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ORIGINAL ARTICLE

Species diversity *of Cladorrhinum* in Argentina and description of a new species, *Cladorrhinum australe*

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Abstract The genus *Cladorrhinum* constitutes a fungal group of prime importance for agriculture and livestock, since some species have biocontrol potential or were shown to promote plant growth and to produce phytases, which are enzymes useful for processing animal feed. We assessed the species diversity of *Cladorrhinum* in Argentina. Strains were identified at the species level by analysis of morphological and physiological characters, as well as by using molecular characters and by sequencing three nuclear DNA loci: internal transcribed spacer regions (ITS), and the 28S ribosomal subunit and β-tubulin genes. *C. bulbillosum* and *C. samala* were detected, and a new species is described as *C. australe*.

Keywords Diversity · Growth rate · Lasiosphaeriaceae · Molecular phylogeny · Sordariomycetes

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Introduction

Soil inhabiting fungi are a diverse group of microorganisms, characterized by adaptation abilities to different environments. They can be saprotrophs, mutualists, or parasites (Chaverri and Samuels 2013; Kernaghan 2013). Species of the genus *Cladorrhinum* Sacc. and Marchal have been extensively reported in agricultural soils (Mouchacca and Gams 1993; Mouchacca 2007). They were found in soil as saprotrophs on dung or plant material (Lewis and Larkin 1998; Madrid et al. 2011), or in roots as endophytes (Gasoni and Stegman de Gurfinkel 1997). Some species have been associated with human and animal opportunistic diseases (Zapater and Scattini 1979; Chopin et al. 1997; Gajjar et al. 2011).

Cladorrhinum species are characterized by rather fast-growing colonies with pale to dark greenish, greyish or brownish pigmentation, and conidiophores consisting typically of intercalary phialides with lateral openings (pleurophialides), usually with a flaring collarette. The conidia are one-celled, hyaline or subhyaline, smooth-walled, commonly globose to dacryoid, and arranged in slimy masses. The genus *Bahupaathra* Subram. and Lodha was synonymised with *Cladorrhinum* (Mouchacca and Gams 1993).

In a revision of the genus by Mouchacca and Gams (1993), five species were accepted, i.e., *C. brunnescens*, *C. bulbillosum*, *C. foecundissimum*, *C. phialophoroides*, and *C. samala*. Two additional species, *C. flexuosum* and *C. microsclerotigenum*, were described by Madrid et al. (2011) based on molecular and phenotypic data. Two other recorded species in Index Fungorum are *C. ricini* Garb and *C. maiae* Bat. The teleomorphic states of the *Cladorrhinum* species are found in *Apiosordaria* Arx and W. Gams and *Cercophora* Fuckel, two members of the Lasiosphaeriaceae (Mouchacca and Gams 1993). According to Udagawa and

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Muroi (1979), the anamorph of *Cercophora* is similar to that of *Podospora*, being *Cladorrhinum* or *Phialophora*. Also, Cai et al. (2006) demonstrated that four species with *Cladorrhinum* anamorphs, *Apiosordaria verruculosa* (C.N. Jensen) Arx and W. Gams, *Cercophora samala*, *Ce. striata*, and *Podospora fimiseda*, are phylogenetically closely related.

According to The International Code of Nomenclature for algae, fungi, and plants (ICN) (Hawksworth 2011), when a taxon is typified by two morphs, the legitimate names compete for priority. As the name *Cladorrhinum* was published in 1885 by Saccardo and Marchal, and the known teleomorphic state was proposed in *Apiosordaria* by Arx and Gams in 1967, the name *Cladorrhinum* has priority over *Apiosordaria*.

The type species of the genus is *C. foecundissimum* Sacc. and Marchal (Marchal 1885), which was described based on a fungus isolated on dung in Belgium (Mouchacca and Gams 1993). It is an ammonia fungus belonging to the early successional phase of fungi involved in the saprotrophic litter-decomposition in soil (EP fungi) (Sagara 1975; Fukiharu and Horigome 1996). *Cladorrhinum foecundissimum* is highly interesting due to its ability to produce phytases, a class of enzymes involved in the release of phosphate from phytate present in cereal grains and oil seeds used in animal feed (Lei and Stahl 2001; Pradhan and Sukla 2005). In addition, some isolates demonstrated a biocontrol activity by reducing plant diseases caused by pathogenic fungi (Lewis et al. 1995; Gasoni and Stegman de Gurfinkel 1997; Gasoni and Stegman de Gurfinkel 2009).

In Argentina, Zapater and Scattini (1979) recorded *C. bulbillosum* Gams and Mouchacca from an eye infection in a child and later, Gasoni and Stegman de Gurfinkel (1997) reported *C. foecundissimum* from soil and symptomless plant tissues.

In previous works conducted in our laboratory (Gasoni 1994), *Cladorrhinum* spp. were isolated repeatedly from soil samples of different areas of Argentina sharing the same niche with pathogenic isolates of *Rhizoctonia* spp. Considering the potential of *Cladorrhinum* strains as a biocontrol agent, and

