New foliicolous species of *Cladosporium* from South America

K. Schubert^{1, 2}, A. Greslebin³, J.Z. Groenewald¹, P.W. Crous¹

Key words

Argentina
Cladosporium
Colombia
Davidiellaceae
foliar diseases
Pinus

Abstract Two new species of *Cladosporium* found on necrotic needles of *Pinus ponderosa* trees in Patagonia, Argentina, are described as *C. chubutense* and *C. pini-ponderosae*. An additional isolate from dead leaves of *Cortaderia* collected in Colombia, which is a sister taxon to the species occurring on *Pinus*, is described as *Cladosporium colombiae*. These species are phylogenetically closely related, but differ from each other and other known species by multilocus sequence data, phenetic characters and culture characteristics.

Article info Received: 7 April 2009; Accepted: 20 April 2009; Published: 1 May 2009.

INTRODUCTION

Exotic conifer plantations in Patagonia occupy about 53 000 ha located in the western limit of the steppe near native forests. *Pinus ponderosa* is the most planted species covering about 40 000 ha. Ponderosa pine has been planted in the region for more than 25 years. During a survey of fungi on *P. ponderosa*, two cercosporoid hyphomycetes were detected growing on necrotic or partially necrotic, 1-yr-old needles. These isolates were consistently associated with needle discoloration and necrosis, although discrete leaf lesions were absent. Phylogenetically, the two species proved to be closely related to another undescribed species occurring on *Cortaderia* leaves in Colombia. Based on their morphology, cultural characteristics and phylogenetic position, all three species could be allocated to the genus *Cladosporium*.

The genus *Cladosporium* is characterised by a unique structure of conidiogenous loci and conidial hila consisting of a central convex dome surrounded by a raised periclinal rim (Roquebert 1981) for which David (1997) introduced the term 'coronate'. This unique feature makes it quite easy to assign isolates to the latter genus which proved to be a sister clade of *Mycosphaerella* s.str. (Braun et al. 2003, Crous et al. 2007a, b, Schubert et al. 2007b) having teleomorphs in *Davidiella*. Employing DNA sequence data of four loci (SSU nrDNA, LSU nrDNA, EF-1 α , RPB2) Schoch et al. (2006) demonstrated that species of *Davidiella* cluster in a separate family (*Davidiellaceae*) from species of *Mycosphaerella* (*Mycosphaerellaceae*), with both families residing in the *Capnodiales* (*Dothideomycetes*).

In recent years, the genus *Cladosporium* has been studied extensively on a morphological (Braun & Schubert 2007, Braun et al. 2006, 2008a,b, Crous et al. 2006, Schubert 2005a,b, Schubert & Braun 2004, 2005a,b, 2007, Heuchert et al. 2005, Schubert et al. 2006) and molecular basis (Arzanlou et al. 2007, Crous et al. 2007a, Schubert et al. 2007a) to establish and refine its generic concept (*Davidiella* teleomorphs, typical

coronate scar structure), and delimit it from morphologically similar genera. Similarly, polyphasic approaches were also undertaken to establish species identities in species complexes such as *C. herbarum* (Schubert et al. 2007b) and *C. sphaerospermum* (Zalar et al. 2007, Dugan et al. 2008). A surprising finding of these studies was the high diversity of species and genotypes that exist in nature in what was formerly accepted to be well-defined species.

The present paper is an additional contribution highlighting the biodiversity in *Cladosporium*. Two new species from pine needles in Argentina, as well as another closely allied sister species occurring on *Cortaderia* leaves collected in Colombia, are described using the protocol outlined by Schubert et al. (2007b).

MATERIALS AND METHODS

Isolates

Single-conidial isolates were obtained from surface-dried herbarium materials, and plated onto 2 % malt extract agar (MEA; 20 g/L Biolab malt extract, 15 g/L Biolab agar). Strains were also inoculated onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), and fresh MEA plates (Crous et al. 2009), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. Ex-type strains of the newly described species are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

DNA isolation, amplification and sequence analysis

Fungal colonies were established on MEA plates, and genomic DNA was isolated as described in Crous et al. (2009). Partial gene sequences were determined as described by Crous et al. (2006) and Schubert et al. (2007b) for actin (ACT), translation elongation factor 1 α (TEF), and part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer, the 5.8S rRNA gene, the second internal transcribed spacer and the 5' end of the 28S rRNA gene (ITS). The nucleotide sequences were generated using both forward and reverse PCR primers to ensure good quality sequences over the entire length of the amplicon. Sequence

¹ CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.

² Botanische Staatssammlung München, Menzinger Strasse 67, D-80638 München, Germany; corresponding author e-mail: konstanze.schubert@gmx.de.

³ Área de Protección Forestal, Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Ruta 259 Km. 4, CC 14, 9200 Esquel, Chubut, Argentina.

Table 1 Cladosporium isolates used for phylogenetic analyses.

