# Contribution to the identification of *Cladosporium* species in the North of Iran Received: 19.05.2014 / Accepted: 08.11.2014

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## Abstract

In a taxonomic survey on *Cladosporium* species in Iran, 35 specimens were collected from different substrates of various localities in Guilan (N. Iran) during 2011–12. Seven species *viz. C. cladosporioides s. str.*, *C. oxysporum, C. halotolerans, C. perangustum, C. pseudocladosporioides, C. sphaerospermum*, and *C. tenuissimum* are identified and the latter five species are new records to Iran.

Keywords: Biodiversity, Davidiella, morphology, species complex, taxonomy

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#### خلاصه

طی سالهای ۹۱-۱۳۹۰، ۳۵ جدایه از جنس Cladosporium از مناطق مختلف استان گیلان جمع آوری شد. براساس صفات ریختشناسی هفت گونه .C. perangustum .C. halotolerans ،C. oxysporum ،C. cladosporioides s. str براساس و . sphaerospermum ،C. pseudocladosporioides شناسایی شدند. پنج گونه آخر برای نخستین بار از ایران گزارش می شوند.

واژههای کلیدی: تاکسونومی، تنوع زیستی، گونه مرکب، مورفولوژی، Davidiella

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# Introduction

The genus Cladosporium Link is one of the largest genera of hyphomycetes and its species are most widespread. Production of abundant branched conidial chains and the ability to grow on various substrates facilitates the dispersal of *Cladosporium* species almost anywhere in the world (David 1997, Braun et al. 2003). David (1997) compiled and published the nomenclatural history of Cladosporium. The history of Cladosporium taxonomy dates back to the description of Dematium herbarum Pers. from rotten wood (David 1997). The first mention of *Cladosporium* as a genus was in 1816 by Link. He assigned D. herbarum Pers. and D. abietinum Pers. to this genus (Heuchert et al. 2005). Up to 1931, 270 species were listed under this genus (Heuchert et al. 2005). Since 1950, more than 130 new species have been described in Cladosporium (Morgan-Jones & McKemy 1990) and Dugan et al. (2004) listed over 700 names under this genus in a world-wide checklist.

First major monograph on the genus Cladosporium was authored by David (1997) where the genus is wellcharacterized by unique structures of the conidiogenous loci and conidial hila. During recent years, the genus was the subject of extensive studied based on morphological and molecular characteristics (Schubert & Braun 2004, Heuchert et al. 2005, Schubert 2005, Schubert et al. 2006, 2007, Zalar et al. 2007, Braun et al. 2008, Bensch et al. 2010, 2012). These studies have led to the introduction of new species, combinations, names and nomen clatural clarifications, especially in C. herbarum, C. sphaerospermum and C. cladosporioides complexes. Based on phylogenetic characters and morphological differences, Bensch et al. (2010) described 22 new species within C. cladosporioides complex indicating that a few of them are host-specific and have restricted geographical distribution.

Some 20 *Cladosporium* species are listed by Ershad (2009) in his book 'Fungi of Iran' that are mostly sporadic reports of single species from various substrata. This study aims to a more comprehensive revision of the genus in Iran considering the new findings and taxonomic changes in recent years.

#### **Materials and Methods**

## - Isolates

Isolates were obtained from different substrates and localities in Guilan province (N Iran), during 2011–12. Single-spore isolates were obtained according to Ho & Ko (1997) and Crous (1998). Colonies were transferred onto Potato Dextrose Agar (PDA), synthetic nutrient-poor agar (SNA) and 2% Malt extract Agar (MA) and incubated under continuous fluorescent light at 25° C to promote sporulation (Crous *et al.* 2009, Bensch *et al.* 2010).

# - Morphology and light microscopy

Colony characteristics were recorded from cultures grown on SNA plates after 7–9 days incubation in darkness at 25° C (Bensch *et al.* 2010). Colony color on MA and PDA (surface and reverse) were recorded after 14 days at 25° C in the dark according to Rayner (1970). Preparations were mounted in Lactic acid and studied under a BH2 Olympus microscope (× 1000 magnification) equipped with a Sony Digital Camera. Measurements of 10–30 conidiophores and 50 conidia were taken in 50% Lactic acid.

