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ORIGINAL ARTICLE

New plectosphaerellaceous species from Dutch garden soil

Alejandra Giraldo[1,2] · Margarita Hernández-Restrepo[1] · Pedro W. Crous[1,2,3]

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Abstract

During 2017, the Westerdijk Fungal Biodiversity Institute (WI) and the Utrecht University Museum launched a Citizen Science project. Dutch school children collected soil samples from gardens at different localities in the Netherlands, and submitted them to the WI where they were analysed in order to find new fungal species. Around 3000 fungal isolates, including filamentous fungi and yeasts, were cultured, preserved and submitted for DNA sequencing. Through analysis of the ITS and LSU sequences from the obtained isolates, several plectosphaerellaceous fungi were identified for further study. Based on morphological characters and the combined analysis of the ITS and *TEF1-α* sequences, some isolates were found to represent new species in the genera *Phialoparvum*, i.e. *Ph. maaspleinense* and *Ph. rietveltiae*, and *Plectosphaerella*, i.e. *Pl. hanneae* and *Pl. verschoorii*, which are described and illustrated here.

Keywords Biodiversity · Citizen Science project · *Phialoparvum* · *Plectosphaerella* · Soil-born fungi

Introduction

Soil is one of the main reservoirs of fungal species and commonly ranks as the most abundant source regarding fungal biomass and physiological activity. Fungal diversity is affected by the variety of microscopic habitats and microenvironments present in soils (Anderson and Domsch 1978; Bills et al. 2004). More than half of the soil mycobiota is composed of basidiomycetes, although they are overlooked by most isolation techniques (Kirk et al. 2001). The remaining fungal biomass is comprised by Ascomycota, Chytridiomycota, Mucorales and Oomycetes (Kirk et al. 2001; Bills et al. 2004; Crous et al. 2019). This observed species richness has been supported by the discovery of previously unrecognised phylogenetic fungal lineages in soil-inhabiting fungi (Tedersoo et al. 2017).

Among Ascomycota, the family Plectosphaerellaceae (Glomerellales, Sordariomycetes) harbours important plant pathogens such as *Verticillium dahliae*, *V. alboatrum* and *Plectosphaerella cucumerina*, but also several saprobic genera usually found in soil, i.e. *Chordomyces*, *Gibellulopsis* and *Sodiomyces* (Domsch et al. 2007; Zare et al. 2007; Carlucci et al. 2012; Grum-Grzhimaylo et al. 2013, 2016). Members of this family are mainly known from their asexual morphs, which are morphologically characterised by simple or verticillate conidiophores with mono- or polyphialidic conidiogenous cells, and 1- or 2-celled elongate conidia arranged in slimy heads or chains, and rarely produced sporodochia or synnemata. The sexual morph is mostly observed in culture, showing perithecial or cleistothecial ascomata, superficial, brown to dark brown, with clavate or saccate asci and hyaline to pale brown ascospores (Giraldo and Crous 2019).

The interest in soil fungi has recently increased due to the high demand for new compounds with application in the biotechnological or pharmaceutical industries (Hujslová et al. 2010), and also because this substratum contains a pool of interesting and undescribed species, especially when samples from diverse habitats are studied (Blackwell 2011; Tedersoo et al. 2014, 2017). Therefore, during 2017, children from different locations in the Netherlands collected soil samples which were

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Alejandra Giraldo
a.giraldo@westerdijkinstitute.nl

1 Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

2 Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State, 339, Bloemfontein 9300, South Africa

3 Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

analysed in order to find new fungal species. This was done during the course of a Citizen Science project, named 'Wereldfaam, een schimmel met je eigen naam' (World fame - a fungus with your name) coordinated by the Westerdijk Fungal Biodiversity Institute and the Utrecht University Museum. Among the examined samples, several plectosphaerellaceous fungi were isolated, some of which have been recently described as new species in the genera *Gibellulopsis* and *Lectera* (Crous et al. 2018), while others represent new species in the genera *Phialoparvum* and *Plectosphaerella*, which are described and illustrated here.

Materials and methods

Sampling and isolation

Protocols for the collection and processing of soil samples are described in Groenewald et al. (2018). After 1 week of incubation on MEA supplemented with penicillin-G and streptomycin, individual colonies were transferred to MEA plates without antibiotics and incubated between 22 and 24 °C for 7–14 days, in order to obtain axenic cultures. Subsequently, each isolate was submitted for DNA extraction and sequencing. All isolates are maintained in the Johanna Westerdijk (JW) collection housed at the Westerdijk Fungal Biodiversity Institute in Utrecht, The Netherlands. Isolates from new and interesting species are also deposited at the CBS Culture Collection at the WI.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from colonies growing on MEA using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), following the manufacturer's protocol. The internal transcribed spacer (ITS) regions and the 5′ end of the 28S nrDNA gene (LSU) were amplified for all isolates with the primer combination ITS5/LR5, and sequenced with the primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990; Vilgalys and Sun 1994), respectively. Additional loci were sequenced for some isolates, including fragments from the protein-coding genes translation elongation factor 1-alpha (*TEF1-α*) and RNA polymerase II second largest subunit (*RPB2*) with the primer sets EF-983F/EF-2218R (Rehner and Buckley 2005) and RPB2-5F2/RPB2-7cR (Liu et al. 1999), respectively. The consensus sequence of each locus was obtained by using the program SeqMan v. 12.1.0 (DNASTAR, Madison, WI, USA).

Species identification

Firstly, the LSU and ITS consensus sequences from each isolate were blasted against sequences available in NCBI (http://blast.ncbi.nlm.nih.gov) and CBS (http://www.westerdijkinstitute.nl/Collections) databases, in order to determine the generic placement of each isolate. Secondly, sequences from all isolates identified as members of the Plectosphaerellaceae were aligned with sequences from ex-type and reference strains of the species currently accepted in the genera (Table 1) to which each isolate belonged. Separate analyses based on ITS or ITS combined with *TEF1-α* were performed for those isolates that were not identified at level species by using the previous approach.

Phylogenetic analysis

Sequences were aligned through MAFFT v. 7 (Katoh et al. 2017), using the default parameters, and were manually corrected in MEGA v. 6.06 (Tamura et al. 2013). Phylogenetic inferences of the individual gene regions and the combined dataset were based on maximum likelihood (ML) and were performed on the CIPRES Science Gateway portal (Miller et al. 2012) using RAxML v. 8.2.10 (Stamatakis, 2014). The default parameters were used, and bootstrap support (BS) was carried out using the rapid bootstrapping algorithm with the automatic halt option. A BS ≥ 70% was considered as statistically significant. Each partition was assessed for incongruence before being concatenated by checking individual phylogenies for conflicts between clades with significant ML support (Mason-Gamer and Kellogg 1996; Wiens 1998). All novel DNA sequences generated in this study were deposited in GenBank and ENA databases (Table 1), while the alignments and the resulting trees were accessioned in TreeBASE (http://www.treebase.org).

Morphological characterisation of novel species

Morphological features from the new species were determined on oatmeal agar (OA), potato carrot agar (PCA), 2% potato dextrose agar (PDA) and 2% malt extract agar (MEA) (recipes in Crous et al. 2019). Cultures were incubated at ca. 24 °C in the dark for 4 weeks. Macroscopic characters and diameters were measured after 14 days of incubation, and the colony colour (surface and reverse) rated after Rayner (1970). Microscopic features were examined from slide cultures and preparations mounted in clear lactic acid from colonies sporulating on the media previously mentioned. Observations were performed with a Zeiss V20 Discovery (Zeiss, Oberkochen, Germany) stereo microscope and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) optics. Photomicrographs and measurements

Table 1 Details of the isolates used in this study. New generated sequences are in bold

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no.				Reference
				LSU	ITS	*TEF1-α*	*RPB2*	
Acrostalagmus luteoalbus	CBS 121214	*Musa sapientum*	Brazil, Minas Gerais, Viçosa	LR025791	LR026662	–	–	Giraldo and Crous (2019)
	JW 1001	Garden soil	The Netherlands, Amsterdam	**LR590267**	**LR590089**	–	–	This study
	JW 1049	Garden soil	The Netherlands, Amsterdam	**LR590269**	**LR590090**	–	–	This study
	JW 10014	Garden soil	The Netherlands, Utrecht	**LR590268**	–	–	–	This study
	JW 22005	Garden soil	The Netherlands, Vlissingen	**LR590271**	**LR590092**	–	–	This study
	JW 22008	Garden soil	The Netherlands, Vlissingen	**LR590272**	**LR590093**	–	–	This study
	JW 22010	Garden soil	The Netherlands, Vlissingen	**LR590273**	**LR590094**	–	–	This study
	JW 22029	Garden soil	The Netherlands, Vlissingen	**LR590274**	**LR590095**	–	–	This study
	JW 57005	Garden soil	The Netherlands, Capelle aan den Ijssel	–	**LR590098**	–	–	This study
	JW 59014	Garden soil	The Netherlands, Utrecht	–	**LR590099**	–	–	This study
	JW 145012	Garden soil	The Netherlands, Wageningen	**LR590270**	**LR590091**	–	–	This study
	JW 234011	Garden soil	The Netherlands, Born	**LR590275**	**LR590096**	–	–	This study
	JW 234012B	Garden soil	The Netherlands, Born	–	**LR590097**	–	–	This study
	JW 263015	Garden soil	The Netherlands, Utrecht	**LR590276**	–	–	–	This study
A. annulatus	DAOMC 212126	Soil and roots	Brazil, Pará, near Belen	GU180646	GU180632	–	–	Réblová et al. (2011)
Brunneochlamydosporium cibotii	CBS 109240[IT]	*Cibotium schiedei*	The Netherlands, Delft	LR025807	LR026678	–	–	Giraldo and Crous (2019)
B. macroclavatum	CBS 101249[T]	Pteridophyte	Mauritius	LR025811	LR026682	–	–	Giraldo and Crous (2019)
	JW 10015 = CBS 145320	Garden soil	The Netherlands, Utrecht	**LR590277**	**LR590100**	**LR594766**	**LR594787**	This study
B. nepalense	CBS 971.72[T]	Soil under *Pinus* sp.	Nepal, Himalaya septentrional	LR025813	LR026684	–	–	Giraldo and Crous (2019)
	CBS 277.89	Soil	Unknown	LR025812	LR026683	–	–	Giraldo and Crous (2019)
	CBS 113254	Scrub sandy soil	The Netherlands, Kwade Hoek	LR025815	LR026686	–	–	Giraldo and Crous (2019)
	CBS 116720	Scrub sandy soil	The Netherlands, Kwade Hoek	LR025816	LR026687	–	–	Giraldo and Crous (2019)
	CBS 116721	Scrub sandy soil	The Netherlands, Kwade Hoek	LR025817	LR026688	–	–	Giraldo and Crous (2019)
	CBS 116722	Scrub sandy soil	The Netherlands	LR025818	LR026689	–	–	Giraldo and Crous (2019)
	JW 5014	Garden soil	The Netherlands, Nieuwegein	**LR590285**	–	–	–	This study
	JW 13002	Garden soil	The Netherlands, Alkmaar	**LR590278**	**LR590101**	–	–	This study
	JW 17010	Garden soil	The Netherlands, Ravenswaaij	**LR590281**	**LR590104**	–	–	This study
	JW 17016	Garden soil	The Netherlands, Ravenswaaij	**LR590282**	**LR590105**	–	–	This study
	JW 17026	Garden soil	The Netherlands, Ravenswaaij	**LR590283**	**LR590106**	–	–	This study
	JW 141012	Garden soil	The Netherlands, Kortenhoef	**LR590279**	**LR590102**	–	–	This study
	JW 147024	Garden soil	The Netherlands, Utrecht	**LR590280**	**LR590103**	–	–	This study
	JW 263017	Garden soil	The Netherlands, Utrecht	**LR590284**	**LR590107**	–	–	This study
Chordomyces albus	CBS 987.87[T]	*Hypogymnia physodes*	Luxembourg	JX158444	DQ825970	–	–	Grum-Grzhimaylo et al. (2013), Zare et al. (2007)
	JW 1007	Garden soil	The Netherlands, Amsterdam	**LR590286**	**LR590108**	–	–	This study
	JW 16023	Garden soil	The Netherlands, Reeuwijk	**LR590290**	**LR590113**	–	–	This study
	JW 25004	Garden soil	The Netherlands, Hooglanderveen	**LR590300**	**LR590123**	–	–	This study
	JW 96001	Garden soil	The Netherlands, Leiden	**LR590301**	**LR590124**	–	–	This study