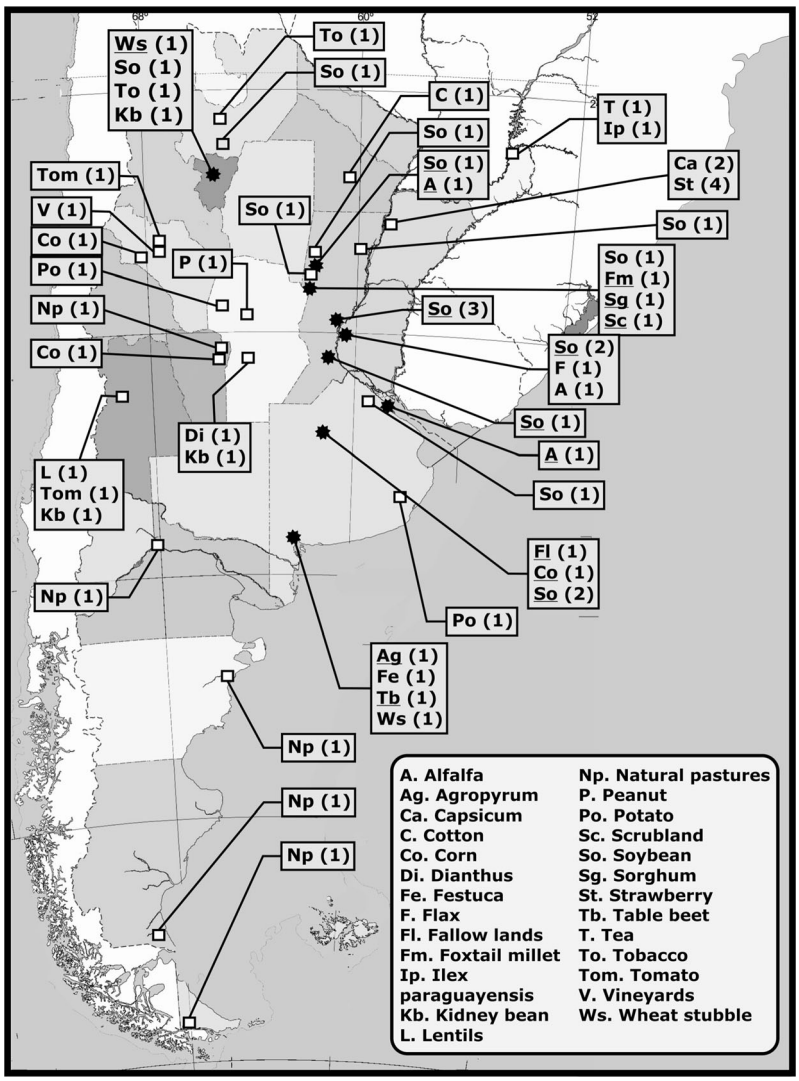


Fig. 1 Map of Argentina showing the location of fields sampled ("□" and "✸"). The symbol "✸" indicates locations from which isolates of *Cladorrhinum* were obtained while the symbol "□" represents locations from which no *Cladorrhinum* isolates were obtained (see Table 1). The name of crops or vegetation is indicated in the *box* and the number of evaluated fields is in *brackets*. For each location, underlined crop names represent crops with positive detection of *Cladorrhinum* species. The boundaries of the provinces are indicated by *dot-dashed lines*

Table 1 Origin, code, and GenBank accession number of strains identified in this study

Location	Species	Isolate code	Habitat	ITS region	β-tubulin	28S region
S M de Tucumán province S 26° 46′ W 65° 14′	*C. samala*	INTA-AR 5	Wheat stubble	KT321043	KT291677	KT312962
Santa Fe province	*C.bulbillosum*	INTA-AR 29	Alfalfa crop	ND	KT291690	ND
Colonia Ana S 30° 09′ W 61° 53′	*C. samala*	INTA-AR 14	Soybean crop	KT321047	KT291682	KT312965
Santa Fe province	*C.bulbillosum*	INTA-AR 75	Sorghum crop	KT321064	KT291702	KT312978
Suardi	*C. samala*	INTA-AR 56	Foxtail millet crop	KT321058	KT291696	KT312974
S 31° 32′ W 61° 57′	*C. samala*	INTA-AR 85	Scrubland	KT321065	KT291703	KT312979
Santa Fe province	*C.bulbillosum*	INTA-AR 157	Soybean crop	KT321077	KT291715	KT312990
Santa Fe Capital	*C. samala*	INTA-AR 112	Soybean crop	KT321075	KT291713	KT312988
S 31° 36′ W 60° 47′	*C. samala*	INTA-AR 156	Soybean crop	KT321076	KT291714	KT312989
Entre Ríos province	*C. australe*	INTA-AR 18	Soybean crop	KT321049	KT291684	ND
Paraná S 31° 44′ W 60° 31′	*C. australe*	INTA-AR 71	Soybean crop	KT321063	KT291701	KT312977
Santa Fe province Rosario S 33° 03′ W 60° 43′	*C. samala*	INTA-AR 168	Soybean crop	KT321078	KT291716	KT312991
Buenos Aires province	*C. samala*	INTA-AR 1[a]	Alfalfa crop	KT321042	KT291676	ND
Castelar	*C. samala*	INTA-AR 7	Alfalfa crop	KT321044	KT291679	KT312963
S 34° 36′ W 58°40′	*C. samala*	INTA-AR 13	Alfalfa crop	KT321046	KT291681	KT312964
	C. samala	INTA-AR 17	Alfalfa crop	KT321048	KT291683	KT312966
	C. samala	INTA-AR 20	Alfalfa crop	ND	KT291686	ND
	C. samala	INTA-AR 23	Alfalfa crop	KT321052	KT291688	KT312967
	C. samala	INTA-AR 25	Alfalfa crop	KT321053	KT291689	KT312968
Buenos Aires province	*C. australe*	INTA-AR 6	Soybean crop	ND	KT291678	ND
9 de Julio	*C. australe*	INTA-AR 12	Corn crop	KT321045	KT291680	ND
S 38° 28′ W 60° 57′	*C. australe*	INTA-AR 19	Corn crop	KT321050	KT291685	ND
	C. australe	INTA-AR 21	Corn crop	KT321051	KT291687	ND
	C. australe	INTA-AR 63	Soybean crop	KT321060	KT291698	ND
	C. australe	INTA-AR 69	Soybean crop	KT321061	KT291699	KT312975
	C. australe	INTA-AR 70	Soybean crop	KT321062	KT291700	KT312976
	C. australe	INTA-AR 95	Soybean crop	KT321067	KT291705	KT312981
	C. australe	INTA-AR 107	Fallow land	KT321072	KT291710	ND
	C. australe	INTA-AR 108	Corn crop	KT321073	KT291711	KT312986
	C. australe	INTA-AR 110	Corn crop	KT321074	KT291712	KT312987
	C.bulbillosum	INTA-AR 51	Soybean crop	KT321056	KT291693	KT312971
	C.bulbillosum	INTA-AR 53	Soybean crop	ND	KT291694	KT312972
	C.bulbillosum	INTA-AR 54	Soybean crop	KT321057	KT291695	KT312973
	C.bulbillosum	INTA-AR 92	Soybean crop	KT321062	KT291704	KT312980
	C.bulbillosum	INTA-AR 102	Fallow land	KT321068	KT291706	KT312982
	C.bulbillosum	INTA-AR 104	Fallow land	KT321069	KT291707	KT312983
	C.bulbillosum	INTA-AR 105	Fallow land	KT321070	KT291708	KT312984
	C.bulbillosum	INTA-AR 106	Fallow land	KT321071	KT291709	KT312985
	C. samala	INTA-AR 59	Soybean crop	KT321059	KT291697	ND
Buenos Aires province	*C. samala*	INTA-AR 32[a]	Roots of *Agropyrum* crop	KT321054	KT291691	KT312969
Bahia Blanca S 38° 42′ W 62° 14′	*C. samala*	INTA-AR 47	Tablebeet	KT321055	KT291692	KT312970