Isolates were identified morphologically based on conidiophores (such as shape, dimorphism, width, ramification and location of the conidiogenous loci), conidia and ramoconidia (formation and surface ornamentation), symptoms, lesions and host-specificity according to David (1997), Heuchert *et al.* (2005), Schubert *et al.* (2007), Zalar *et al.* (2007) and Bensch *et al.* (2010, 2012). All examined specimens are preserved at the Fungal Collection of the Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran (GUM).

## **Results and Discussion**

Seven species viz. C. cladosporioides, C. halotolerans, C. oxysporum, C. perangustum, C. pseudocladosporioides, C. sphaerospermum, and C. tenuissimum were identified. A description and illustrations for all species are presented. *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, Contr. Knowl. Genus *Cladosporium*: 57 (1952)

Colonies on PDA attaining 6–7 cm diameter after 14 days, surface olivaceous-gray and reverse olivaceousblack with white margin, regular, aerial mycelium abundant, sporulation profuse. On MA reaching 8 cm diameter after 14 days, surface pale olivaceous-gray and reverse iron-gray with white margins, aerial mycelium loose to dense, sporulation profuse.

On SNA plates mycelium immersed and superficial, loosely branched, 1-4 µm wide, septate, mostly without swellings and constriction at septa but sometimes distinctly constricted due to swellings, subhyaline to pale or medium olivaceous-brown, smooth minutely verruculose or verruculose, to walls unthickened, sometimes anastomosing of few hyphae. Conidiophores arising terminally and laterally from hyphae, solitary, erect, straight to slightly flexuous, cylindrical-oblong, non-nodulose, unbranched or rarely branched, 27–160 (-170) × (2.5–)3–4  $\mu$ m, with up to 8 septa, pale to medium olivaceous-brown, smooth to verruculose-verrucose, walls slightly thickened. Conidiogenous cells integrated, mostly terminal, sometimes intercalary with short lateral denticulate outgrowths just below a septum, cylindrical-oblong, sometimes slightly geniculate, up to  $40(-42)\mu m \log_{10}$ with 1-4(-5) loci at the apex, sometimes subdenticulate to denticulate, protuberant, 1-2(-2.5) µm diameter, thickened. Ramoconidia cylindrical-oblong, 20-40(-44)  $\times$  (2.5–)3–4 µm, 0–1-septate, base broadly truncate 2-3.5 µm wide. Conidia catenate, in branched chains, branching in all directions, up to 9-10 conidia (mostly up to 6 conidia) in the unbranched terminal part of the chain, small terminal conidia obovoid, occasionally subglobose,  $3.5-5 \times (2-)2.5-3 \mu m$ , aseptate, apex usually broadly rounded, intercalary conidia ovoid, limoniform to ellipsoid-ovoid,  $5-11 \times 2.5-3.5(-4) \mu m$ , aseptate, with 1-3 distal hila, rarely up to 4, apex and base often rounded or attenuated, secondary ramoconidia ellipsoidovoid, subcylindrical to cylindrical-oblong or somewhat irregular,  $8-30(-32) \times (2.5-)3-4(-4.5) \mu m$ , with 0-2-septa, not constricted at septa, with 2-4(-5) distal

hila crowded at the apex, sometimes olivaceous-brown, almost smooth or coarsely verruculose to verrucose, hila more or less conspicuous, subdenticulate to denticulate, (0.5-)1-1.5 µm diameter, thickened, occasionally microcyclic conidiogenesis occurring (Fig. 1).

Specimens examined: Iran: Guilan province, Rudbar, on fallen wood of Olea europaea., 19 Sept. 2011, coll. M. Salimi (CC133); Somaesara, on dead Aphis sp., 18 Jun. 2012, coll. S.A. Khodapararst (H1136); Praesh Kooh, on necrotic tissues of living leaves of Citrus sp., 18 Aug. 2012, coll. A.R. Amirmijani (CC134); Markazi province, Mahallat, on living leaves of Dianthus sp. as a secondary invader, 08 Feb. 2012, coll. J. Bagheri (H1137); Guilan province, Rasht, on necrotic tissues of leaves of Quercus sp., 05 Aug. 2011, coll. A.R. Amirmijani (H1138); on necrotic tissues of leaves of Citrus sp., 07 Aug. 2011, coll. A.R. Amirmijani (H1139); on decaying fruit of Cucurbita sp., 26 Jul. 2012, coll. S.A. Khodapararst (CC135); on lesions and leaf spot of Nerium oleander., 06 Aug. 2012, coll. A.R. Amirmijani (H1140); on decaying bulb of Allium cepa, 11 Jul. 2011, coll. A.R. Amirmijani (CC136).