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
	JW 143002	Garden soil	The Netherlands, Utrecht	**LR590287**	**LR590109**	–	–	This study
	JW 144015	Garden soil	The Netherlands, Herten	**LR590288**	**LR590110**	–	–	This study
	JW 149016	Garden soil	The Netherlands, Utrecht	–	**LR590111**	–	–	This study
	JW 160002	Garden soil	The Netherlands, Houten	**LR590289**	**LR590112**	–	–	This study
	JW 187003	Garden soil	The Netherlands, Soest	**LR590291**	**LR590114**	–	–	This study
	JW 189008	Garden soil	The Netherlands, Weert	**LR590292**	**LR590115**	–	–	This study
	JW 200003	Garden soil	The Netherlands, Houten	**LR590293**	**LR590116**	–	–	This study
	JW 211012	Garden soil	The Netherlands, Utrecht	**LR590294**	**LR590117**	–	–	This study
	JW 211015	Garden soil	The Netherlands, Utrecht	**LR590295**	**LR590118**	–	–	This study
	JW 232015	Garden soil	The Netherlands, Nederweert	**LR590296**	**LR590119**	–	–	This study
	JW 232019	Garden soil	The Netherlands, Nederweert	**LR590297**	**LR590120**	–	–	This study
	JW 234007	Garden soil	The Netherlands, Born	**LR590298**	**LR590121**	–	–	This study
	JW 234017	Garden soil	The Netherlands, Born	**LR590299**	**LR590122**	–	–	This study
C. antarcticus	CBS 120045[T]	Soda soil	Russia, Kulunda Steppe, Altai	KJ443109	KJ443241	–	–	Grum-Grzhimaylo et al. (2016)
	JW 20003	Garden soil	The Netherlands, 's-Gravenhage	**LR590304**	**LR590127**	–	–	This study
	JW 95011	Garden soil	The Netherlands, Warmond	**LR590305**	**LR590128**	–	–	This study
	JW 96013	Garden soil	The Netherlands, Leiden	**LR590306**	**LR590129**	–	–	This study
	JW 125029	Garden soil	The Netherlands, Amersfoort	**LR590302**	**LR590125**	–	–	This study
	JW 150004	Garden soil	The Netherlands, Liempde	**LR590303**	**LR590126**	–	–	This study
Furcasterigmium furcatum	CBS 122.42[T]	Dune sand under *Calystegia soldanella*	France, Normandie, Pointe du Siège	LR025838	LR026709	–	–	Giraldo and Crous (2019)
	JW 12002	Garden soil	The Netherlands, Breda	**LR590307**	**LR590130**	–	–	This study
	JW 96018	Garden soil	The Netherlands, Leiden	–	**LR590133**	–	–	This study
	JW 125004	Garden soil	The Netherlands, Amersfoort	**LR590308**	**LR590131**	–	–	This study
	JW 170003	Garden soil	The Netherlands, Utrecht	**LR590309**	**LR590132**	–	–	This study
Fuscohypha expansa	CBS 103.95	Soil	Brazil	LR025844	LR026714	LR026411	–	Giraldo and Crous (2019)
	CBS 418.89[T]	Tuber of Dioscorea sp.	Martinique	LR025845	LR026715	LR026412	–	Giraldo and Crous (2019)
Gibellulopsis aquatica	CBS 117131[T]	Cloud water	France	LR025850	LR026720	–	–	Giraldo and Crous (2019)
G. catenata	CBS 113951[T]	Cervical swab of mare	Germany	LR025851	LR026721	–	–	Giraldo and Crous (2019)
G. fusca	CBS 308.38	*Apium graveolens*	Germany, Giessen	LR025852	LR026722	–	–	Giraldo and Crous (2019)
	CBS 402.80	*Aegopodium podagraria*	The Netherlands, Baarn	LR025853	LR026723	–	–	Giraldo and Crous (2019)
	CBS 560.65[T]	Soil	India, Banaras	LR025854	LR026724	–	–	Giraldo and Crous (2019)
	CBS 747.83	*Apium graveolens*	The Netherlands	LR025855	LR026725	–	–	Giraldo and Crous (2019)
	JW 40010	Garden soil	The Netherlands, Goes	–	**LR590633**	–	–	This study
	JW 40011	Garden soil	The Netherlands, Goes	**LR590312**	**LR590135**	–	–	This study
	JW 122019	Garden soil	The Netherlands, Leiden	**LR590310**	**LR590253**	–	–	This study
	JW 214001	Garden soil	The Netherlands, Nijkerk	**LR590311**	**LR590134**	–	–	This study
G. nigrescens	CBS 179.40	Wrapping material	The Netherlands, Rotterdam	LR025857	LR026727	–	–	Giraldo and Crous (2019)
	CBS 455.51	*Solanum tuberosum*	United Kingdom	LR025858	LR026728	–	–	Giraldo and Crous (2019)
	CBS 469.64		Denmark, Klippinge	LR025859	LR026729	–	–	

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
		Seedling of *Linum usitatissimum*						Giraldo and Crous (2019)
	CBS 470.64	*Medicago sativa*	France	LR025860	LR026730	–	–	Giraldo and Crous (2019)
	CBS 100833	Soil	Israel, Lahav	LR025864	LR026734	–	–	Giraldo and Crous (2019)
	CBS 100844	*Solanum tuberosum*	Israel, Kerem-Shalom	LR025865	LR026735	–	–	Giraldo and Crous (2019)
	CBS 100829	*Solanum tuberosum*	Israel, Kerem-Shalom	LR025862	LR026732	–	–	Giraldo and Crous (2019)
	CBS 100832	Soil	Israel, Lahav	LR025863	LR026733	–	–	Giraldo and Crous (2019)
	CBS 110719	Sandy soil	The Netherlands, Kwade Hoek	LR025866	LR026736	–	–	Giraldo and Crous (2019)
	CBS 119666	Nail	The Netherlands	LR025867	LR026737	–	–	Giraldo and Crous (2019)
	CBS 120949[NT]	Soil under lawn	The Netherlands, Baarn	LR025868	LR026738	–	–	Giraldo and Crous (2019)
	CBS 123176	Moisture damaged building insulator wool	Finland	LR025869	LR026739	–	–	Giraldo and Crous (2019)
	JW 4001	Garden soil	The Netherlands, Breda	**LR590352**	**LR590175**	–	–	This study
	JW 4003	Garden soil	The Netherlands, Breda	**LR590353**	**LR590176**	–	–	This study
	JW 18031	Garden soil	The Netherlands, Valkenswaard	**LR590323**	**LR590146**	–	–	This study
	JW 22012	Garden soil	The Netherlands, Vlissingen	**LR590330**	**LR590153**	–	–	This study
	JW 24012	Garden soil	The Netherlands, Utrecht	**LR590341**	**LR590164**	–	–	This study
	JW 26002	Garden soil	The Netherlands, Soest	**LR590343**	**LR590166**	–	–	This study
	JW 26005	Garden soil	The Netherlands, Soest	**LR590344**	**LR590167**	–	–	This study
	JW 26011	Garden soil	The Netherlands, Soest	**LR590345**	**LR590168**	–	–	This study
	JW 26030	Garden soil	The Netherlands, Soest	**LR590346**	**LR590169**	–	–	This study
	JW 27001	Garden soil	The Netherlands, Vleuten	**LR590349**	**LR590172**	–	–	This study
	JW 27033	Garden soil	The Netherlands, Vleuten	**LR590350**	**LR590173**	–	–	This study
	JW 29026	Garden soil	The Netherlands, Oude Wetering	**LR590351**	**LR590174**	–	–	This study
	JW 48001	Garden soil	The Netherlands, De Bilt	**LR590354**	**LR590177**	–	–	This study
	JW 52010	Garden soil	The Netherlands, Driebergen-Rijsenburg	**LR590355**	**LR590178**	–	–	This study
	JW 67017	Garden soil	The Netherlands, Zandvoort	**LR590356**	**LR590179**	–	–	This study
	JW 79018	Garden soil	The Netherlands, Kapel Avezaath	**LR590357**	**LR590180**	–	–	This study
	JW 80012	Garden soil	The Netherlands, Gouda	**LR590358**	**LR590181**	–	–	This study
	JW 80013	Garden soil	The Netherlands, Gouda	**LR590359**	**LR590182**	–	–	This study
	JW 85004	Garden soil	The Netherlands, Eibergen	**LR590360**	**LR590183**	–	–	This study
	JW 96006	Garden soil	The Netherlands, Leiden	**LR590361**	**LR590184**	–	–	This study
	JW 96011	Garden soil	The Netherlands, Leiden	**LR590362**	**LR590185**	–	–	This study
	JW 126006	Garden soil	The Netherlands, Bunschoten-Spakenburg	**LR590313**	**LR590136**	–	–	This study
	JW 130017	Garden soil	The Netherlands, Utrecht	**LR590314**	**LR590137**	–	–	This study
	JW 132013	Garden soil	The Netherlands, Meteren	**LR590315**	**LR590138**	–	–	This study
	JW 143007	Garden soil	The Netherlands, Utrecht	**LR590316**	**LR590139**	–	–	This study
	JW 143017	Garden soil	The Netherlands, Utrecht	**LR590317**	**LR590140**	–	–	This study
	JW 158004	Garden soil	The Netherlands, Houten	**LR590318**	**LR590141**	–	–	This study
	JW 163008	Garden soil	The Netherlands, Amersfoort	**LR590319**	**LR590142**	–	–	This study
	JW 169002	Garden soil	The Netherlands, Utrecht	**LR590320**	**LR590143**	–	–	This study
	JW 171009	Garden soil	The Netherlands, Baarn	**LR590321**	**LR590144**	–	–	This study
	JW 176011	Garden soil	The Netherlands, Utrecht	**LR590322**	**LR590145**	–	–	This study
	JW 185004	Garden soil	The Netherlands, Ophemert	**LR590324**	**LR590147**	–	–	This study