Anamorph	Teleomorph	Accession number ¹	Host	Country	Collector	Source	GenBank numbers ² (ITS, TEF, ACT)
<i>Cladosporium antarcticum</i>	–	CBS 690.92*	<i>Calophaea regalis</i>	Antarctica	C. Möller	Schubert et al. 2007b	EF679334, EF679405, EF679484
<i>Cladosporium bruhnei</i>	<i>Davidiella allicina</i>	CBS 157.82	<i>Quercus robur</i>	Belgium	–	Schubert et al. 2007b	EF679336, EF679407, EF679486
		CBS 161.55	Man, sputum	The Netherlands	–	Schubert et al. 2007b	EF679338, EF679409, EF679488
<i>Cladosporium chubutense</i>	–	CBS 121624*; CPC 12211	<i>Hordeum vulgare</i>	Belgium	J.Z. Groenewald	Schubert et al. 2007b	EF679350, EF679425, EF679502
<i>Cladosporium cladosporioides</i> complex	–	CBS 124457*; CPC 13979; CIEFAP 321	<i>Pinus ponderosa</i>	Argentina	A. Greslebin	This study	FJ936158, FJ936161, FJ936165
	–	CBS 170.54	<i>Arundo</i> , leaf	United Kingdom	–	Zalar et al. 2007	DQ780940, FJ936162, EF101352
	–	CBS 673.69	Air	The Netherlands	–	Schubert et al. 2007b	EF679353, EF679428, EF679505
<i>Cladosporium colombiae</i>	<i>Davidiella</i> sp.	CBS 109082	<i>Silene maritima</i>	United Kingdom	A. Aptroot	Schubert et al. 2007b	EF679354, EF679429, EF679506
<i>Cladosporium herbaroides</i>	–	CPC 11606	<i>Musa</i> sp.	India	M. Arzanlou	Schubert et al. 2007b	EF679355, EF679430, EF679507
<i>Cladosporium herbarum</i>	–	CBS 274.80B*	<i>Cortaderia</i> sp.	Colombia	W. Gams	This study	FJ936159, FJ936163, FJ936166
	–	CBS 121626*; CPC 12052; EXF-1733	Hypersaline water from salterns	Israel	P. Zalar	Schubert et al. 2007b	EF679357, EF679432, EF679509
<i>Cladosporium tassiana</i>	–	CBS 121621*; CPC 12177	<i>Hordeum vulgare</i>	The Netherlands	P.W. Crous	Schubert et al. 2007b	EF679363, EF679440, EF679516
	–	CPC 12181	<i>Hordeum vulgare</i>	The Netherlands	P.W. Crous	Schubert et al. 2007b	EF679367, EF679444, EF679520
	–	CPC 12183	<i>Hordeum vulgare</i>	The Netherlands	P.W. Crous	Schubert et al. 2007b	EF679368, EF679445, EF679521
<i>Cladosporium iridis</i>	<i>Davidiella macrospora</i>	CBS 107.20	<i>Iris</i> sp.	–	–	Schubert et al. 2007b	EF679369, EF679446, EF679522
	–	CBS 138.40*	<i>Iris</i> sp.	The Netherlands	–	Schubert et al. 2007b	EF679370, EF679447, EF679523
<i>Cladosporium macrocarpum</i>	<i>Davidiella macrocarpa</i>	CBS 299.67	<i>Triticum aestivum</i>	Turkey	–	Schubert et al. 2007b	EF679372, EF679450, EF679526
	–	CBS 121811*; CPC 12755	<i>Spinacia oleracea</i>	USA	L. du Toit	Schubert et al. 2007b	EF679376, EF679454, EF679530
	–	CPC 12756	<i>Spinacia oleracea</i>	USA	L. du Toit	Schubert et al. 2007b	EF679377, EF679455, EF679531
<i>Cladosporium ossifragi</i>	–	CBS 842.91*	<i>Narthecium ossifragum</i>	Norway	M. di Menna	Schubert et al. 2007b	EF679381, EF679459, EF679535
	–	CBS 843.91	<i>Narthecium ossifragum</i>	Norway	M. di Menna	Schubert et al. 2007b	EF679382, EF679460, EF679536
<i>Cladosporium pini-ponderosae</i>	–	CBS 124456*; CPC 13980; CIEFAP 322	<i>Pinus ponderosa</i>	Argentina	A. Greslebin	This study	FJ936160, FJ936164, FJ936167
<i>Cladosporium pseudiridis</i>	–	CBS 116463*; ICMP 15579	<i>Iris</i> sp.	New Zealand	C.F. Hill	Schubert et al. 2007b	EF679383, EF679461, EF679537
<i>Cladosporium ramotenellum</i>	–	CBS 121628*; CPC 12043; EXF-454	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007b	EF679384, EF679462, EF679538
	–	CPC 12047; EXF-967	Air conditioning system	Slovenia	P. Zalar	Schubert et al. 2007b	EF679385, EF679463, EF679539
<i>Cladosporium sinuosum</i>	–	CBS 121629*; CPC 11839; ICMP 15819	<i>Fuchsia excorticata</i>	New Zealand	A. Blouin	Schubert et al. 2007b	EF679386, EF679464, EF679540
<i>Cladosporium sp.</i>	–	CBS 300.96	Soil along coral reef coast	Papua New Guinea	A. Aptroot	Zalar et al. 2007	DQ780352, EU570259, EF101385
<i>Cladosporium sphaerospermum</i>	–	CBS 109.14; ATCC 36950	<i>Carya illinoensis</i> leaf scale	USA	–	Zalar et al. 2007	DQ780350, EU570260, EF101384
	–	CBS 193.54*; ATCC 11289; IMI 49637	Human nails	The Netherlands	G.A. de Vries	Zalar et al. 2007	DQ780343, EU570261, EU570269
	–	CBS 102045; EXF-2524; MZKI B-1066	Hypersaline water	Spain	P. Zalar	Zalar et al. 2007	DQ780351, EU570262, EF101378
	–	CPC 12476	<i>Ambrosia artemisiifolia</i>	Germany	J. Nitzsche	Dugan et al. 2008	EU570255, EU570264, EU570271
<i>Cladosporium spinulosum</i>	–	CPC 13995; CAMS 000750	Thatch	South Africa	–	Dugan et al. 2008	EU570257, EU570266, EU570273
	–	CBS 102044	Hypersaline water from salterns	Slovenia	S. Soujak	Schubert et al. 2007b	EF679387, EF679465, EF679541
<i>Cladosporium subinflatum</i>	–	CBS 11907*; CPC 12040; EXF-334	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007b	EF679388, EF679466, EF679542
<i>Cladosporium subtilissimum</i>	–	CBS 121630*; CPC 12041; EXF-343	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007b	EF679389, EF679467, EF679543
	–	CBS 113753	Bing cherry fruits	USA	F. Dugan	Schubert et al. 2007b	EF679396, EF679474, EF679550
	–	CBS 113754*	Grape berry	USA	F. Dugan	Schubert et al. 2007b	EF679397, EF679475, EF679551
<i>Cladosporium tenellum</i>	–	CPC 12044; EXF-462	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007b	EF679398, EF679476, EF679552
	–	CBS 121634*; CPC 12053; EXF-1735	Hypersaline water from salterns	Israel	P. Zalar	Schubert et al. 2007b	EF679401, EF679479, EF679553
	–	CPC 11813	<i>Phyllactinia</i> sp. on <i>Corylus</i> sp.	USA	D. Glawe	Schubert et al. 2007b	EF679399, EF679477, EF679553
<i>Cladosporium variabile</i>	<i>Davidiella variabile</i>	CBS 121636*; CPC 12751	<i>Spinacia oleracea</i>	USA	L. du Toit	Schubert et al. 2007b	EF679402, EF679480, EF679556
	–	CPC 12753	<i>Spinacia oleracea</i>	USA	L. du Toit	Schubert et al. 2007b	EF679403, EF679481, EF679557

