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Four new *Geosmithia* species from bark beetles infesting indigenous South African trees

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ABSTRACT

Over the past two decades, numerous *Geosmithia* fungi have been isolated from the bodies and galleries of wood–boring beetles. However, this genus of asexual *Sordariomycetes* remains taxonomically and ecologically understudied, especially in the Southern Hemisphere. In South Africa, two prior surveys reported *Geosmithia* species from bark beetles, but neither thoroughly investigated species identities. In this study, we collected bark beetles from native trees in the Western Cape Province of South Africa and isolated, identified and described their associated *Geosmithia* species. *Geosmithia* spp. previously collected in South Africa were also re-examined. The ITS sequences of *Geosmithia* isolates from 13 beetle taxa infesting 10 host species were considered. Additional gene regions, *BT*, *EF1a* and *RPB2*, were sequenced for a subset of isolates. Four previously described species, *G. flava*, *G. langdonii*, *G. omnicola* and *G. pumila* were identified by phylogenetic analyses. Additionally, four novel taxa were identified and are here described as *G. capensis*, *G. multisociorum*, *G. oroboidis* and *G. stellenboschiana*. *Geosmithia multisociorum* appears to be a generalist associated with multiple beetle–host combinations in the Northern and Southern Hemisphere, whereas *G. oroboidis* is currently known only from a single beetle and tree host in South Africa. South African isolates of *G. capensis* and *G. stellenboschiana* appeared to be restricted to *Lanurgus* spp. and *Hypothenemus* sp. beetles, respectively, but both species are also known from beetles and hosts in other countries.

1. Introduction

Geosmithia (*Sordariomycetes: Hypocreales*) is an ecologically and taxonomically understudied genus of globally distributed filamentous fungi (Kolařík and Hulcr, 2023). The genus was historically considered part of *Penicillium* (*Eurotiomycetes: Eurotiales*), due to the similarities of their conidiophores (Pitt, 1979). Species are asexual, characterised by hydrophobic spores and conidia produced in long chains (Kolarík et al., 2004, 2005). The dry spores of *Geosmithia* species enable airborne dispersal and represent an anomaly for a genus commonly isolated from wood–boring beetles and their galleries. Their apparent lack of entomochoric adaptations implies that these fungi do not rely on insects for dispersal. However, the consistency with which certain assemblages of *Geosmithia* species are isolated from specific beetle populations suggests a strong link (Kolařík and Jankowiak, 2013; Kolařík and Kirkendall, 2010; Kolařík et al., 2008; Kubátová et al., 2004).

While some *Geosmithia* species have been isolated from soil and plant material or debris in the absence of obvious beetle inhabitants (Kolarík et al., 2004; Pitt and Hocking, 2009), over 30 studies have reported *Geosmithia* species from beetles (Kolařík and Hulcr, 2023). Isolations are frequently made directly from adult beetles, larvae and eggs as well as indirectly from their galleries and frass (Kolařík and Hulcr, 2023; Kubátová et al., 2004). The vast majority of *Geosmithia* isolations have been from phloem-feeding scolytine beetles (*Curculionidae: Scolytinae*; Kolařík and Hulcr, 2023), of which conifer-infesting beetles, such as *Phloeosinus* spp., have yielded a large variety of taxa (Hernández-García et al., 2020; Jankowiak et al., 2014; Kolařík et al., 2017; Kolařík and Jankowiak, 2013).

Fungal-bark beetle symbioses with detrimental impacts on tree health are well known. These include the well-studied bark beetle

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symbioses with ophiostomatoid fungi in the Orders *Ophiostomatales* and *Microascales*, which are responsible for diseases such as Dutch Elm Disease (De Beer et al., 2022; Santini and Faccoli, 2015) and Ceratocystis wilt of various hosts (De Beer et al., 2014; Roux and Wingfield, 2013) respectively. In contrast, only one detrimental *Geosmithia*-beetle symbiosis is known; that of the walnut twig beetle, *Pityophthorus juglandis* and *G. morbida* causing Thousand Cankers Disease of black walnut, *Juglans nigra* (Kolařík et al., 2011; Tisserat et al., 2009). Other than a small number of *Geosmithia* species that have been described as primary and auxiliary associates of ambrosia beetles (Kolařík et al., 2015; Kolařík and Kirkendall, 2010), the role that most *Geosmithia* species play in the nutrition and other aspects of the ecology of their beetle associates remains unclear (Kolařík and Hulcr, 2023).

Numerous studies have considered *Geosmithia* species in the Northern Hemisphere (Kolařík and Hulcr, 2023) and several of these have included extensive surveys (e.g. Huang et al., 2019; Kolařík et al., 2017; Kolařík and Jankowiak, 2013; Kolařík et al., 2007, 2008). In contrast, knowledge of these fungi and their vectors in the Southern Hemisphere is limited. Single records are available from Australia (Kolařík and Hulcr, 2023; Pitt, 1979; Sakalidis et al., 2011), Brazil (Crous et al., 2018), New Zealand, Peru, and the Seychelles (Kolarík et al., 2004). Of these, only the Seychelles isolate was from a bark beetle. In South Africa, a single targeted survey identified five *Geosmithia* species from four different bark beetles infesting unhealthy or dead *Virgilia* trees (Machingambi et al., 2014). A recent South African survey of fungi from bark beetles in the genus *Lanurgus* (*Coleoptera: Scolytinae*) infesting *Widdringtonia* trees yielded numerous *Geosmithia* isolates representing different taxa (Basson et al., 2024), but these were not identified at the species level.

Given the number of *Geosmithia* taxa that have been isolated in surveys of only two native tree genera in South Africa (Basson et al., 2024; Machingambi et al., 2014), it seems likely that a great diversity of *Geosmithia* species has yet to be discovered in the country. Furthermore, the rate of *Geosmithia* species discovery, both in the Northern Hemisphere and in South African surveys has surpassed species descriptions. According to Kolařík and Hulcr (2023), at least 99 *Geosmithia* species have been documented, but only 32 have been described. The remaining ≥67 species are recognised phylogenetically with only a numerical identity. This study was prompted by the unstudied diversity and undescribed species of *Geosmithia* in South Africa as well as the need for species descriptions in this genus. The overall aim was to isolate, identify, and describe *Geosmithia* species from native bark beetle-infested trees in the Western Cape Province of South Africa, and also to re-examine the isolates collected by Basson et al. (2024) and Machingambi et al. (2014).

2. Materials and methods

2.1. Sampling and fungal isolation

Geosmithia isolates previously collected from beetles on two *Widdringtonia* (Basson et al., 2024) and three *Virgilia* species (Machingambi et al., 2014) were obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Bark beetle-infested tissues of five additional native tree species, representing five different families, were also collected (Table 1). Collection of bark beetles and isolation of *Geosmithia* species was performed as described by Basson et al. (2024). Briefly, plant material was placed in emergence cages, from which emerging bark beetles were collected. Additionally, the bark was removed from plant material to expose and directly remove beetles from their galleries. A subset of the collected beetles was stored in 98 % ethanol at −80 °C for identification and reference purposes. Beetle identifications followed those presented in the previous publications (Basson et al., 2024; Machingambi et al., 2014). Those collected from additional hosts in this study were identified by J. Hulcr, School of Forest, Fisheries, and Geomatics Sciences, University of Florida.

Fungi were isolated from living bark beetles, by (i) rolling individual beetles across the surface of 2 % Malt Extract Agar (MEA, Biolab, South Africa) or (ii) shaking a single beetle in 100 μl sterile deionised water before spreading the solution onto Potato Dextrose Agar (PDA, Biolab, South Africa). After incubation at room temperature, fungal colonies resembling *Geosmithia* were purified by transferring hyphal tips to clean agar plates. Cultures were subsequently assigned to phenotypic groups based on texture, colour and pattern.

2.2. Fungal identification

Representative isolates from each *Geosmithia* phenotypic group, representing all 10 tree hosts considered in this study, were used for

Table 1
Species of South African native host trees and beetles from which the *Geosmithia* isolates considered in this study were obtained.