ND Sequences not available

[a] The strains INTA-AR 1 and INTA-AR 32, were formerly referred to as S8 and A32, respectively (Gasoni and Stegman de Gurfinkel 1997)

taking into account the multiple crops and climatic regions from which they were sampled, we explored the diversity of *Cladorrhinum* isolates.

In this paper, we present a polyphasic taxonomic study of isolated strains based on morphological, physiological, and molecular data. The diversity of species of *Cladorrhinum* is reported, and a new species is proposed.

Materials and methods

Biological material

Soils under several cultivated crops and native pastures were sampled from 58 fields in 15 provinces of Argentina, ranging in latitude from S 24° 59′ to S 54° 48′ (Fig. 1, Table 1). Ten samples of soil for each field were collected at each location and stored at 4 °C in polypropylene bags, until used in the laboratory.

Cladorrhinum isolates were obtained from soil samples following the modified bait technique developed by Ko and Hora (1971). Twenty-five autoclaved table beet glomeruli were placed on each soil sample in Petri dishes for 48 h. Then they were washed with sterilized tap water for 2 h and placed on 2 % water agar. After 16 h of incubation, the tips of emerging hyphae were transferred onto Petri dishes containing potato dextrose agar (PDA, Oxoid, USA). The plates were then incubated during 5–7 days at 25 °C and the pure cultures were examined microscopically to a preliminary *Cladorrhinum* identification. Cultures were preserved on PDA plates at 4 °C and in tubes containing the preservation media developed by Butler (1980), at −18 °C.

Fragments of roots were also picked up from healthy plants of different crops to explore the presence of *Cladorrhinum* isolates. Small pieces of roots were surface sterilized by immersion in 70 % ethanol for 1 min, then in 2 % NaOCl for 1 min, dried on tissue paper, and plated on 2 % water agar.

The isolates were deposited at the fungal collection of the Instituto de Microbiología y Zoología Agrícola, Instituto Nacional de Tecnología Agropecuaria, Argentina, under the indicated INTA-AR designation numbers and are available by particular agreements.

Morphological characterization and growth rate

For micromorphology studies, isolates were grown for 7 days on oatmeal agar (OMA, Oxoid, USA) in Petri dishes (approximately 20 ml) at 27 °C under UV light. (Mouchacca and Gams 1993). Microscopic mounts were made in water, lactic acid, or in Phloxin B from the colonies and bubbles and excess of material were removed. Characters were observed from material that was first wetted in distilled water or 3 % KOH. Approximately 40 measurements were made of each structure for each isolate, using Image Pro-Plus software (2000). In order to confirm the characters observed, micro-cultures were prepared as follows: a small piece of the fresh culture was cut from the leading edges of the fungal colony and placed on a sterilized microscope slide supported on a glass triangle (made

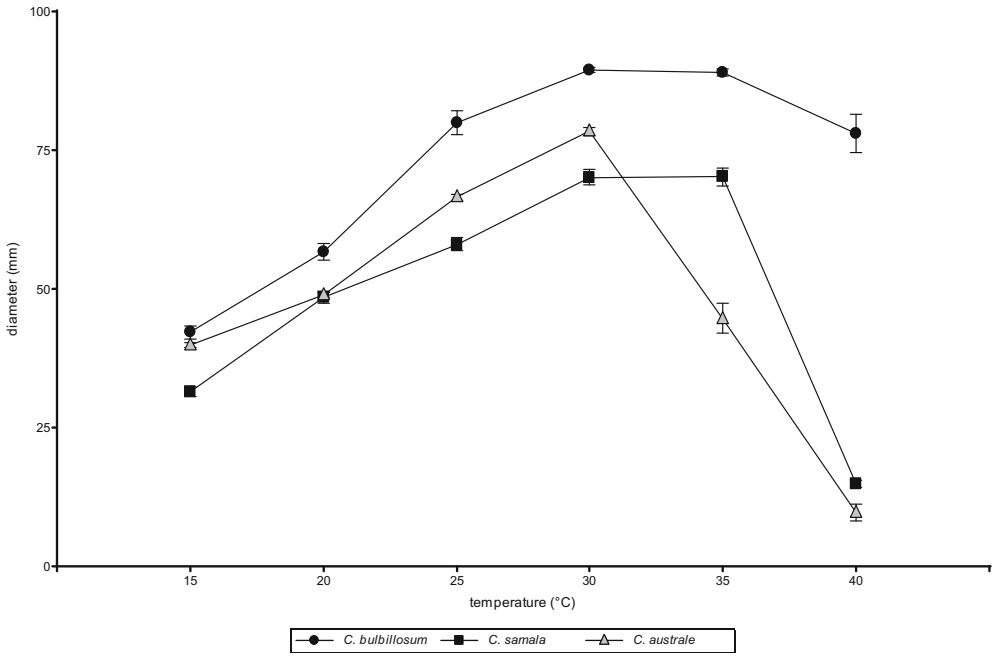


Fig. 2 Colony diameter of species of *Cladorrhinum* after 3 days of incubation on MEA at 15, 20, 25, 30, 35, and 40 °C. Ref: "▲" *Cladorrhinum australe*, "●" *C. bulbillosum*; "■" *C. samala*