¹ ATCC: American Type Culture Collection, Virginia, USA; CAMS: SERA's Centre for Applied Mycological Studies, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CBS: Centraalbureau voor Schimmecultures, Utrecht, The Netherlands; CIEFAP: Centro de Investigación y Extensión Forestal Andino Patagónico, Argentina; CPC: Culture collection of Pedro Crous, housed at CBS; EXF: Extremophilic Fungi Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, CAB International, Egham, Surrey, UK; MZKI: Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia; NRRC: Agricultural Research Culture Collection, Peoria, Illinois, USA.

² ACT: partial actin gene, TEF: partial translation elongation factor 1- α gene, ITS: internal transcribed spacer region with 5.8S rRNA gene.

* Ex-type cultures

Table 2 Statistical parameters describing the sequence alignments and phylogenetic analysis of three different loci and the combined alignment.

Parameter	ITS ¹	ACT ¹	TEF ¹	Combined	P value
<i>Parsimony information</i>					
Number of alignment positions including gaps	495	219	380	1094	
Number of parsimony informative characters	37	105	181	323	
Number of variable and parsimony-uninformative characters	100	24	51	175	
Number of constant characters	358	90	148	596	
<i>Calculated parsimony measures</i>					
Tree length (TL)	169	371	750	Not determined	
Consistency Index (CI)	0,947	0,671	0,591	Not determined	
Retention Index (RI)	0,963	0,856	0,833	Not determined	
Rescaled Consistency Index (RC)	0,911	0,574	0,492	Not determined	
Number of equally most parsimonious trees	4	1000	12	Not determined	
<i>Partition homogeneity test</i>					
ITS & ACT & TEF					0.004
ITS & ACT					0.941
ITS & TEF					0.95
ACT & TEF					0.001

¹ ACT: partial actin gene, TEF: partial translation elongation factor 1- α gene, ITS: internal transcribed spacer regions with 5.8S rRNA gene.

Fig. 1 The first of four equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate branches present in the strict consensus tree and type strains are indicated in **bold**. The tree was rooted to sequences of *Cercospora beticola* strain CBS 116456 (GenBank accession numbers AY840527, AY840458, AY840494, respectively).

Fig. 2 The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ACT sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate branches present in the strict consensus tree and type strains are indicated in **bold**. The tree was rooted to sequences of *Cercospora beticola* strain CBS 116456 (GenBank accession numbers AY840527, AY840458, AY840494, respectively).

data obtained from Schubert et al. (2007b) and Zalar et al. (2007) were used as reference data for the alignments (Table 1). Subsequent sequence alignment and phylogenetic analyses followed the methods of Crous et al. (2006). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and the alignment in TreeBASE (www.treebase.org).

Morphology

Freehand sections of dried needles with conidiophores were examined microscopically, mounted in 5 % KOH plus 1 % aqueous phloxine and distilled water.

Microscopic observations of the isolates were made from colonies cultivated for 7 d under continuous near-ultraviolet light

at 25 °C on SNA. Preparations were mounted in Shear's solution (Crous et al. 2009). To study conidial development and branching patterns, squares of transparent adhesive tape (Titan Ultra Clear Tape, Conglom Inc., Toronto, Canada) were placed on conidiophores growing in the zone between the colony margin and 2 cm inwards, and mounted between two drops of Shear's solution under a glass cover slip. Conidial terminology follows that of Schubert et al. (2007b). Wherever possible, 30 measurements ($\times 1\,000$ magnification) were made of conidia with the extremes of spore measurements given in parentheses. For culture characteristics colonies were cultivated on PDA, SNA and MEA for 14 d at 25 °C in the dark, after which the surface and reverse colours were rated using the charts of Rayner (1970).

Fig. 3 The first of 12 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the TEF sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate branches present in the strict consensus tree and type strains are indicated in **bold**. The tree was rooted to sequences of *Cercospora beticola* strain CBS 116456 (GenBank accession numbers AY840527, AY840458, AY840494, respectively).

RESULTS

Phylogeny

The manually adjusted concatenated alignment contained 43 sequences (including the outgroup sequence) and the three loci were represented by a total of 1 094 characters including alignment gaps that were used in the analyses (Table 2). The result of the partition homogeneity test (P value = 0.004) indicated that the three loci were not congruent and therefore the sequence data were analysed as separate alignments. The ITS alignment was congruent with both the ACT and the TEF alignments (Table 2), but the ACT and TEF alignments not with each other (P value = 0.001). This can in part be explained by the two isolates of *C. spinulosum* not clustering together in the TEF phylogeny (Fig. 3).

ITS alignment — Four equally most parsimonious trees, the first of which is shown in Fig. 1, were obtained from the parsimony analysis of the ITS alignment. Neighbour-joining analysis using three substitution models (uncorrected 'p', Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies to one another and to Fig. 1 (data not shown).

