Nuytsia The journal of the Western Australian Herbarium

https://florabase.dbca.wa.gov.au/nuytsia/
https://doi.org/10.58828/nuy01059

Nuytsia 34: 203–219
Published online 26 October 2023

Marasmius australotrichotus (Marasmiaceae), a new setose species from Australia, and an intriguing range extension for *M. paratrichotus*

Frances E. Guard[1], John Dearnaley[2], Teresa Lebel[3,4], Matt D. Barrett[5] and Neale L. Bougher[6]

[1] University of Southern Queensland, Toowoomba, Queensland 4350
[2]School of Agriculture & Environmental Science, Faculty of Health, Engineering & Sciences, University of Southern Queensland, Toowoomba, Queensland 4350
[3]State Herbarium of South Australia, Hackney Road, Adelaide, South Australia 5000
[4]Royal Botanic Gardens Victoria, Private Bag 2000, Victoria 3141
[5]Australian Tropical Herbarium, James Cook University, PO Box 6811, Cairns, Queensland 4870
[6]Western Australian Herbarium, Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983
[1]Corresponding author, email: franguard@icloud.com

Abstract

Guard, F.E., Dearnaley, J., Lebel, T., Barrett, M.D. & Bougher, N.L. *Marasmius australotrichotus* (Marasmiaceae), a new setose species from Australia, and an intriguing range extension for *M. paratrichotus*. *Nuytsia* 34: 203–219 (2023). Based on morphological characters and molecular analysis of the nrITS regions, *Marasmius australotrichotus* F.E.Guard, J.Dearnaley & T.Lebel, the first known Australian species in sect. *Sicci*, ser. *Spinulosi* is described. The distribution of *M. paratrichotus* is extended from the West African island nation of São Tomé and Príncipe (ST&P) to Christmas Island (CI) and northern Western Australia (WA). Classification, distinguishing characters, habitat variability, geographic distribution and possible dispersal mechanisms are discussed.

Introduction

Marasmius Fr. is a widespread genus of basidiomycete fungi, particularly well-represented in the tropics and subtropics. *Marasmius* species are usually saprotrophs, and most are small to medium-sized fungi (1–20 mm pileus diameter), decomposing twigs and leaf litter in rainforest, wet sclerophyll forest, and disturbed habitats including road verges, gardens and lawns. Traditionally, *Marasmius* was divided into sections and subsections by morphological features (Singer 1986; Antonin & Noordeloos 2010). Antonin and Noordeloos recognised four sections: *Marasmius, Hygrometrici, Globulares* and *Sicci* (including series *Atrorubentes*, *Haematocephali*, *Leonini* and *Spinulosi*) in *Marasmius sensu stricto*. Their work was based on morphology alone, and while recognising that phylogenetic data would be required to clarify the genus, they preserved the older concepts. Section *Sicci* Singer, subsect. *Siccini* Singer, ser. *Spinulosi* was erected to accommodate *Marasmius* species with non-insititious stipes, dextrinoid hyphae, and pileal, stipe and/or hymenial setae (Clémençon 1982). The presence of setae was the distinguishing feature of this series. Section *Sicci* Singer, subsect. *Siccini* Singer, ser. *Atrorubentes* (Desjardin & Horak 1997) was erected to correct some confusion caused by Singer misnaming the series *Actinopus*, and to include species without pileal or hymenial setae,

ISSN 2200-2790 (Online)
ISSN 0085-4417 (Print)

but with pruinose stipes produced by irregular, cylindrical, cystidioid or setoid cells, and absence of *Siccus*-type broom cells from the stipe.

The type species of ser. *Spinulosi*, *M. cohaerens* (Pers.) Cooke & Quél., was originally collected from Germany, and it is now considered to occur across Europe and North America (Antonin & Noordeloos 2010). Other species in ser. *Spinulosi* sensu Singer have been reported from Africa (Shay *et al.* 2017; Grace *et al.* 2019), south-east Asia (Tan *et al.* 2009; Wannathes *et al.* 2009), Korea (Antonín *et al.* 2012), Far-eastern Russia (Kiyashko *et al.* 2014) and Argentina (Niveiro *et al.* 2018). These vary in the presence of pleurocystidia, being present in many but absent in others, e.g., the 'trichotus' group. Some taxa have smooth cells (*Globulares*-type) in the pileipellis, while others have *Siccus*-type broom cells. Caulocystidia are variable and within closely related taxa may include *Siccus*-type broom cells, transitional cells with narrow body and sparse elongated stellate branches, irregular, cylindrical blunt cells, intermixed with long, lanceolate, thick-walled setae (Figure 1). The morphological distinction between ser. *Spinulosi* with setoid caulocystidia and ser. *Atrorubentes* with obtuse caulocystidia was not always clear as transitional forms have been shown to occur (Desjardin & Horak 1997; Antonin 2007).

Tan *et al.* (2009), Wannathes *et al.* (2009), Shay *et al.* (2017) and Grace *et al.* (2019) have produced a large body of molecular data based on nrITS gene region for *Marasmius* sensu stricto. Their findings confirmed that while nrITS is very useful for delimiting species and confirming closely related morphogroups, it does not support the morphology-based infrageneric classification. None of the series in the traditional concept of sections *Globulares* and *Sicci* are monophyletic in these studies. However, a recent study by Oliveira *et al.* (2020), which focussed on sect. *Globulares* (*Globulares-Sicci* complex), based on a larger global dataset, using both nrITS and the more conserved marker, LSU, showed that historical 'ser. *Spinulosi*' species appeared in at least three unrelated clades, grouping with *M. cohaerens*, *M. purpureostriatus* and *M. atrorubens*. That study demonstrated the existence of several monophyletic supra-specific groups, equating to Singer's 'stirpes', a classification level below series for grouping morphologically similar species (Singer 1976). Oliveira *et al.* (2020) raised subsection *Spinulosi* J.S.Oliveira & Moncalvo, with type species *M. cohaerens* within series *Cohaerentes* J.S.Oliveira & Moncalvo, and placed series *Atrorubentes* Desjardin & E.Horak emend. J.S.Oliveira & Moncalvo in subsection *incertae sedis*. In their study, series *Atrorubentes* included *M. trichotus* Corner.

Marasmius trichotus, described from Singapore (Corner 1996), is a distinctive SE Asian species, with currently known distribution from India to Papua New Guinea with a habitat preference for tropical rainforest. Its morphological characters include conspicuous golden setae on the pileus and golden to rusty-brown setae on the stipe (Corner 1996; Desjardin & Horak 1997; Wannathes *et al*. 2009). More recently, Grace *et al*. (2019) described *M. paratrichotus* C.L.Grace, Desjardin & B.A.Perry from a single collection in the West African island nation of São Tomé and Príncipe (ST&P). It is similar to *M. trichotus*, but with a dull, velvety pileus and pubescent stipe.

Extensive sampling of *Marasmius* species in north-eastern Australia in 2001, 2018 and 2021, combined with unique opportunities to collect fungi in the remote Kimberley region of Western Australia and Christmas Island, revealed several collections which had distinct setae on the pileus and stipe. While morphologically similar to *M. trichotus*, our analyses show that some of the collections represent a new taxon, *M. australotrichotus sp. nov.*, which we describe here, while two are an intriguing range extension for *M. paratrichotus*.