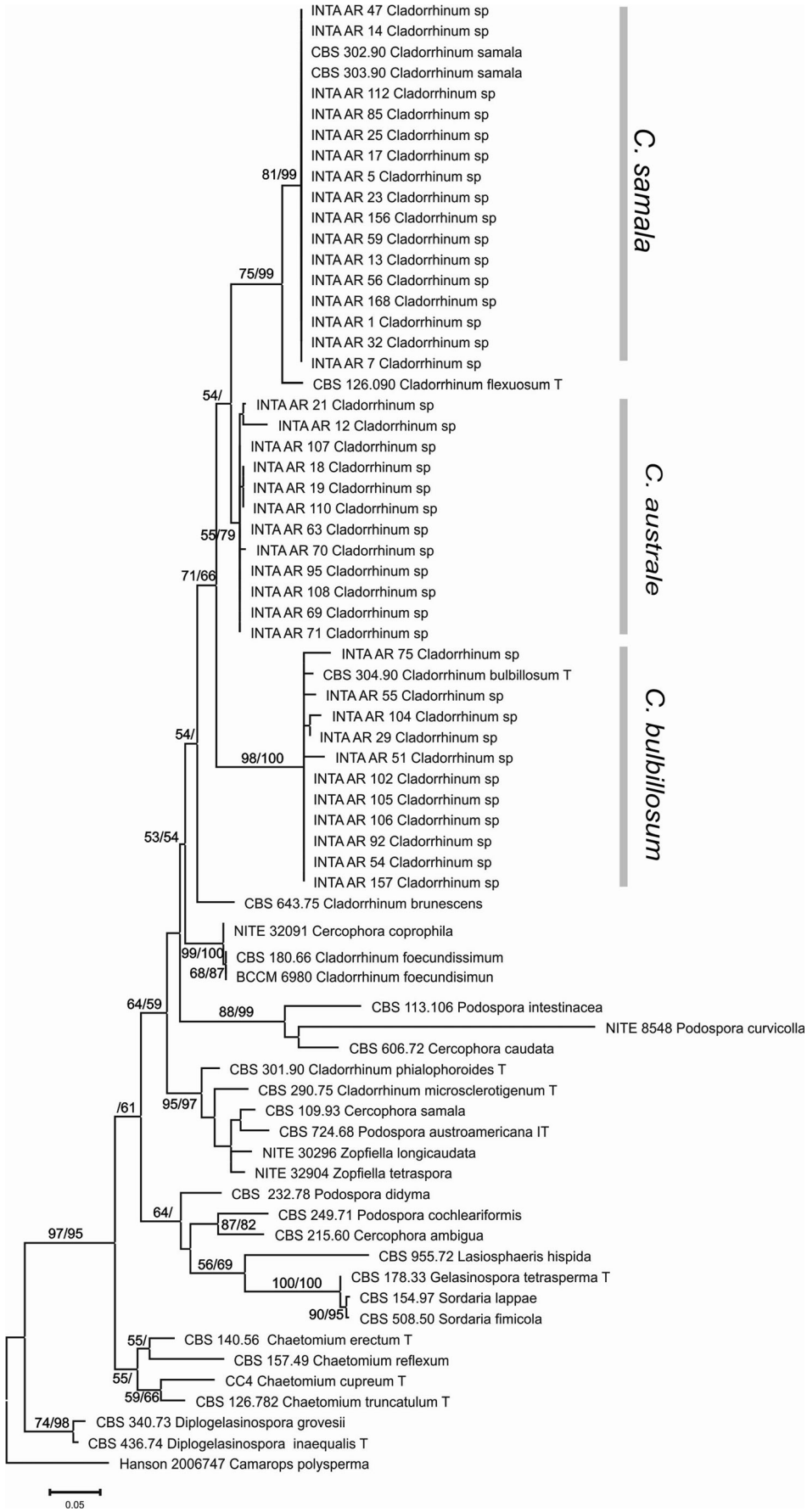


Fig. 3 The tree with the highest log likelihood (−3443.9659) based on ITS region is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 415 positions in the final dataset. The *numbers in the branches* indicate the bootstrap value, on the left are given those obtained from Maximum Likelihood and in the right are given those obtained from Parsimony analyses. T = Type, IT = Isotype, NT = neotype

of a 5-mm-diameter glass rod) placed in the lower dish of a petri plate. The square was covered with a sterilized cover glass. A shallow layer of water was added to the dish to provide high humidity. Once covered with the lid, the plate was incubated at 28 °C until the cover glass was filled with mycelia. Replicate plates were prepared for each of the fungal isolates. The slides were then observed with an Olympus BX51 microscope and the fungal features were examined.

Growth rate trials were performed in darkness on 2 % malt extract agar (MEA, Oxoid, USA), according to Mouchacca and Gams (1993). Cultures of the Argentine strains of *Cladorrhinum* species identified were grown at six different temperatures: 15, 20, 25, 30, 35, and 40 °C. Growth was recorded by measuring the diameter of colonies, every 24 h for 3 days. Each growth-rate trial was repeated three times, and the results of the three replicates were averaged.

DNA extraction, PCR amplification and sequencing

Isolates were grown on potato dextrose broth for 4–5 days and the extraction of genomic DNA was performed using the Qiagen DNeasy Plant Mini kit (Qiagen, California, USA) following the manufacturer's instructions. The ITS region was amplified by PCR using the fungal-specific primers ITS1 and ITS4 (White et al. 1990). The 28S rDNA was amplified with primers LR0R (5′-ACCCGCTGAACTTAAGC-3′) (Bunyard et al. 1994) and LR6 (5′-CGCCAGTTCTGCTTACC-3′) (Vilgalys and Hester 1990). A 365 bp fragment of the β-tubulin gene was amplified with primers Bt2a (5′-GGT AACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson 1995). Although the β-tubulin gene is poorly represented for *Cladorrhinum* in the NCBI database, we have included this marker because it is frequently used for fungal studies (Walker et al. 2012; Kruys et al. 2015).

The amplification reactions were performed in 50 μl volume, and the PCR cycling parameters were optimized for each reaction. PCR products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide. PCR products were then purified using the Promega Wizard SV Gel and PCR Clean-Up System kit (Promega, Wisconsin, USA) following the manufacturer's instructions. The PCR products were used as template for cycle sequencing with primers used for PCR amplification. Additional sequences of the 28S rDNA were obtained with reverse primers LR3 (5′-CCGTGTTTCAAGACGGG-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) (Vilgalys and Hester 1990). Sequences generated were deposited in GenBank (Table 1).