Tree host species	Family	Beetle species	Location	GPS	*Geosmithia* spp.	Reference
Olea europaea subsp. *africana* (Wild olive)	*Oleacea*	*Lanurgus jubatus*	Stellenbosch	33°56′17.6″S 18°52′36.6″E	2	This study
Podalyria calyptrata (Sweetpea bush)	*Fabaceae*	*Ctonoxylon* sp.	Franschhoek Pass	33°56′10.6″S 19°09′46.4″E	1	“
Podocarpus latifolius (Real yellowwood)	*Podocarpaceae*	*Lanurgus podocarpi*	Groenkop, George	33°54′40.0″S 22°33′06.0″E	2	“
Searsia angustifolia (Willow karee)	*Anacardiaceae*	*Hypothenemus* sp.	Stellenbosch	33°56′17.6″S 18°52′36.6″E	4	“
Sideroxylon inerme (White milkwood)	*Sapotaceae*	*Xyloctonus maculatus*	Kalk Bay, Cape Town	34°07′38.6″S 18°26′41.1″E	1	“
Widdringtonia cedarbergensis (Clanwilliam cedar)	*Cupressaceae*	*Lanurgus* sp. 1, *Lanurgus* sp. 3	Cederberg	32°21′43.0″S 19°04′10.0″E	2	Basson et al. (2024)
W. nodiflora (Mountain cedar)	“	*Lanurgus* sp. 4	Franschhoek Pass	33°56′10.6″S 19°09′46.4″E	1	“
Virgilia divaricata (Pink Keurboom)	*Fabaceae*	*Cryphalini* sp. 1, *Elattoma* sp. 1	Storms River	33°57′55.9″S 23°55′54.3″E	2	Machingambi et al. (2014)
V. oroboides subsp. *ferruginea* (Brown-Haired Keurboom)	“	*Cryphalini* sp. 1	George	33°57′57.9″S 23°55′58.7″E	1	“
V. o. subsp. *oroboides* (Cape Keurboom)	“	*Scolytoplatypus fasciatus*, *Cryphalini* sp. 1, *Elattoma* sp. 1, *Hapalogenius fuscipennis*, *Liparthrum* sp. 1	Betty’s Bay; Kirstenbosch Botanical Garden; Silvermine Nature Reserve, Cape Town	34°20′51.0″S 18°55′29.4″E; 33°59′11.3″S 18°25′34.4″E; 34°05′27.9″S 18°25′17.6″E	5	“

species identification. DNA was isolated from young (3-5-day old) fungal cultures using the Prepman Ultra Sample Preparation Reagent (Thermo Fisher Scientific, MA, USA) and the ITS region was amplified with primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). The 20 µl PCR reaction contained 10 µl Ampliqon Taq DNA Polymerase Master Mix RED (Biomol, Germany), 0.4 µM of each primer, and 1.5 µl of the Prepman DNA extraction. Reaction conditions were 5 min initial denaturation at 95 °C, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 90 s elongation at 72 °C. This was followed by a final elongation step of 10 min at 72 °C. Amplicons were sequenced at the Central Analytical Facilities (CAF), Stellenbosch University, South Africa.

Incorporating *Geosmithia* reference sequences obtained from GenBank (Table S1), a Maximum Likelihood (ML) ITS tree was computed using MAFFT v7.525 (Katoh and Standley, 2013) for sequence alignment and trimAl v1.4. rev22 for alignment curation (Capella-Gutiérrez et al., 2009). Appropriate substitution models were identified with Modeltest-NG 0.1.7 (Darriba et al., 2020) and the phylogeny was determined with RAxML-NG 1.2.2 (Kozlov et al., 2019), applying 1000 bootstrap replicates. Based on the ITS phylogeny, additional gene regions were sequenced for a subset of isolates, using the same PCR mixture as for ITS and adjusting only the annealing temperature in the PCR reaction. These were *Beta-tubulin* (*BT*) with primers T1/Bt2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997) at Ta = 53 °C, the *Elongation Factor (EF)1a* large intron with primers EF1-728 F/EF2 (Carbone and Kohn, 1999) at Ta = 53 °C, the *EF1a* large exon with primers EF1-983 F/EF1-2218 R (Rehner and Buckley, 2005) at Ta = 55 °C and *RNA Polymerase II* (*RPB2*) with primers RPB2-5F2/fRPB2-7cR (Liu et al., 1999; Sung et al., 2007) at Ta = 57 °C.

ML phylogenies for individual regions and the partitioned concatenated dataset were constructed as described above. According to the AIC, nucleotide substitution models GTR + I + G4, TPM1uf + I + G4, TIM3+I + G4 and GTR + G4 were applied to the ITS, *BT*, *EF1a* exon and *RPB2* partitions, respectively. Bayesian Inference was performed on the cancatenated dataset in MrBayes 3.2.7a (Ronquist et al., 2012) from a random starting tree, sampling across GTR model space (nst = mixed) for each of the four partitions. Two MCMC runs of four chains were run for 10 million generations. Runs were sampled every 5000 generations and the default 25 % was discarded as burn-in. The GTR substitution models with the highest posterior probabilities for the ITS, *BT, EF1a* exon and *RPB2* partitions were M_{134} = 123141 (PP = 0.304), M_{45} = 111231 (PP = 0.329), M_{36} = 122232 (PP = 0.175) and M_{15} = 121121 (Kimura, 1980; PP = 0.304).

2.3. Morphological examinations

Isolates grown on 2 % MEA and incubated at optimum temperature for 14 days were used to study morphological characteristics. Fruiting structures were mounted in water, which was replaced with 85 % lactic acid for further observation. Microscopes (Nikon Eclipse Ni, SMZ18), mounted with a Nikon DS-Ri2 camera, and imaging software (Nikon NIS-Elements) were used to study the structures. Twenty-five structures were measured for the conidiogenous apparatus: penicilli (between stipes and conidia), stipes, rami (first branch), metulae (branch-bearing phialides) and conidiogenous cells (phialides), 50 structures were measured for conidia, and 10 structures were counted for the number of whorls. The dimensions of the structures were presented in min–max (average ± SD).

For the growth study, mycelial plugs (5 mm diam.) were obtained from 7-day-old cultures. The plugs were placed at the centre of 90 mm Petri dishes containing 2 % MEA. Three replicates per strain were kept in incubators with temperatures ranging from 5 to 35 °C, in 5 °C intervals. For each Petri dish, two diameters were measured at 8-, 14- and 28-days incubation or just before mycelia growing at the optimum temperature reached the edge of the Petri dish. Average daily growth was calculated for each temperature. The plates were left in incubators for 28 days for characterization. Strains kept at 5 °C and 35 °C that did not show any growth after 28 days were re-incubated at their optimal temperatures for another 14 days to assess viability. Colours were described according to the ISCC-NBS system. Type specimens were lodged at the H.G.W.J. Schweickerdt herbarium (PRU) and ex-type cultures were deposited at the culture collection (CMW-IA) of Innovation Africa in the University of Pretoria, Hatfield, Pretoria, South Africa.

3. Results

3.1. Beetle identification and Geosmithia isolations

Each of the five native tree hosts collected in this study yielded a single bark beetle species from which fungal isolations were made (Table 1). The beetles included a *Ctonoxylon* sp., a *Hypothenemus* sp., two species of *Lanurgus* and *Xyloctonus maculatus*. The *Geosmithia* isolates from the studies of Basson et al. (2024) and Machingambi et al. (2014) represented an additional eight bark beetle species, three on *Cupressaceae* and five on *Fabaceae* hosts. No overlap in beetle species was found among the different genera of hosts, although the same beetle taxa infested the three *Virgilia* species investigated by Machingambi et al. (2014).

Eight *Geosmithia* species were identified (Table 2), of which seven were isolated from the five tree hosts collected during this study. *Geosmithia* sp. A (Machingambi et al., 2014), described here as *G. oroboidis,* was the only previously collected species that was not re-isolated. The greatest diversity of *Geosmithia* species was encountered on the *Cryphalini* sp. 1 beetle from *Virgilia oroboides* subsp. *oroboides* and *Hypothenemus* sp. collected from *Searsia angustifolia*. Both yielded four *Geosmithia* taxa from a single beetle species (Table 1). A maximum of two *Geosmithia* taxa were isolated from each of the remaining beetle species. Of the eight species identified, only *G. oroboidis* was seemingly beetle- and host-specific. Six others were isolated from at least two different beetle species and host trees in this study, whereas, *G. stellenboschiana* has also been found outside of South Africa.

3.2. Phylogenetic analyses

The ITS phylogeny (Fig. S1A) grouped the *Geosmithia* isolates into seven clades that were further explored. As previously noted (Kolařík and Hulcr, 2023), phylogenetic resolution of closely related *Geosmithia* species with ITS data alone was poor, but most relationships were resolved by considering sequence data for the additional gene regions. The individual gene trees (Figs. S1B–E) and the concatenated phylogeny (Fig. 1) identified three previously described species, *G. langdonii*, *G. omnicola*, and *G. pumila* as well as isolates belonging to the *G. microcorthyli* species complex. Four novel species were resolved. Of these, *G. capensis* and *G. oroboidis*, were sufficiently distinct from other taxa to be resolved using only ITS sequence data, whereas *G. multisociorum* and *G. stellenboschiana* required data for additional gene regions to delineate the species boundaries.

None of the phylogenies could confidently distinguish between *G. microcorthyli* and the two phylogenetic species, *Geosmithia* sp. 8 and *Geosmithia* sp. 48. Investigation of the individual alignments did not reveal any differences between *Geosmithia* sp. 8 and *Geosmithia* sp. 48 in the ITS or *EF1a* exon regions available for the latter species. Compared to *G. microcorthyli*, the portions of the ITS and *RPB2* regions that were investigated were not variable among reference sequences. Polymorphism was detected at one position in the *BT* alignment and three positions in the *EF1a* exon alignment. None of the South African isolates nor other Northern Hemisphere isolates shared these polymorphisms. We, therefore, conclude that the South African isolates in this clade are distinct from *G. microcorthyli* and likely represent *Geosmithia* sp. 8, which may be synonymous with *Geosmithia* sp. 48.

For the concatenated phylogenetic tree, bootstrapping converged after 400 replicates. The LogLikelihood of the final ML tree was

Table 2
Identity and metadata of the *Geosmithia* strains isolated in this study.