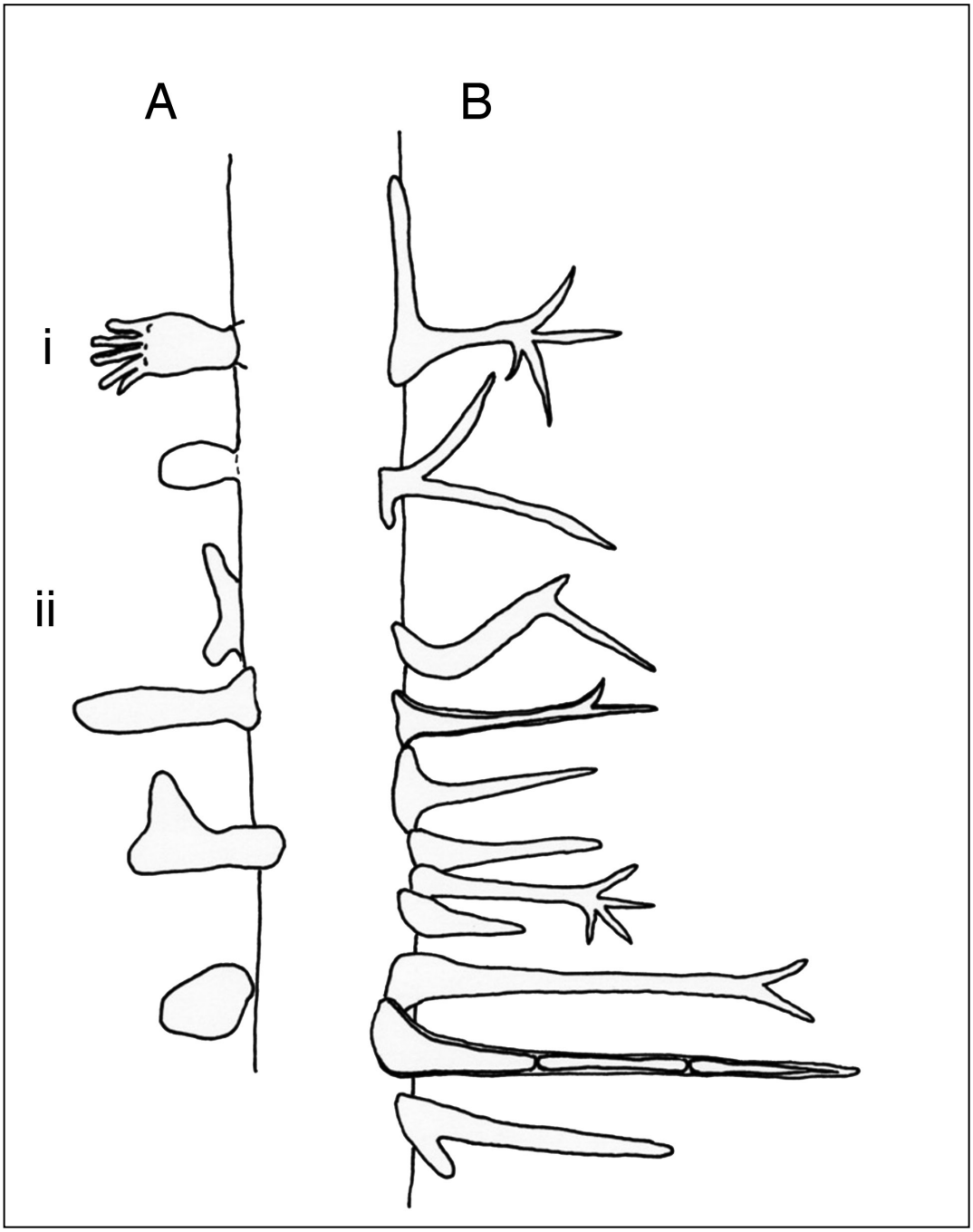

Figure 1. Variability of stipe cystidia within *Marasmius australotrichotus* and *M. paratrichotus* and usage of terms '*caulocystidia*' and '*caulosetae*'. A. *Caulocystidia* – i. *Siccus*-type broom cells with terminal digitate projections and ii. irregular shaped cystidia with obtuse apices. B. *Caulosetae* – lanceolate, bifurcate, branched, stellate cystidia with acute apices. In this study all cells with long pointed apices are termed *caulosetae*.

Materials and methods

Sampling protocols

Collections of *M. australotrichotus* were made in far north Queensland (2018, 2021 and 2022) under Queensland Department of Environment and Science Permits No. WITK18734918-1 to 2021 and P-PTUKI-100021825-1 to 2023. One collection of *M. paratrichotus* was made by M.D. Barrett at Theda Station, north Kimberley, WA, under Western Australian Scientific Purposes license SW008226 in 2003 and the other by N.L. Bougher and K. Ruthrof on Christmas Island in 2016. Field work on Christmas Island was carried out under Commonwealth areas Permit AU-COM2015-295 and Christmas Island National Park Permit CINP_2015_16_3.

These collections were supplemented with herbarium material from the Queensland Herbarium (BRI) and the National Herbarium of Victoria (MEL) including further specimens of *M. australotrichotus* from far north Queensland and south-east Queensland, as well as one from the Northern Territory. The Australian specimens of *M. paratrichotus* are held in the Western Australian Herbarium (PERTH).

Morphological protocols

Fresh collections were photographed, described and a sample placed in silica gel prior to DNA analysis. The basidiomes were then dried using an *Ezidri* Snackmaker FD500 (Hydraflow Industries Ltd, Upper Hutt, NZ) food dehydrator at the lowest setting for at least 12 hours. Spore prints were made where possible. Macroscopic features including details of pileus, lamellae, stipe, substrate and habit were recorded from the fresh material. Colours were recorded according to the Flora of British Fungi Colour Identification Chart (Royal Botanic Gardens 1969).

Microscopic characters of hand-sectioned dried material were examined using a Prism Optical (Model EX-30T) compound microscope with Tucsen GT12 camera (Tucsen Photonics Co., China) with a 100× objective. Sections were rehydrated and examined in Congo Red in water, in 5% potassium hydroxide (KOH) or Melzer's Reagent. Microscopic details were recorded with Mosaic V2.0 software (http://www.tucsen.com). For spore measurement, the abbreviation Q refers to the length/width ratio, Q_m the mean of Q values. Values in square brackets are standard deviation [SD] for mean measurements of length and width and Q_m values. Caulocystidia include *Siccus*-type broom cells and irregular, smooth, terminally obtuse, cylindrical cells. Lanceolate, solitary, branched or stellate cells with acute apices are termed caulosetae in this paper, and transitional forms are also termed caulosetae, if they have acute apices (Figure 1).

Molecular protocols

Samples from each collection were ground with 2 lead pellets in a bead mill (Fast Prep-24™ 5G, MP Biomedicals, Ca., USA). Genomic DNA was extracted using the E.Z.N.A. Forensic DNA Extraction Kit (Omega Bio-tek Inc., Norcross, GA), following the standard protocol for hair, nails and feathers, apart from substituting 0.8 µL β mercapto-ethanol for 20 µL 1M DTT and using 50 µL elution buffer instead of 100 µL for a more concentrated extract. The nuclear ribosomal internal transcribed spacer region (ITS1+5.8S+ITS2) was amplified using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) in a polymerase chain reaction (PCR) mixture of 1 µL (10 pmol/ µL) of each primer, 12.5 µL My Taq Red Mix, 0.8 µL of 10% bovine serum albumin (BSA), 7.7 µL sterile water and 2 µL DNA extract. The nuclear ribosomal large subunit (nrLSU) gene region was amplified using LR7 and

LROR primers (Vilgalys & Hester 1990) in a PCR mixture of 1 µL (10 pmol/ µL) of each primer, 12.5 µL My Taq Red Mix, 0.8 µL 10% BSA, 7.7 µL sterile water and 2 µL DNA extract. The PCR thermal cycling conditions included 35 cycles of 95°C for 1 minute, 51°C for 1 minute and 72°C for 1 minute, with a final extension step of 72°C for 10 minutes for the ITS, and 95°C for 1 minute, 48°C for 1 minute, 72°C for 1 minute, with a final extension step of 72°C for 10 minutes for LSU. Successful PCR amplification was checked by gel electrophoresis. PCR products were sent to Macrogen, Seoul, for Sanger sequencing using the original amplification primers.

