New foliicolous species of \textit{Cladosporium} from South America

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\textbf{Key words}
Argentina
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\textbf{Abstract}
Two new species of \textit{Cladosporium} found on necrotic needles of \textit{Pinus ponderosa} trees in Patagonia, Argentina, are described as \textit{C. chubutense} and \textit{C. pini-ponderosa}. An additional isolate from dead leaves of \textit{Cortaderia} collected in Colombia, which is a sister taxon to the species occurring on \textit{Pinus}, is described as \textit{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.

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\section*{INTRODUCTION}

Exotic conifer plantations in Patagonia occupy about 53 000 ha located in the western limit of the steppe near native forests. \textit{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 \textit{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 \textit{Cortaderia} leaves in Colombia. Based on their morphology, cultural characteristics and phylogenetic position, all three species could be allocated to the genus \textit{Cladosporium}.

The genus \textit{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 \textit{Mycosphaerella} s.str. (Braun et al. 2003, Crous et al. 2007a, b, Schubert et al. 2007b) having teleomorphs in \textit{Davidiella}. Employing DNA sequence data from four loci (SSU nrDNA, LSU nrDNA, EF-1\textalpha, \textit{C. sphaero-}

\begin{itemize}
\item \textit{Cortaderia} sphaero-
\item \textit{Cortaderia} colombiae
\item Phenetic characters and culture characteristics.
\item MATERIALS AND METHODS
\subsection*{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).

\subsection*{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\textalpha (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
| Anamorph | Teleomorph | Accession number1 | Host  | Country           | Collector | Source                          | GenBank numbers2 (ITS, TEF, ACT) |
|----------|------------|------------------|-------|-------------------|-----------|---------------------------------|----------------------------------|
| Cladosporium antarcticum | – | CBS 690.92* | Caloplaca regalis | Antarctica | C. Möller | Schubert et al. 2007b | EF679334, EF679405, EF679484 |
| Cladosporium brunnei | Davidiella aliena | CBS 157.82 | Quercus robur | Belgium | – | Schubert et al. 2007b | EF679336, EF679407, EF679486 |
| | | CBS 161.55 | Man. sp. | The Netherlands | – | Schubert et al. 2007b | EF679338, EF679409, EF679488 |
| | | CBS 121624; CPC 12211 | Hordeum vulgare | Belgium | J.Z. Groenewald | Schubert et al. 2007b | EF679350, EF679425, EF679502 |
| | Cladosporium chubutense | – | CBS 124457*; CPC 13979; CIEFAP 321 | Pinus ponderosa | Argentina | A. Greslebin | This study | FJ036158, FJ036161, FJ036165 |
| | | | | | | | |
| | Cladosporium cladosporioides complex | – | CBS 170.54 | Arundo, leaf | United Kingdom | – | Zalar et al. 2007 | DG0789040, FJ036162, FJ036153 |
| | | | | | | | |
| | Cladosporium colombiae | – | CBS 774.80* | Corticivora sp. | Colombia | W. Gams | This study | FJ036159, FJ036163, FJ036166 |
| | Cladosporium herbarum | Davidiella tassiana | CBS 121621*; CPC 12177 | Hordeum vulgare | The Netherlands | P.W. Crous | Schubert et al. 2007b | EF679377, EF679450, EF679530 |
| | | | | | | | |
| | Cladosporium macrocarpum | Davidiella macrocarpa | CBS 299.67 | Tetritium aestivum | Turkey | – | Schubert et al. 2007b | EF679372, EF679450, EF679526 |
| | | | | | | | |
| | Cladosporium ossifragi | – | CBS 842.91* | Narthecium ossifragum | Norway | M. di Menza | Schubert et al. 2007b | EF679378, EF679450, EF679530 |
| | | | | | | | |
| | Cladosporium pan-pennonseae | – | CBS 124456*; CPC 13980; CIEFAP 322 | Pinus ponderosa | Argentina | A. Greslebin | This study | FJ036160, FJ036164, FJ036167 |
| | | | | | | | |
| | Cladosporium ramotenellum | CBS 116463*; ICMP 15579 | Iris sp. | New Zealand | C.F. Hill | Schubert et al. 2007b | EF679380, EF679461, EF679530 |
| | | | | | | | |
| | Cladosporium sphaerospermum | – | CBS 119907*; CPC 12040; EXF-334 | Hypersaline water from salterns | Slovenia | P. Zalar | Schubert et al. 2007b | EF679382, EF679463, EF679530 |
| | | | | | | | |
| | Cladosporium subtilissimum | CBS 113754* | Grape berry | USA | F. Dugan | Schubert et al. 2007b | EF679389, EF679476, EF679530 |
| | | | | | | | |
| | Cladosporium variabile | Davidiella variabile | CBS 121636*; CPC 12751 | Spinacia oleracea | USA | L. du Toit | Schubert et al. 2007b | EF679390, EF679477, EF679530 |