Species (ITS clade)	CMW Strain	Isolate	Beetle vectors	Tree host	Locality[a]	ITS[b]	*TEF1-α Intron*	*TEF1-α Exon*	*BT*	*RPB2*
G. capensis sp. nov. (C3)	64002[T]	WR01	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032691	–	–	PQ048054	PQ045591
	64003	WR02	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032694	–	–	–	–
	64004	WR06	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032697	–	–	PQ048055	PQ045592
	64005	WR09	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032677	–	–	–	–
	64007	WR13	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032683	–	–	PQ048056	PQ045593
	64008	WR15	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032686	–	–	PQ048057	PQ045594
		WR17	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032692	–	–	–	–
	–	5_WNbn001	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032705	–	–	–	–
	–	6_WWT018	*Lanurgus* sp. 1	*Widdringtonia cedarbergensis*	CB	PQ032706	PQ045565	–	–	PQ045578
	59299	7_WWB009	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032707	PQ045567	–	PQ048050	PQ045588
	59302	9_WWB002	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032699	PQ045566	–	PQ048052	PQ045590
	59310	8_WWB001	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032708	PQ045564	–	PQ048053	PQ045586
	–	26_WNbn002	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032714	–	–	–	–
G. flava (C6)	40726	NM80	*Hapalogenius fuscipennis*	*V. oroboides* subsp. *oroboides*	BB	KJ513210*	–	–	–	–
	40727	NM37	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	SMNR	KJ513209*	–	–	–	–
	40728	NM79	*Hapalogenius fuscipennis*	*V. oroboides* subsp. *oroboides*	BB	KJ513208*	–	–	–	–
	40745	NM109	*Cryphalini* sp. 1	*Virgillia divaricata*	SR	KJ513211*	–	–	–	–
	64006	WR11	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032680	–	PQ048075	–	–
	64009	WR16	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032689	–	PQ048076	PQ048058	PQ045595
	64010	WR18	*Lanurgus jubatus*	*Olea europaea* subsp. *africana*	SB	PQ032695	–	PQ048077	PQ048059	PQ045596
G. langdonii (C5.2)	64016	WR36	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032687	PQ045575	PQ048079	PQ048063	PQ045601
	64019	WR48	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032682	PQ045574	PQ048085	PQ048066	PQ045603
	–	WR19	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032698				
	–	WR43	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032693				
	–	WR46	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032688				
	–	WR47	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032679				
	59275	3_PL015	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032703	PQ045572	PQ048072	PQ048048	PQ045587
		1_PL03	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032701				
	–	2_PL016	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032702	PQ045571	–	PQ048043	PQ045577
	–	24_PL018	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032713	PQ045573	–	–	–
G. multisociorum sp. nov. (C7)	40739[T]	NM93	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	BB	KJ513226*	–	PQ048070	PQ048047	–
	40740	NM98	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	BB	KJ513227*	–	–	–	–
	59296	10_WM001	*Xyloctonus maculatus*	*Sideroxylon inerme*	KB	PQ032709	–	PQ048073	–	–
	59300	15_WWB010	*Lanurgus* sp. 3	*Widdringtonia cedarbergensis*	CB	PQ032712	PQ045563	PQ048074	PQ048051	PQ045563
	64013	WR32	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032684	–	PQ048082	PQ048062	PQ045563
	64014	WR33	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032719	–	PQ048083	–	PQ045563
	64015	WR34	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032718	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

Species (ITS clade)	CMW Strain	Isolate	Beetle vectors	Tree host	Locality[a]	ITS[b]	*TEF1-α Intron*	*TEF1-α Exon*	*BT*	*RPB2*
	-	WR35	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032720	–	–	–	PQ045576
	–	11_WN013	*Lanurgus* sp. 4	*Widdringtonia nodiflora*	FP	PQ032710	–	–	–	–
	–	28_WM002	*Xyloctonus maculatus*	*Sideroxylon inerme*	KB	PQ032716	–	–	–	–
	–	29_WM003	*Xyloctonus maculatus*	*Sideroxylon inerme*	KB	PQ032717	–	–	–	–
	–	30_WM004	*Xyloctonus maculatus*	*Sideroxylon inerme*	KB	PQ032700	–	–	–	–
	–	4_WN023	*Lanurgus* sp. 4	*Widdringtonia nodiflora*	FP	PQ032704	–	–	–	–
	59312	14_WWT036	*Lanurgus* sp. 1	*Widdringtonia cedarbergensis*	CB	PQ032711	–	–	–	–
G. oroboidis sp. nov. (C1)	40732^{T}	NM105	*Scolytoplatypus fasciatus*	*V. oroboides* subsp. *oroboides*	BB	KJ533336*	–	PQ048068	–	–
	40741	NM005	*Scolytoplatypus fasciatus*	*V. oroboides* subsp. *oroboides*	BB	KJ533337*	–	–	–	–
	40742	NM006	*Scolytoplatypus fasciatus*	*V. oroboides* subsp. *oroboides*	BB	KJ533338*	–	PQ048071	–	–
G. omnicola (C4)	40733	NM102	*Elattoma* sp. 1	*V. oroboides* subsp. *oroboides*	BB	KJ513217*	–	–	–	–
	40734	NM110	*Liparthrum* sp. 1	*V. oroboides* subsp. *oroboides*	BB	KJ513215*	–	PQ048069	PQ048046	–
	40735	NM111	*Liparthrum* sp. 1	*V. oroboides* subsp. *oroboides*	BB	KJ513216*	–	–	–	–
	64017	WR38	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032690	–	–	PQ048064	PQ045602
	64021	27_PyS	*Ctonoxylon* sp.	*Podalyria calyptrata*	FP	PQ032715	PQ045570	–	PQ048042	PQ045605
G. pumila (C2)	40729	NM60	*Elattoma* sp. *1*	*V. divaricata*	SR	KJ513230*	–	–	–	PQ045580
	40730	NM83	*Hapalogenius fuscipennis*	*V. oroboides* subsp. *oroboides*	BB	KJ513231*	–	–	–	PQ045581
	40731	NM91	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	BB	KJ513253*	–	–	PQ048045	PQ045582
	40736	NM62	*Cryphalini* sp. *1*	*V. oroboides* subsp. *ferruginea*	GE	KJ513234*	–	–	–	–
	40737	NM71	*Hapalogenius fuscipennis*	*V. oroboides* subsp. *oroboides*	BB	KJ513229*	–	–	–	–
	40738	NM90	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	BB	KJ513254*	–	–	–	–
	40743	NM2	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	BB	KJ513232*	–	–	–	PQ045583
	40744	NM6	*Cryphalini* sp. *1*	*V. oroboides* subsp. *oroboides*	KBG	KJ513233*	–	–	–	PQ045584
	40746	NM101	*Hapalogenius fuscipennis*	*V. oroboides* subsp. *oroboides*	BB	KJ513258*	–	–	–	–
	59297	21_PL010	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032676	PQ045568	–	PQ048049	PQ045585
	–	18_PL020	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032674	PQ045569	–	PQ048044	PQ045579
	–	23_PL005	*Lanurgus podocarpi*	*Podocarpus latifolius*	GE	PQ032675	–	–	–	–
G. stellenboschiana sp. nov. (C5.1)	64011	WR20	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032678	–	PQ048078	PQ048060	PQ045597
	64012^{T}	WR28	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032681	–	PQ048081	PQ048061	PQ045598
	64018	WR45	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032696	–	PQ048084	PQ048065	–
	64020	WR49	*Hypothenemus* sp.	*Searsia angustifolia*	SB	PQ032685	–	PQ048080	PQ048067	PQ045604

[a] BB = Betty's Bay; CB = Cederberg; FP = Franschhoek Pass; GE = George; KB = Kalk Bay, Cape Town; KBG = Kirstenbosch Botanical Garden; SMNR = Silver Mine Nature Reserve, Cape Town; SB = Stellenbosch; SR = Storm's River. See Table 1 for more information.

[b] Asterisks (*) indicate ITS sequences generated by Machingambi et al. (2014). All other sequences were generated in this study.

−16499.706459. The Bayesian analysis reached a maximum standard deviation of split frequencies <0.01, with an average Estimated Sample Size <1000 and a Potential Scale Reduction Factor value of 1.00, indicating convergence of the runs.

3.3. Taxonomy

Geosmithia capensis Aylward, Marinc., M.J. Wingf & Roets, sp. nov. Fig. 2.

MycoBank: MB855500.

Etymology: "Capensis" (from the cape), refers to its common occurrence in the Western Cape Province of South Africa.

Diagnosis: *Geosmithia capensis* is phylogenetically distinct from other known species based on the ITS or any single gene region.