ACT alignment — One thousand equally most parsimonious trees, the first of which is shown in Fig. 2, were obtained from the parsimony analysis of the ACT alignment. Neighbour-joining analyses using three substitution models (uncorrected 'p', Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies to one another, but differed with regard to the order of the clades in the *C. herbarum* complex shown in Fig. 2 (data not shown). The high number of equally

parsimonious trees can be attributed to the many possible arrangements of the clades in the *C. herbarum* complex (based on the strict consensus branches shown in Fig. 2).

TEF alignment — Twelve equally most parsimonious trees, the first of which is shown in Fig. 3, were obtained from the parsimony analysis of the TEF alignment. Neighbour-joining analyses using three substitution models (uncorrected 'p', Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies to one another, but differed with regard to the order of the clades in the *C. herbarum* complex shown in Fig. 3 (data not shown).

Taxonomy

Based on their distinct morphological and molecular characters, three new *Cladosporium* species are described below.

Cladosporium chubutense K. Schub., Gresl. & Crous, *sp. nov.*
— MycoBank MB509557; Fig. 4–6

Differt a *C. oreodaphnes* conidiophoris non-nodulosis et leniter latoribus, (4–)5–8(–9) μm latis, et a *C. antarctico* mycelio non dimorpho, conidiis in vitro 0–1-septatis, locis conidiogenis et hilis latoribus, (0.5–)0.8–2(–2.2) μm diam (in vitro).

Etymology. Refers to Chubut, a province of Argentina, where the species was collected.

In vivo: Isolated from needles becoming necrotic from the top to the base, no discrete leaf lesions formed. *Caespituli* first punctiform, dark brown, distributed along the stomatal lines in upper and under sides, then coalescing and forming elongated, erumpent, black stromata. *Mycelium* internal, subcuticular to intraepidermal, occasionally external, superficial, hyphae unbranched to occasionally branched, 2–6(–8) μm wide, pluriseptate, often in short succession, slightly constricted, often appearing to be darkened, pale olivaceous to pale olivaceous-brown, smooth to minutely verruculose, walls unthickened or almost so, becoming regularly or irregularly swollen, up to 14 μm diam, walls more thickened at swellings, sometimes forming ropes. *Stromata* small to large, 20–75 μm diam or confluent, compact, dense, several layers deep, substomatal to intraepidermal, pseudoparenchymatous, composed of swollen hyphal cells, subglobose to somewhat angular, 5–10 μm diam, medium to dark brown, walls thickened. *Conidiophores* macronematous, fasciculate, in small to large, loose to dense fascicles, spider-like, emerging through stomata or erumpent through the cuticle,

arising from stromata, erect, straight to flexuous, subcylindrical, not geniculate or only slightly so, sometimes subnodulose due to small lateral swellings, unbranched or rarely once branched towards the apex, 28–120 $\times$ (4–)5–8(–9) μm , often slightly to distinctly attenuated towards the apex, base often wider or swollen, up to 10 μm wide, pluriseptate, septa often in short succession, sometimes slightly constricted, medium to dark olivaceous-brown or brown, paler at apices, smooth to minutely verruculose or irregularly and distinctly roughened, rugose, outer walls seeming to detach irregularly, especially towards the apex, walls thickened, often distinctly 2-layered, up to 1.5 μm wide, sometimes enteroblastically proliferating. *Conidiogenous cells* integrated, terminal and intercalary, subcylindrical, not geniculate, sometimes subnodulose with loci situated on small lateral shoulders, 6–32 μm long, with a single or several conspicuous loci, somewhat crowded towards the apex, 1–2(–2.5) μm diam, somewhat thickened and darkened-refractive. *Conidia* solitary or catenate, in unbranched or branched chains, straight, subglobose, obovoid, ellipsoid to subcylindrical, 4.5–19 $\times$ 4.5–7(–8) μm (av. $\pm$ SD: 11.1 $\pm$ 3.5 $\times$ 5.8 $\pm$ 1.0), 0–1(–2)-septate, rarely with three septa, septum more or less median, sometimes slightly constricted, with age becoming sinuous, pale to medium olivaceous-brown, almost smooth to minutely verruculose, walls somewhat thickened, attenuated towards apex and base, often broadly rounded at the distal end, sometimes slightly pointed, with a single, two, or rarely three hila at the distal end, conspicuous, 1–2(–2.5) μm diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis not observed.

In vitro (on SNA): *Mycelium* mainly immersed, sometimes superficial; hyphae mainly unbranched, 1–4 μm wide, septate, septa occasionally darkened, without any swellings and constrictions, subhyaline to pale dingy brown or greyish brown, almost smooth to somewhat irregularly rough-walled, walls unthickened. *Conidiophores* macronematous and micronematous, arising terminally from ascending hyphae, or sometimes laterally from plagiotropous hyphae, solitary, erect, straight or slightly flexuous, subcylindrical to cylindrical, slightly to distinctly geniculate-sinuous towards the apex, sometimes subnodulose with loci situated on small lateral shoulders, often several times, unbranched or once branched, 10–200 $\times$ (1.5–)2.5–4 μm , septate, not constricted at septa, greyish brown to olivaceous-brown, smooth to minutely verruculose or verruculose, especially towards the apex, walls only slightly

Fig. 4 *Cladosporium chubutense* (HAL 2323 F). Fascicle of conidiophores and conidia in vivo. — Scale bar = 10 μm . K. Schubert del.

Fig. 5 *Cladosporium chubutense* (CBS 124457 = CPC 13979). Conidiophores and conidia in vitro. — Scale bar = 10 μm . K. Schubert del.