Sequence editing was done within Geneious Prime 2022.1.1. (https://www.geneious.com) and initial alignment was performed using MAFFT (Katoh & Standley 2013). Sequences were trimmed, then edited manually. A BLAST search against the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) was used to determine closest sequence matches. The final alignment included sequences from taxa in ser. *Spinulosi* sensu Singer and ser. *Atrorubentes* as proposed by Oliveira *et al.* (2020). Newly generated sequences (in **bold**) used in the construction of the phylogenetic tree are shown in Table 1.

Maximum likelihood phylogenetic analysis was conducted using RAxML 8.2.11 (Stamatakis 2014) with the GTRGAMMA model using default parameters and 1,500 rapid bootstrap (BS) replicates. Bayesian analysis was performed with Mr Bayes 3.2.6 (Huelsenbeck 2001) using the substitution model GTR and Metropolis Coupled (MCMC) settings, for 50,000 iterations. New sequences were registered with GenBank.

Results

The nrITS phylogeny of the *Marasmius* taxa sampled for this study is shown in Figure 2. The eight samples of the putative new species *M. australotrichotus* formed a monophyletic clade, with ML bootstrap support of 97% and posterior probability (PP) of 1.0. All sequences of *M. australotrichotus* were 98.94–99.55% identical with each other. The sister species to *M. australotrichotus* was resolved as *M. neotrichotus* Niveiro, N.A.Ramírez & Antonin from Argentina (Niveiro *et al*. 2018) with low to moderate support (56% BS, 0.97 PP). The two new specimens of *Marasmius paratrichotus* were resolved as a clade with the type specimen from São Tomé, with ML bootstrap support (BS) of 97% and posterior probability (PP) of 0.95. The sequences of *M. paratrichotus* were 99.77–99.96% identical with each other. *M. paratrichotus* had a close sister relationship to *M.* aff. *trichotus* from India (73% BS, 0.94 PP), and also *M. trichotus* from Thailand, (97% BS, 1.0 PP for the combined clades). The type species for ser. *Spinulosi*, *M. cohaerens* belonged to a clade distant to the taxa of this study (Figure 2).

Table 1. Dataset of 53 *Marasmius* species used in the phylogenetic analysis for this study. New sequences in **bold.**

Species	Sect./Series Traditional classification	Collection #	GenBank # ITS/LSU
M. atrorubens	*Sicci/Atrorubentes*	JO528	KP635207
M. atrorubens	*Sicci/Atrorubentes*	JO489	KP635206
M. australotrichotus	***Sicci/Spinulosi***	**AMW1F**	**OQ725921**
M. australotrichotus	***Sicci/Spinulosi***	**PIF26879**	**OQ725926**
M. australotrichotus	***Sicci/Spinulosi***	**GMB-2014**	**KP012696**
M. australotrichotus	***Sicci/Spinulosi***	**SMF 3296**	**OQ725923/OQ725916**
M. australotrichotus	***Sicci/Spinulosi***	**PIF27894**	**OQ725925/OQ725917**

Species	Sect./Series Traditional classification	Collection #	GenBank # ITS/LSU
M. australotrichotus	***Sicci/.Spinulosi***	**F2021072**	**OQ725920/OQ725914**
M .australotrichotus	***Sicci/Spinulosi***	**Cribb-249**	**OQ725927**
M. australotrichotus	***Sicci/Spinulosi***	**MDB F2022110**	**OQ725924**
M. chrysoblepharioides	*Sicci/Spinulosi*	SI-15-24	MF683956
M. cohaerens	*Sicci/Spinulosi*	TENN 067870	KF774179
M. cohaerens	*Sicci/Spinulosi*	TENN 067916	KF774178
M. cohaerens	*Sicci/Spinulosi*	BRNM 695761	GU266260
M. cohaerens	*Sicci/Spinulosi*	LE295982	KF774174
M. cohaerens var. mandshuricus	*Sicci/Spinulosi*	LE295986	KF774171
M. cohaerens var. lachnophyllus	*Sicci/Spinulosi*	iNAT:16190382	MZ197975
M. corrugatiformis	*Sicci/Atrorubentes*	Buyck 97.425	KX148981
M. dendrosetosus	*Sicci/Spinulosi*	JES 205	NR158833
M. dendrosetosus	*Sicci/Spinulosi*	JES 211	KX148996
M. elegans	*Sicci/ Atrorubentes*	JAC13253	OQ282799
M. elegans	*Sicci/ Atrorubentes*	JAC10928	OQ282785
M. grandisetulosus	*Sicci/Spinulosi*	DED 8225	KX953743
M. grandisetulosus	*Sicci/Spinulosi*	DED 8257	KX953744
M. grandiviridis	*Globulares*	NW152	EU643514
M. hinnuleus	*Sicci/Haematocephali*	JES 217	KX148988
M. hypophaeus	*Sicci/Haematocephali*	NW285	EU935484
M. iras	*Sicci/Atrorubentes*	NW276	EU935486
M. iras	*Sicci/Atrorubentes*	NW375	EU935487
M. katangensis	*Sicci/Atrorubentes*	JES 227	KX148991
M. laticlavatus	*Globulares*	NW412	EU643511
M. longisetosus	*Sicci/Spinulosi*	JO248	JX424040
M. longisetosus	*Sicci/Spinulosi*	SP 417470	NR154160
M. megistus	*Sicci/Leonini*	JES 163	KX148992
M. neotrichotus	*Sicci/Spinulosi*	SI-7-13	MF683958
M. nummularius	*Sicci/Spinulosi*	NW396	EU935493
M. nummularius	*Sicci/Spinulosi*	JES121	KX148979
M. ochroleucus	*Sicci/Atrorubentes*	LE 295978	KF912952

Figure 2. Phylogenetic analysis for *Marasmius australotrichotus* and *M. paratrichotus* inferred from a Bayesian analysis of the nrITS region (ITS1, 5.8S rDNA and ITS2). ML bootstrap proportions and Bayesian posterior probabilities are shown as BS/PP respectively. Nodes receiving support values greater than 95/0.95 are represented by **bold** branches. *Marasmius* sequences indicated in red type are *M. australotrichotus sp. nov.* from this study, and in blue type *M. paratrichotus*, including the holotype KX935749 from São Tomé. New sequences are in bold. The traditional classification is included as follows: SA = Subsect. *Siccini*, ser. *Atrorubentes*, SH = ser. *Haematocephali*, SL = ser. *Leonini*, SS = ser. *Spinulosi*, G = *Globulares*. The new classification as proposed by Oliveira is added where possible.

Taxonomy

Marasmius australotrichotus F.E.Guard, Dearnaley & T.Lebel, *sp. nov.*

Type: Queensland, Trinity Beach, S 16° 47’ 52”, E 145° 41’ 09”, 31 December 2022, *M.D. Barrett* MDB F2022110 (*holo*: BRI AQ1039752). [MB 848444].