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
	JW 189031	Garden soil	The Netherlands, Weert	**LR590325**	**LR590148**	–	–	This study
	JW 191001	Garden soil	The Netherlands, Amersfoort	**LR590326**	**LR590149**	–	–	This study
	JW 191003	Garden soil	The Netherlands, Amersfoort	**LR590327**	**LR590150**	–	–	This study
	JW 210002	Garden soil	The Netherlands, Heerenveen	**LR590328**	**LR590151**	–	–	This study
	JW 214006	Garden soil	The Netherlands, Nijkerk	**LR590329**	**LR590152**	–	–	This study
	JW 221006	Garden soil	The Netherlands, Houten	**LR590331**	**LR590154**	–	–	This study
	JW 221021	Garden soil	The Netherlands, Houten	**LR590332**	**LR590155**	–	–	This study
	JW 221027	Garden soil	The Netherlands, Houten	**LR590333**	**LR590156**	–	–	This study
	JW 221030	Garden soil	The Netherlands, Houten	**LR590334**	**LR590157**	–	–	This study
	JW 234005	Garden soil	The Netherlands, Born	**LR590335**	**LR590158**	–	–	This study
	JW 234009	Garden soil	The Netherlands, Born	**LR590336**	**LR590159**	–	–	This study
	JW 234014	Garden soil	The Netherlands, Born	**LR590337**	**LR590160**	–	–	This study
	JW 234016	Garden soil	The Netherlands, Born	**LR590338**	**LR590161**	–	–	This study
	JW 234022	Garden soil	The Netherlands, Born	**LR590339**	**LR590162**	–	–	This study
	JW 236004	Garden soil	The Netherlands, Maasbracht	**LR590340**	**LR590163**	–	–	This study
	JW 244014	Garden soil	The Netherlands, Jorwert	**LR590342**	**LR590165**	–	–	This study
	JW 261004	Garden soil	The Netherlands, Utrecht	**LR590347**	**LR590170**	–	–	This study
	JW 269002	Garden soil	The Netherlands, Utrecht	**LR590348**	**LR590171**	–	–	This study
G. serrae	CBS 125.79	Soil	New Zealand, Havelock North	LR025870	LR026740	–	–	Giraldo and Crous (2019)
	CBS 175.75	*Solanum tuberosum*	Germany	LR025871	LR026741	–	–	Giraldo and Crous (2019)
	CBS 290.30[T]	Human eye	Italy	LR025872	LR026742	–	–	Giraldo and Crous (2019)
	CBS 345.39	Wood pulp	Sweden	LR025873	LR026743	–	–	Giraldo and Crous (2019)
	CBS 383.66	*Beta vulgaris* var. altissima	Canada, Quebec	LR025874	LR026744	–	–	Giraldo and Crous (2019)
	CBS 387.35	*Amaranthus tricolour*	Italy	LR025875	LR026745	–	–	Giraldo and Crous (2019)
	CBS 392.89	Seed of *Abelmoschus esculentus*	Cuba, Santiago de las Vegas	LR025876	LR026746	–	–	Giraldo and Crous (2019)
	CBS 493.82A	Soil of *Glycine max*	Argentina, Misiones, Cerro Azul	LR025878	LR026748	–	–	Giraldo and Crous (2019)
	CBS 493.82B	Seed	Argentina, Buenos Aires, Castelar	LR025879	LR026749	–	–	Giraldo and Crous (2019)
	CBS 493.82C	Seed	Argentina, Chaco, Las Brenas	LR025880	LR026750	–	–	Giraldo and Crous (2019)
	CBS 493.82D	Seed	Argentina, Buenos Aires, Castelar	LR025881	LR026751	–	–	Giraldo and Crous (2019)
	CBS 565.78A	Oidium sp.	Russia, Odessa	LR025882	LR026752	–	–	Giraldo and Crous (2019)
	CBS 565.78B	*Cercospora beticola*	Moldavia	LR025883	LR026753	–	–	Giraldo and Crous (2019)
	CBS 565.78C	Erysiphe sp.	Russia, Astrakhan	LR025884	LR026754	–	–	Giraldo and Crous (2019)
	CBS 892.70[T]	Goldfish (*Carassius auratus*)	Brazil, Recife	LR025885	LR026755	–	–	Giraldo and Crous (2019)
	CBS 100826	*Solanum tuberosum*	Israel, Gilat	LR025886	LR026756	–	–	

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
								Giraldo and Crous (2019)
	CBS 100827	Soil in cotton field	Israel, Ramat-David	LR025887	LR026757	–	–	Giraldo and Crous (2019)
	CBS 100830	Soil	Israel, Ein-Shemer	LR025888	LR026758	–	–	Giraldo and Crous (2019)
	CBS 100831	Soil	Israel, Ein-Shemer	LR025889	LR026759	–	–	Giraldo and Crous (2019)
	CBS 101221	Soil in cotton field	Israel, Ein-Shemer	LR025890	LR026760	–	–	Giraldo and Crous (2019)
	CBS 109724	Human blood	Greece, Thessaloniki	LR025891	LR026761	–	–	Giraldo and Crous (2019)
	CBS 120008	Leaf of *Musa* sp.	India, Bangoan, W.-Bengal	LR025892	LR026762	–	–	Giraldo and Crous (2019)
	CBS 120177	*Solanum tuberosum*	Japan	LR025893	LR026763	–	–	Giraldo and Crous (2019)
	JW 38004	Garden soil	The Netherlands, Sevenum	**LR590365**	**LR590188**	–	–	This study
	JW 191018	Garden soil	The Netherlands, Amersfoort	**LR590363**	**LR590186**	–	–	This study
	JW 234023	Garden soil	The Netherlands, Born	**LR590364**	**LR590187**	–	–	This study
G. simonii	JW 132008^T = CBS 144923	Garden soil	The Netherlands, Gelderland, Meteren	MK047517	MK047467	–	**LR594788**	Crous et al. (2018), This study
	JW 132005	Garden soil	The Netherlands, Gelderland, Meteren	**LR590366**	**LR590189**	–	**LR594789**	This study
Lectera capsici	CBS 142534^T	*Capsicum annuum*	Malaysia	KY979825	KY979770	–	–	Crous et al. (2017a)
L. colletotrichoides	IMI 332702	*Cicer arietinum*	Egypt	LR025895	JQ647428	–	–	Cannon et al. (2012), Giraldo and Crous (2019)
L. humicola	IMI 265740^T	Soil	Brazil	LR025896	JQ647449	–	–	Cannon et al. (2012), Giraldo and Crous (2019)
L. longa	IMI 181698^T	*Triticum* sp.	Australia	LR025897	JQ647448	–	–	Cannon et al. (2012), Giraldo and Crous (2019)
L. nordwiniana	JW 46012 = CBS 144922	Garden soil	The Netherlands, Güeldres, Arnhem	MK047513	MK047463	MK047551	MK047572	Crous et al. (2018)
	JW 231009^T = CBS 144921	Garden soil	The Netherlands, Friesland, Leeuwarden	MK047511	MK047461	MK047549	MK047570	Crous et al. (2018)
	JW 231013	Garden soil	The Netherlands, Friesland, Leeuwarden	MK047512	MK047462	MK047550	MK047571	Crous et al. (2018)
L. phaseoli	IMI 366179^T	*Phaseolus vulgaris*	Ethiopia	LR025898	JQ693168	–	–	Cannon et al. (2012), Giraldo and Crous (2019)
Monilochaetes infuscans	CBS 379.77	*Ipomoea batatas*	New Zealand, South Auckland, Mangere	GU180645	LR026764	–	–	Réblová et al. (2011), Giraldo and Crous (2019)
	CBS 869.66	*Ipomoea batatas*	South Africa, Eastern Cape, Gamtoos	GU180639	GU180626	–	–	Réblová et al. (2011)
Musidium stromaticum	CBS 135.74F	Rhizosphere of *Musa* sp.	Philippines	LR025925	LR026790	–	–	Giraldo and Crous (2019)
	CBS 863.73T	Root and rhizome of *Musa sapientum*	Honduras	HQ232143	DQ825969	–	–	Summerbell et al. (2011), Zare et al. (2007)
Phialoparvum bifurcatum	CBS 299.70B^T	Soil	Belgium, Heverlee	LR025931	LR026793	–	–	Giraldo and Crous (2019)

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
***P. rietveltiae* sp. nov.**	JW 211005^{T} = CBS 145322	Garden soil	The Netherlands, Utrecht	**LR590367**	**LR590191**	–	**LR594790**	This study
***P. maaspleinense* sp. nov.**	JW 266001^{T} = CBS 145321	Garden soil	The Netherlands, Utrecht	**LR590368**	**LR590190**	–	**LR594791**	This study
Plectosphaerella alismatis	CBS 113362^{ET}	*Alisma plantago-aquatica*	The Netherlands, Pijnenburg near Soest	LR025932	LR026794	LR026489	–	Giraldo and Crous (2019)
P. citrullae	CBS 131740	Root of *Cucumis melo*	Italy, Foggia, Torre Bianca	LR025933	LR026795	LR026490	–	Giraldo and Crous (2019)
	CBS 131741^{T}	Root of *Citrullus lanatus*	Italy, Foggia	LR025934	LR026796	LR026491	–	Giraldo and Crous (2019)
P. cucumerina	CBS 137.33^{NT}	*Nicotiana tabacum*	England, Bristol	LR025935	LR026797	LR026492	–	Giraldo and Crous (2019)
	CBS 137.37^{T}	Paper	Italy	LR025936	LR026798	LR026493	–	Giraldo and Crous (2019)
	CBS 286.64	*Nicotiana tabacum*	Belgium, Heverlee	LR025938	LR026800	LR026495	–	Giraldo and Crous (2019)
	CBS 355.36	Root of *Viola tricolour*	The Netherlands	LR025939	LR026801	LR026496	–	Giraldo and Crous (2019)
	CBS 367.73	*Viola odorata*	Egypt	LR025940	LR026802	LR026497	–	Giraldo and Crous (2019)
	CBS 400.58	*Solanum esculentum*	Canada	LR025941	LR026803	LR026498	–	Giraldo and Crous (2019)
	CBS 619.74	*Leaf of Pyrus malus*	Switzerland, Basel	LR025943	LR026805	LR026500	–	Giraldo and Crous (2019)
	CBS 101014	*Arabidopsis thaliana*	Switzerland	LR025945	LR026807	LR026502	–	Giraldo and Crous (2019)
	CBS 101958	Endophyte in leaf and stem of *Galium spurium*	Canada, Alberta	LR025946	LR026808	LR026503	–	Giraldo and Crous (2019)
	CBS 131739^{NT}	*Collar of Cucumis melo*	Italy, Foggia, Borgo Cervaro	LR025947	LR026809	LR026504	–	Giraldo and Crous (2019)
	JW 4021	Garden soil	The Netherlands, Breda	**LR590375**	**LR590198**	–	**LR594792**	This study
	JW 29030	Garden soil	The Netherlands, Oude Wetering	**LR590374**	**LR590197**	–	**LR594793**	This study
	JW 79002	Garden soil	The Netherlands, Kapel Avezaath	**LR590376**	**LR590199**	–	**LR594794**	This study
	JW 79010	Garden soil	The Netherlands, Kapel Avezaath	**LR590377**	**LR590200**	–	–	This study
	JW 144006	Garden soil	The Netherlands, Herten	**LR590369**	**LR590192**	–	–	This study
	JW 144016	Garden soil	The Netherlands, Herten	**LR590370**	**LR590193**	–	–	This study
	JW 198006	Garden soil	The Netherlands, Amsterdam	**LR590371**	**LR590194**	–	**LR594795**	This study
	JW 236009	Garden soil	The Netherlands, Maasbracht	**LR590372**	**LR590195**	–	–	This study
	JW 261010	Garden soil	The Netherlands, Utrecht	**LR590373**	**LR590196**	–	–	This study
P. delsorboi	CBS 116708^{T}	*Curcuma alismatifolia*	Italy, Portici	LR025948	LR026810	LR026505	–	Giraldo and Crous (2019)
***P. hanneae* sp. nov.**	JW181001^{T} = CBS 144925	Garden soil	The Netherlands, Leiden	**LR590378**	**LR590201**	**LR594767**	–	This study
P. humicola	CBS 423.66^{T}	Soil	Zaire, Katanga	LR025949	LR026811	LR026506	–	Giraldo and Crous (2019)
P. melonis	CBS 489.96^{T}	Root of *Cucurbita melo*	Japan, Shizuoka, Asaba-chou	LR025950	LR026812	LR026507	_	Giraldo and Crous (2019)
	CBS 525.93	*Cucumis melo*	Spain	LR025951	LR026813	LR026508	_	Giraldo and Crous (2019)
P. oligotrophica	CBS 440.90	Soil	Brazil, Pará	LR025952	LR026814	LR026509	–	Giraldo and Crous (2019)
P. oratosquillae	NJM 0662^{T}	Mantis shrimp (*Oratosquilla oratoria*)	Japan, Yamaguchi	_	AB425974	_	_	Duc et al. (2009)
	NJM 0665	Mantis shrimp (*Oratosquilla oratoria*)	Japan, Yamaguchi	_	AB425975	_	_	Duc et al. (2009)
P. pauciseptata	CBS 131744	Collar of Cucumis melo	Italy, Foggia	LR025953	LR026815	LR026510	_	Giraldo and Crous (2019)
	CBS 131745^{T}	Root of *Solanum esculentum*	Italy, Apulia, Rignano Garganico	LR025954	LR026816	LR026511	–	Giraldo and Crous (2019)
P. pauciseptata	JW 83023	Garden soil	The Netherlands, Ridderkerk	**LR590379**	**LR590202**	**LR594768**	**LR594796**	This study
P. plurivora	CBS 261.89	Soil	Germany	LR025958	LR026820	LR026515	–	Giraldo and Crous (2019)
	CBS 292.66	Soil	The Netherlands	LR025960	LR026822	LR026517	–	Giraldo and Crous (2019)
	CBS 386.68	Wheat field soil	The Netherlands, Oostelijk Flevoland	LR025961	LR026823	LR026518	–	Giraldo and Crous (2019)
	CBS 131742^{T}	Apex of *Asparagus officinalis*	Italy, Apulia, Borgo Cervaro	LR025967	LR026829	LR026524	–	Giraldo and Crous (2019)
	JW 5012	Garden soil	The Netherlands, Nieuwegein	**LR590412**	**LR590237**	–	–	This study
	JW 5035	Garden soil	The Netherlands, Nieuwegein	**LR590413**	**LR590238**	**LR594769**	**LR594797**	This study
	JW 13024	Garden soil	The Netherlands, Alkmaar	**LR590382**	**LR590205**	**LR594770**	–	This study