Type: South Africa: Western Cape Province, Stellenbosch, banks of the Eerste River. Isolated from *Lanurgus jubatus* infesting *Olea europaea*

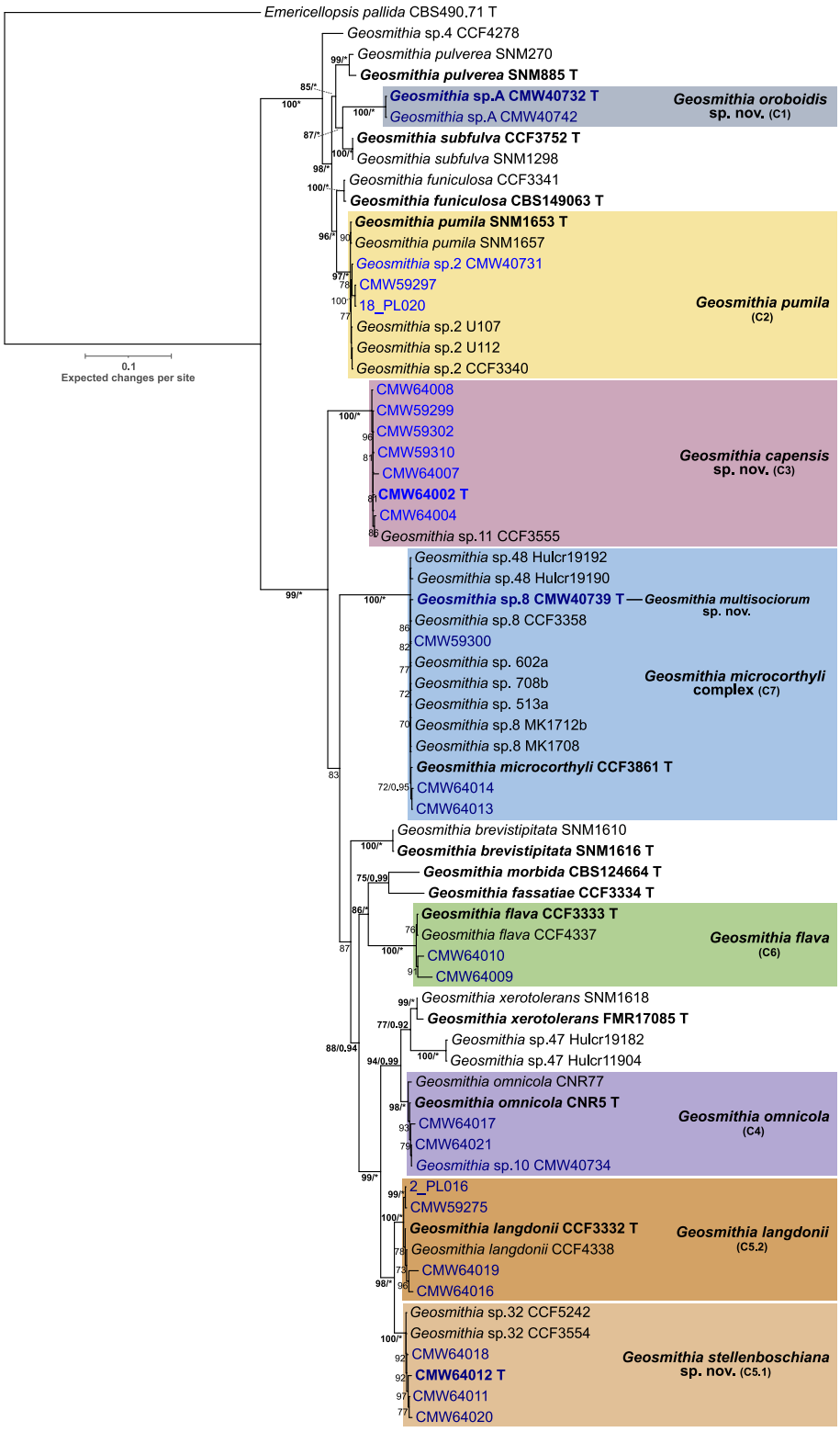

Fig. 1. Maximum Likelihood phylogeny of the concatenated ITS, *BT*, *EF1a* exon and *RPB2* regions of a subset of South African isolates (blue). Clades containing species considered in this study are highlighted and labelled C1-C7, according to the clades on the phylogenies of individual regions (Fig. S1). *Geosmithia* type strains (T), including those assigned as types in this study, are shown in bold. Isolates of Machingambi et al. (2014) are labelled according to their original identifications. Branch support values before the slash represent Transfer Bootstrap Expectation (TBE) support values ≥ 70 %. Bayesian Inference posterior probabilities (PP) above 0.9 are indicated on main branches after the slash, with asterisks denoting a PP of 1.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

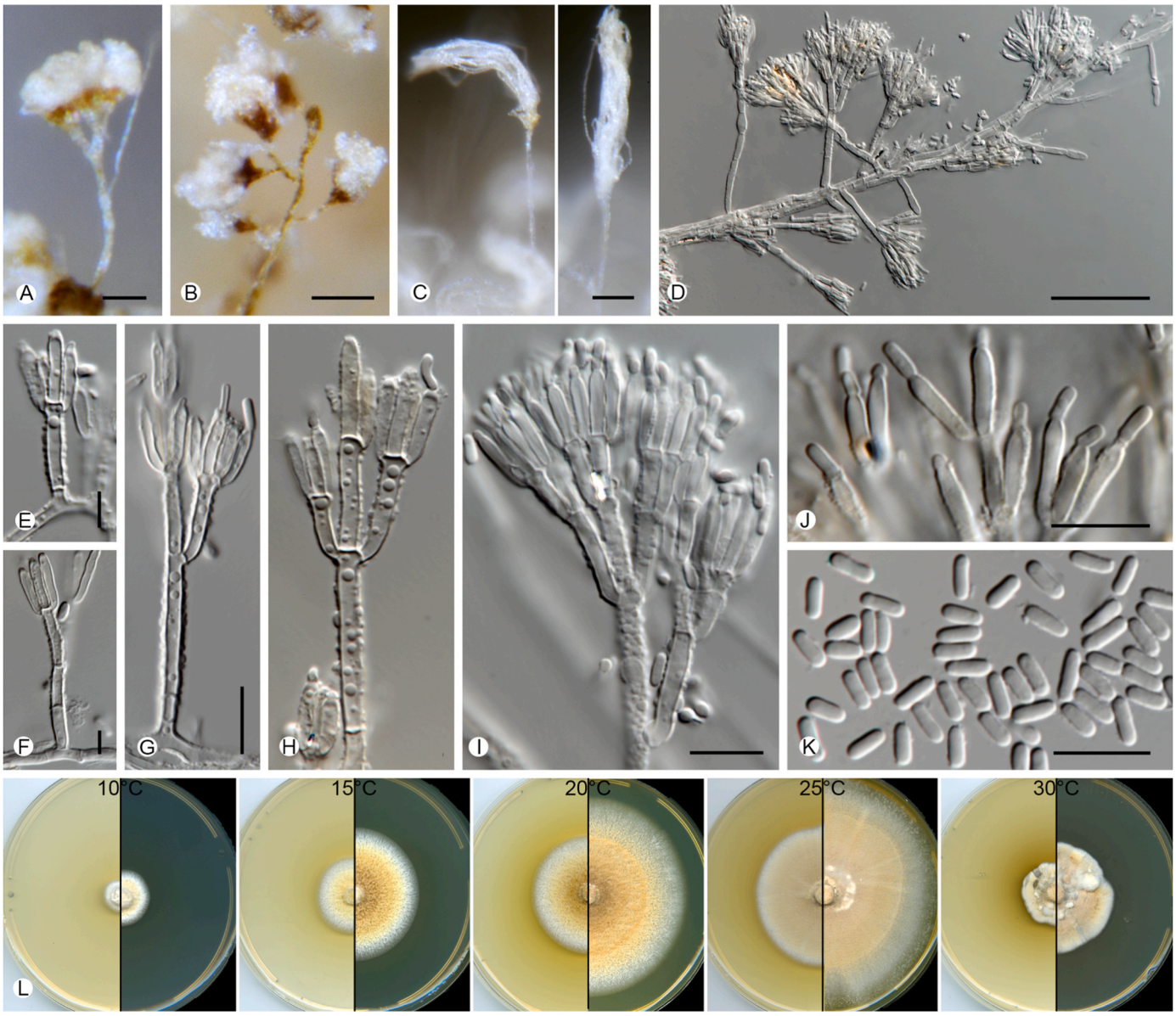

Fig. 2. Micrographs of *Geosmithia capensis* sp. nov (ex-holotype, CMW-IA 6985, CMW 64002). A–C. Conidiophores bearing chained conidia, showing pigmented exudates. D. Conidiophores borne on aerial funiculose mycelia. E–I. Conidiophores and penicilli. J. Conidiogenous cells (phialides). K. Conidia. L. Cultures on 2 % MEA at different temperatures for 14 days (left, white background) and 28 days (right, black background). Scale bars: B–D = 50 μm; A = 25 μm; G–K = 10 μm, E, F = 5 μm.

subsp. *africana*. June 2023. *W. Rippon, WR01* (Holotype PRU(M) 4605, stored in a metabolically inactive state; ex-holotype culture CMW-IA 6985, CMW 64002). GenBank: PQ032691 (ITS); PQ048054 (*BT*); PQ045591 (*RPB2*).

Description: *Sexual morph* not observed. *Asexual morph* penicillium-like. *Conidiophores* upright from vegetative hyphae on surface of media or on funiculose aerial mycelia, simple to branched; *stipes* hyaline to pale yellow with age, verruculose, simple or often branched irregularly, 22–174 (81.8 ± 22.44) μm long; *penicilli* terminal, appressed or somewhat loosely packed, getting pigmented with age, branched in 1–4 tiers including conidiogenous cells, 20–55 (36.9 ± 10.16) μm long; *rami* hyaline to pale yellow with age, verruculose, 11–20 (15.5 ± 2.33) μm long, in whorls of 2–4; *metulae* verruculose, 7–14 (10.4 ± 1.71) μm long, in whorls of 3–5. *Conidiogenous cells* phialidic, cylindrical, narrowed at apex, smooth or verruculose, 8–11 (9.2 ± 0.95) μm long, in whorls of 4–6. *Conidia* hyaline, aseptate, mostly cylindrical with round apex and truncate base, straight or occasionally slightly curved, occasionally ellipsoidal, 4–6 × 1.5–3 (4.8 ± 0.40 × 1.9 ± 0.26) μm, in basipetal chain. Funiculose aerial mycelia becoming yellowish with age. Pigmented exudate droplets on penicilli and stipes.