Pileus 5–15 mm diam., apricot (47) to rust (13) becoming orange-yellow with rusty tawny (14) centre, convex, campanulate, almost applanate, occasionally umbonate, smooth with wavy margin, surface finely velutinous, dry, *flesh* very thin (<1 mm), off-white. *Lamellae* free to adnexed, sub-close, 14–16, with 3–4 tiers of lamellulae, cream (4D) to buff (52), non-marginate. *Stipe* 25–60 (–80) × 0.5–1 mm, central, reddish-brown strigose base, pale brown mid-section and cream apex, hollow, cartilaginous, surface may appear smooth but under a hand lens is finely hispid with brownish hairs. (Figure 3)

Basidiospores 10.5–13 × 3.5–4.5 µm, mean 11.9 [± 0.69 SD] × 3.9 [± 0.33 SD] µm, Q = 2.49–3.71, Q_m = 3.08 [± 0.29 SD], (n = 50 spores from 4 specimens), narrow ellipsoid in face view, often flattened adaxially and slightly curved in profile, smooth, hyaline, thin-walled, inamyloid. *Basidia* three to four-spored, rare, 21–23 × 6–7.5 µm. *Basidioles* 20–24 × 5–7 µm, fusiform, occasionally clavate. *Cheilocystidia Siccus*-type broom cells, often forming a sterile edge, cylindrical, clavate, occasionally branched, main body, 10–19 × 5–9 µm, with multiple apical setulae, 2.5–6.5 × 1–1.5 µm, apices of setulae obtuse to sub-acute. *Lamellar trama* composed of thin-walled hyphae, 4–5 µm diam., inamyloid to faintly dextrinoid. *Pileal trama* hyphae 4–7.5 µm diam., thin-walled, faintly dextrinoid. *Pileipellis* a hymeniderm of two cell types: 1. common *Siccus*-type broom cells, hyaline, inamyloid, cylindrical, clavate, sometimes thick-walled, main body 13.5–22 × 5–9 µm, with multiple apical erect to slightly divergent thick-walled setulae, 4–6(–8) × 1–1.5 µm, and; 2. moderately common lanceolate setae, 50–130(–166) µm length, 4–6 µm mid-shaft diam., mostly thick-walled, up to 2 µm thick, rarely with cross-walls, apices acute, bases bulbous to boot-shaped. *Pleurocystidia* absent. *Stipe trama* dextrinoid, composed of parallel hyphae, 5–6 µm diam. *Caulosetae* very common, inamyloid, lanceolate, with thick walls to 1.5 µm, occasionally forked or branched 30–95(–200) µm, and rarely stellate, apices acute and bases broad and irregular to boot-shaped, branches to 45 µm length; and occasional thick-walled, blunt, irregular caulocystidia, 20–35 × 10 µm. *Clamp connections* present in all tissues. (Figure 4)

Other specimens examined. QUEENSLAND: Gap Creek Reserve, Brisbane, 5 Jan. 2008, *A.B. Cribb* Cribb-246 (BRI AQ1023825) and Cribb-249 (BRI AQ1023828); Mt Coot-tha, Brisbane, 24 Mar. 2021, *N. Fechner* NAF210304 (BRI AQ1021623); Irvinebank Hotel, Herberton, 20 Feb. 2001, *P.I. Forster, R. Booth & A.M. Young* PIF26879 (BRI AQ669468); Rex Range, Julatten, 1 Dec. 2001, *P.I. Forster, R. Booth & A.M. Young* PIF27894 (BRI AQ553476); Townsville, 28 Feb. 1997, *B.A. Fuhrer* 2084 (MEL 2037493); Davies Creek National Park, 13 Feb. 2021, *F.E. Guard, R. Palmer & T. Lebel* F2021058 (BRI AQ1033129); Clacherty Rd, Julatten, 17 Feb. 2021, *T. Lebel & J. Dearnaley* F2021072 (BRI

Figure 3. *Marasmius australotrichotus* (F2021058) field photo of sporing bodies. Scale bar = 10mm. Inset image of stipe. Scale bar = 2mm. Inset images of pileal surface and lamellae. Scale bar = 10mm. Photographs F.E. Guard.

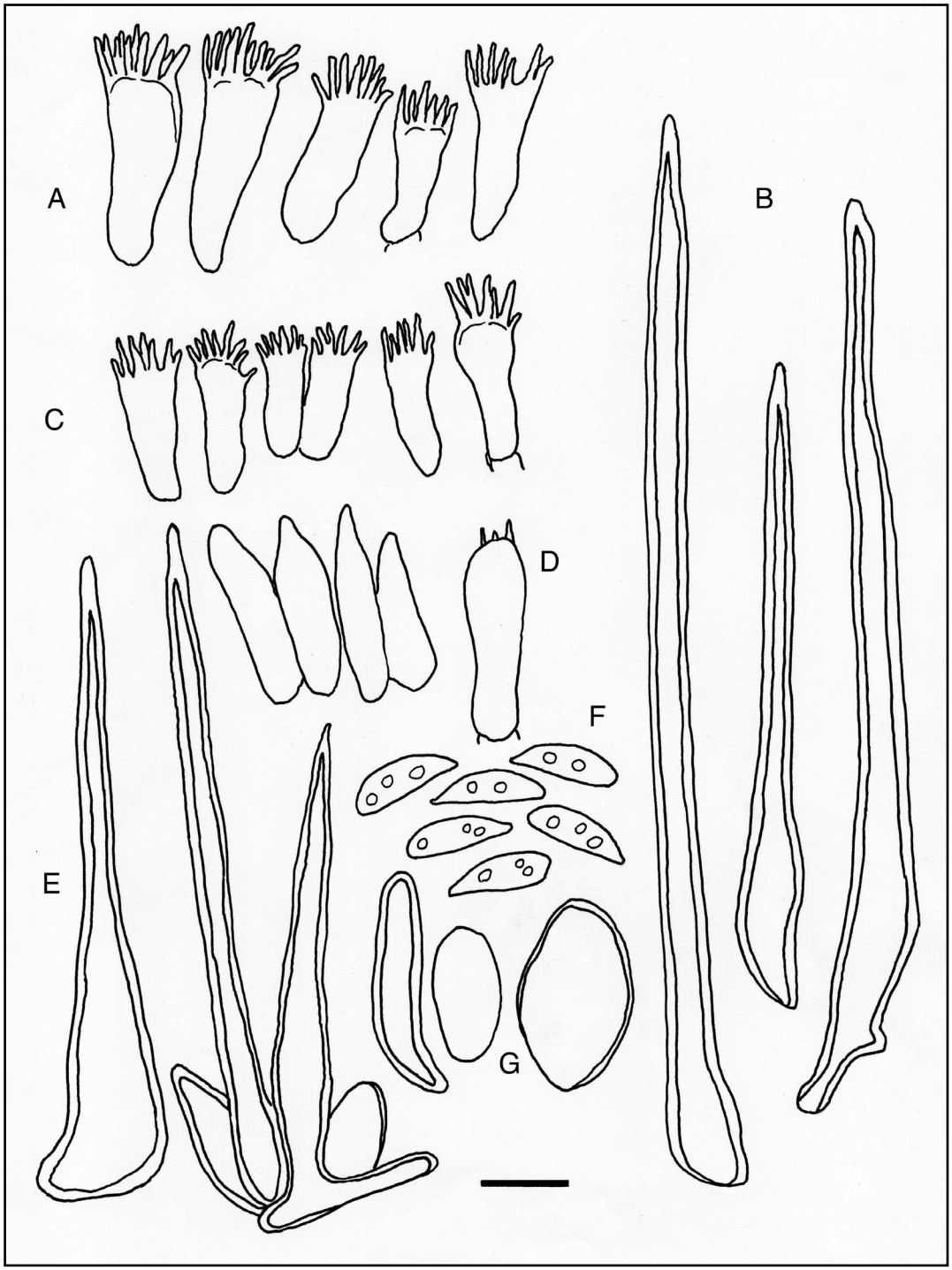

Figure 4. *Marasmius australotrichotus* (MDB F2022110) micromorphology. A – pileipellis *Siccus*-type broom cells, B – pileosetae, C – cheilocystidia, D – basidioles and basidium, E – caulosetae, F – spores, G – caulocystidia. Scale bar = 10 µm.