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
	JW 18012	Garden soil	The Netherlands, Valkenswaard	**LR590391**	**LR590214**	–	–	This study
	JW 26001	Garden soil	The Netherlands, Soest	**LR590399**	**LR590224**	–	–	This study
	JW 29002	Garden soil	The Netherlands, Oude Wetering	**LR590401**	**LR590226**	**LR594771**	**LR594798**	This study
	JW 29017	Garden soil	The Netherlands, Oude Wetering	**LR590402**	**LR590227**	–	–	This study
	JW 29025	Garden soil	The Netherlands, Oude Wetering	**LR590403**	**LR590228**	–	–	This study
	JW 40004	Garden soil	The Netherlands, Goes	**LR590404**	**LR590229**	–	–	This study
	JW 40005	Garden soil	The Netherlands, Goes	**LR590405**	**LR590230**	–	–	This study
	JW 43013	Garden soil	The Netherlands, Zoelen	**LR590406**	**LR590231**	–	–	This study
	JW 44009	Garden soil	The Netherlands, Utrecht	**LR590407**	**LR590232**	–	–	This study
	JW 44018	Garden soil	The Netherlands, Utrecht	**LR590408**	**LR590233**	–	–	This study
	JW 44021	Garden soil	The Netherlands, Utrecht	**LR590409**	**LR590234**	–	–	This study
	JW 48008	Garden soil	The Netherlands, De Bilt	**LR590410**	**LR590235**	–	–	This study
	JW 50045	Garden soil	The Netherlands, Houten	**LR590411**	**LR590236**	–	–	This study
	JW 80004	Garden soil	The Netherlands, Geertruidenberg	**LR590414**	**LR590239**	–	–	This study
	JW 124026	Garden soil	The Netherlands, Amersfoort	**LR590380**	**LR590203**	–	–	This study
	JW 127008	Garden soil	The Netherlands, Utrecht	**LR590381**	**LR590204**	–	–	This study
	JW 132001	Garden soil	The Netherlands, Meteren	**LR590383**	**LR590206**	–	–	This study
	JW 137005	Garden soil	The Netherlands, Tilburg	**LR590384**	**LR590207**	–	–	This study
	JW 141016	Garden soil	The Netherlands, Kortenhoef	**LR590385**	**LR590208**	–	–	This study
	JW 144008	Garden soil	The Netherlands, Herten	**LR590386**	**LR590209**	–	–	This study
	JW 146011	Garden soil	The Netherlands, Heemstede	**LR590387**	**LR590210**	–	–	This study
	JW 152008	Garden soil	The Netherlands, Utrecht	**LR590388**	**LR590211**	–	–	This study
	JW 154005	Garden soil	The Netherlands, De Meern	**LR590389**	**LR590212**	–	–	This study
	JW 161013	Garden soil	The Netherlands, Utrecht	**LR590390**	**LR590213**	–	–	This study
	JW 190001	Garden soil	The Netherlands, Wijk bij Duurstede	**LR590392**	**LR590215**	**LR594772**	**LR594799**	This study
	JW 192005	Garden soil	The Netherlands, Woerden	**LR590393**	**LR590216**	–	–	This study
	JW 198008	Garden soil	The Netherlands, Amsterdam	**LR590394**	**LR590217**	–	–	This study
	JW 198019	Garden soil	The Netherlands, Amsterdam	**LR590395**	**LR590218**	–	–	This study
	JW 200004	Garden soil	The Netherlands, Houten	–	**LR590219**	–	–	This study
	JW 211020	Garden soil	The Netherlands, Utrecht	**LR590396**	**LR590220**	–	–	This study
	JW 211027	Garden soil	The Netherlands, Utrecht	**LR590397**	**LR590221**	–	–	This study
	JW 230016	Garden soil	The Netherlands, Maarssen	**LR590398**	**LR590222**	–	–	This study
	JW 255003	Garden soil	The Netherlands, Ede	–	**LR590223**	**LR594773**	**LR594800**	This study
	JW 266007	Garden soil	The Netherlands, Utrecht	**LR590400**	**LR590225**	–	–	This study
P. populi	CBS 139623^T	Branch of *Populus nigra*	Germany, Kuestrin-Kietz, Brandenburg	KR476783	KR476750	LR026527	_	Crous et al. (2015) Giraldo and Crous (2019)
	CBS 139624	*Populus nigra*	Germany, Kuestrin-Kietz, Brandenburg	MH878144	KR476751	LR026528	_	Crous et al. (2015), Giraldo and Crous (2019)
P. ramiseptata	CBS 131743	Collar of Citrullus lanatus	Italy, Foggia	LR025969	LR026831	LR026529	_	Giraldo and Crous (2019)
	CBS 131861^T	Root of Solanum esculentum	Italy, Apulia, Rignano Garganico	LR025970	LR026832	LR026530	_	Giraldo and Crous (2019)
P. sinensis	ACCC 39144	Stem of Citrullus lanatus	China, Hebei	KX527892	KX527889	_	_	Su et al. (2017)
	ACCC 39145^T	Stem of Cucumis melo	China, Hebei	KX527891	KX527888	_	_	Su et al. (2017)
***P. verschoorii* sp. nov.**	JW 13004 = CBS 144924^T	Garden soil	The Netherlands, Alkmaar	**LR590476**	**LR590240**	**LR594774**	_	This study
	JW 13006	Garden soil	The Netherlands, Alkmaar	**LR590415**	**LR590241**	**LR594775**	**LR594801**	This study
	JW 62006	Garden soil	The Netherlands, Delft	**LR590426**	**LR590252**	**LR594776**	**LR594802**	This study

Table 1 (continued)

Species	Isolate nr.	Source	Locality	GenBank/ENA accession no. LSU	ITS	*TEF1-α*	*RPB2*	Reference
	JW 143005	Garden soil	The Netherlands, Utrecht	**LR590416**	**LR590242**	**LR594777**	**LR594803**	This study
	JW 146007	Garden soil	The Netherlands, Heemstede	**LR590417**	**LR590243**	**LR594778**	–	This study
	JW 146009	Garden soil	The Netherlands, Heemstede	**LR590418**	**LR590244**	**LR594779**	–	This study
	JW 146016	Garden soil	The Netherlands, Heemstede	**LR590419**	**LR590245**	**LR594780**	–	This study
	JW 150001	Garden soil	The Netherlands, Liempde	**LR590420**	**LR590246**	**LR594781**	–	This study
	JW 170008	Garden soil	The Netherlands, Utrecht	**LR590421**	**LR590247**	**LR594782**	–	This study
	JW 191043	Garden soil	The Netherlands, Amersfoort	**LR590422**	**LR590248**	**LR594783**	**LR594804**	This study
	JW 208020	Garden soil	The Netherlands, Hilversum	**LR590423**	**LR590249**	**LR594784**	–	This study
	JW 230011	Garden soil	The Netherlands, Maarssen	**LR590424**	**LR590250**	**LR594785**	**LR594805**	This study
	JW 232007	Garden soil	The Netherlands, Nederweert	**LR590425**	**LR590251**	**LR594786**	**LR594806**	This study

ACCC, Agricultural Culture Collection of China, Beijing, China; *CBS*, Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; *DAOMC*, Canadian Collection of Fungal Cultures, Canada; *IMI*, International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; *JW*, Johanna Westerdijk Culture Collection, Utrecht, The Netherlands; *NJM*, Nippon Veterinary and Life Science University, Tokyo, Japan; *T*, ex-type; *ET*, ex-epitype; *IT*, ex-isotype; *NT*, ex-neotype; *ENA*, European Nucleotide Archive; *LSU*, large subunit of the nrDNA; *ITS*, internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; *TEF1-α*, translation elongation factor 1-alpha; *RPB2*, RNA polymerase II second largest subunit. Accession numbers of sequences generated in this study are in bold

were taken with a Nikon DS-Ri2 digital camera (Nikon, Tokyo, Japan) using the NIS-elements imaging software v. 4.3. The length and width of at least 30 randomly selected structures were measured, and the extreme values calculated. Morphological descriptions and taxonomic information for the new species were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

Results

A total of 293 soil samples were analysed, and nearly 3000 fungal isolates were obtained. Among them, 176 were identified as members of Plectosphaerellaceae according to the BLAST search results using the LSU and/or ITS sequences. The combined ITS and LSU dataset comprises 197 sequences including the ones generated in the present study from soil isolates, together with reference sequences of Plectosphaerellaceae taxa download from GenBank, and the outgroup *Monilochaetes infuscans* CBS 379.77 and CBS 869.96 (Fig. 1). The RAxML tree showed BS values higher than 95% for all the generic clades, except for *Gibellulopsis* and *Brunneochlamydosporum* that were not fully supported (Fig. 1). The majority of the isolates were distributed among *Plectosphaerella* spp. ($n = 61$) and *Gibellulopsis* spp. ($n = 60$). They were followed by *Chordomyces* spp. ($n = 22$), *Acrostalagmus luteoalbus* ($n = 15$) and *Brunneochlamydosporium* spp. ($n = 9$). The remaining isolates were identified as *Furcasterigmium furcatum* ($n = 4$), *Lectera nordwiniana* ($n = 3$) and *Phialoparvum* spp. ($n = 2$) (Table 1). Since the species boundaries were not resolved with this analysis for *Brunneochlamydosporium*, *Chordomyces*, *Gibellulopsis*, *Phialoparvum* and *Plectosphaerella*, subsequent phylogenetic analyses were carried out (Figs. 2, 3, 4 and 5).