Culture characteristics: Optimum growth on 2 % MEA in 8 d at 25 °C reaching 1.52 mm/d, followed by 20 °C (1.36 mm/d), 15 °C (0.82 mm/d), 30 °C (0.65 mm/d) and 10 °C (0.23 mm/d). No growth at 5 °C and 35 °C; 5 °C cultures and two out of three 35 °C cultures grew back after re-incubated at 25 °C. Cultures (28 day-old) fertile; *shape* circular; *margin* entire (10–25 °C), lobate (30 °C); *elevation* flat (20–30 °C) to raised (10–15 °C); *texture* fluffy (10–15 °C), velvety becoming fluffy towards centre with patches of yellowish white aerial mycelia (20 °C), velvety with zonation or sectoring (25–30 °C); *colour* above yellowish white becoming paler towards edges (10 °C), dark orange yellow becoming paler towards edges (15 °C), strong yellowish brown becoming paler towards edges (20 °C), light yellowish brown becoming paler towards edges (25 °C), yellowish white with light yellowish brown sectors (30 °C); *density* medium (10–30 °C); *pigmentation on media* vivid

yellow to brilliant yellow (15–30 °C).

Hosts: *Adansonii gregorii*; *Lanurgus jubatus* infesting *Olea europaea* subsp. *africana*; *Lanurgus* spp. infesting *Widdringtonia cedarbergensis*; *Phloeosinus thujae* infesting *Chamaecyparis pisifera* and *Thuja occidentalis*; *Phleotribus scarabeiodes* infesting *O. europaea*; *Pseudopityophthorus minutissimus* and *Scolytus intricatus* infesting *Quercus* spp.; *Scolytus carpini* infesting *Carpinus betulus*.

Distribution: Eastern Europe; Mediterranean Europe; Southeastern USA; Western Australia; Western Cape Province, South Africa.

Additional specimen examined: South Africa: Western Cape Province, Cederberg, Algeria; 32°21′43.0″S, 19°04′10.0″E. 19 October 2021. Isolated from *Lanurgus* sp. 3 infesting *Widdringtonia cedarbergensis*. *R.J. Basson*, *WW0b1G017BB-i001C003* (culture CMW-IA 6984, CMW 59302). GenBank: PQ032699 (ITS); PQ048052 (*BT*); PQ045590 (*RPB2*); PQ045566 (*EF1a* intron).

Notes: *Geosmithia capensis* is described from isolates in the Western Cape Province of South Africa but forms a monophyletic clade (clade C3) with two Hungarian isolates of "*Geosmithia* sp. 11" that were well-supported (≥96 %) in all individual trees. The two isolates of "*Geosmithia* sp. 11" were reported from *Scolytus intricatus* beetles on *Quercus* spp. (Kolařík et al., 2008). An isolate collected from *Adansonii gregorii* bark in the Kimberley region of Western Australia also resolved within this clade using ITS data (Fig. S1A; Sakalidis et al., 2011). Additional Northern Hemisphere isolates are not available, but this taxon reportedly also occurs in the Mediterranean (Kolařík et al., 2008) and southeastern USA (Huang et al., 2017).

Geosmithia multisociorum Aylward, Marinc., M.J. Wingf & Roets, sp. nov. Fig. 3.

MycoBank: MB855501.

Etymology: "Multisociorum", referring to its wide range of tree hosts and beetle associates.

Diagnosis: *Geosmithia multisociorum* is closely related to *G. microcorthyli*. They can be differentiated by a few SNPs in the *BT* (1) and *EF1a* exon (3) regions and by conidial shape: *G. multisociorum* (ellipsoidal to cylindrical) and *G. microcorthyli* (globose).

Type: South Africa: Western Cape Province, Betty's Bay, Harold Porter National Botanical Garden. Isolated from *Cryphalini* sp. 1 infesting *V. oroboides*. 2012. *N.M. Machingambi*, *NM93* (Holotype PRU(M) 4604, stored in a metabolically inactive state; ex-holotype culture CMW-IA 6981, CMW 40739). GenBank: KJ513226 (ITS); PQ048047 (*BT*); PQ048070 (*EF1a* exon).

Description: *Sexual morph* not observed. *Asexual morph* penicilliumlike. *Conidiophores* upright borne on vegetative hyphae on surface of media or on funiculose aerial mycelia, simple or branched; *stipes* verruculose, straight or infrequently curved, occasionally attenuated towards base, 9–150 (60 ± 33.5) μm long; *penicilli* often loosely appressed,

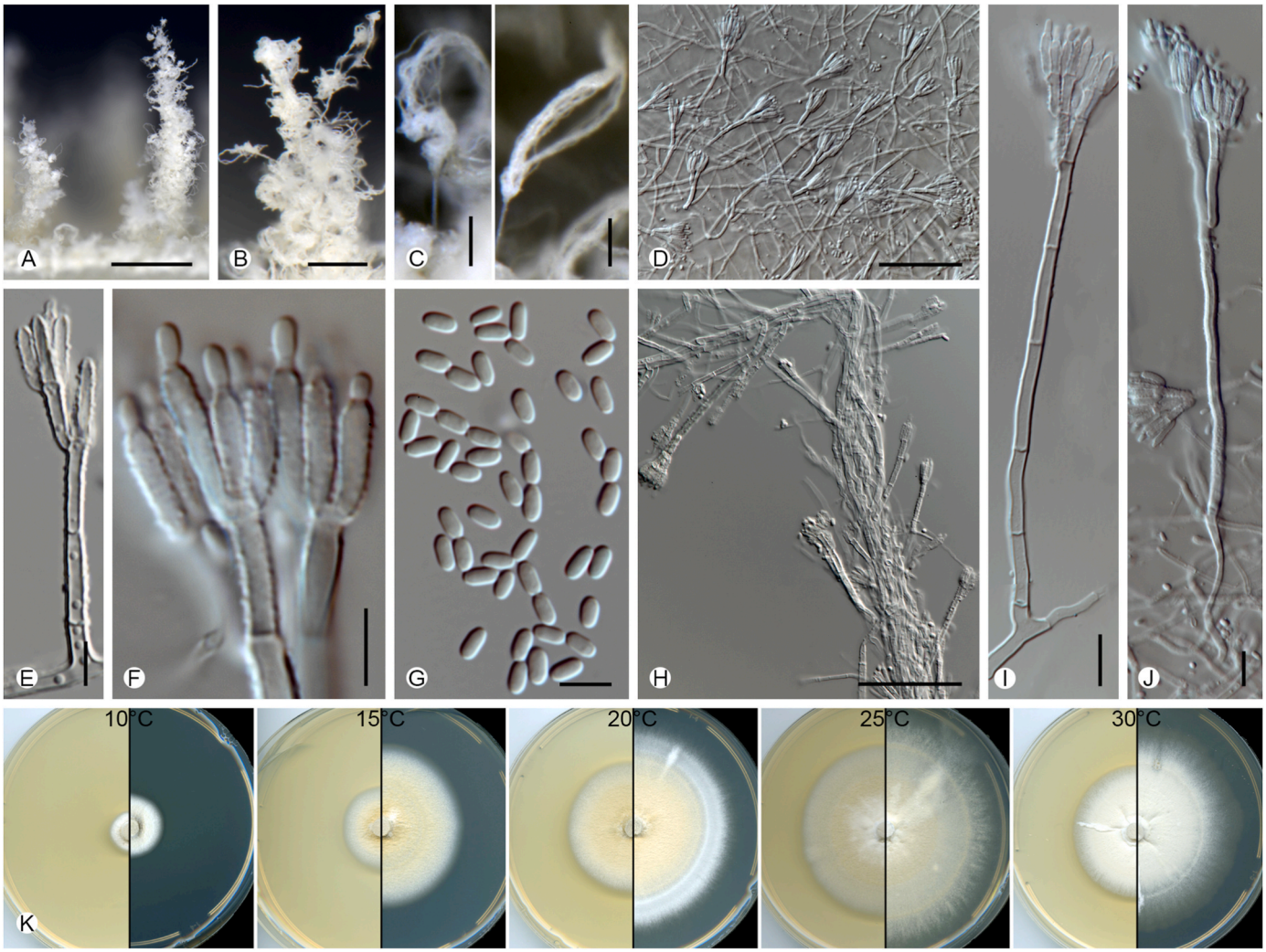

Fig. 3. Micrographs of *Geosmithia multisociorum* sp. nov (ex-holotype, CMW-IA 6981, CMW 40739). A, B. Conidiophores born on funiculose mycelia. C. Single conidiophore with chained conidia. D. Simple conidiophores on surface of media. E. Verruculose conidiophore and conidial apparatus. F. Conidiogenous cells (phialides). G. Conidia. H. Conidiophores on aerial funiculose mycelia. I. Upright conidiophore. J. Conidiophore with stipe attenuating towards the base. K. Colony on 2 % MEA in 14 days (left, white background) and 28 days (right, black background). Scale bars: A = 500 μm; B = 100 μm; C, D, H = 50 μm; I, J = 10 μm; E–G = 5 μm.

asymmetric, in 1–3 tiers including conidiogenous cells, 15.5–44 (26.8 ± 9.19) µm long; *rami* verruculose, 9–19 (12.3 ± 2.77) µm long, in whorls of 2–3; *metulae* verruculose, 7–12 (9.6 ± 1.16) µm long, whorls of 2–5. *Conidiogenous cells* phialidic, verruculose, 7–13 (9.1 ± 1.42) µm, in whorls of 3–6. *Conidia* hyaline, aseptate, ellipsoidal to cylindrical with round ends, smooth, 3–5 × 2–3 (3.66 ± 0.35 × 2.03 ± 0.23) µm, in basipetal chains.