Fig. 4 The tree with the highest log likelihood (−3566.0780) based on β-tubulin gene is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 68 nucleotide sequences. There were a total of 301 positions in the final dataset. The *numbers in the branches* indicate the bootstrap value, on the left are given those obtained from Maximum Likelihood and in the right are given those obtained from Parsimony analyses. T = Type, NT = Neotype

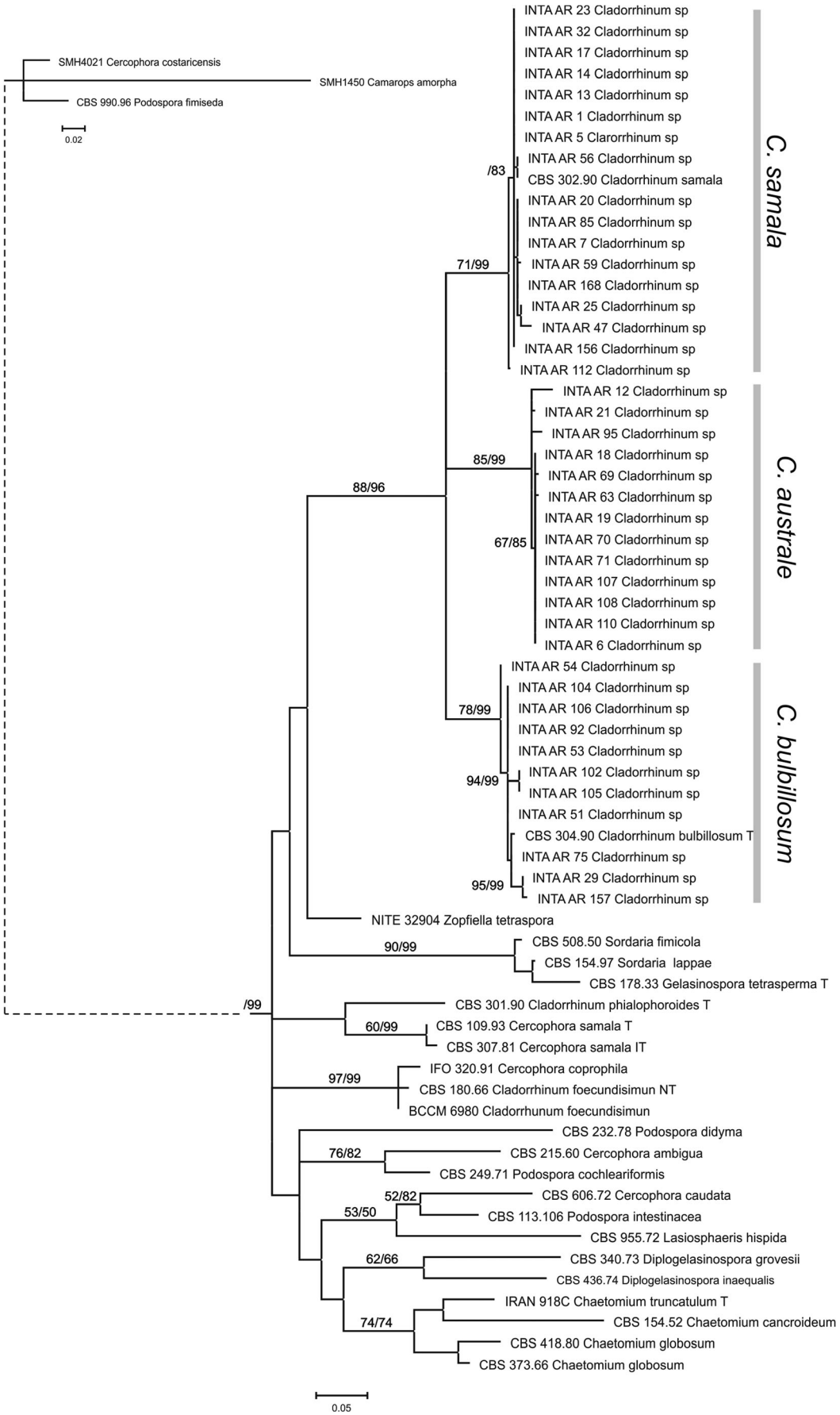
Sequence analysis

Sequences for each native isolate of *C. australe*, *C. samala,* and *C. bulbillosum* were generated in this work, and the reference sequences were obtained from GenBank according to the information provided by the literature about the fungal family (Table 2, Online Resources 1). The sequences were aligned using Clustal W (Thompson et al. 1994). The alignments were deposited in TreeBASE (Submission ID 17898, 17916, 17917). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura et al. 2013). For the matrix based on ITS sequences, the evolutionary history was inferred by using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model (Kimura 1980), for β-tubulin and 28S rDNA data, the relationship was inferred based on the Tamura-Nei model (Tamura et al. 2013). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. Additionally Maximum parsimony (MP) analyses were conducted with the same program. The trees were obtained using a tree bisection and reconnection (TBR) branch swapping strategy with random addition of sequences. In all cases the bootstrap support values were calculated from 1000 replicates.

Results

Identification

In a total of 550 soil and root tissue samples evaluated, 41 *Cladorrhinum* isolates were obtained. Amplified sequences of 28S rDNA, β-tubulin gene, and ITS regions were highly similar to the corresponding sequences reported for *Cladorrhinum* species (Cai et al. 2006 and Madrid et al. 2011). Twenty-eight of the 41 isolated strains were identified as belonging to previously described species: 11 to *C. bulbillosum* and 17 to *C. samala*. The isolate INTA AR 32 was the only one obtained from *Agropyrum* roots (Table 1). The remaining 13 isolates were phylogenetically distinct from the *Cladorrhinum* species previously described.


