungal biodiversity can be increased by ecological monitoring of plant pathogens. We describe three previously unknown species of *Kordyana* on native Australian *Commelinaceae*, two of which co-occurred with the exotic and weedy *T. fluminensis*. *Kordyana spectabilis*, *K. luteoalba*, and *K. occidentalis* were discovered as a direct consequence of surveys before and after the release of *K. brasiliensis*. These three novel species are the first published records of *Kordyana* spp. on native *Commelinaceae* in Australia. Several other native Australian *Commelinaceae* species remain to be studied to determine if they also are hosts of *Kordyana* species. The collection of fresh specimens of *Kordyana* is required to further clarify the taxonomy, the intraspecific morphological variability, and distribution of these pathogens both in Australia and globally.

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**Author contribution** Isabel Zeil-Rolfe, Ben Gooden, Gavin Hunter, and Celeste Linde contributed to study conception and design. Data collection was undertaken by Isabel Zeil-Rolfe and Ben Gooden and data analysis by Isabel Zeil-Rolfe and Celeste Linde. The first draft of the manuscript was written by Isabel Zeil-Rolfe and Roger Shivas and all authors commented and edited the previous versions. The final manuscript was read and approved by all authors.

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**Data Availability** All sequence data generated in this study was deposited on GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Alignment of phylogeny is available in Supplementary Information S1.

## Declarations

**Competing interests** The authors declare no competing interests.

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