Culture characteristics: Optimum growth on 2 % MEA in 8 d at 25 °C reaching 1.82 mm/d, followed by 20–30 °C (1.44 mm/d), 15 °C (0.9 mm/d), 10 °C (0.37 mm/d). No growth at 5 °C and 35 °C; 5 °C and 35 °C cultures grew back after re-incubation at 25 °C. Cultures (28 day-old) fertile; *shape* circular; *margin* entire (10–30 °C); *elevation* flat (10–30 °C); *texture* velvety to powdery (10–30 °C) with some fluffy sectors (15–25 °C); *colour* above yellowish white (10 °C), yellowish grey becoming greyish yellow towards with some sectors yellowish white (15–25 °C), yellowish white becoming paler towards edges (30 °C); *density* dense (10 °C), sparse to medium towards centre (15–30 °C); *pigmentation on media* absent.

Hosts: *Cryphalini* sp. infesting *Virgilia oroboides*; *Hylesinus* spp. infesting *Fraxinus excelsior*; *Hypothenemus* sp. infesting *Searsia angustifolia*; *Kissophagus hederae* infesting *Hedera helix*; *Lanurgus* spp. infesting *Widdringtonia* spp.; *Phloeosinus dentatus* infesting *Juniperus virginiana*; *Phloeosinus* sp. infesting *Cupressus sempervirens*; *P. thujae* infesting *Chamaecyparis pisifera*; *Scolytus carpini* infesting *Carpinus betulus*; *S. intricatus* infesting *Quercus* spp.; *S. mali* infesting *Malus domestica*; *S. rugulosus* infesting *Prunus* spp.; *Xyloctonus maculatus* infesting *Sideroxylon inerme*.

Distribution: Eastern and southern Europe; Georgia, USA; Israel; Western Cape Province, South Africa.

Additional specimen examined: South Africa: Western Cape Province, Cederberg, Algeria. 19 October 2021. Isolated from *Lanurgus* sp. 3 infesting *Widdringtonia cedarbergensis*. *R.J. Basson, WW0b1G001BB-f001C006* (culture CMW-IA 6983, CMW 59300). GenBank: PQ032712 (ITS); PQ048051 (*BT*); PQ045563 (*RPB2*); PQ048074 (*EF1a* exon); PQ045563 (*EF1* intron).

Notes: *Geosmithia multisociorum* was recovered from five different hosts and six different beetles across the Western Cape (Table 2). It is closely related to the primary ambrosial fungus *G. microcorthyli*, but conidial shape differentiates them. It was found to be con-specific with two *Geosmithia* spp. From the Northern Hemisphere, "*Geosmithia* sp. 8" from *Scolytus intricatus* infesting *Quercus* spp. in Europe (Kolařík et al., 2008) and "*Geosmithia* sp. 48" from *Phloeosinus dentatus* infesting *Juniperus virginiana* in the USA (Huang et al., 2019).

Geosmithia oroboidis Aylward, Marinc., M.J. Wingf & Roets, sp. nov. Fig. 4.

MycoBank: MB855502.

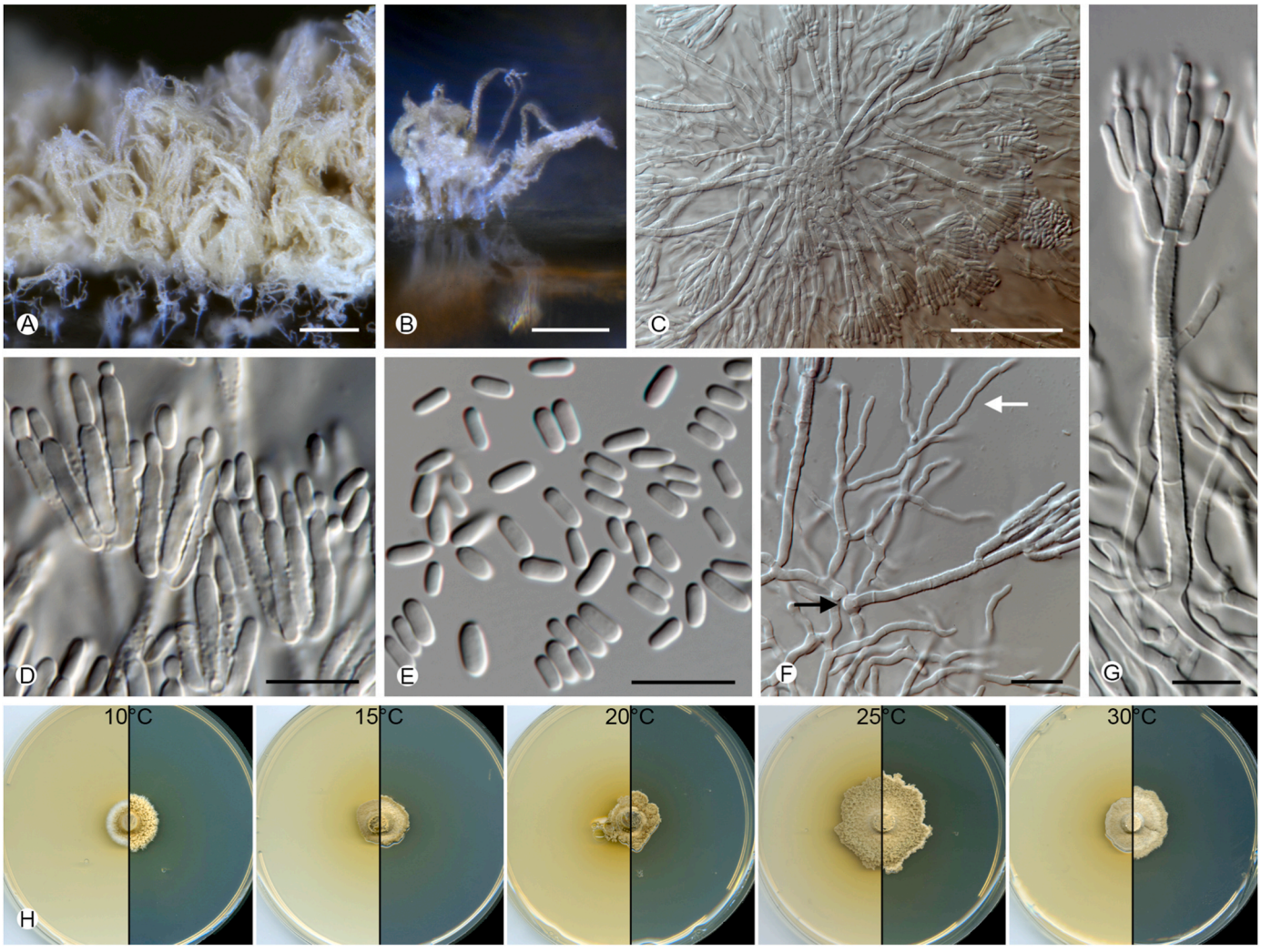

Fig. 4. Micrographs of *Geosmithia oroboidis* sp. Nov (ex-holotype, CMW-IA 6980, CMW 40732) (A, B) Conidiophores and chained conidia directly borne on 2 % MEA (C) Conidiophore cluster (D) Verruculose conidiogenous cells (phialides) (E) Conidia (F) Conidiophore originating from an inflated basal cell (black arrow) and undulate vegetative hyphae (white arrow) (G) Upright conidiophore (H) Colonies on 2 % MEA at different temperatures at 14 days (left, white background) and 28 days (right, black background). Scale bars: A, B = 100 µm; C = 50 µm; D–G = 10 µm.

Etymology: Referring to its host tree, *Virgilia oroboides*.

Diagnosis: *Geosmithia oroboidis* is morphologically distinguishable from closely related taxa by its larger conidial dimensions (3–6 × 2–3 μm), the presence of soluble pigmentation at all temperatures and slower growth (18–26 mm/8 d, 20–30 mm/14 d) at optimum temperature: *G. funiculosa* (1.6–5 × 0.9–2.5 μm, 39–48 mm/14 d), *G. pulverea* (2.1–5.1 × 1.1–2.0 μm, 30–37 mm/8 d), *G. pumila* (1.5–2.9 × 0.9–1.9 μm, 25–33 mm/8 d) and *G. subfulva* (1.1–2.2 × 1.0–1.7 μm, 24–36 mm/8 d). Phylogenetically, it is distinguished from its closest relatives *G. subfulva* and *G. pulverea* with either ITS or *EF1a* exon sequences. Five SNPs in the ITS region and 19 SNPs and one indel in the *EF1a* exon are unique to *G. oroboidis*.