AQ1033131); The Fort Bushland Reserve, Oxley, Brisbane, 25 Mar. 2017, *M. Prance* 20170327MP4 (BRI AQ1023218); Meiers Rd, Indooroopilly, Brisbane, 16 Feb. 1998, *A.M. Wood* AMW1F (BRI AQ662716). NORTHERN TERRITORY: Palmerston, 21 Jan. 2014, *G.M. Bonito, M.D. Barrett, T. Lebel & C.N. Barrett* GMB469 (MEL 2382816).

Habit, habitat & distribution. Generally single, rarely caespitose, occurring in groups on leaf litter in *Eucalyptus/Acacia/Allocasuarina* forests. Also occurring on grassy road verges and in mulched garden beds from tropical to subtropical habitats. The species occurs in native vegetation but is also moderately common in suburban and disturbed areas. It has been found in Far North Queensland from Julatten to Townsville, South East Queensland, suburban Brisbane, and the Top End region of the Northern Territory.

Conservation status. Because of the wide distribution across multiple habitat types, *M. australotrichotus* is not considered to be under threat.

Etymology. The epithet is from the Latin *australis*, referencing its southerly global distribution, and acknowledges its close morphological and phylogenetic relationship to *Marasmius trichotus* (Corner 1996).

Notes. *Marasmius australotrichotus* has macroscopic features resembling the well-known Australian temperate species, *M. elegans* (Cleland) Grgur. However, *M. australotrichotus* has long, thin, velutinous stipe and small, velutinous orange-brown pileus, whereas *M. elegans* (Grgurinovic 1997; Bougher & Syme 1998) is more robust with smooth stipe 20–50 × 3–5 mm, (25–60(–80) × 0.5–1 mm in *M. australotrichotus*) and smooth pileus 12–50 mm diam. (10–40 mm diam. in *M. australotrichotus*), making them distinguishable in the field. *Marasmius elegans* also differs by lacking pileosetae and caulosetae. *Marasmius elegans* belongs to Sect. *Sicci*, ser. *Leonini* (sensu Singer 1976) and is phylogenetically distant from *M. australotrichotus* (Figure 2). The recently described *M. vagus* F.E.Guard, M.D.Barrett & Farid (Crous *et al*. 2020) is also similar, and has a similar distribution to *M. australotrichotus*, but is more robust (pileus 10–40 mm diam., stipe 3–5 mm diam., compared to pileus 5–15 mm diam. and stipe 0.5–1 mm diam. in *M. australotrichotus*), and always has white to cream stipe for its full length (reddish brown at the base in *M. australotrichotus*). It is also distinct phylogenetically as shown in Figure 2. Phylogenetic analysis of nrITS gene region using RAxML and Bayesian analyses places the new taxon in a distinct highly supported (BS 97% /PP 1.0) clade, with its sister species *M. neotrichotus* (Niveiro *et al.* 2018) from Argentina with low to moderate support (BS 56% /PP 0.97) (Figure 2).

Marasmius paratrichotus C.L.Grace, Desjardin & B.A.Perry, *Phytotaxa* 414(2): 75–77, figs 14, 15 (2019). *Type*: AFRICA. São Tomé, N 00°24.374', E 06°37.092', 17 April 2008, *D.E. Desjardin* DED 8248 (*holo*: SFSU). [MB 830250]

Background. *Marasmius paratrichotus* was described from the west African island nation of São Tomé and Príncipe (ST&P) by Grace *et al.* (2019). It forms a sister clade to *M. trichotus* (Figure 2) described from Singapore by Corner (1996). A revised description is provided here, incorporating details from the new Australian collections.

Pileus ~6 mm diam., orange, drying brownish, convex, broadly campanulate, to applanate, weakly striate, surface dull, dry, suede to velutinous. *Lamellae* adnexed, sub-distant (12–14) with 3 tiers lamellulae, white, non-marginate. *Stipe* 20–30 × 1 mm, central, cylindrical, cartilaginous, pale above and orange to brown in lower half, dry, pubescent with cream to orange strigose base. (Figure 5)

Basidiospores 10–11 × 3.5–4 µm, mean 10.5 × 3.5 µm, Q = 2.68–3.20, Q_m 2.96 (n = 30, measured from Christmas Island collection, narrow ellipsoid to lacrymoid, smooth, hyaline, inamyloid, thin-walled. Only one spore (11 × 4.5 µm) was found in the Kimberley collection. *Basidia* rare 18 × 5.5 µm. *Basidioles* abundant, narrow fusiform, ventricose-rostrate 21–25 × 5.5–7.5 µm. *Cheilocystidia* abundant *Siccus*-type broom cells, main body 7.5–22 × 5–7.5 µm, cylindrical, narrowly clavate, occasionally subglobose, with multiple apical setules 2.5–7.5 × 0.5–1.5 µm, thin-walled, apices sub-acute. *Pleurocystidia* absent. *Pileal* and *lamellar trama* interwoven, hyphae 2–5 µm diam., thin-walled, hyaline, dextrinoid. *Pileipellis* a hymeniderm of two cell types: 1. *Siccus*-type broom cells, main body 7–20 × 3.5–9.5 µm, broadly clavate, cylindrical, sometimes thick-walled, refractile, occasionally lobed with multiple apical setules 2.5–7 × 1–2 µm, rarely forked, thin and thick-walled, and 2. *Pileosetae* 45–87 × 5–9 µm, cylindrical lower half, lanceolate distal half, thick-walled, walls 1.5 µm thick, bulbous base. *Stipe* trama dextrinoid, hyphae parallel, 5–7.5 µm diam. *Caulosetae* 41–85(–140) × 5.5–8.5 µm, lanceolate, occasionally geniculate, with bulbous or boot-shaped base to 20 µm wide, thick-walled to 1.5 µm, with some thick-walled septa (common in CI collection), inamyloid becoming yellowish-brown with maturity. *Caulocystidia* of two types: 1. uncommon *Siccus*-type broom cells in upper stipe, main body 8.5–14 × 5–6.5 µm, clavate to cylindrical, with sparse apical setules 3–7 × 0.5–1 µm, occasionally forked, usually thin-walled, (not observed in CI collection); and 2. rare, smooth, irregular thin-walled caulocystidia 15–21 × 6.5–10 µm. *Clamp connections* present in all tissues. (Figure 6)

Specimens examined. WESTERN AUSTRALIA: Theda Station, north Kimberley, 25 Jan. 2003, *M.D. Barrett* MDB F26/03 (PERTH 09594582); AUSTRALIAN TERRITORY: Christmas Island, Territory Day Park Nature Trail loop track, 18 Feb. 2016, *N.L. Bougher & K. Ruthrof* NLB 1255 (PERTH 08827117).

Notes. This species has only been found twice in Australia and its Territories. However, the remoteness of its tropical distribution means little survey effort has been made in other likely habitats. The addition of two collections (GenBank ON715786 & OQ725928) that are >99% similar to the ST&P collection, (GenBank KX953749 Grace *et al.* 2019), in the nrITS region is important in confirming the validity of the species as distinct from *M. trichotus*. *Marasmius paratrichotus* is morphologically similar to *M. trichotus*, which has an Asian and Oceania distribution including Thailand (Wannathes *et al*. 2009) and Papua New Guinea (Desjardin & Horak 1997). *Marasmius paratrichotus* is also very similar to *M. australotrichotus* and is morphologically differentiated only by a shorter, paler stipe (20–30 mm

Figure 5. *Marasmius paratrichotus* (NLB 1255) A – sporing bodies in situ, scale bar =10 mm and B – pileosetae. Scale bar =10 µm. Photographs N.L. Bougher.