Fig. 6 *Cladosporium chubutense* (CBS 124457 = CPC 13979). a–e. Macro- and micronematous conidiophores with conidia; f–g. conidial chains. — Scale bars = 10 µm.

thickened, about 0.5 µm wide. *Conidiogenous cells* integrated, terminal and intercalary, subcylindrical to cylindrical, slightly to distinctly geniculate, once or several times, non-nodulose but the whole apical cell occasionally inflated, ellipsoid, 10–57 µm long, conidiogenous loci conspicuous, often crowded towards the apex, up to six or more loci per cell, 1–2(–2.2) µm diam, somewhat thickened and darkened-refractive. *Ramoconidia* subcylindrical, 19–34(–38) × (3.5–)4–5 µm, aseptate, concolorous with the tips of conidiophores. *Conidia* catenate, in branched chains, branching in all directions, up to three or four conidia in the unbranched parts, small terminal conidia obovoid to ellipsoid, 4–8 × 2.5–4 µm (av. ± SD: 5.9 ± 1.2 × 3.0 ± 0.5), aseptate, broadly rounded at the apex, hila 0.5–1 µm diam, intercalary conidia ellipsoid-ovoid, 7–14 × 3–4.5 µm (av. ± SD: 9.9 ± 2.0 × 3.9 ± 0.5), aseptate, with a single sometimes up to three distal hila, hila 0.8–1.5 µm diam, secondary ramoconidia ellipsoid-ovoid to subcylindrical, 13–27(–34) × 4–5 µm (av. ± SD: 19.8 ± 5.4 × 4.4 ± 0.4), 0–1-septate, not constricted at septa, septa median or somewhat in the upper half, with up to four distal hila, pale to medium greyish brown or olivaceous-brown, minutely verruculose to usually verruculose, occasionally distinctly verrucose, walls unthickened or only slightly so, hila conspicuous, (0.5–)0.8–2(–2.2) µm diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis sometimes generating conidia irregular in outline.

Culture characteristics — Colonies on PDA attaining 50–61 mm diam after 1 mo, dull green to dark grey-olivaceous, reverse iron-grey, velvety to powdery, margin white, broad, glabrous,

aerial mycelium pale olivaceous-grey, diffuse, loose, only few areas covered, growth regular, flat, without conspicuous exudates, sporulation profuse. Colonies on MEA reaching 26–37 mm diam after 1 mo, olivaceous to greenish olivaceous forming concentric zones, reverse iron-grey to greenish black, velvety, margin white, narrow, regular, feathery, aerial mycelium pale olivaceous-grey, sparse, growth flat, sometimes with a crater-like structure in the centre or wrinkled, without conspicuous exudates, sporulation profuse.

Specimens examined. ARGENTINA, Chubut, dpto. Languineo, Rio Pico, Camelia property, 44° 8' 30" S, 71° 26' 40" W, on needles of *Pinus ponderosa* (Pinaceae), 12 Jan. 2005, A. Greslebin, mixed infection with *Cladosporium macrocarpum* and an alternarioid hyphomycete, HAL 2323 F; Apr. 2007, A. Greslebin, holotype (dried culture) CBS H-20209; isotype BAFC 51695; cultures ex-type CBS 124457 = CIEFAP 321 = CPC 13979.

Notes — This species has been recorded on dead and living needles of *Pinus ponderosa* in *Pinus* plantations in Patagonia (Argentina). Its biology remains unclear since no pathogenicity test has been carried out. On the natural host *Cladosporium chubutense* resembles *C. oreodaphnes* known from *Oreodaphne foetens* in Germany (Schubert 2005b) but is distinct in having non-nodulose or only subnodulose, shorter but somewhat wider conidiophores in vivo, 28–120 × (4–)5–8(–9) µm (vs 40–230 × 3.5–7 µm in *C. oreodaphnes*). *Cladosporium antarcticum* is also similar but easily distinguishable by its dimorphic mycelium, 0–3-septate conidia and its narrower conidiogenous loci and hila, 0.8–1.5(–2) µm (Schubert et al. 2007b).

Table 3 Unique nucleotide differences (colour-coded per species) and their base positions observed in three loci between the three *Cladosporium* species described in this study. Sequences of *Cladosporium cladosporioides* strain CBS 109082 were used as references to calculate base positions, which do not include spaces caused by alignment gaps (indicated by hyphens). To simplify the table, only characters that are different between the three novel species are shown. See Table 1 for the definition of the strain and locus abbreviations and for complete strain information.

Species	ITS	ACT								
	378 ^b	75 ^a	76 ^b	80 ^b	116 ^a	151–152 ^c	165 ^a	169 ^b	178 ^{a,b}	193 ^b
<i>C. cladosporioides</i>	A	C	C	C	T	–	A	T	C	C
<i>C. chubutense</i>	T	C	C	C	T	T–	A	G	T	G
<i>C. colombiae</i>	A	C	C	C	T	T–	A	G	T	G
<i>C. pini-ponderosae</i>	A	T	G	G	C	TCT	G	T	G	C

Species	TEF												
	57 ^a	112 ^c	125 ^{a,b}	136 ^{a,b}	141 ^a	157 ^b	158 ^a	159 ^a	165 ^a	168 ^b	172 ^b	175 ^a	259 ^b
<i>C. cladosporioides</i>	C	C	T	G	G	C	T	C	T	A	G	C	C
<i>C. chubutense</i>	T	–	C	T	C	C	T	T	C	C	G	T	C
<i>C. colombiae</i>	T	C	A	C	T	C	T	T	C	C	G	T	C
<i>C. pini-ponderosae</i>	C	C	C	T	C	G	C	C	T	A	C	C	G

^a Transition.
^b Transversion.
^c Insertion/deletion.

In addition to the newly described *C. chubutense*, both the teleomorph and anamorph of the widespread saprobic species *C. macrocarpum* co-occur on the infected needles of *Pinus ponderosa*. The latter species may represent a secondary invader following infection with *C. chubutense* or both species may be saprobic invaders. Phylogenetically, *C. chubutense* is closely related to *C. colombiae* based on ACT and ITS sequences, but differs from it at one position on ITS and four positions on TEF (Fig. 1–3, Table 3).