Type: South Africa: Western Cape Province, Betty's Bay, Harold Porter National Botanical Garden; 34°20′51.0″S, 18°55′29.4″E. 2012. Isolated from *Scolytoplatypus fasciatus* infesting *Virgilia oroboides* subsp. o*roboides*. *N.M. Machingambi NM105* (Holotype PRU(M) 4603, stored in a metabolically inactive state; ex-holotype culture CMW-IA 6980, CMW 40732). GenBank: KJ533336 (ITS); PQ048068 (*EF1a* exon).

Description: *Sexual morph* not observed. *Asexual morph* penicillium-like. *Conidiophores* upright from vegetative hyphae mostly on surface of media, occasionally on inflated basal cells, verruculose, mostly simple; *stipes* verruculose, straight, 15–73 (45.6 ± 16.18) μm long; *penicilli* terminal, branched in 1–3 tiers including conidiogenous cells, 13–41 (27.8 ± 7.02) μm long, laterally appressed, asymmetric or symmetric; *rami* verruculose, 10–15 (12.7 ± 1.21) μm long, 2–4 in wholrs; *metulae* smooth or verruculose, 7–16 (10.1 ± 1.82) μm long, 2–5 in whorls. *Conidiogenous cells* phialidic, hyaline, smooth or verruculose, cylindrical with narrowed tip, 9–15 (11.1 ± 1.40) μm long, 3–7 in whorls. *Conidia* hyaline, pale yellow-shade in mass, cylindrical with round apex and pointed base to ellipsoidal, straight or slightly curved, 3–6 × 2–3 (4.9 ± 0.53 × 2.1 ± 0.23) μm, in basipetal chain. Some vegetative hyphae undulate. Funiculose mycelia scarce.

Culture characteristics: Optimum growth on 2 % MEA in 8 d at 25 °C reaching 1.05 mm/d, followed by 20 °C (0.6 mm/d), 30 °C (0.58 mm/d), 15 °C (0.54 mm/d) and 10 °C (0.39 mm/d). No growth at 5 °C and 35 °C; 5 °C cultures grew back when re-incubated at 25 °C but 35°C-cultures did not grow back. Daily growth rate declined by 76, 69, 80 % after 8 days at 20 °C, 25 °C and 30 °C, respectively. Cultures (28 day-old) fertile; *shape* circular; *margin* entire (10 °C), irregular with lobate (15–30 °C), no submerged hyphae at margins; *elevation* raised (10–30 °C); *texture* velutinous (20–30 °C) to fluffy (10–15 °C); *colour* above light yellowish brown (10 °C), yellowish grey with greyish yellowish brown edges (15 °C), yellowish grey with dark greyish yellow edges or patches (20 °C), greyish yellow (25 °C), yellowish grey (30 °C); *density* dense becoming furrowed (30 °C); *pigmentation on media* deep orange yellow (15–30 °C), inconspicuous.

Host: *Ellatoma* sp. on *Virgilia oroboides* subsp. f*erruginea*; *Scolytoplatypus fasciatus* infesting *V. oroboides* subsp. o*roboides*.

Distribution: Western Cape Province, South Africa.

Additional specimen examined: South Africa: Western Cape Province, Betty's Bay, Harold Porter Botanical Garden; −34.333581, 18.916811.2011. Isolated from *Scolytoplatypus fasciatus* infesting *Virgilia oroboides*. *N.M. Machingambi, NM005* (culture CMW-IA 6982, CMW 40742). GenBank: KJ533338 (ITS); PQ048071 (*EF1a* exon).

Notes: *Geosmithia oroboidis* was isolated by Machingambi et al. (2014) as "*Geosmithia* sp. A″ from *Scolytoplatypus fasciatus* on *V. oroboides* subsp. o*roboides* in the Harold Porter National Botanic Garden, Betty's Bay, South Africa. Additional collections were reported from an *Ellatoma* sp. on *V. oroboides* subsp. f*erruginea* in George. This taxon is distinct from all previously described *Geosmithia* species, being resolved with 100 % support with ITS and *EF1a* exon sequences (clade C1 in Fig. 1 and S1). *Geosmithia oroboidis* showed a close affinity to *G. funiculosa* (Crous et al., 2022), *G. pulverea, G. pumila*, and *G. subfulva* (Zhang et al., 2022) in both morphological and phylogenetic analyses. Their cultures are mostly velvety and have yellow to brown shades. *Geosmithia funiculosa* is widely distributed in Europe on various bark beetle species, whereas the other three species were collected in China from the galleries of *Scolytus semenovi*, *Dinoderus* sp., and *Ernoporus japonicus*, respectively. The larger conidial dimensions (3–6 × 2–3 μm), no growth at 5 °C or 35 °C, and the presence of pigmentation on 2 % MEA differentiates *G. oroboidis* from its close phylogenetic relatives. The CMW-IA 6982 (CMW 40742) culture showed less pigmentation and fruiting structures than the ex-holotype (CMW-IA 6980, CMW 40732).

Geosmithia stellenboschiana Aylward, Marinc., M.J. Wingf & Roets, sp. nov. Fig. 5.

MycoBank: MB855503.

Etymology: Referring to the town of Stellenbosch where this the holotype of this species was collected.

Diagnosis: *Geosmithia stellenboschiana* is a sister taxon to *G. langdonii* and can be distinguished by multiple SNPs in the *BT* (12 SNPs), *EF1a* exon (13) and *RPB2* (31) gene regions.

Type: South Africa: Western Cape Province, Stellenbosch, Banks of the Eerste River. Isolated from *Hypothenemus* sp. infesting *Searsia angustifolia*. June 2023. *W. Rippon, WR28* (Holotype PRU(M) 4606, stored in a metabolically inactive state; ex-holotype culture CMW-IA 6987, CMW 64012). GenBank: PQ032681 (ITS); PQ048061 (*BT*); PQ045598 (*RPB2*); PQ048081 (*EF1a* exon).

Description: *Sexual morph* not observed. *Asexual morph* penicillium-like. *Conidiophores* upright borne on vegetative hyphae on surface of media or on funiculose aerial mycelia, mostly simple, occasionally branched; *stipes* verruculose, 0–3-septate, 10–117 (36.4 ± 25.5) μm long; *penicilli* terminal, mostly branched in 2–3 tiers including conidiogenous cells, occasionally in 4 tiers, 14–37 (22.4 ± 6.64) μm long, mostly appressed, asymmetric, occasionally irregularly branched; *rami* verruculose, infrequently absent, 9–15 (11.1 ± 1.46) μm long, in whorls of 2–4; *metulae* verruculose, 7–11 (9.1 ± 1.03) μm long, in whorls of 2–5. *Conidiogenous cells* phialidic, verruculose, cylindrical, narrowed at the tip, 7–12 (9.2 ± 1.42) μm long, in whorls of 2–6. *Conidia* hyaline, aseptate, oblong to ellipsoidal, cylindrical with narrowed tip, borne in basipetal chains, 4–7 × 2–3 (4.8 ± .0.78 × 2.5 ± 0.28) μm, in basipetal chains.

Culture characteristics: Optimum growth on 2 % MEA in 8 d at 20–25 °C reaching 1.9 mm/d, followed by 15 °C (1.09 mm/d), 30 °C (0.78 mm/d), 10 °C (0.51 mm/d) and 5 °C (0.17 mm/d in 14 d). No growth at 35 °C, one of three 35 °C cultures grew back after re-incubated at 25 °C. Cultures (28 day-old) fertile; *shape* circular; *margin* (10–30 °C); *elevation* umbonate (10–30 °C); *texture* fluffy becoming woolly towards centre (10–25 °C), velvety becoming fluffy towards centre with cottony patches (30 °C); *colour* above yellowish white with white sectors (10–30 °C); *density* medium, dense in some sectors; *pigmentation on media* absent; sectoring common at all temperatures.

Hosts: *Hypothenemus* sp. infesting *Searsia angustifolia*; *Phloeosinus sequoiae* infesting *Sequoia sempervirens*; *P. thujae* infesting *Chamaecyparius pisifera*; *Phleoeotribus scarabeoides* infesting *Olea europea*.

Distribution: California, USA; Czech Republic; Spain; Western Cape Province, South Africa.

Additional specimen examined: South Africa: Western Cape Province, Stellenbosch, Banks of the Eerste River, June 2023. Isolated from *Hypothenemus* sp. infesting *Searsia angustifolia*. *W. Rippon, WR20* (culture CMW-IA 6986, CMW 64011). GenBank: PQ032678 (ITS); PQ048060 (*BT*); PQ045597 (*RPB2*); PQ048078 (*EF1a* exon).

Notes: *Geosmithia stellenboschiana* formed a sister clade with *G. langdonii*, which was isolated from *Scolytus intricatus* in the Czech Republic (Kolarík et al., 2005). The two "*Geosmithia* sp. 32" isolates from *Phloeosinus* spp. on *Cupressaceae* in the Czech Republic (Kolařík et al., 2008) and California (Kolařík et al., 2017) were found to be con-specific with *G. stellenboschiana*. This species, therefore, occurs on at least two genera of bark beetles on both gymnosperm and angiosperm hosts. *Geosmithia stellenboschiana* cultures did not show yellow shades nor pigmentation on 2 % MEA like *G. langdonii* and its *in vitro* conidial dimensions (4–7 × 2–3 μm) were larger than *G. langdonii* (3–5.5 × 1.5–3.5 μm).