The *Brunneochlamydosporium* dataset comprises ITS and *TEF1-α* sequences of 19 isolates including the outgroups (Fig. 2). This combined analysis showed *B. nepalense* ($n = 7$) as the most common species, followed by *B. macroclavatum* ($n = 1$). The ITS phylogeny represented in Fig. 3 shows the species distribution from all the isolates belonging to *Chordomyces*, *Lectera* and *Phialoparvum*. In the *Chordomyces* clade (95% BS), most of the isolates ($n = 17$) were grouped with the type of *C. albus* CBS 987.87 (76%), while the remaining isolates ($n = 5$) were identified as *C. antarticus*. The three isolates of *Lectera* were confirmed as *L. nordwiniana*, but the two *Phialoparvum* isolates were genetically different from the type of *Ph. bifurcatum* CBS 299.70B, representing two putative new species, introduced in the 'Taxonomy' section.

The phylogenetic analysis shown in Fig. 4 encompasses the ITS sequences from 106 isolates belonging to *Gibellulopsis* (including the ex-type strains from the different

Gibellulopsis species), and *Furcasterigmium* and *Musidium* as outgroups (Fig. 4). According to this phylogeny, the majority of the soil isolates were identified as *G. nigrescens* ($n = 50$), followed by *G. fusca* ($n = 5$), *G. serrae* (formerly *G. piscis*) ($n = 3$) and *G. simonii* ($n = 2$).

A preliminary phylogenetic analysis based on the ITS region was carried out for *Plectosphaerella* (data not shown). Species were distributed in *Pl. plurivora* ($n = 37$), *Pl. cucumerina* ($n = 9$) and *Pl. pauciseptata* ($n = 1$) (Table 1). However, 14 isolates were distributed in two different lineages (with 1 and 13 isolates, respectively), phylogenetically distant from the other species. Subsequently, isolates of each clade were selected based in the similarity of their ITS regions for further combined analysis of ITS and *TEF1-α* sequences. This analysis included 55 soil isolates and 32 reference strains (Fig. 5), and confirmed the identity of the isolates placed in the *Pl. plurivora* (77% BS) and *Pl. cucumerina* clades (88% BS). Furthermore, this analysis revealed that 13 isolates were nested in a highly supported clade (99% BS), phylogenetically distant from the remaining species in the genus, representing a potentially new species. Isolate JW 181001 was placed in a single branch related (71% BS) but different from *Pl. alismatis*, *Pl. delsorboi*, *Pl. melonis* and *Pl. sinensis*. The two new species are described in the 'Taxonomy' section below.

Taxonomy

Based on the molecular results and the morphological observations, four new species are described and illustrated here, i.e. *Phialoparvum maaspleinense*, *Ph. rietveltiae*, *Plectosphaerella hanneae* and *Pl. verschoorii.*

Phialoparvum maaspleinense Hern.-Restr. & Giraldo López, *sp. nov.* MycoBank MB 831346 (Fig. 6a, b, e–j).

Etymology. Named after 'Maaspleinschool' from Utrecht, where the soil sample was collected by the students Ouail Zaim and Mohamed Bidari.

Mycelium consisting of branched, septate, smooth, hyaline and thick-walled hyphae, 1.5–2.5 μm wide. *Conidiophores* solitary, erect, arising directly from vegetative hyphae or ropes of hyphae, unbranched or poorly branched. *Phialides* lateral, terminal, subulate, hyaline, thick- and smooth-walled, 10–42 × 1.5–3 μm, with cylindrical collarette and conspicuous periclinal thickening at the conidiogenous locus; adelophialides commonly present, intercalary and terminal, up to 4 μm long; polyphialides with two conidiogenous loci are commonly present. *Conidia* arranged in slimy heads, cylindrical, sometimes with slightly truncate base, 1-celled, hyaline, thick- and smooth-walled, 3.5–5 × 2 μm. *Sexual morph* unknown.

Culture characteristics — After 14 days at ca. 24 °C on PDA reaching 45–48 mm diam., flat, membranous, aerial mycelium sparse, dirty white, reverse uncoloured. On OA reaching 40–42 mm diam., flat, pulverulent, rosy buff, margin effuse, reverse buff to honey. On MEA reaching 37–38 mm diam., radially folded, velvety at centre, pulverulent toward the periphery, dirty white, margin entire, reverse uncoloured.

Typus. The Netherlands, Utrecht province, Utrecht, Maaspleinschool, from garden soil, 2017, coll. O. Zaim & M. Bidari, isol. A. Giraldo (holotype CBS H-23910, cultures ex-type CBS 145321 = JW 266001).

Notes: The monotypic genus *Phialoparvum* was recently proposed based on *Ph. bifurcatum,* recovered from soil in Belgium (Giraldo and Crous 2019). *Phialoparvum bifurcatum* and the new species, *Ph. maaspleinense* can be morphologically distinguished based on phialide length (up to 15 μm in *Ph. bifurcatum* vs up to 42 μm in *Ph. maaspleinense*; Giraldo and Crous 2019) and the frequent production of intercalary and terminal adelophialides in the latter species (Fig. 6h, i).

Phialoparvum rietveltiae Hern.-Restr. & Giraldo López, *sp. nov.* MycoBank MB 831347 (Fig. 6c, d, k–n).

Etymology. Named after Emma Rietvelt, who collected the soil sample.

Mycelium consisting of branched, septate, hyaline and thick-walled hyphae, 1–2 μm wide. *Conidiophores* solitary, erect, arising directly from vegetative hyphae or ropes of hyphae, unbranched or poorly branched. *Phialides* lateral, terminal, subulate to ampulliform, hyaline, thick- and smooth-walled, 6–11(− 17) × 1–2.5 μm, with cylindrical collarette and conspicuous periclinal thickening at the conidiogenous locus, adelophialides sometimes present, up to 4 μm long; polyphialides with two conidiogenous loci are commonly present. *Conidia* arranged in slimy heads, cylindrical, with slightly pointed ends, 1-celled, hyaline, thick- and smooth-walled, 3.5–5 × 1.5–2 μm. *Sexual morph* unknown.

Culture characteristics — After 14 days at ca. 24 °C on PDA reaching 30–33 mm diam., flat, membranous, aerial mycelium sparse or absent, dirty white, margin entire, reverse uncoloured. On OA reaching 28–30 mm diam., flat, pulverulent to velvety, dirty white to buff, margin rhizoid, reverse uncoloured. On MEA reaching 24–29 mm diam., umbonate, radially folded, membranous to felty, buff, entire margin, reverse uncoloured.

Typus. The Netherlands, Utrecht province, Utrecht, from garden soil, 2017, coll. E. Rietvelt, isol. A. Giraldo (holotype CBS H-23911, cultures ex-type CBS 145322 = JW 211005).

Notes: *Phialoparvum bifurcatum* and *Ph. rietveltiae* are morphologically similar in phialide shape and length (8–15 μm in *Ph. bifurcatum* vs 6–17 μm in *Ph. rietveltiae*). However, the latter species has longer conidia (3.5–5 μm in *Ph. rietveltiae* vs 2.8–4.4 μm in *Ph. bifurcatum*) and does not produce melanin precipitations in culture (Giraldo and Crous 2019).

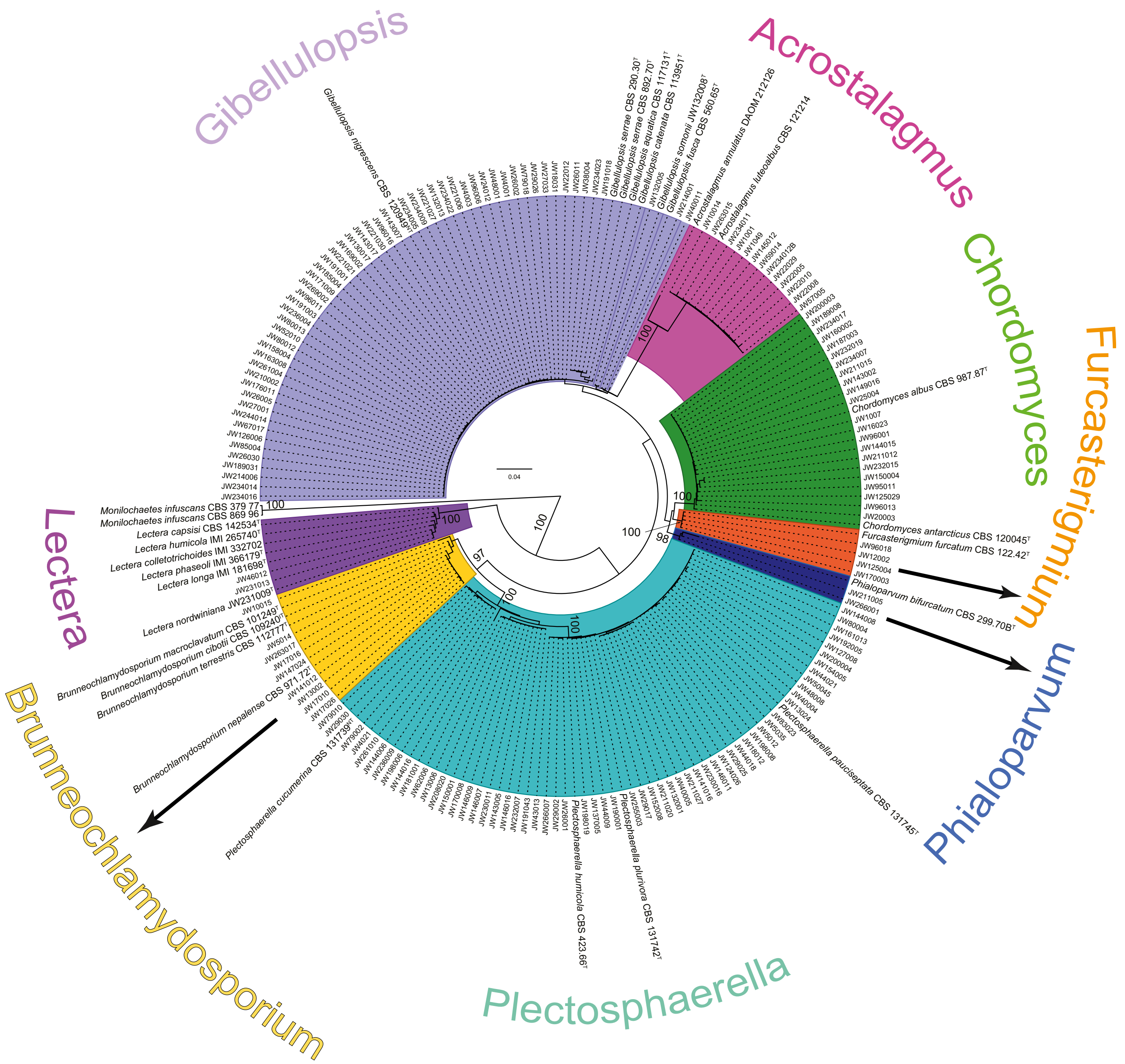

Fig. 1 Maximum composite likelihood tree based on partial sequences from the ITS and LSU from all the soil isolates placed in Plectosphaerellaceae. Colour boxes indicate the generic clade. Bootstrap support values above 70% are shown at the genus nodes (internal values are not shown). ^{T}Ex-type, ^{IT}Ex-isotype, ^{NT}Ex-neotype

Plectosphaerella hanneae Giraldo López & Hern.-Restr., *sp. nov.* MycoBank MB 831348 (Fig. 7a–d).

Etymology. Named after Hanne de Levita, who collected the soil sample.