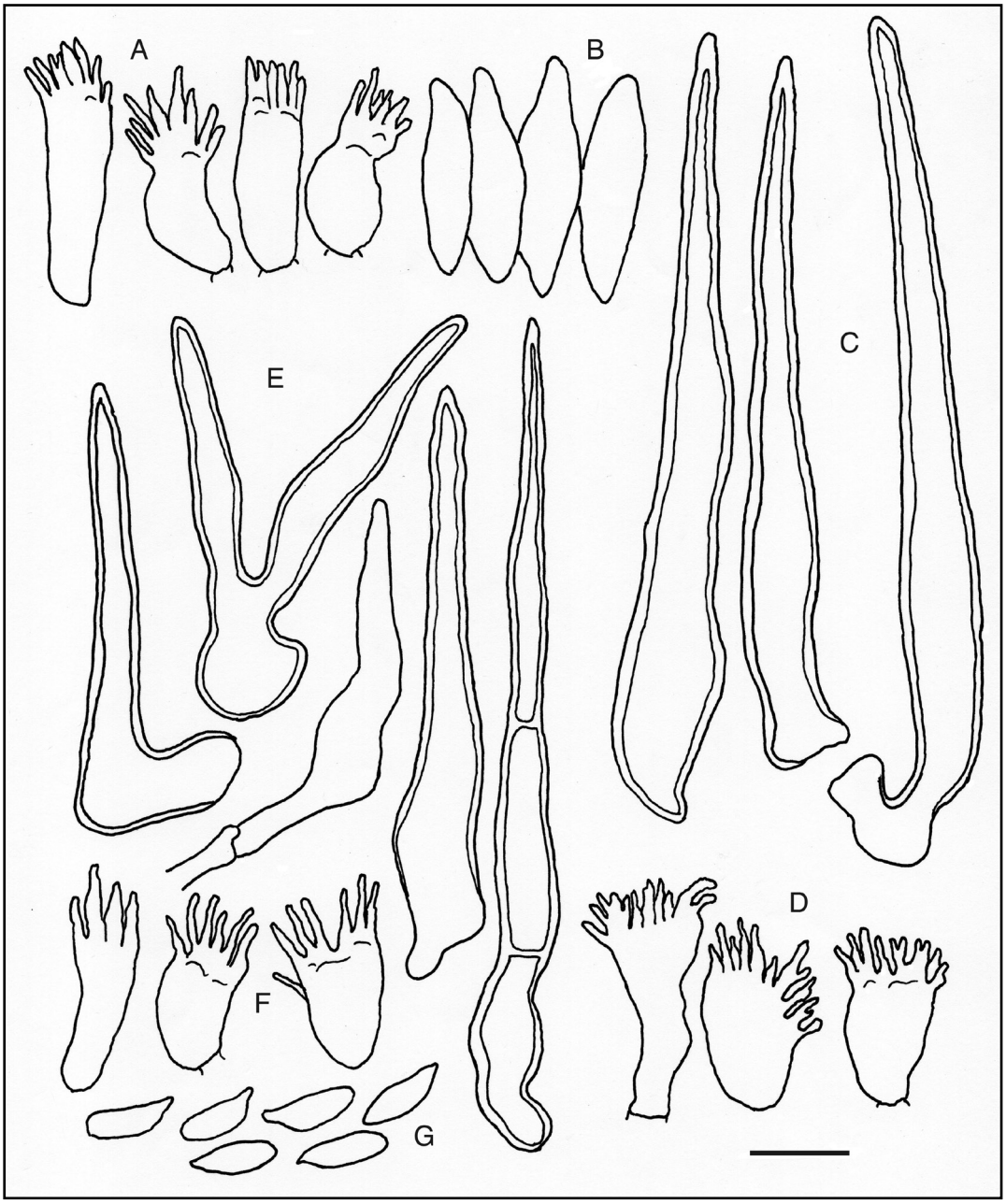

Figure 6. *Marasmius paratrichotus* (MDB F26/03 and NLB 1255) micromorphology. A – cheilocystidia, B – basidioles, C – pileosetae, D – pileipellis *Siccus* type broom cells, E –caulosetae, F – caulocystidia, G – spores. Scale bar = 10 µm.

cf. 25–80 mm in *M. australotrichotus*), white (*cf.* cream in *M. australotrichotus*) lamellae and slightly smaller spores (mean 10.5 µm (CI), 11.3 µm (ST&P) *cf.* 12 µm in *M. australotrichotus*). Should the distribution of the two taxa be found to overlap, care will be needed to separate them in the field. Phylogenetically, they form two distinct well-supported, non-sister clades (Figure 2).

Informative morphological characters used by Grace *et al*. (2019) in determining their new species in comparison to *M. trichotus* are: 1. Pileosetae are generally shorter in *M. paratrichotus* (<125 µm vs up to 300 µm and up to 1000 µm on pileal margin in *M. trichotus*); 2. while spores overlap in size, they are generally shorter in *M. paratrichotus* (mean 10.5 × 3.5 µm (CI) and 11 × 4.3 µm (ST&P) vs 12.5 × 3.5 µm); and 3. the occurrence of caulocystidia in the form of *Siccus*-type broom cells, while rare, is a particular feature of *M. paratrichotus* and not *M. trichotus*. However, these were not found in the CI specimen.

Corner (1996) described the stipe of *M. trichotus* as 'wholly puberulous, scurfy', yet did not mention lanceolate caulosetae in his description of the taxon and lanceolate caulosetae were not found in re-examination of the type specimen of *M. trichotus* by Niveiro (2018). Instead, he described caulocystidia consisting of modified broom cells with a narrow basal body and 2–6 setiform projections 45–70 µm long. Desjardin (1997), however, described lanceolate caulosetae up to 1000 µm long in the montane rainforest specimens of *M. trichotus* from Papua New Guinea, and Wannathes *et al.* (2009) found them to be 22–213 × 4–7 µm in the Thai collection. Caulosetae in *M. paratrichotus* are of overlapping, but generally shorter length than in *M. trichotus* (41–170 × 5.5–10.5(–14) µm), lanceolate, occasionally geniculate.

We note that the two Indian collections identified here as *M.* aff. *trichotus* (Dutta unpubl., GenBank MF189039 & MF189040) show greater similarity in nrITS analysis to *M. paratrichotus* than to *M. trichotus* from Thailand (99.09 & 99.18% vs 97.2 to 97.53% in this study). However, they remain on a separate branch in both Bayesian and Maximum Likelihood (RAxML) analyses of the nrITS region. It was argued by Grace *et al*. (2019) that the Indian sequences identified as *M. trichotus* were misidentified and that they represented *M. paratrichotus*. With the addition of the two sequences from the Australian region in this study, molecular differences remain, and indicate that the Indian specimens may represent yet another taxon, separate from both *M. paratrichotus* and *M. trichotus.*

Discussion

Support for monophyly of the traditional sect. *Sicci* (within the *Globulares*-*Sicci* complex), subsect. *Siccini*, ser. *Spinulosi* and *Atrorubentes* was low as noted in the introduction. Members of ser. *Atrorubentes*, *Haematocephali* and *Leonini* lay within the broader ser. *Spinulosi* clade. In 2020 Oliveira *et al.* proposed a new supra-specific classification using phylogenetic, ecological and morphological characteristics to recognise more stringent groups within sect. *Globulares*. A number of new taxa have been described since that proposal and this study includes some of them (*M. australotrichotus*, *M. neotrichotus*, *M. paratrichotus* and *M.* aff. *trichotus* among others), in the phylogeny (Figure 2). This has been an opportunity to test the newly raised subsections and series. Separating the 'cohaerens' group of northern hemisphere setose species of *Marasmius* is logical. This was catered for by raising subsect. *Spinulosi*, ser. *Cohaerentes* (Oliveira *et al.* 2020). However, the next phylogenetic group consisted of a set of unrelated subclades, termed subsect. *Incertae sedis*. Oliveira's series *Atrorubentes* (sensu Oliveira *et al.* 2020), includes species traditionally in ser. *Atrorubentes* and ser. *Spinulosi.* While all the species in this study fall into ser. *Atrorubentes* sensu Oliveira *et al.* (2020), with high phylogenetic support, morphologically they are very diverse. Members in the 'trichotus' group – *M. trichotus*, *M. australotrichotus*, *M. paratrichotus* and *M. neotrichotus* – are all similar, including having small to medium basidiomes (4–15(–23) mm diam.), medium hyaline clavate spores, (9–)10–13(–15) × 3–4.5 µm. They have setae on pileus and stipe, but not in the hymenium and lack pleurocystidia. Caulocystidia (as defined in this paper) are sparse and inconspicuous or absent. These common features suggest that this group could form another series. However, further collections and data are needed.