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 Schimmelcultures, Utrecht, The Netherlands; CIEFAP: Centro de Investigacion y Extension Forestal Andino Patagonico, Argentina; CPC: Culture collection of Pedro Crous, housed at CBS; EXF: Extremehabitats Fungi Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ICMP: International Collection of Microorganisms from Plants, from Plant Research, Private Bag 90170, Auckland, New Zealand; MTC: International Mycological Institute, CABI Biocentre, Egham, Berkshire, London, 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 |
|-----------|-----------|-----------|-----------|----------|------------|
| **Parsimony information** | | | | | |
| 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 | | |
| **Calculated parsimony measures** | | | | | |
| 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 | | |
| **Partition homogeneity test** | | | | | 0.004 |
| ITS & ACT & TEF | 0.941 | | | | |
| ITS & ACT | | | | | 0.95 |
| ITS & TEF | | | | | 0.963 |
| 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).
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 (×1000 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).
RESULTS

Phylogeny

The manually adjusted concatenated alignment contained 43 sequences (including the outgroup sequence) and the three loci were represented by a total of 1094 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 sequence 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 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 sequence 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
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— MycoBank MB509557; Fig. 4–6

Differs from C. oreodaphnes conidiophoris non-nodulosis et leniter laticibus, (4–)5–8–(9) µm latis, et a C. antarcticomyco non dimorpho, conidios in vitro 0–1-septatis, locis conidiogenis et hilis latioribus, (0.5–)0.8–2(–2.2) µm latis, et a mycelio non dimorpho, conidiis in vitro 0–1-septatis, locis conidiogenis et hilis latioribus, (0.5–)0.8–2(–2.2) µm latis (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, substromatal 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 × (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 × 4.5–7(–8) µm (av. ± SD: 11.1 ± 3.5 × 5.8 ± 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; micropyctic 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 × (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

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

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**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.
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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, Carnelia 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).

Fig. 6 Cladosporium chubutense (CBS 124457 = CPC 13979). a–e. Macro- and micronematous conidiophores with conidia; f–g. conidial chains. — Scale bars = 10 µm.
In addition to the newly described \textit{C. chubutense}, both the teleomorph and anamorph of the widespread saprobic species \textit{C. macrocarpum} co-occur on the infected needles of \textit{Pinus ponderosa}. The latter species may represent a secondary invader following infection with \textit{C. chubutense} or both species may be saprobic invaders. Phylogenetically, \textit{C. chubutense} is closely related to \textit{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, \textit{sp. nov.} — MycoBank MB509558; Fig. 7–9

Differs a \textit{Laguminicola} conidios laticus, (3–)4–(8–9) µm latus, conidiophori ecrassitunicati, saepe bistriotat, et a \textit{C. chubutensis} conidios irregulariter rugosis, catenis longioribus, conidiis usque ad 9 in catenis terminalibus non ramosis.

Etymology. Named after its host, \textit{Pinus ponderosa}.

In vivo: Isolated from needles becoming necrotic from the top to the base, no discrete leaf lesions formed. \textit{Caespmutili} 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. \textit{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, olive-coloured to brown, rounded cells, 8–15 µm diam. \textit{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 olive-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. \textit{Conidiogenous cells} integrated, mostly terminal but also intercalary, subhyaline to pale brown, genulate, polyblastic, proliferation sympodial with several conidiogenous loci situated terminally or laterally on small shoulders, catenated, loci protuberant, denticulate, thickened and darkened-refractive. \textit{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, hilum conspicuous, 1–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis observed.