SMH4021 Cercophora costaricensis
SMH1450 Camarops amorpha
CBS 990.96 Podospora fimiseda
0.02
INTA AR 23 Cladorrhinum sp
INTA AR 32 Cladorrhinum sp
INTA AR 17 Cladorrhinum sp
INTA AR 14 Cladorrhinum sp
INTA AR 13 Cladorrhinum sp
INTA AR 1 Cladorrhinum sp
INTA AR 5 Clarorrhinum sp
INTA AR 56 Cladorrhinum sp
/83
CBS 302.90 Cladorrhinum samala
INTA AR 20 Cladorrhinum sp
INTA AR 85 Cladorrhinum sp
INTA AR 7 Cladorrhinum sp
71/99
INTA AR 59 Cladorrhinum sp
INTA AR 168 Cladorrhinum sp
INTA AR 25 Cladorrhinum sp
INTA AR 47 Cladorrhinum sp
INTA AR 156 Cladorrhinum sp
INTA AR 112 Cladorrhinum sp
C. samala
INTA AR 12 Cladorrhinum sp
INTA AR 21 Cladorrhinum sp
INTA AR 95 Cladorrhinum sp
85/99
INTA AR 18 Cladorrhinum sp
INTA AR 69 Cladorrhinum sp
88/96
INTA AR 63 Cladorrhinum sp
INTA AR 19 Cladorrhinum sp
67/85
INTA AR 70 Cladorrhinum sp
INTA AR 71 Cladorrhinum sp
INTA AR 107 Cladorrhinum sp
INTA AR 108 Cladorrhinum sp
INTA AR 110 Cladorrhinum sp
INTA AR 6 Cladorrhinum sp
C. australe
INTA AR 54 Cladorrhinum sp
INTA AR 104 Cladorrhinum sp
78/99
INTA AR 106 Cladorrhinum sp
INTA AR 92 Cladorrhinum sp
INTA AR 53 Cladorrhinum sp
94/99
INTA AR 102 Cladorrhinum sp
INTA AR 105 Cladorrhinum sp
INTA AR 51 Cladorrhinum sp
CBS 304.90 Cladorrhinum bulbillosum T
INTA AR 75 Cladorrhinum sp
95/99
INTA AR 29 Cladorrhinum sp
INTA AR 157 Cladorrhinum sp
C. bulbillosum
NITE 32904 Zopfiella tetraspora
90/99
CBS 508.50 Sordaria fimicola
CBS 154.97 Sordaria lappae
CBS 178.33 Gelasinospora tetrasperma T
/99
CBS 301.90 Cladorrhinum phialophoroides T
60/99
CBS 109.93 Cercophora samala T
CBS 307.81 Cercophora samala IT
97/99
IFO 320.91 Cercophora coprophila
CBS 180.66 Cladorrhinum foecundisimun NT
BCCM 6980 Cladorrhunum foecundisimun
CBS 232.78 Podospora didyma
76/82
CBS 215.60 Cercophora ambigua
CBS 249.71 Podospora cochleariformis
52/82
CBS 606.72 Cercophora caudata
53/50
CBS 113.106 Podospora intestinacea
CBS 955.72 Lasiosphaeris hispida
62/66
CBS 340.73 Diplogelasinospora grovesii
CBS 436.74 Diplogelasinospora inaequalis
IRAN 918C Chaetomium truncatulum T
74/74
CBS 154.52 Chaetomium cancroideum
CBS 418.80 Chaetomium globosum
CBS 373.66 Chaetomium globosum
0.05

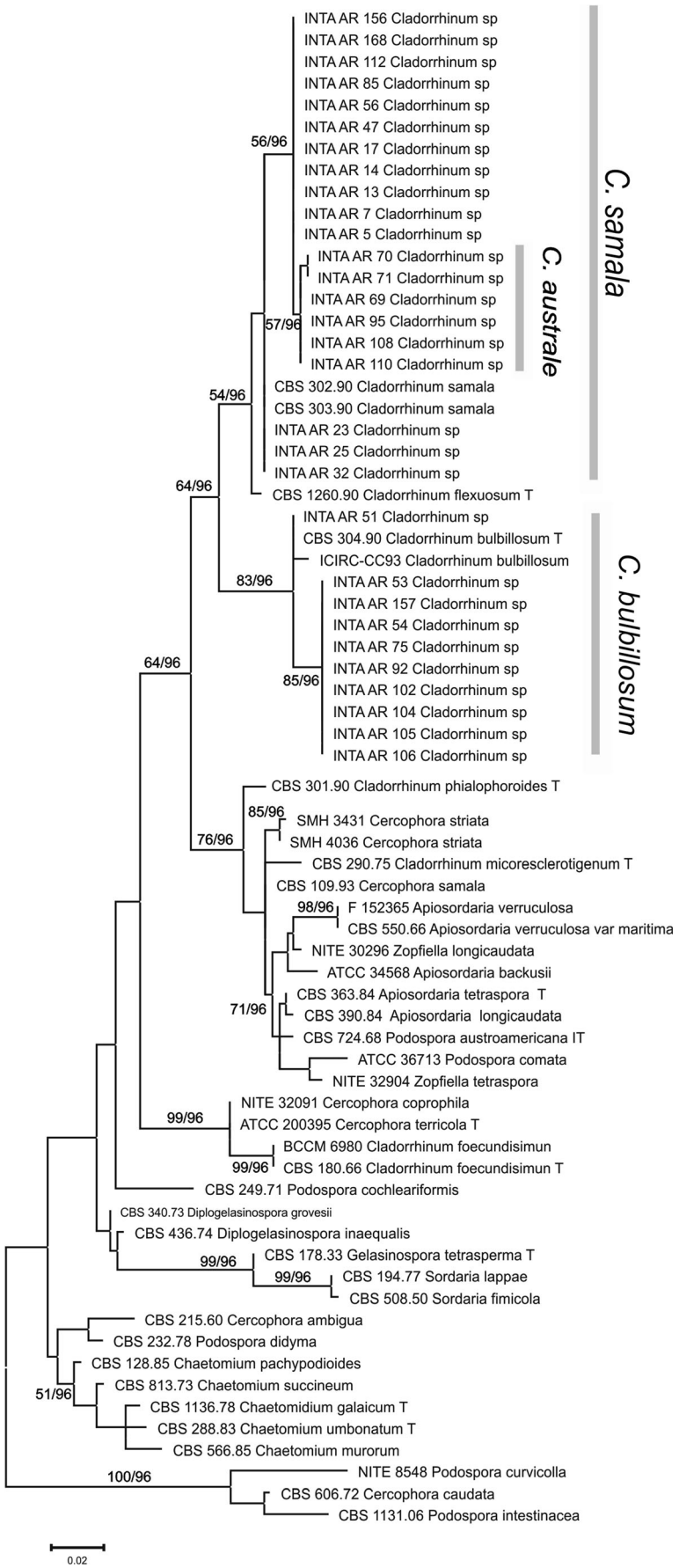


Fig. 5 The tree with the highest log likelihood (−1843.4270) based on 28S rDNA sequences is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 71 nucleotide sequences. There were a total of 412 positions in the final dataset. The *numbers in the branches* indicate the bootstrap value, on the left are given those obtained from Maximum Likelihood and in the right are given those obtained from Parsimony analyses. T = Type, IT = Isotype