Marasmius australotrichotus has a distribution in tropical and subtropical Queensland and the Northern Territory (Figure 7), which mirrors some other saprotrophic species of fungi, e.g., *M. vagus* (Crous *et al*. 2020). While it is found in rainforest margins (QLD: Julatten) and *Eucalyptus*/*Allocasuarina* forest (QLD: Davies Creek National Park), it is also found in much more disturbed areas, such as road verges, suburban lawns and even mulched gardens (e.g., Kuranda, Indooroopilly, Mt Coot-tha). It has been mistaken for the more common *M. elegans*, but examination with a hand lens readily separates the two (see *Notes* above). Aerial dispersal of spores is likely for *M. australotrichotus*; however, human movement of the species cannot be discounted.

The distribution of *M. paratrichotus* is more unusual and intriguing, with known populations almost 11,000 km apart on São Tomé, Gulf of Guinea in West Africa and Christmas Island, near Java in the Indian Ocean, with a further population another 2,300 km east in the Kimberley, WA. Figure 7 shows this distribution in relation to its sister species. To date, it has been found on woody debris under baobabs (*Adansonia* sp.) in a coastal xerophytic habitat (ST&P) (Grace *et al*. 2019), among leaf litter in tropical monsoon savanna grass/lawn (WA - this study, M.D. Barrett) and on rotting wood in marginal rainforest (CI - this study, N.L. Bougher). All three sites are in tropical latitudes (0–14°S). It would appear from this study and earlier work that habitat is not an informative feature within the clade or in distinguishing between *M. paratrichotus* and its sister, *M. trichotus.*

São Tomé and Principe are ancient volcanic islands in the Cameroon Volcanic Line, which appeared about 30 Mya. Volcanism persisted until recently (0.1–0.036 Mya) (Melo *et al*. 2022). There is a history of repeated colonisation and extinction of organisms, which have largely originated from continental Africa. Dispersal to the islands is postulated to have been aerial (wind borne), avian, or by rafts of vegetation floating in freshwater plumes from the three large rivers that drain into the Gulf of Guinea, namely the Congo, Ogooué, and Niger Rivers (Ceríaco *et al*. 2022; Desjardin & Perry 2022). According to Desjardin and Perry (2022), the rich fungal biodiversity of tropical Africa is understudied, and under-documented. Antonin's (2007) monograph of *Marasmius* of tropical Africa, includes collections from the west African nations of Cameroon, Sierra Leone and Democratic Republic of Congo. He described five species in sect. *Sicci,* ser. *Spinulosi*, none of which are morphologically similar to *M. paratrichotus* and none were sequenced. Further sampling across continental tropical

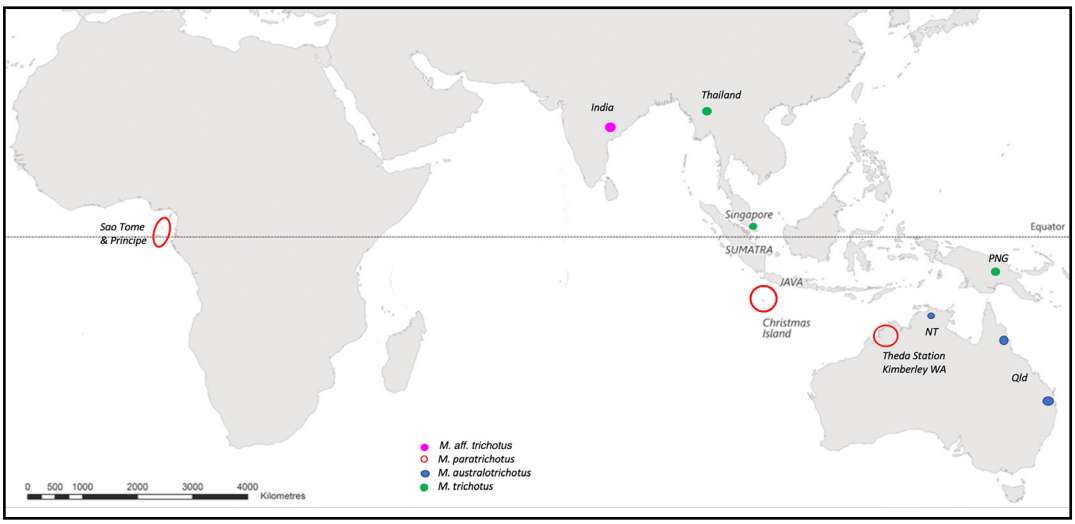

Figure 7. Global distribution of *Marasmius trichotus*, *M.* aff. *trichotus*, *M. paratrichotus* and *M. australotrichotus*.

Africa may produce relatives of species from São Tomé including *M. paratrichotus*, but otherwise its nearest known relatives are in India.

Christmas Island is another ancient oceanic volcanic island 350 km south of Java, having arisen 44 Mya (James & McAllan 2014). Like other isolated islands it has a high degree of endemism (James *et al*. 2019). It is also the breeding ground for a number of tropical pelagic birds, e.g., Abbott's booby, which ranges widely across the Indian Ocean and, in recent times, bred on some of the western Indian Ocean islands near Madagascar (Carboneras 1992). Other migratory shorebirds occasionally visit the island from the East Asian-Australasian flyway (James & McAllan 2014). These and other visiting terrestrial birds may have carried spores of *M. paratrichotus* to the island. Other possible means of spore dispersal include the prevailing winds, with the northwest monsoon (December to April) from the Indo-Malayan region, and the dry season (May to November) south east trade winds from Australia (James & McAllan 2014).

The Kimberley region of northwest Australia has a monsoonal climate and rich fungal diversity much of which remains to be studied (Barrett 2018). It is part of the Australian Monsoon Tropics (AMT) biome, which extends from WA across the Top End region of Northern Territory to Cape York and shares a number of fungal flora across that region, e.g., *Marasmius vagus* (Crous *et al*. 2020). *Marasmius australotrichotus* occurs in NT and far north Qld but has not yet been discovered in WA. It is quite possible that both *M. australotrichotus* and *M. paratrichotus* will be found in suitable habitats across the AMT. Further surveys may produce new populations of both taxa there or on other islands across the Indian Ocean.

The possibility of human dispersal cannot be discounted, with exploration, settlement, trade and more recently tourism ensuring a constant flow of humans around the globe, including even these seemingly remote and distant places. Molecular data show only two base pairs difference between the nrITS sequences from the three locations of *M. paratrichotus*. It is impossible to determine from such a small sample where the species originated. Further surveys are needed to extend knowledge of the distribution of both taxa.