Cladosporium pini-ponderosae K. Schub., Gresl. & Crous,
sp. nov. — MycoBank MB509558; Fig. 7–9

Differt a *C. leguminicola* conidiis latoribus, (3–)4–8(–9) µm latis, conidiophoris crassitunicatis, saepe bistratis, et a *C. chubutensi* conidiis irregulariter rugosis, catenis longioribus, conidiis usque ad 9 in catenis terminalibus non ramosis.

Etymology. Named after its host, *Pinus ponderosa*.

In vivo: Isolated from needles becoming necrotic from the top to the base, no discrete leaf lesions formed. *Caespituli* punctiform, sometimes coalescing, dark brown, distributed along the stomatal lines in upper and under sides of the needles but more abundant on the lower side. *Mycelium* internal, immersed, but also external, superficial, composed of septate, smooth, subhyaline to pale brown, thin to slightly thick-walled hyphae, 2.5–6(–8) µm diam, hyphae somewhat constricted at septa, often swollen, forming substomatal, pseudoparenchymatous stromata, small to extended, 50–135 µm diam or even larger, several layers deep, composed of thick-walled, olivaceous to brown, rounded cells, 8–15 µm diam. *Conidiophores* in small to large dense fascicles, arising from stromata, emerging through stomata, subcylindrical, sinuous, slightly geniculate due to sympodial proliferation and slightly tapered towards the apices, mostly unbranched, rarely branched, 12–70(–100) × 3.5–8 µm, 0–3-septate, rarely forming more septa, pale to dark olivaceous-brown, paler towards apices, walls brown, thick-walled, often 2-layered (two distinct wall layers visible), darkened and thickened towards the base, often enteroblastically proliferating, once or twice. *Conidiogenous cells* integrated, mostly terminal but also intercalary, subhyaline to pale brown, geniculate, polyblastic, proliferation sympodial with several conidiogenous loci situated terminally or laterally on small shoulders, cicatrised, loci protuberant, denticulate, thickened and darkened-refractive. *Conidia* single or catenate, in unbranched or branched chains, ovoid, ellipsoid to subcylindrical, 5–20(–31) × (3–)4–8(–9) µm (av. ± SD: 13.1 ± 5.9 × 5.7 ± 1.5), 0–3-septate, pale brown to

brown, slightly thick-walled, almost smooth to usually verruculose, sometimes verrucose, hila conspicuous, 1–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis observed.

In vitro (on SNA): *Mycelium* immersed and superficial; hyphae unbranched or loosely branched, 1.5–5(–8) µm wide, septate, without any constrictions or swellings, subhyaline to pale greyish brown or dingy-brown, smooth to irregularly rough-walled to verruculose or walls covered by polysaccharide-like material, walls unthickened or only slightly thickened. *Conidiophores* macronematous, arising terminally from ascending or laterally from plagiotropous hyphae, solitary, sometimes in pairs of two, erect, straight or slightly flexuous, subcylindrical to cylindrical-oblong, sometimes slightly geniculate towards the apex, once or twice, unbranched, sometimes once branched, rarely twice, non-nodulose, 14–190 × (2.5–)3.5–5.5 µm, septate, not constricted at septa, branches as short lateral outgrowths just below a septum, later becoming longer, greyish brown or dingy brown, sometimes paler towards the apex, almost smooth to minutely verruculose to irregularly rough-walled, walls thickened, 0.5–1(–1.5) µm wide, sometimes even appearing to be 2-layered (two distinct wall layers visible), not or only very slightly attenuated towards the apex. *Conidiogenous cells* integrated, mainly terminal, sometimes intercalary, subcylindrical to cylindrical-oblong, sometimes slightly geniculate towards the apex, 14–45 µm long, conidiogenous loci mostly crowded at the apex, (1–)2–4(–6) loci, broadly truncate, central convex

Fig. 7 *Cladosporium pini-ponderosae* (BAFC 51696). Fascicle of conidiophores arising from extended stromata and conidia. — Scale bar = 10 µm. A. Greslebin del.

Fig. 8 *Cladosporium pini-ponderosae* (CBS 124456 = CPC 13980). Conidiophores and conidia. — Scale bar = 10 μ m. K. Schubert del.

dome not very prominent, 1.5–2.5 μ m diam, somewhat thickened and darkened-refractive. *Ramoconidia* cylindrical-oblong, not attenuated towards the base, 20–45 $\times$ 3.5–5(–5.5) μ m, 0–1(–3)-septate, not constricted, concolorous with the tips of conidiophores, walls thickened, base unthickened, broadly truncate. *Conidia* catenate, in unbranched or branched chains, branching in all directions, up to nine conidia in the terminal chain, small terminal conidia obovoid to ellipsoid-ovoid, 5–6 $\times$ 2.5–4(–4.5) μ m (av. $\pm$ SD: 5.5 $\pm$ 0.5 $\times$ 3.3 $\pm$ 0.6), broadly rounded at the apex, slightly attenuated towards the base, aseptate, hila 0.5–1(–1.2) μ m diam, intercalary conidia ellipsoid-ovoid, fusiform, sometimes rostrate towards the distal end, attenuated towards apex and base, 6–15(–22) $\times$ 3–4(–5) μ m (av. $\pm$ SD: 9.9 $\pm$ 3.8 $\times$ 3.8 $\pm$ 0.5), aseptate, rarely 1-septate, mostly with a single but sometimes up to three distal hila, hila 0.8–1.5 μ m diam, secondary ramoconidia fusiform to subcylindrical, 10–30(–36) $\times$ 3.5–5 μ m (av. $\pm$ SD: 20.3 $\pm$ 6.4 $\times$ 4.3 $\pm$ 0.4), 0–1(–2)-septate, very rarely 3-septate, not constricted at septa, septa sometimes slightly darkened, becoming somewhat sinuous with age, with up to four distal hila, attenuated towards apex and base, pale to medium greyish brown or dingy brown, small terminal conidia and young conidia subhyaline, verruculose to very irregularly rough-walled, younger conidia almost smooth, walls appear to be very thick-walled, lumen distinct, very pale between inner and outer wall, hila broadly truncate, 0.5–2.5 μ m diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring.