Morphology and growth rates

The cultural studies of Argentine strains of *C bulbillosum*, *C. samala,* and the new species showed differences among the species. The colonies of the 28 strains of *C. samala* and *C. bulbillosum* showed very little aerial mycelium on MEA. Figure 2 shows data corresponding to the growth of the three species on MEA at different temperatures.

The strains assigned to *C. samala* showed fast-growing colonies. After 3 days of incubation, they reached 55–65 mm diameter at 25 °C, and 65–70 mm diameter at both 30 °C and 35 °C. The optimal temperature for growth ranged between 30 and 35 °C. The growth was very limited at 40 °C (4–17 mm). The mycelium showed greenish to ochraceous-brown tints, mainly in submerged parts, with clusters of sub-hyaline and branched vegetative hyphae, 4–7 μm wide. The conidiogenous cells (2.2–3.7 μm long) were intercalary and monophialidic. The fertile hyphae showed terminal subhyaline, phialides (4.5–11×2.5–4 μm), and lateral openings, with distinct collarettes, 1.5–4 μm wide. The conidia were hyaline, dacryoid to globose, 3.5–3.7 long, and 2.7–2.9 μm wide, smooth-walled, and gathered in slimy balls 11–12.8 μm wide.

The strains of *C. bulbillosum* showed a faster growth rate, and maintained a high growth rate at 40 °C, reaching the border of the Petri dish after 48 h at 30 and 35 °C (Fig. 2). The submerged mycelium with rather thick-walled and runner hyphae was hyaline while the aerial mycelium was ochraceous, with patches of conidia producing hyphae. Intercalary phialides presented distinct flaring collarettes over a single, lateral, conidiogenous opening. The conidia were hyaline, subglobose, 3.3–3.5×2.8–2.9 μm, smooth-walled, and aggregated in slimy balls. The colonies showed a high production of microsclerotia, with no sign of tissue differentiation.

The strains belonging to the new species showed overall features similar to those of *C. samala* except for often basally truncate conidia not aggregating in conspicuous slimy balls and absence of dark sterile setiform hyphae. They also differed in reaching 70–75 mm diameter in 3 days at 30 °C and the growth slower at 35 °C and very limited growth at 40 °C (Fig. 2).

Phylogenetic analysis

Among the three loci evaluated, ITS regions and β-tubulin gene presented the better resolution and congruence (Figs. 3 and 4). The tree topology for each region was congruent in the methods of tree construction (MP and ML) and the obtained tree with ML was selected for representation for each gene region.

The sequences of strains identified by morphological characters as belonging to *C bulbillosum*, clustered with the sequences corresponding to the ex-type isolate (CBS 304.90). Similarly, the clade corresponding to *C. samala* can be recognized in topologies recovered with the ITS region and β-tubulin gene.

The new species is clearly recognized in analyses using ITS and β-tubulin regions, although the analysis based on the 28S rDNA region (Fig. 5) did not recover clearly the clade corresponding to the new taxon, probably due to low amount of informative characters.

The clade representing the new taxon clustered within clade "A" proposed by Cai et al. (2006) and extended by Madrid et al. (2011) though it is not clearly recovered in all regions studied.

The 13 strains with morphological and physiological characters that did not fit with the previous described species were included in one single clade. Based on these results, a new species of *Cladorrhinum* is proposed, namely *Cladorrhinum australe*.

Taxonomy

Cladorrhinum australe Gasoni, **sp. nov.**

(Figs. 6–12)

MycoBank MB813037

Etym.: refers to geographical location of the species.

C. samalae simile, *sed differt conidiis minoribus et augmento majore post 3 dies ad 30 °C celeriterque decrescenti post 3 dies ad 35 °C.*

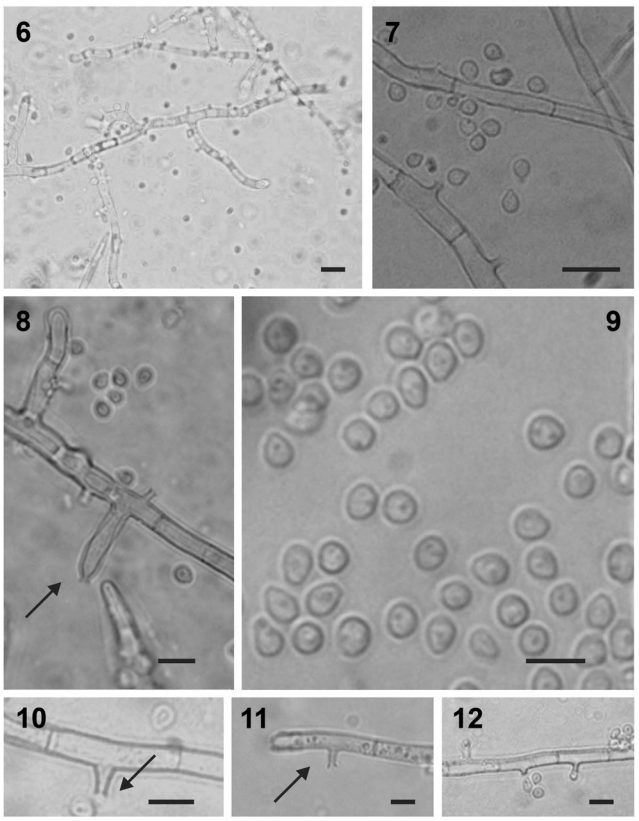


Fig. 6–12 *Cladorrhinum australe*. 6–8 Conidiophores and conidia. 9 Detail of conidia. 10–12 Detail of conidiogenous cells and collarettes. *Scale bars*: 6, 7=10 μm; 8, 9, 10, 11, 12=5 μm

Specimen examined: **Argentina**: Buenos Aires Province: 9 de Julio, 19°14′33.6″N, 98°38′ 29.4″E, 982 m, July, 2011, Martinez (HOLOTYPE: INTA-AR 70). A complete list of Argentinean isolates examined is given in Table 1.