Acknowledgements

We thank curatorial staff at the Queensland Herbarium (BRI), National Herbarium of Victoria (MEL) and Western Australian Herbarium (PERTH) for their assistance. We acknowledge funding sources including the Australasian Systematic Botany Society for financial support in fieldwork and molecular analysis and ABRS grant 4-EHOJ161 for field work assistance in Qld and NSW. We acknowledge assistance from Dr Katinka Ruthrof (then Murdoch University) and project staff of MINTOPE (Mining to Plant Enterprises Project), Alasdair Grigg and staff at CI Parks Australia, and Christmas Island Phosphates (Phosphate Resources Ltd.). Cecilia Myers is thanked for assistance with field work in the Kimberley in 2003. Sincere thanks are extended to referees for their very useful comments.

References

Antonin, V. (2007). *Fungus flora of tropical Africa* (ed. Degreef, J., Vol. 1). (National Botanic Garden, Belgium.)

Antonin, V. & Noordeloos, M. (2010). *A monograph of marasmioid and collybioid fungi in Europe*. (IHW-Verlag 2010, Eching, Berlin.)

Antonín, V., Ryoo, R. & Shin, H-D. (2012). Marasmioid and gymnopoid fungi of the Republic of Korea. 4. *Marasmius* sect. *Sicci*. *Mycological Progress* 11: 615–638.

Barrett, M.D. (2018). Fungi of the Kimberley. *In*: McGlashan, H., Coate, K., Gresham, J. & Hart, R. (eds.) *The natural world of the Kimberley*. pp. 38–42. (Kimberley Soc. Inc., Perth.)

Bougher, N.L. & Syme, K. (1998). *Fungi of southern Australia*. (University of Western Australia Press: Perth.)

Carboneras, C. (1992). *Family Sulidae (Gannets and Boobies). In*: del Hoyo, J., Elliott, A. & Sargatal, J. (eds.) *Handbook of the birds of the world* (Vol. 1): *Ostrich to Ducks.* pp. 312–325. (Lynx Edicions, Barcelona.)

Ceríaco, L.M.P., Santos, B.S., de Lima, R.F., Bell, R.C., Norder, S.J. & Melo, M. (2022). Physical geography of the Gulf of Guinea oceanic islands. *In*: Ceríaco, L.M.P., de Lima, R.F., Melo, M., & Bell, R.C. (eds.) *Biodiversity of the Gulf of Guinea oceanic islands: science and conservation*. pp. 13–36. (Springer International Publishing, Cham, Switzerland.)

Clémençon H. (1982). Kompendium der Blätterpilze. II. *Marasmius*. *Zeitschrift für Mykologie* 48: 3–16.

Corner, E. (1996). The agaric genera *Marasmius, Chaetocalathus, Crinipellis, Heimiomyces, Resupinatus, Xerula* and *Xerulina* in Malesia. *Nova Hedwigia, Beihefte* 111: 1–175.

Crous, P.W., Wingfield, M. & Chooi, Y.-H. (2020). Fungal Planet description sheets: 1042–1111, *Marasmius vagus* F.E. Guard, M.D. Barrett & Farid sp. nov. (sheet1091). *Persoonia* 44: 301–459. Retrieved from https://www.fungalplanet.org/content/pdf-files/FungalPlanet1091.pdf

Desjardin, D.E. & Horak, E. (1997). *Marasmius* and *Gloiocephala* in the south Pacific region: Papua New Guinea, New Caledonia and New Zealand taxa. Part 1: Papua New Guinea and New Caledonia taxa. *In*: Petrini, O., Petrini, L.E. & Horak, E. (eds.) *Taxonomic monographs of Agaricales 2, Bibliotheca Mycologica* 168: 1–152.

Desjardin, D.E. & Perry, B.A. (2022). Fungi of São Tomé and Príncipe Islands: basidiomycete mushrooms and allies. *In*: Ceríaco, L.M.P., de Lima, R.F., Melo, M., & Bell, R.C. (eds.) *Biodiversity of the Gulf of Guinea oceanic islands: science and conservation*. pp. 189–216. (Springer International Publishing, Cham, Switzerland.)

Gardes, M. & Bruns, T. (1993). ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2): 113–118.

Grace, C.L., Desjardin, D.E., Perry, B.A. & Shay, J.E. (2019). The genus *Marasmius* (Basidiomycota, Agaricales, Marasmiaceae) from Republic of Sao Tome and Principe, West Africa. *Phytotaxa* 414(2): 55–104.

Grgurinovic, C.A. (1997). *Larger fungi of South Australia*. (The Botanic Gardens of Adelaide and State Herbarium: Adelaide.)

Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754–755.

James, D., Green, P.T., Humphreys, W. & Woinarski, J. (2019). Endemic species of Christmas Island, Indian Ocean. *Records of the Western Australian Museum* 34(2): 55–114.

James, D.J. & McAllan, I.A. (2014). The birds of Christmas Island, Indian ocean: a review. *Australian Field Ornithology* 31: S1–S175 (Supplement).

Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability, *Molecular Biology and Evolution* 30(4): 772–780.

Kiyashko, A.A., Malysheva, E.F., Antonin, V., Svetasheva, T.Y. & Bulakh, E.M. (2014). Fungi of the Russian Far East 2. New species and new records of *Marasmius* and *Cryptomarasmius* (Basidiomycota). *Phytotaxa* 186(1): 1–28.

Melo, M., Ceríaco, L.M. & Bell, R.C. (2022). Biogeography and evolution in the oceanic islands of the Gulf of Guinea. *In*: *Biodiversity of the Gulf of Guinea oceanic islands.* pp. 141–170. (Springer International Publishing, Cham, Switzerland.)

Niveiro, N., Michlig, A., Ramírez, N.A., Salvador-Montoya, C.A., Pérez, M.L., Albertó, E.O. & Antonín, V. (2018). Two new setose species of Marasmius from the Paraná riparian forest in Argentina. *Cryptogamie, Mycologie* 39(4): 483–506.

Oliveira, J.J.S., Sanchez-Ramirez, S. & Capelari, M. (2014). Some new species and new varieties of *Marasmius* (Marasmiaceae, Basidiomycota) from Atlantic rainforest areas of São Paulo State, Brazil. *Mycological Progress* 13(3): 923–949.

Oliveira, J.J.S., Moncalvo, J.M., Margaritescu, S. & Capelari, M. (2020). A morphological and phylogenetic

evaluation of *Marasmius* sect. *Globulares* (Globulares-Sicci complex) with nine new taxa from the neotropical Atlantic Forest. *Persoonia* 44(1): 240–277.

Royal Botanic Gardens, Edinburgh (1969). *Flora of British fungi: colour identification chart*. (Her Majesty's Stationery Office: Edinburgh.)

Shay, J.E., Desjardin, D.E., Perry, B.A., Grace, C.L. & Newman, D.S. (2017). Biodiversity and phylogeny of *Marasmius* (Agaricales, Basidiomycota) from Madagascar. *Phytotaxa* 292(2): 101–149.

Singer, R. (1976) Marasmieae (Basidiomycetes - Tricholomataceae). *Flora Neotropica* 17: 1–347.

Singer, R. (1986) The Agaricales in modern taxonomy. (Koenigstein, Koeltz scientific books: Federal Republic of Germany)

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313.

Tan, Y.-S., Desjardin, D., Perry, B., Vikineswary, S. & Noorlidah, A. (2009). *Marasmius* sensu stricto in peninsular Malaysia. *Fungal Diversity* 37: 9–100.

Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatially amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology* 172(8): 4238–4246.

Wannathes, N., Desjardin, D., Hyde, K., Perry, B. & Lumyong, S. (2009). A monograph of *Marasmius* (Basidiomycota) from northern Thailand based on morphological and molecular (ITS sequences) data. *Fungal Diversity* 37: 209–306.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18(1): 315–322.