In vitro (on SNA): \textit{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. \textit{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. \textit{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

### Table 3

| Species            | ITS         | ACT          |
|--------------------|-------------|--------------|
| \textit{C. cladosporioides} | 378<sup>a</sup> | 75<sup>a</sup>, 76<sup>b</sup>, 80<sup>b</sup>, 116<sup>b</sup> | 151–152<sup>a</sup>, 165<sup>a</sup>, 169<sup>b</sup>, 178<sup>b</sup>, 193<sup>b</sup> |
| \textit{C. colombiae} | T          | A – T        |
| \textit{C. pini-ponderosae} | A          | T – G        |

<sup>a</sup> Transition.<br><sup>b</sup> Transversion.<br><sup>c</sup> Insertion/deletion.
dome not very prominent, 1.5–2.5 µm diam, somewhat thickened and darkened-refractive. Ramoconidia cylindrical-oblong, not attenuated towards the base, 20–45 × 3.5–5(–5.5) µm, 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 ovoid to ellipsoid-ovoid, 5–6 × 2.5–4(–4.5) µm (av. ± SD: 5.5 ± 0.5 × 3.3 ± 0.6), broadly rounded at the apex, slightly attenuated towards the base, aseptate, hila 0.5–1(–1.2) µm diam. Intercalary conidia ellipsoid-ovoid, fusiform, sometimes rostrate towards the distal end, attenuated towards apex and base, 6–15(–22) × 3–4(–5) µm (av. ± SD: 9.9 ± 3.8 × 3.8 ± 0.5), aseptate, rarely 1-septate, mostly with a single but sometimes up to three distal hila, hila 0.8–1.5 µm diam, secondary ramoconidia fusiform to subcylindrical, 10–30(–36) × 3.5–5 µm (av. ± SD: 20.3 ± 6.4 × 4.3 ± 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 µm 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. 8 Cladosporium pini-ponderosae (CBS 124456 = CPC 13980). Conidiophores and conidia. — Scale bar = 10 µm. K. Schubert del.

Fig. 9 Cladosporium pini-ponderosae (CBS 124456 = CPC 13980). a–d. Conidiophores with conidial chains; e–g. conidia. — Scale bars = 10 µm.
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 and with swellings, up to 8 µm diam, subhyaline to 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, Alumínio, Lagos Marmol prop., 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. — Myco- Bank MB059559; Fig. 10, 11

Differs a *C. ramotenello* ramosidnus nullis, conidios terminalibus non-globosis, angustioribus, 2.5–3(–4) µm lat., conidios intercalarios ovordibus, lomyn-formibus et ellipsoidibus, asetatis, breviseris et teneri angustioribus, 6–9 × (2.5–)3–3.5 µm, ramodidnsecundaris breviseribus, 8–17(–23) µm longis, a–(1–2)–septatis; et a *Subtilissimo* conidios intercalarios breviseribus et teneri angustioribus, 6–9 × (2.5–)3–3.5 µm, conidios secundaris breviseribus 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 mm 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; micronematous 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 obvoid, 4–6.5 × 2.5–3–(4) µm (av. ± SD: 4.7 ± 0.8 × 3.2 ± 0.5), intercalary conidia ovoid, limenform to ellipsoid-ovoid, 6–9 × (2.5–)3–3.5 µm (av. ± SD: 7.0 ± 0.8 × 3.2 ± 0.3), asetate, attenuated towards apex and base, secondary ramoconidia ellipsoid to subcyldindrical, 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 conidogenesis 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), dep. 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) conidigenous loci per conidigenous 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 4.5–6.5 × 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-
Cladosporium colombiae, 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, 2–4(–4.5) µm wide, ellipsoid to subcylindrical, 0–1-septate, longer and wider intercalary conidia, 8–15 × 3–4(–4.5) µm, and 0–3-septate, longer secondary ramoconidia, 17–35 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, 9–18 × 3–4(–6) µm, and longer and wider secondary ramoconidia, (13–)17–32(–37) × 3–5(–6) µm (Schubert et al. 2007b). Originally the strain was identified and stored as Cladosporium 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 genulate conidiophores in vitro and somewhat shorter and wider aseptate ramoconidia (19–34(–38) × (3.5–)4–5 µm vs 20–40(–55) × 1.5–4 µm 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.

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