Mycelium consisting of branched, septate, smooth, hyaline and thin-walled hyphae, up to 2 μm wide. *Conidiophores* solitary, erect, unbranched, hyaline, smooth-walled, sometimes radiating out from sterile coils formed by the mycelium. *Phialides* lateral or terminal, subulate, hyaline, smooth- and thick-walled, occasionally borne on short cylindrical subtending cells, 16–32 × 2–4.5 μm, with conspicuous cylindrical collarette and periclinal thickening at the conidiogenous locus, sometimes with percurrent proliferation; adelophialides rarely present, up to 15 μm long. *Conidia* arranged in slimy heads, (0-)1-septate, cylindrical to ellipsoidal with pointed apex and slightly truncate base, inequilateral, with inner plane flat and outer plane convex, guttulate, hyaline, thin- and smooth-walled; septate conidia abundant, 9–12.5 × 2.5–3.5 μm; aseptate conidia scarce, 9–10.5 × 3–3.5 μm. *Chlamydospores* and *sexual morph* absent.

Culture characteristics — After 14 days at ca. 24 °C on PDA reaching 76–77 mm diam., flat, membranous, dirty white

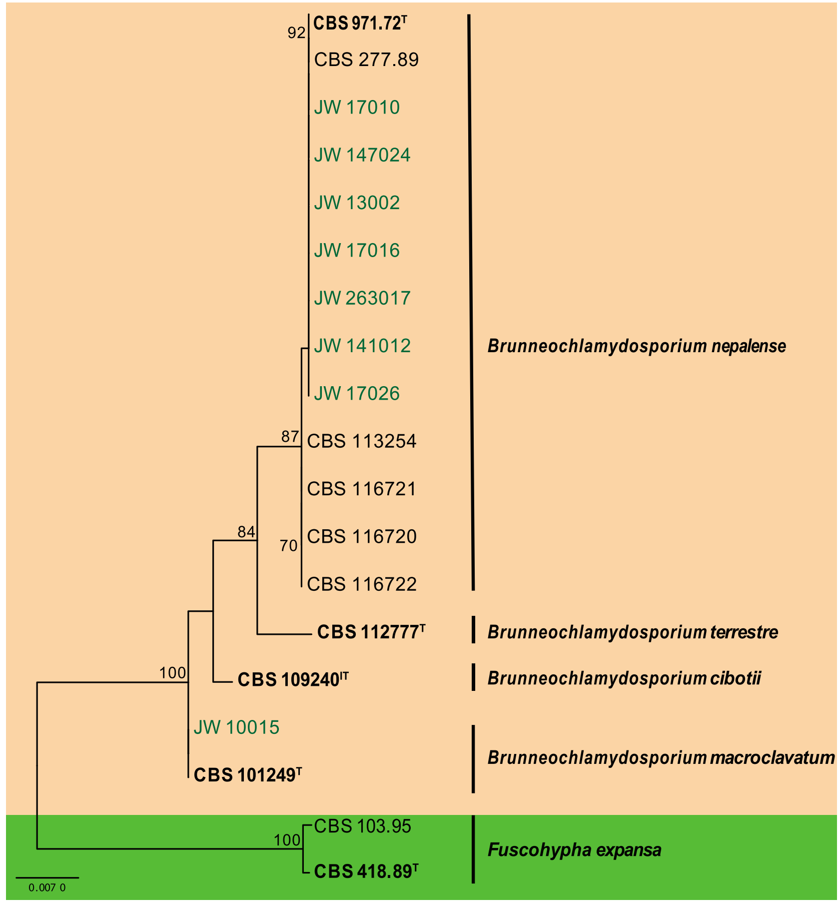

Fig. 2 Maximum composite likelihood tree based on ITS and *TEF1-α* from *Brunneochlamydosporium* species. Colour boxes indicate the generic clade. Bootstrap support values above 70% are shown at the nodes. JW isolates are in green font and the ex-type strains in bold. ^{T}Ex-type, ^{IT}Ex-isotype

to pale luteous, aerial mycelium sparse or absent, with concentric rings, reverse uncoloured. On OA reaching 58–60 mm diam., flat, glabrous, pale luteous with ochreous border, aerial mycelium sparse at the periphery, reverse uncoloured.

Typus. The Netherlands, South Holland, Leiden, from garden soil, 2017, coll. H. de Levita, isol. A. Giraldo (holotype CBS H-23737, cultures ex-type CBS 144925 = JW 181001).

Notes: According to phylogenetic inference from the ITS and *TEF1-α* loci (Fig. 5), *Pl. hanneae* occupies a single branch in the clade containing *Pl. alismatis*, *Pl. delsorboi*, *Pl. melonis* and *Pl. sinensis* (71% BS). With the exception of *Pl. delsorboi* and *Pl. hanneae,* all the species in that clade produce hyaline chlamydospores in chains (Su et al. 2017). *Plectosphaerella delsorboi* differs in having longer phialides (30–50 μm; Antignani et al. 2008) than those of *Pl. hanneae* (16–32 μm).

Plectosphaerella verschoorii Giraldo López & Hern.-Restr., *sp. nov.* MycoBank MB 831349 (Fig. 7e–i).

Etymology. Named after Brent Verschoor, who collected the soil sample.

Mycelium consisting of branched, septate, smooth, hyaline and thin-walled hyphae, up to 2 μm wide. *Conidiophores* solitary, erect, unbranched or poorly branched, hyaline, smooth-walled. *Phialides* lateral or terminal, subcylindrical to subulate, hyaline, smooth- and thick-walled, 16–29 × 1.5–3 μm, with conspicuous cylindrical collarette and periclinal thickening at the conidiogenous locus; adelophialides, rarely present; ampulliform, up to 14 μm long. *Conidia* arranged in slimy head, (0-)1-septate, cylindrical to ellipsoidal with pointed apex and slightly truncate base, or broadly ellipsoidal, guttulate*,* hyaline, thin- and smooth-walled; septate conidia abundant*,* sometimes slightly constricted at the septum, 8–11.5 × 2–3 μm; aseptate conidia scarce, 3–8.5 × 2–3 μm. *Chlamydospores* and *sexual morph* absent.

Culture characteristics — After 14 days at ca. 24 °C on PDA reaching 76–77 mm diam., flat, membranous, flesh at centre, pale luteous at periphery, aerial mycelium sparse, with concentric rings, dirty white, reverse uncoloured. On OA reaching 70–75 mm diam., flat, glabrous, honey, reverse uncoloured.

Typus. The Netherlands, North Holland, Alkmaar, from garden soil, 2017, coll. B. Verschoor, isol. A. Giraldo (holotype CBS H-23738, cultures ex-type CBS 144924 = JW 13004).

Additional specimens examined: The Netherlands, Limburg, Nederweert, from garden soil, 2017, coll. M. van Meijl, isol. A. Giraldo, JW 232007; North Brabant, Liempde,

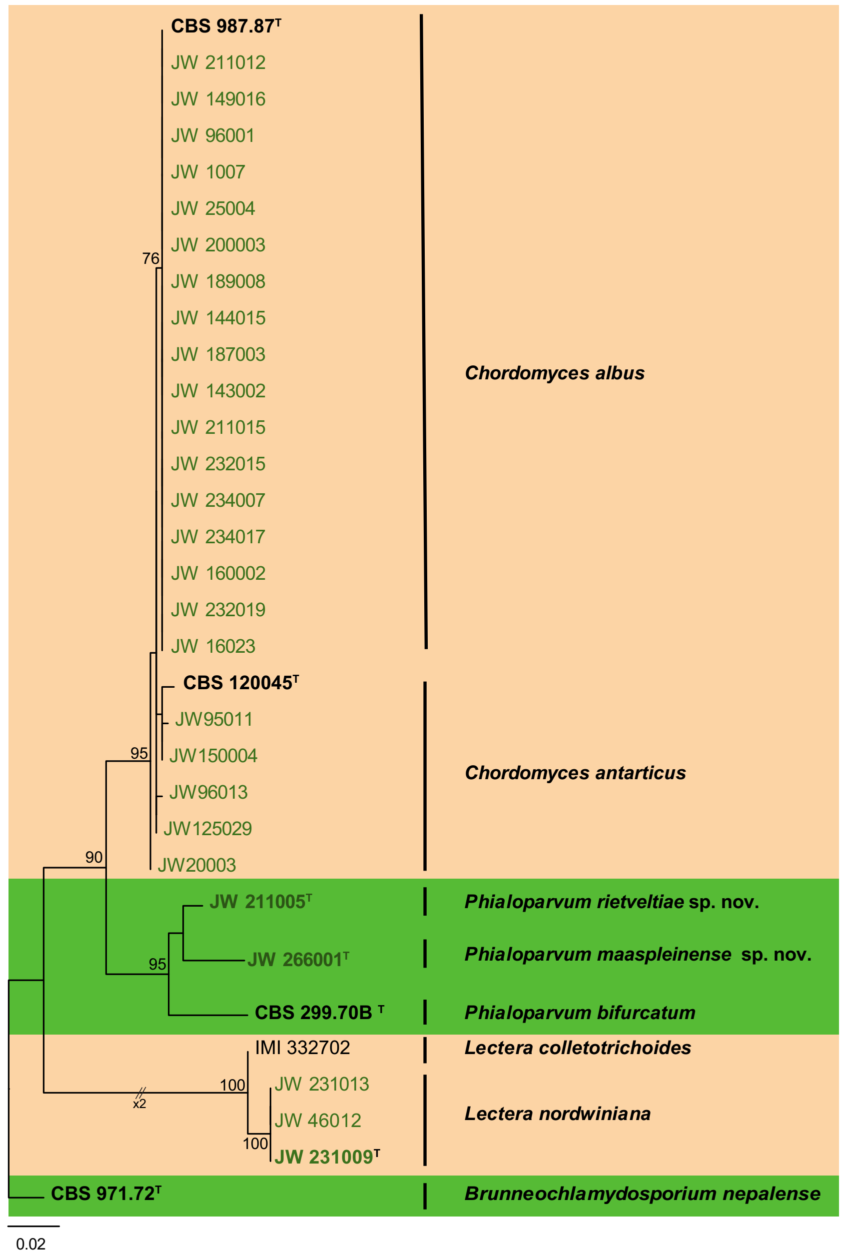

Fig. 3 Maximum composite likelihood tree based on ITS from *Chordomyces*, *Lectera* and *Phialoparvum* species. Colour boxes indicate the generic clade. Bootstrap support values above 70% are shown at the nodes. JW isolates are in green font and the ex-type strains in bold. ^{T}Ex-type

from garden soil, 2017, coll. L. & V. Gijzen, isol. A. Giraldo, JW 150001; North Holland, Alkmaar, from garden soil, 2017, coll. Brent Verschoor, isol. A. Giraldo, JW 13006; Heemstede, from garden soil, 2017, coll. Alfons Vaessen, isol. A. Giraldo, JW 146007, JW 146009, JW 146016; Hilversum, from garden soil, 2017, coll. J.A.L. Keyes-Rens, isol. A. Giraldo, JW 208020; South Holland, Delft, from garden soil, 2017, E. & M. Bordes, isol. A. Giraldo, JW 62006; Utrecht, Amersfoort, from garden soil, 2017, coll. T. & K. Wesselink, isol. A. Giraldo, JW 191043; Maarssen, from garden soil, 2017, coll. Y. & F. van der Ouderaa, isol. A. Giraldo, JW 230011; Utrecht, from garden soil, 2017, coll. G. Bleijlevens, isol. A. Giraldo, JW 143005; from garden soil, 2017, coll. J.P. van Eesteren, isol. A. Giraldo, JW 170008.

Notes: *Plectosphaerella verschoorii* forms a well-supported clade (99% BS, Fig. 5), phylogenetically different from other *Plectosphaerella* species. This species was collected in five provinces from The Netherlands (i.e. Limburg, North Brabant, North and South Holland, and Utrecht).