Cladosporium cladosporioides s. lat. is one of the largest complexes among Cladosporium species. The members of this group are very common and widespread saprobs (Bensch *et al.* 2010). C. cladosporioides s. str. is well-distinguished from other Cladosporium species by its long branched chain of conidia in the unbranched terminal part of the chain and wider ramoconidia (3–5  $\mu$ m) (Bensch *et al.* 2010, 2012). According to Bensch *et al.* (2012) conidiogenous cell attaining up to 30  $\mu$ m but in the Iranian specimens examined had longer conidiogenous cells (up to 42  $\mu$ m).

*Cladosporium cladosporioides s. lat.* has been frequently reported from many substrates in Iran (Ershad 2009), however, we identified and described this species according to new concepts provided recently based on molecular and morphological characteristics (Bensch *et al.* 2010, 2012).



Fig. 1. *Cladosporium cladosporioides*: A. Conidiophore, B. Conidia, C. Ramoconidia, D. Denticulate loci at the apex of conidiophore and small terminal conidia (Bars =  $10 \mu m$ ).

*Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cimerman, Stud. Mycol. 58: 172 (2007)

Colonies on PDA attaining 5–6 cm diameter after 14 days, surface olivaceous-gray and reverse olivaceousblack, margins white, regular, aerial mycelium absent, growth flat with a somewhat elevated colony centre, sporulation profuse. On MA attaining 5 cm diameter after 14 days, surface pale olivaceous-gray and reverse olivaceous-gray, margins white, colony centre furrowed, aerial mycelium absent, sporulation profuse.

On SNA plates mycelium slightly superficial and sparingly branched, (1-)2-3(-4) µm wide, pluriseptate, pale brown or pale olivaceous-brown, almost smooth or minutely verruculose, walls unthickened. Conidiophores solitary, arising laterally and terminally from hyphae, erect, straight to somewhat flexuous, narrowly cylindrical-oblong, non-nodulose, rarely attenuated towards the apex, usually unbranched, (28-)30-140(-150)  $\times$  (2–)2.5–3 µm, with up to 4 septa, septa often appearing darkened, septa not constricted, pale olivaceous-brown, smooth to minutely verruculose, walls unthickened. Conidiogenous cells integrated, terminal or sometimes also intercalary, cylindrical, (8-)9-25(-30) µm long, usually neither geniculate nor nodulose, up to three protuberant, subdenticulate or denticulate conidiogenous loci, 1-1.5 µm diameter. Ramoconidia rarely occurring,  $16-20 \times 2.5(-3) \mu m$ , with three septa, base truncate, about 2.5 µm wide. Conidia catenate, in branched chains, conidial chains branching in all directions, terminal chains with up to 5-6 conidia, small terminal conidia globose or subglobose,  $2-2.5(-3) \times 2-3$ µm, aseptate, intercalary conidia subglobose, ovoid or ellipsoid,  $(3-)4-10 \times (2-)2.5-3 \mu m$ , aseptate or rarely one septate, pale to medium brown, minutely verruculose or verruculose, secondary ramoconidia ellipsoid, fusiform,  $8-24(-27) \times 2-2.5(-3) \mu m$ , 0-1(-3)-septate, mostly 1-septate, not constricted at septa, septa often somewhat darkened, pale to medium brown, almost smooth or minutely verruculose, walls unthickened, with 3-4 distal hila, hila protuberant, subdenticulate or denticulate, (0.5-)1-1.5 µm diameter, thickened and darkened, microcyclic conidiogenesis not occurring (Fig. 2).

Specimens examined: Iran: Guilan province, Rasht, on lesions and necrotic tissues of *Aloe* sp. as a secondary invader, 06 Jul. 2011, coll. S.V. Taherian (H1141); Praesh Kooh, on decaying stem and leaves of *Citrus sinensis* L. 19 Aug. 2012, coll. A.R. Amirmijani (CC137); Rasht, on *Pedilanthus* sp. infected with powdery mildew, as a secondary invader, 18 Jun. 2012, K. Sharifi (CC138). *Cladosporium halotolerans* is possibly cosmopolitan (Zalar *et al.* 2007 & Bensch *et al.* 2012). This species belongs to the *C. sphaerospermum* complex and morphologically is very close to this species but it differs from *C. sphaerospermum* by having slender conidia (2.5–3 µm). This is the first report of this species from Iran.