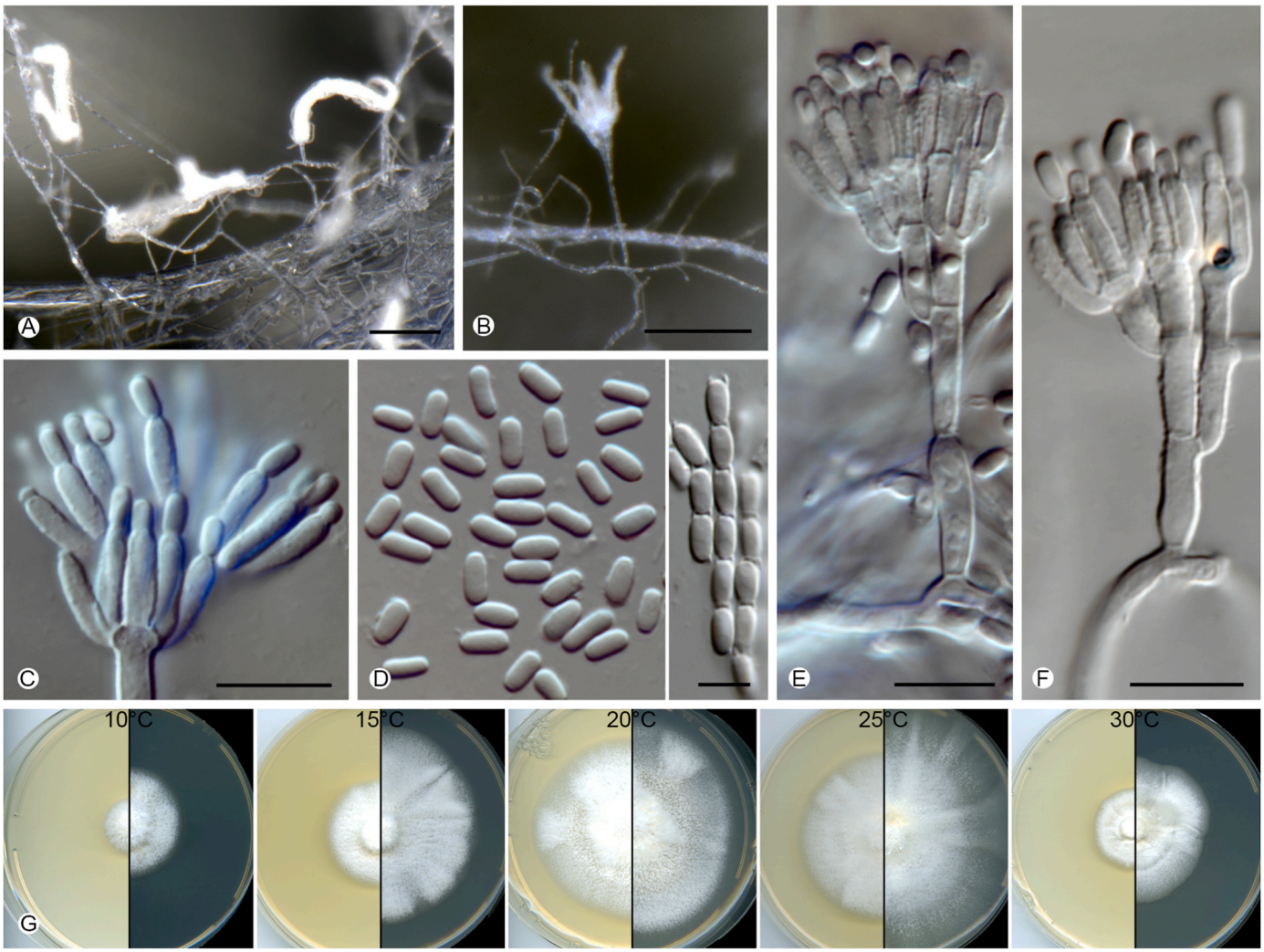

Fig. 5. Micrographs of *Geosmithia stellenboschiana* sp. nov (ex-holotype, CMW-IA 6987, CMW 64012). A. B. Conidiophores with chained conidia borne on aerial funiculose mycelia or single hypha. C. Conidiogenous cells (phialides). D. Conidia. E, F. Verruculose conidiophores. G. Culture on 2 % MEA at different temperatures for 14 days (left, white background) and 28 days (right, black background). Scale bars: A, B = 100 μ; C, E, F = 10 μm; D = 5 μm.

4. Discussion

This is only the second study specifically considering *Geosmithia* species in the Southern Hemisphere. It also represents the first *Geosmithia* species descriptions from South Africa. Only one other species has been described from the Southern Hemisphere (Crous et al., 2018). That species, *G. carolliae*, was isolated from the wings of a bat in Brazil, but based on ITS sequences, it likely also occurs on *Hypoborus ficus* beetles infesting *Ficus carica* in the Mediterranean region (Kolařík et al., 2007). "*Geosmithia* sp. A″ in the study of Machingambi et al. (2014), described as *G. oroboidis* in the present study, therefore, remains the only *Geosmithia* species known exclusively from the Southern Hemisphere, as noted by Kolařík and Hulcr (2023).

The *Geosmithia* species described in this study were obtained from beetles infesting various native South African trees, representing two orders of gymnosperms and four angiosperm orders. The three species known from the Northern Hemisphere, *G. langdonii*, *G. omnicola* and *G. pumila*, were obtained from multiple beetle–host combinations, consistent with their previously established generalist nature (Kolařík and Hulcr, 2023; Pepori et al., 2015; Zhang et al., 2022). *Geosmithia multisociorum*, described in this study, was also isolated from a variety of beetles and plant hosts and was the most widely occurring of all taxa thus far encountered in South Africa. This is even though the closely related Northern Hemisphere isolates, referred to as *Geosmithia* sp. 8 and *Geosmithia* sp. 48, are known from single beetle species, *Scolytus intricatus* and *Phloeosinus dentatus*, on *Quercus* spp. and *Juniperus virginiana*, respectively (Huang et al., 2019; Kolařík et al., 2008). Along with the multiple beetle vectors and tree hosts, the global distribution of this taxon, which includes South Africa, Europe, and the USA, points to its status as a generalist *Geosmithia* species.

In contrast to *G. capensis* and *G. multisociorum*, *G. oroboidis* appeared highly specialised, associating with a specific beetle and host tree. The *G. capensis* isolates collected in this study were all associated with only *Lanurgus* spp. beetles, although occurring on both an angiosperm and coniferous host tree. Other specialist *Geosmithia* species are known from specific host genera, e.g. *G. morbida* from *Jugulans* spp. (Kolařík et al., 2011) and *G. ulmaceae* from *Ulmus* spp. hosts (Kolařík and Hulcr, 2023; Pepori et al., 2015) or restricted to specific geographic areas, such as *G. funiculosa* in Europe (Crous et al., 2022). Further surveys will be required to determine whether the species described in this study, specifically *G. oroboidis*, represent host-specific or geographically restricted taxa.

Geosmithia multisociorum showed only minor differences to *G. microcorthyli* in the *BT* and *EF1a* exon sequences. *Geosmithia microcorthyli* is a primary ambrosial symbiont of a *Microcorthylus* sp. on *Cassia grandis* (Kolařík and Kirkendall, 2010) and the distinction between bark and ambrosial beetle symbionts presents a strong argument to distinguish between these species. Although ambrosial fungi may associate

with more than one related beetle species (Saucedo-Carabez et al., 2018), we know of no examples where the same ambrosial fungus is associated with phylogenetically different beetles. Along with the gene sequences, this also suggests that the isolates obtained from *Phloeosinus* sp. in Israel and deposited on GenBank as *G. microcorthyli* (Meshram et al., 2022) are the same as *G. multisociorum*.

The nature of the association between the *Geosmithia* species and bark beetles investigated in this study is largely unknown. Basson et al. (2024) considered the occurrence frequency of various fungal taxa associated with *Lanurgus* spp. beetles on *Widdringtonia* trees. They found that the *Geosmithia* species from the *Lanurgus* sp. 4 on *W. nodiflora* stems, described here as *G. multisociorum*, was isolated from the beetle almost 90 % of the time. In contrast, *G. capensis*, was isolated from only 36 % of the galleries of the *W. cedarbergensis* stem beetle *Lanurgus* sp. 3 and from 8 % of the galleries of *Lanurgus* sp. 1 on the same host. Both *Geosmithia* species were also isolated from other native South African host trees and beetles considered in this study.

Similar to other surveys that have preceded it, this study illustrates that collections from previously unexplored regions, hosts, and beetles will continue to yield novel *Geosmithia* taxa. The interspecific diversity of *Geosmithia* species in the Northern Hemisphere has received focused attention in the past 20 years (Kolařík and Hulcr, 2023) and the diversity found in South Africa appears to be similarly vast. South Africa's rich diversity of vascular plants and understudied insect diversity, especially in the Cape Floristic Region of the Western Cape (Manning and Goldblatt, 2012), suggests that many other *Geosmithia* species will likely be found on native trees throughout the region. Although knowledge regarding *Geosmithia* species diversity continues to expand, the ecological role of these beetle-associated fungi, specifically of the non-ambrosial species, remains uncertain. Focused attention is required to understand the nature of the association between these fungi and their beetle associates, their impact on host trees and their global distribution patterns.

CRediT authorship contribution statement

Janneke Aylward: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Formal analysis. **Seonju Marincowtiz:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Renier J. Basson:** Investigation. **William Rippon:** Investigation. **Michael J. Wingfield:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Francois Roets:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2024.09.006.

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