Morphologically, *Plectosphaerella verschoorii* resembles *Pl. citrullae*, *Pl. delsorboi, Pl. hanneae* and *Pl. plurivora* in lacking polyphialides. However, it can be distinguished from *Pl. plurivora* by the scarce production of aseptate conidia; from *Pl. delsorboi* and *Pl. citrullae* by having shorter phialides (up to 29 μm in *Pl. verschoorii,* up to 50 μm in *Pl. delsorboi,* up to 60 μm in *Pl. citrullae*; Carlucci et al. 2012; Liu et al. 2013), and from *Pl. hanneae* by producing smaller aseptate conidia (3–8.5 ×

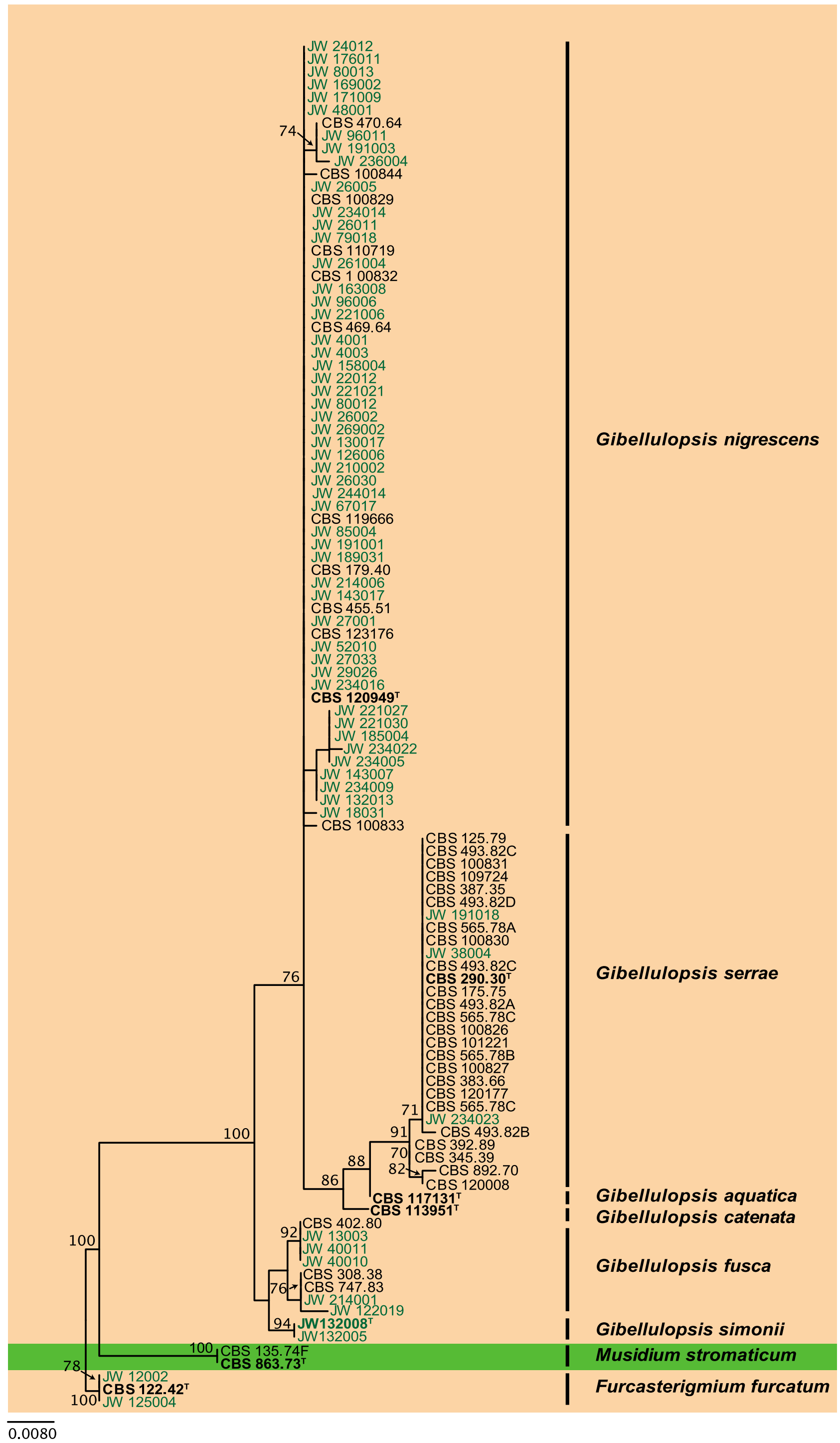

Fig. 4 Maximum composite likelihood tree based on partial sequences from ITS from *Gibellulopsis* species. Colour boxes indicate the generic clade. Bootstrap support values above 70% are shown at the nodes. JW isolates are in green font and the ex-type strains in bold. ^{T}Ex-type

2–3 μm in *Pl. verschoorii* vs. 9–10.5 × 3–3.5 μm in *Pl. hanneae, this study*).

Discussion

Although there are no specific studies exploring the diversity of the plectosphaerellaceous species from soil, a survey about fungi from soda soils in Asia (Armenia, Kazakhstan, Mongolia and Russia) and Africa (Kenia and Tanzania) revealed several isolates representing four genera of Plectosphaerellaceae (Grum-Grzhimaylo et al. 2016). That study showed *Sodiomyces* spp. and *A. luteoalbus* as obligate and facultative alkaliphilic fungi, respectively, while *C. antarticus* and *V. zaregamsianum* seemed to be alkalitolerant species. In our set of samples, we did not find

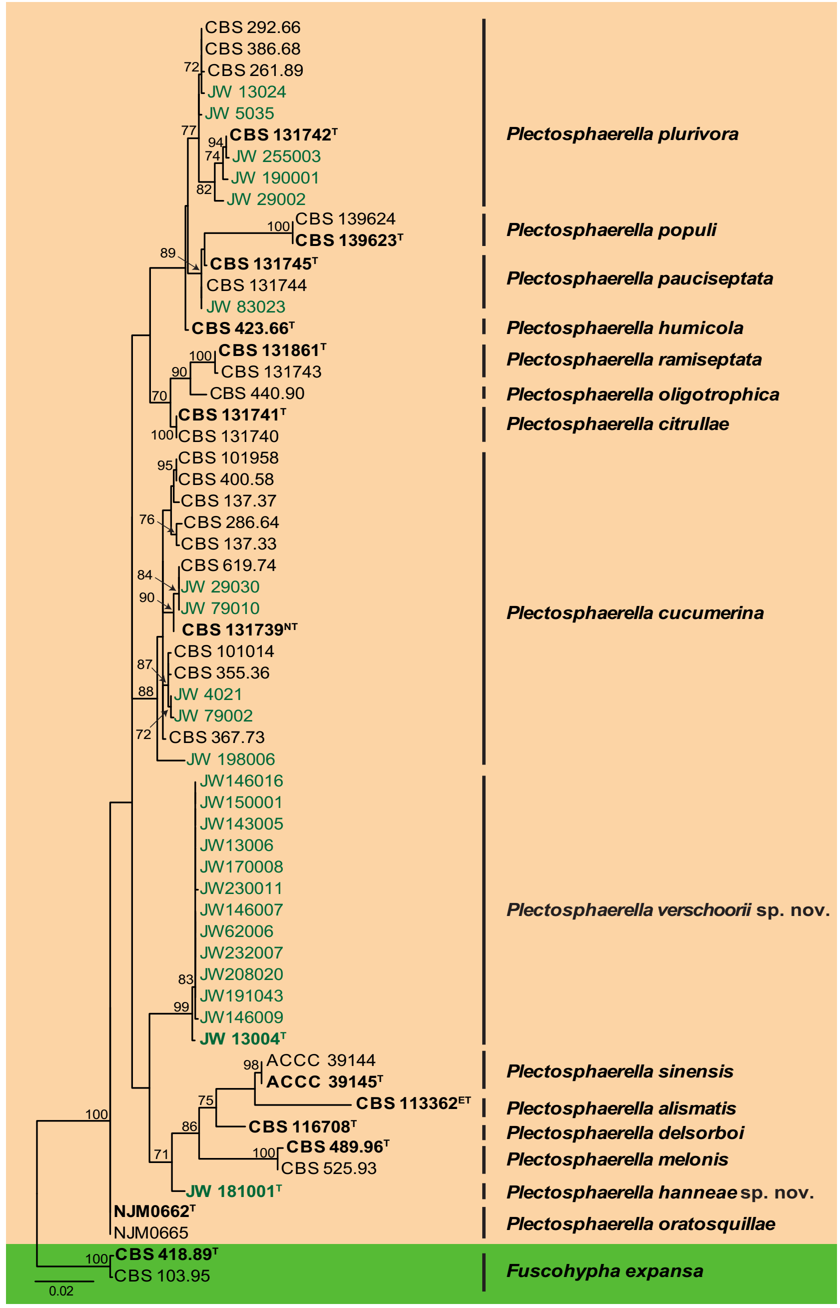

Fig. 5 Maximum composite likelihood tree based on ITS and *TEF1-α* from *Plectosphaerella* species. Colour boxes indicate the generic clade. Bootstrap support values above 70% are shown at the nodes. JW isolates are in green font and the ex-type strains in bold. [T]Ex-type, [ET]Ex-epitype, [NT]Ex-neotype

Sodiomyces spp. nor *V. zaregamsianum*, probably due of the media used for primary isolation. However, in our study, *A. luteoalbus* and *C. antarticus* were represented by 13 and five isolates respectively (Figs. 1 and 3 and Table 1). Additionally, we obtained several isolates ($n = 17$) of *C. albus*, which was recently described from a lichen (*Hypogymnia physodes*) and human sputum (Giraldo et al. 2017), but also reported from soil in Belgium, France, Germany and the Netherlands (Giraldo and Crous 2019).

In addition, we have identified several isolates from genera recently added to *Plectosphaerellaceae*, i.e. *Brunneochlamydosporium*, *Furcasterigmium*, *Lectera* and *Phialoparvum* (Cannon et al. 2012; Giraldo and Crous 2019). *Brunneochlamydosporium* was proposed to accommodate *Acremonium nepalense*, *Gliocladium cibotii* and the new species *B. macroclavatum* and *B. terrestre*. *Brunneochlamydosporium nepalense* and *B. macroclavatum* were represented in our study with seven and one isolate respectively. According to Prenafeta-Boldú et al. (2014), the former species seems to be a common soil-borne fungus in the Netherlands, being present in Gelderland, North and South Holland and Utrecht provinces, while *B. macroclavatum* was isolated from a fern, and the flowering plant, *Aphelandra* sp. (Acanthaceae), but not from soil (Giraldo and Crous 2019).