Fig. 2. *Cladosporium halotolerans*: A. Conidiophore, B & C. Small terminal Conidia and Secondary ramoconidia (Bars = 10 µm).

Cladosporium oxysporum Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 362 (1869)

Colonies on PDA attaining 7–8 cm diameter after 14 days, surface pale olivaceous-gray and reverse olivaceous-black, margins white and glabrous, aerial mycelium abundant, sporulation profuse. Colonies on MA attaining 7 cm diameter after 14 days, surface pale olivaceous-gray and reverse olivaceous-gray to black, margins colorless to whitish, glabrous, regular, aerial mycelium abundant, sporulation more or less profuse.

On SNA plates mycelium internal and superficial, hyphae loosely branched, 1-3.5(-5) µm wide, septate, rarely constricted at septa, subhyaline to pale olivaceous, darker towards the base of the conidiophores, pale olivaceous-brown, smooth to vertucose specially at the base of conidiophores, walls unthickened. Conidiophores solitary, arising terminally and laterally from hyphae, erect, straight to slightly flexuous, distinctly torulose and nodulose, up to 7 nodes per conidiophore, swellings (4-)4.5-6 µm wide, unbranched, sometimes with short lateral denticulate or long branches (up to 30 µm long), conidiophores long, (30-)50-610(-700) µm, (2.5-)3-4 µm wide, at the base up to 5 µm wide, occasionally slightly attenuated towards the apex, pluriseptate, not constricted at septa, olivaceous-brown, sometimes dark brown, often paler at the apex, smooth, walls somewhat thick-walled, about 0.5(-1) µm wide. Conidiogenous cells integrated, terminal and intercalary, with a single node, conidiogenous loci confined to these swellings, with 1-4(-5) loci per node, (11-)13-37(-48) µm long, loci conspicuous, subdenticulate, (<1-)1-1.5 µm diameter, somewhat thickened. Ramoconidia rarely occurring,  $35-44 \times 3-4.5$  with 0-3 septa, base broadly truncate, 3-4 µm wide. Conidia catenate, in branched

chains, up to seven conidia in the terminal unbranched part of the chain, branching in all directions, small terminal conidia subglobose to obovoid,  $(1.5-)2-3 \times$ 3-4(-5) µm, aseptate, apex rounded, intercalary conidia ovoid, limoniform to ellipsoid,  $(6-)8-11(-14) \times 3-4(-$ 4.5)  $\mu$ m, aseptate, rarely 1-septate, with 2–4(–6) distal hila, attenuated towards apex and base, secondary ramoconidia ellipsoid to subcylindrical, (9-)11-22(-27)  $\times$  3–4(–4.5) µm, 0–1(2)-septate, not constricted at septa, pale olivaceous to pale olivaceous-brown, smooth, walls unthickened, with up to 4-5 distal hila, subdenticulate, 1 - 1.5(-2)μm diameter thickend. microcyclic conidiogenesis occurring with conidia forming secondary short or long conidiophores (Fig. 3).

Specimens examined: Iran: Guilan province, Komule, on leaves of *Citrus* sp. infected with sooty moulds as a secondary invader, 18 Aug. 2012, coll. A.R. Amirmijani (CC139, CC140); Malat, 18 Aug. 2012, coll. A.R. Amirmijani (CC141); Rasht, on *Pedilanthus* sp. infected with powdery mildew, 18 Jun. 2012, coll. K. Sharifi (CC142); Rasht-Fuman road, on dead parts of living leaves of *Acer velutinum*, 13 Aug. 2011, coll. A.R. Amirmijani (H1142); Bandbon-e-Vajargah, on leaves of *Citrus* sp. infected with sooty molds as a secondary invader, 18 Aug. 2012, coll. A.R. Amirmijani (CC143), (CC144); Paresh Kooh, 18 Aug. 2012, coll. A.R. Amirmijani (CC145).

Bensch et al. Based on (2010,2012) Cladosporium oxysporum is close to C. herbarum complex by having torulose and nodulose conidiophores but it is easily distinguishable from this complex by its relatively small, smooth to slightly verrucose conidia. Morphologically it belongs to the C. cladosporioides s. lat. species complex that is distinguishable from other memebers of this group by its small terminal, subglobose conidia. This species is a rather common and widespread saprobic hyphomycete that has been frequently reported from various materials including dead parts of leaves and stems of herbaceous and woody plants in Iran (Ershad 2009).