Culture characteristics — Colonies on PDA attaining 65–73 mm diam after 1 mo, grey-olivaceous to olivaceous-grey, reverse olivaceous-grey to iron-grey, velvety to powdery, margin white, narrow, glabrous to feathery, regular, entire edge to

Fig. 9 *Cladosporium pini-ponderosae* (CBS 124456 = CPC 13980). a–d. Conidiophores with conidial chains; e–g. conidia. — Scale bars = 10 μ m.

slightly undulate, aerial mycelium formed, pale olivaceous-grey, somewhat fluffy, especially in colony centre, growth flat, without conspicuous exudates, sporulation profuse. Colonies on MEA reaching 64–74 mm diam after 1 mo, olivaceous-grey to grey-olivaceous due to profuse sporulation, greenish grey towards the margin, velvety, margin white, narrow, regular, glabrous, radially furrowed, growth flat, often folded, without conspicuous exudates.

Specimen examined: ARGENTINA, Neuquén, Aluminé, Lagos Marmol property, 39° 22' 52" S, 71° 5' 38" W, on needles of *Pinus ponderosa* (Pinaceae), Jan. 2005, A. Greslebin, holotype CBS H-20210; isotypes BAFC 51696, HAL 2322 F; cultures ex-type CBS 124456 = CIEFAP 322 = CPC 13980.

Notes — As for *C. chubutense*, this species was recorded on dead and living needles of *Pinus ponderosa* in pine plantations in Patagonia (Argentina). However, *C. chubutense* differs from *C. pini-ponderosae* in forming both macro- and micronematous conidiophores in culture which are slightly to distinctly geniculate towards the apex and somewhat narrower in vitro ((1.5–)2.5–4 µm vs (2.5–)3.5–5.5 µm in *C. pini-ponderosae*). The unbranched terminal conidial chains are much shorter, consisting of up to three conidia (in *C. pini-ponderosae* up to nine conidia in the unbranched parts of the conidial chains); the surface ornamentation is quite different in being minutely verruculose to verruculose or occasionally verrucose with the conidial walls unthickened or only slightly so. Furthermore, *C. chubutense* is slower-growing and possesses different cultural characteristics. *Cladosporium leguminicola*, described from pods of *Phaseolus vulgaris* from Spain, resembles *C. pini-ponderosae* on the natural host but is distinct in having narrower conidia (3–5.5 µm vs (3–)4–8(–9) µm in *C. pini-ponderosae*) and thin-walled or only slightly thickened conidiophores (Braun & Schubert 2007). Although almost identical on ITS, *C. pini-ponderosae* is phylogenetically distinct from *C. chubutense* and *C. colombiae* based on its ACT and TEF sequence (Fig. 1, 3, Table 3).

Cladosporium colombiae K. Schub. & Crous, sp. nov. — MycoBank MB509559; Fig. 10, 11

Differt a *C. ramotenello* ramoconidiis nullis, conidiis terminalibus non-globosis, angustioribus, 2.5–3(–4) µm latis, conidiis intercalariis ovoidibus, limoniiformibus et ellipsoidibus, aseptatis, brevioribus et leniter angustioribus, 6–9 × (2.5–)3–3.5 µm, ramoconidiis secundariis brevioribus, 8–17(–23) µm longis, 0–1(–2)-septatis; et a *C. subtilissimo* conidiis intercalariis brevioribus et leniter angustioribus, 6–9 × (2.5–)3–3.5 µm, conidiis secundariis brevioribus et angustioribus, 8–17(–23) × (2.5–)3–3.5 µm.

Etymology. Refers to the country where it was collected, Colombia.

In vitro (on SNA): Mycelium immersed and superficial; hyphae branched, 1–5 µm wide, septate, sometimes constricted at septa and with swellings, up to 8 µm diam, subhyaline to olivaceous or olivaceous-brown, smooth to minutely verruculose or irregularly rough-walled, sometimes covered by polysaccharide-like material, wart-like, rugose, therefore irregular in outline, often forming ropes or loose aggregations. **Conidiophores** macro- and micronematous, arising terminally or laterally from ascending or plagiotropous hyphae, erect, straight to flexuous, solitary or in pairs of two; macronematous conidiophores cylindrical-oblong, non-nodulose, unbranched or occasionally branched, 25–105 × 3–4(–4.5) µm, often slightly attenuated towards the apex, 0–4(–5)-septate, not constricted at septa, sometimes in short succession, pale to medium olivaceous-brown, sometimes even dark olivaceous-brown, smooth to often minutely verruculose, especially towards the apex, walls slightly thickened; micronematous conidiophores filiform, narrower, paler, often only as peg-like lateral outgrowth of hyphae, unbranched, 10–135 × 2–2.5 µm, septate, subhyaline to pale olivaceous-brown, smooth to minutely verruculose, walls unthickened. **Conidio-**

genous cells integrated, terminal, rarely intercalary, cylindrical-oblong or filiform, 10–37 µm long, usually with only a single apical locus, sometimes with 2–3 loci, then subdenticulate, 1–2 µm diam, thickened and darkened-refractive. **Conidia** catenate, in long branched chains, up to 10 conidia in the unbranched part, small terminal conidia obovoid, 4–6.5 × 2.5–3(–4) µm (av. ± SD: 4.7 ± 0.8 × 3.2 ± 0.5), intercalary conidia ovoid, limoniiform to ellipsoid-ovoid, 6–9 × (2.5–)3–3.5 µm (av. ± SD: 7.0 ± 0.8 × 3.2 ± 0.3), aseptate, attenuated towards apex and base, secondary ramoconidia ellipsoid to subcylindrical, sometimes clavate, 8–17(–23) × (2.5–)3–4 µm (av. ± SD: 13.8 ± 3.4 × 3.5 ± 0.4), 0–1(–2)-septate, not constricted at the median septum, pale to medium olivaceous-brown, smooth to minutely verruculose or often irregularly rough-walled, walls somewhat thickened, about 0.5 µm thick, attenuated towards apex and base, with 2–4(–5) distal hila, subdenticulate, 0.8–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis sometimes occurring.