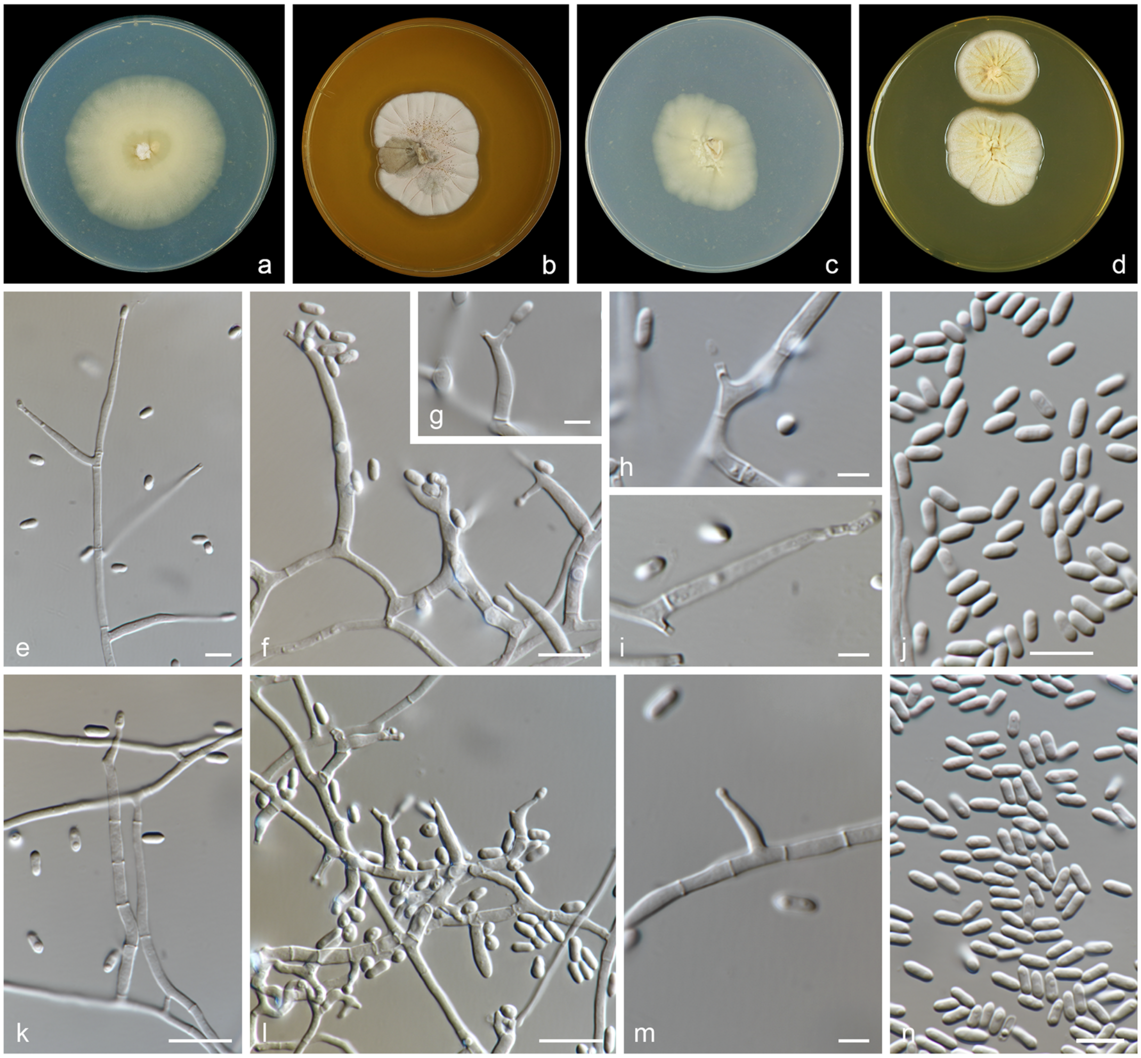

Fig. 6 *Phialoparvum* spp. **a**, **b**, **e**–**j** *Phialoparvum maaspleinense* (ex-type CBS 145321 = JW 266001). **a**, **b** Colonies on PDA and MEA respectively, after 14 days at ca. 24 °C. **e**, **f** Monophialides and conidia. **g** Polyphialide and conidium. **h**, **i** Adelophialides and conidia. **j** Conidia. **c**, **d**, **k**–**n** *Phialoparvum rietveltiae* (ex-type CBS 145322 = JW 211005). **c**, **d** Colonies on PDA and MEA respectively, after 14 days at ca. 24 °C. **k** Monophialides and conidia. **l** Polyphialides and conidia. **m** Adelophialide. **n** Conidia. Scale bars: **g**–**i**, **m** = 5 μm. **e**, **f**, **j**–**l**, **n** = 10 μm

Furcasterigmium furcatum was established based on *Acremonium furcatum*, and it was represented in our study with four isolates collected from North Brabant, South Holland and Utrecht provinces. Like *B. nepalense*, this species was recovered from soil in the Netherlands, but is also present in soils from France, Germany and Italy (Gams 1971; Giraldo and Crous 2019).

Two soil samples of Friesland and Gederland provinces revealed three isolates of *Lectera* that turned out to represent the new species, *L. nordwiniana* (Crous et al. 2018). Although most of the species in *Lectera* are plant pathogens, including *L. colletotrichoides* and *L. capsici* (Crous et al. 2017a), the former species is commonly isolated from soil and plant litter (Cannon et al. 2012). Another species recently described from soil is *L. humicola* from Brazil (Giraldo and Crous 2019).

In this study, *Gibellulopsis* and *Plectosphaerella* were the dominant genera, with *G. nigrescens* and *Pl. plurivora* as the most common species from each respective genus. In some studies of fungal biodiversity in soil, *Gibellulopsis* and *Plectosphaerella* are occasionally recovered (Hujslová et al. 2010; Duran et al. 2019; Wentzel et al. 2019). According to the data shown by Giraldo and Crous (2019), *Gibellulopsis* spp. inhabit soils around the world, with *G. nigrescens* recovered from Israel, the Netherlands, New Zealand and the UK. Species of *Plectosphaerella* are more common on plants than in soil, except for *Pl. plurivora*, which is present in soils of different countries in Northern Europe (Belgium, the Netherlands and Germany), and *Pl. humicola* and *Pl. oligotrophica* from Democratic Republic of the Congo, Brazil and China, respectively (Liu et al. 2013; Giraldo and Crous 2019). In our study, a single isolate (JW 83023) was

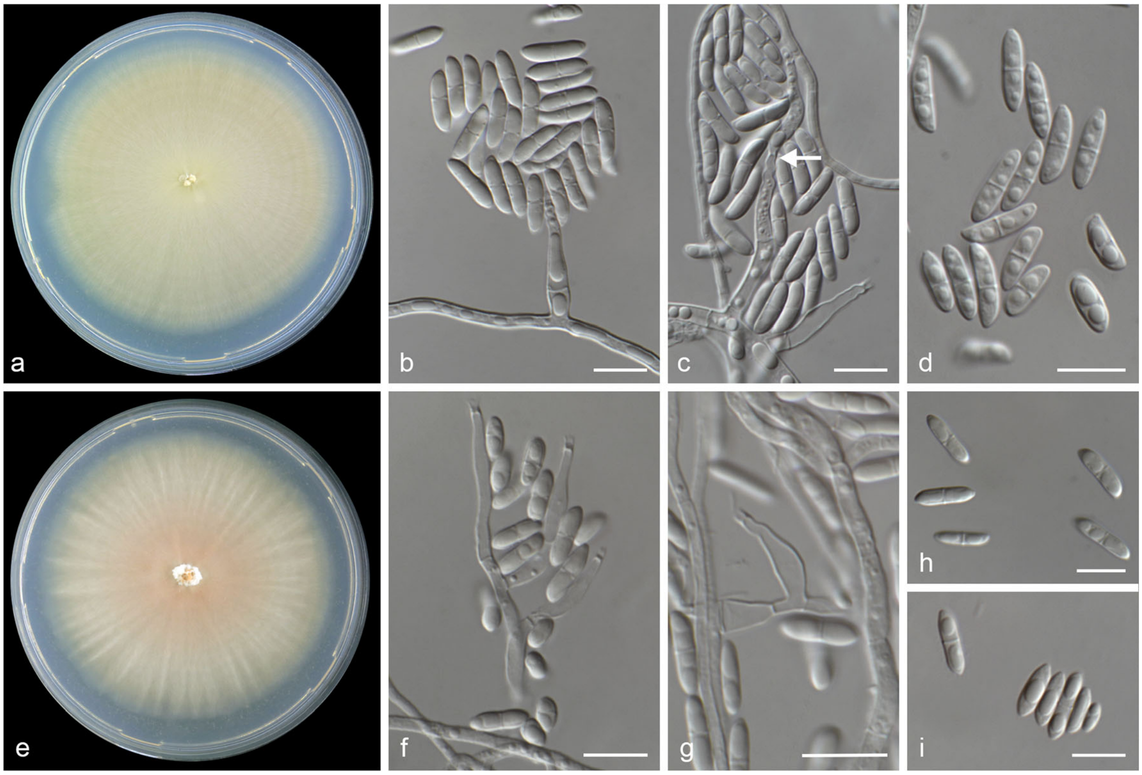

Fig. 7 *Plectosphaerella* spp. **a**–**d** *Plectosphaerella hanneae* (ex-type CBS 144925 = JW 181001). **a** Colony on PDA after 14 days at ca. 24 °C. **b** Monophialide and conidia. **c** Monophialides with a percurrent proliferation (arrow) and conidia. **d** Septate and aseptate conidia. **e**–**i** *Plectosphaerella verschoorii* (ex-type CBS 144924 = JW 13004). **e** Colony on PDA after 14 days at ca. 24 °C. **f** Monophialides and conidia. **g** Adelophialide and conidia. **h**, **i** Septate and aseptate conidia. Scale bars = 10 µm

identified as *Pl. pauciseptata*, known thus far from *Citrullus lanatus*, *Cucumis melo*, *C. sativus* and *Solanum esculetum* in Italy (Carlucci et al. 2012). Therefore, this is the first time that this species has been recovered from soil.

Other studies have described *G. nigrescens* from soil samples in Korea (Nguyen et al. 2018) and China (Wu et al. 2013), where it was also shown to be the cause of wilt of sugar beet (Zhou et al. 2017) and alfalfa (Hu et al. 2011). In Europe, this species and *Plectosphaerella* spp. have been found in saline and acidic soils in the Czech Republic (Hujslová et al. 2010), and inhabiting in good quality soils (as Gleyic Chernozem, Fluvic Cambisol, Cambic Leptosol) in Poland (Grządziel and Gałązka 2019). In a survey about microfungal community in cultivated soils, *Pl. cucumerina* and *A. luteoalbus* (as *Verticillium tenerum*) were found in low proportions in soils from different locations of the Eskişehir province in Turkey (Demirel et al. 2005). In Argentina, *Pl. plurivora* was found in rhizosphere soil, showing an antagonistic effect against eggs of the plant-parasitic nematode *Nacobbus aberrans* (Sosa et al. 2018), and *Plectosphaerella* sp. was recovered from farmland in the Tibetan Plateau (Li et al. 2012).

The studies based in Next Generation Sequence generally show a higher diversity of soil fungi compared with those based on culture-dependent methods (Tedersoo et al. 2014, 2017; Wardle and Lindahl 2014). The latter approach usually overlooks the true diversity, mainly because of the low percentage of fungi growing on artificial media. However, through this Citizen science project, we have explored the fungal diversity of Dutch soils, which has revealed a rich species diversity. Some of the taxa obtained represent new species, genera and even families, which have been described here or in other publications (Crous et al. 2017b, 2018; Groenewald et al. 2018), while others are still awaiting description (unpubl. data). As a result of this project, around 3000 fungal isolates were obtained from 293 soil samples. Among them, 386 isolates were yeast fungi which were treated by Groenewald et al. (2018), who identified 67 species distributed over 40 genera (including basidiomycetous and ascomycetous) including six new species in the genera *Hanseniaspora*, *Ogataea*, *Pichia*, *Saccharomycopsis*, *Trichomonascus* and *Zygoascus.* Regarding filamentous fungi, new taxa have been described in different and unrelated genera, including *Acaulium*, *Collariella*, *Conioscypha*, *Fusarium*, *Fusicolla*, *Gamsia*, *Gibellulopsis*, *Lasionectria*, *Lectera*, *Leptodiscella*, *Parasarocladium*, *Phaeoisaria*, *Sarocladium*, *Striaticonidium*, *Talaromyces*, *Umbelopsis*, *Vandijckella* (incl. *Vandijckella* gen. nov. and Vandijckellaceae fam.nov.) and *Verhulstia trisororum* (incl.

Verhulstia gen. nov.). These results support previous findings concluding that soil contains numerous undescribed fungal taxa (Hujslová et al. 2010; Tedersoo et al. 2014, 2017; Wentzel et al. 2019) and highlights the importance to continue with these kinds of studies in order to generate isolates that can be available in public databases and can be freely used by other researchers.

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