Fig. 3. *Cladosporium oxysporum*: A. Conidiophore, B. Conidia and microcyclic conidiogenesis, C. Branches of conidiophore, D. Ramoconidium (Bars: A = 50 and  $B-D = 10 \mu m$ ).

*Cladosporium perangustum* Bensch, Crous & U. Braun, Stud. Mycol. 67: 65 (2010)

Colonies on PDA attaining up to 7 cm diameter after 14 days, surface gray-olivaceous and reverse irongray to olivaceous-black, margins white and glabrous, aerial mycelium diffuse, sporulation profuse. Colonies on MA reaching up to 8 cm diameter after 14 days, surface gray-olivaceous and reverse olivaceous-gray to iron-gray, margins white and glabrous, aerial mycelium abundant, sporulation profuse.

On SNA plates mycelium internal and superficial, hyphae filiform, loosely branched, (1-)1.5-3(-4) µm wide, septate, sometimes slightly constricted at septa, sometimes irregular due to intercalary swellings and constrictions, occasionally slightly geniculate-sinuous, subhyaline to pale olivaceous, smooth to verruculose specially at the base of conidiophores, walls unthickened. Conidiophores solitary, arising terminally and laterally from hyphae, erect, straight or slightly flexuous, narrowly cylindrical-oblong, without any torulose or nodulose, unbranched, occasionally branched, once or several times, branches short, peg-like or up to 44 µm long, conidiophores  $17-138(-160) \times (2-)2.5-3.5(-4)$ µm, 0-6-septate, usually not constricted at septa, occasionally septa darkened, subhyaline, pale olivaceous, walls at the base of conidiophores, vertuculose and towards the apex smooth or almost so, walls unthickened or slightly thickened, about 0.5 µm wide. Conidiogenous cells integrated, mainly terminal, narrowly cylindricaloblong, sometimes geniculate-sinuous, non nodulose, up to 42 µm long, with up to 4-5 apically crowded loci, conspicuous, subdenticulate to denticulate,  $1-1.5(-2) \mu m$ diameter, thickened. Ramoconidia cylindrical-oblong,  $(17-)25-29(-36) \times 2.5-3 \mu m$ , aseptate, rarely 1-septate, base truncate, 2-2.5(-3) µm wide, sometimes slightly

darkened. Conidia numerous, catenate, in branched chains, branching in all directions, 1-4(-5) conidia in the terminal unbranched part of the chain, small terminal conidia, subglobose or ovoid to obovoid,  $3-4(-4.5) \times$ 2-2.5(-3) µm, apex broadly rounded or slightly attenuated, intercalary conidia ovoid, limoniform to somewhat ellipsoid, fusiform or subcylindrical,  $(4-)5-12(-15) \times 2-3(-3.5)$ μm, 0(-1)-septate, attenuated towards apex and base, with 1-3 distal hila, secondary ramoconidia narrowly ellipsoid to cylindricaloblong,  $(8-)9-30(-33) \times 2-3(-4)$ , 0-1(-2) septa, with up to 4-5 distal hila, pale olivaceous-brown, smooth or almost so to finely verruculose, thin-walled, subdenticulate to hila conspicuous, denticulate, (<1-)1-1.5(-2) µm diameter, microcyclic conidiogenesis occurring (Fig. 4).

Specimens examined: Iran: Guilan province, Malat, on dead parts of living leaves of *citrus* sp., 18 Aug. 2012, coll. A.R. Amirmijani (CC125); Parshkuh, 18 Aug. 2012, coll. A.R. Amirmijani (CC126); Rasht, on home plastic material, 06 Sept. 2011, coll. S.A. Khodaparast (H1143); Siyahroud, on dead parts of stem of *Phragmites* sp., 03 Aug. 2011, coll. A.R. Amirmijani (H1144).

According to Bensch *et al.* (2010) *Cladosporium perangustum* is a common and widespread saprobic species that belongs to *C. cladosporioides s. lat.* Due to its globose or subglobose terminal conidia *C. perangustum* is close to *C. sphaerospermum*, but it is distinct by its slender conidiophores, ramoconidia and secondary ramoconidia (Zalar *et al.* 2007).

Morphologically, *C. cladosporioides s. str.* differs from this species by smooth and wider conidia, wider conidiophores, ramoconidia, secondary ramoconidia and hila (Bensch *et al.* 2012). This is the first report of this species from Iran.