Culture characteristics — Colonies on PDA grey-olivaceous, reverse iron-grey to greenish blue, fluffy to felty, margin feathery, aerial mycelium high, fluffy, growth low convex to convex, without prominent exudates, sporulation profuse. Colonies on MEA smoke-grey to grey-olivaceous and pale olivaceous-grey with small dots of olivaceous-grey towards margins, whitish or glaucous-grey towards margins, reverse iron-grey, velvety to woolly-felty, margins colourless to white, somewhat feathery, growth flat, exudates few and small but conspicuous, sporulation profuse.

Specimen examined. COLOMBIA, Páramo de San Cayetano, isol. from a dead leaf of *Cortaderia* (Poaceae), depos. May 1980, isol. W. Gams, holotype CBS H-10374, formerly stored as *Cladosporium tenuissimum*; culture ex-type CBS 274.80 B.

Notes — Although *C. colombiae* is phylogenetically closely related to *C. chubutense* described in this study (see notes under *C. chubutense*), the morphology of these two species is quite distinct. *Cladosporium chubutense* possesses slightly to often distinctly geniculate, longer conidiophores with several (up to six) conidiogenous loci per conidiogenous cell; the conidia are formed in short chains (3–4 in the unbranched part) compared with those of *C. colombiae* (up to 10 conidia in the unbranched part), and the minutely verruculose to verruculose conidia are longer and wider (small terminal conidia 4–8 × 2.5–4 µm vs 4–6.5 × 2.5–3(–4) µm in *C. colombiae*; intercalary conidia 7–14 × 3–4.5 µm vs 6–9 × (2.5–)3–3.5 µm and secondary ramo-

Fig. 10 *Cladosporium colombiae* (CBS 274.80 B). Conidiophores and conidia. — Scale bar = 10 µm. K. Schubert del.

Fig. 11 *Cladosporium colombiae* (CBS 274.80 B). a–d. Conidiophores and conidia; e. conidial chain. — Scale bars = 10 µm.

conidia $13\text{--}27(-34) \times 4\text{--}5$ µm vs $8\text{--}17(-23) \times (2.5\text{--})3\text{--}4$ µm in *C. colombiae*). *Cladosporium ramotenellum*, isolated from air and hypersaline water, is morphologically somewhat similar but easily distinguishable by having wider, usually globose to subglobose or ovoid small terminal conidia, $2\text{--}4(-4.5)$ µm wide, ellipsoid to subcylindrical, 0–1-septate, longer and wider intercalary conidia, $8\text{--}15 \times 3\text{--}4(-4.5)$ µm, and 0–3-septate, longer secondary ramoconidia, $17\text{--}35$ µm long (Schubert et al. 2007b). *Cladosporium subtilissimum*, a species belonging to the herbarum complex is also morphologically comparable to *C. colombiae* but differs by forming longer and somewhat wider intercalary conidia, $9\text{--}18 \times 3\text{--}4(-6)$ µm, and longer and wider secondary ramoconidia, $(13\text{--})17\text{--}32(-37) \times 3\text{--}5(-6)$ µm (Schubert et al. 2007b). Originally the strain was identified and stored as *C. tenuissimum*. The latter species is characterised by very long conidiophores, usually longer than 100 µm up to $500(-800)$ µm (Heuchert et al. 2005), which makes it easy to distinguish it from *C. colombiae*.

The biology of the new species remains unclear with respect to its possible role as a saprobe or pathogen.

DISCUSSION

The three species introduced herein were collected in South America. Molecular analyses of three loci showed that they are phylogenetically closely related, but due to their deviating morphology and different cultural characteristics they are treated and described as distinct species. It is quite possible that they have evolved from a common ancestor in South America; however, whether this was due to the introduction of such a founder to the continent or whether it is speciation of an existing species on the continent is unclear. More isolates need to be collected from South America and from diverse hosts to establish an acceptable hypothesis. Until now the number of isolates in the *Cladosporium* database at the CBS originating from South America is quite limited. This fact may also be a reason for the clustering of the three species, and may change when additional isolates from this continent are included in the alignments.

From the infected pine needles collected in the province of Chubut, from which the new species *C. chubutense* was isolated, two additional isolates were made which proved to belong to the herbarum complex. These isolates (CPC 12484, CPC 12485) which have already been treated as *Cladosporium* spp. in Schubert et al. (2007b) are morphologi-

cally indistinguishable from *C. subtilissimum* in culture, but are genetically different, clustering apart from the latter species. *Cladosporium chubutense* is distinct from *C. subtilissimum* by having usually slightly to distinctly geniculate conidiophores in vitro and somewhat shorter and wider aseptate ramoconidia ($19\text{--}34\text{--}(38) \times (3.5\text{--})4\text{--}5\text{ }\mu\text{m}$ vs $20\text{--}40\text{--}(55) \times 1.5\text{--}4\text{ }\mu\text{m}$ in *C. subtilissimum*).

This study once again strongly supports the value of implementing a polyphasic approach to species identification and treatment. The availability of sequence data from cultured isolates, especially for such an important and relatively poorly studied genus as *Cladosporium*, is crucial in shaping our understanding of speciation, species richness and diversity in these genera. Towards this goal we will continue our attempts to build an extensive database of polyphasic characters for the identification of *Cladosporium* species by including as many cultures as we can in our studies, and to epitypify existing species where possible.

Acknowledgements The authors thank Ms Mieke Starink-Willemse, Marjan Vermaas and Arien van Iperen (CBS) for technical support. Prof. dr U. Braun (Martin-Luther-Univ., Halle, Germany) is thanked for checking the Latin diagnoses.

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