Colonies on MEA 80–85 mm diam. in 96 h at 25 °C, 6–8 mm at 40 °C, brown (according to Ridgway 2006; Plate XVI, old gold and olive lake), flat, with scarce aerial mycelium, producing conidial tufts at the centre, reverse (3A2). Vegetative hyphae septate, branched, hyaline to pale olive, 5–8 μm wide. Microsclerotia not observed. Conidiophores semimacronematous, septate, profusely branched, branches usually flexuous and densely entangled, often ending in sterile cells with obtuse apices, pale olivaceous to pale brown, smooth, length indeterminate, 2.5–3.4 μm wide. Phialides mostly intercalary, short, light olive to light brown, with one conidiogenous opening (1 μm wide), bearing a conspicuous collarette; subcylindrical to lageniform. Conidia one-celled, subhyaline to pale olive-brown, smooth- and thin-walled, mostly globose to dacryoid, 3.1–3.3×2.5–2.7 μm, often truncate at the base.

Sexual state: not seen.

Distribution: Buenos Aires and Entre Ríos provinces.

Discussion

This paper presents the results of an extensive survey of the genus *Cladorrhinum* aiming at studying its species diversity in Argentina. *Cladorrhinum* strains were recovered from cultivated soils, in places spanning a difference of 12 grades of latitude from the S 26° 46′ to the S 38° 42′ latitude over a distance of 1400 km. Such distance implies a great edaphic and ecological variation among places, although most of them correspond to temperate climates.

Previous reports indicated the presence of *C. samala* and *C. bulbillosum* in Argentina (Gasoni 1994; Gasoni and Stegman de Gurfinkel 1997; Zapater et al. 1979). In 1997, Gasoni and Stegman de Gurfinkel reported the presence of *C. foecundissimum* in Argentina. However, this finding was reconsidered here and strains S8 and A32 reported by these authors (respectively INTA-1 and INTA 32, in Table 1) were reassigned to *C. samala*, following the morphological, physiological, and molecular studies performed on these strains in this study. Among the isolates obtained in the survey conducted in Argentina, *C. bulbillosum* was isolated from four different locations, in soils under crops of soybean, alfalfa, and sorghum. Isolates belonging to *C. samala* were obtained in eight localities, in soils covered by crops of soybean, alfalfa, foxtail millet, and table beet, indicating that *C. samala* is the more widespread species, regardless of the type of crop.

The new species, *Cladorrhinum australe* Gasoni, shows morphological characteristics consistent with the *Cladorrhinum* genus concept according to Mouchacca and Gams (1993). These authors separated the species into two groups, on the basis of the shape of conidia. Those producing mostly globose to dacryoid conidia such as *C. brunnescens* W. Gams, *C. bulbillosum*, *C. foecundissimum* (anamorphs of *Apiosordaria* spp.) and another group that include those taxa with predominantly ellipsoid conidia belong to the group of *C. phialophoroides* Mouch. & W. Gams and the anamorphs of *Cercophora samala* Udagawa & Muroi (1979).

The species within each group often cannot be distinguished by the size of their conidiogenous cells and conidia. However, they are recognized by differences in other phenotypic features, such as the ability to produce microsclerotia, the proportion of intercalary vs. terminal phialides, and growth profiles (Mouchacca and Gams 1993; Domsch et al. 2007). Nevertheless, the features of *C. australe* isolates did not fully match those of the known *Cladorrhinum* species. Indeed, they are morphologically characterized by light olive to light brown pigmentation and fertile hyphae composed by cells with distinct flaring collarettes bearing a neck, resembling *C. foecundissimum* in lacking microsclerotia, the olivaceous tints in the mycelia, and the morphology of conidia but differing in growth rate and the optimal temperature for growth. *Cladorrhinum australe* differs from *C. samala* and *C. bulbillosum* in producing flat colonies, with scarce aerial mycelium, with conidial tufts at the centre, and conidia often truncated at the base. Sterile dark septate hyphae and slimy balls are not observed in strains assigned to *C. australe*.

According to Madrid et al. (2011), considering the convergent morphology in the genus *Cladorrhinum*, the characterization of any potentially new species within this genus should include phylogenetic analyses. Taking into account the low variability of the available 28S rDNA sequences, we used a multigene approach based additionally on the ITS regions and the β-tubulin gene.

The phylogenetic analyses of the Argentine strains, including the sequence references showed a high congruence with the species accepted on the basis of morphological characters. The trees obtained using 28S rDNA, β-tubulin gene, and ITS regions, clustered the sequences belonging to isolates of *C. samala*, *C. bulbillosum,* and *C. australe* with high support. Variation within each of these clades was considered intraspecific variation according to the concept of Taylor et al. (2000), which proposes that transition from concordance to incongruity among branches of the simultaneous analysis of three gene genealogies can be used to diagnose species. Also, morphological data do not support a taxonomical subdivision among isolates within each species.

In the present study, even though an extended survey was performed in different regions, only *C. samala*, *C. bulbilosum,* and the new species, *C. australe*, were isolated. The latter has been registered only in two localities and under two crops, soybean and corn. Even though we collected a large number of soil samples in the different regions of Argentina, the *Cladorrhinum* strains were only obtained from cultivated soils. Therefore, the agricultural management seems to be the key factor for the establishment of these species. Through this study, we noticed that the areas from where we have successfully isolated the strains of *Cladorrhinum*,

conspicuously are congruent with the course of important routes frequently used for transportation of grains. This suggests that their distribution in Argentina could be facilitated by anthropic activities.

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