Fig. 4. *Cladosporium perangustum*: A. Conidiophore, B. Conidia, C. Secondary ramoconidia (Bars = 10 μm).

*Cladosporium pseudocladosporioides* Bensch, Crous & U. Braun, Stud. Mycol. 67: 71 (2010)

Colonies on PDA attaining 7–8 cm diameter after 14 days, surface gray-olivaceous and reverse iron-gray to olivaceous-black, margins regular, glabrous, aerial mycelium abundant, sporulation profuse. Colonies on MA attaining 8.5 cm diameter after 14 days, surface gray-olivaceous and reverse iron-gray, margins white and glabrous, aerial mycelium abundant, sporulation profuse.

On SNA plates mycelium immersed and superficial; hyphae unbranched or sparingly branched, 1–4 (–4.5) µm wide, septate, sometimes constricted at septa, subhyaline to pale olivaceous, smooth to minutely verrucose, mainly at the base of conidiophores, sometimes irregular in outline due to swellings and constrictions, sometimes cells swollen, up to 5.5 µm wide. Conidiophores solitary, arising terminally and laterally from hyphae, erect, straight, cylindricaloblong, non-nodulose, sometimes geniculate-sinuous, unbranched or branched, branches often short denticlelike lateral outgrowth just below a septum or long about 70 µm, conidiophores (20–)52–137(–178) × (2–)2.5–3.5 µm, up to 5 septa, pale to pale medium olivaceousbrown, sometimes paler towards the apex, smooth, at the base finely vertuculose, walls slightly thickened, about 0.5 µm wide. Conidiogenous cells integrated, terminal, seldom intercalary, narrowly cylindrical-oblong, sometimes geniculate, (8-)17-40 µm long, with up to seven loci crowded at or towards the apex, in intercalary cells loci situated on small lateral peg-like outgrowths, 1-2 loci, conspicuous, subdenticulate, 1-1.5(-2) µm diameter, thickened. Ramoconidia cylindrical-oblong,  $17-46 \times (2.5-)3-4 \mu m$ , 0-2-septate, pale olivaceousbrown, smooth, base broadly truncate, 2.5-3.5 µm wide, unthickened or slightly thickened. Conidia very numerous, catenate, in branched chains, branching in all directions with 4-5 conidia in the terminal unbranched part of the chain, small terminal conidia obovoid, ovoid to ellipsoid, sometimes subglobose,  $3-5(-5.5) \times$  $(1.5-)2-3 \mu m$ , apex rounded or attenuated towards apex and base, intercalary conidia ovoid, limoniform to ellipsoid or subcylindrical,  $5-11(-14) \times (2-)2.5-3(-4)$  $\mu$ m, 0(-1)-septate, slightly attenuated towards apex and base, with 2-3(-4) distal hila, secondary ramoconidia

ellipsoid-ovoid to subcylindrical or cylindrical-oblong, (7–)8–24(–31) × (2–)2.5–3.5(–4)  $\mu$ m, 0–1(–2)-septate, septum often somewhat in the lower half, pale olivaceous to pale olivaceous-brown, smooth or almost so, walls unthickened, with up to 5 distal hila, conspicuous, subdenticulate, 1–1.5(– <2)  $\mu$ m diameter, microcyclic conidiogenesis rarely occurring (Fig. 5).

Specimens examined: Iran: Guilan province, Malat, on fallen leaves of *Citrus* sp., 18 Aug. 2012, coll. A.R. Amirmijani (CC127); Rasht, from air, 09 Aug. 2011, coll. A.R. Amirmijani (CC128); on necrotic tissues of living leaves of *Robinia* sp., 25 Oct. 2012, coll. A.R. Amirmijani (H1145); Sangar, on leaf spot of *Rubus* sp., 05 Aug. 2011, coll. A.R. Amirmijani (H1146).

Cladosporium pseudocladosporioides belongs to C. cladosporioides s. lat. and according to Bensch et al. (2010, 2012) this species is morphologically and phylogenetically very close to *C. cladosporioides* but clearly distinct from it by forming a separate lineage in phylogenetic analyses. Bensch *et al.* (2010) have shown it possibly representing a complex containing cryptic species and indicated the distinction between the two species only based on morphology is so difficult.

Cladosporium pseudocladosporioides differs from C. cladosporioides in having shorter and somewhat narrower, 0-1(-2)-septate secondary ramoconidia, narrower conidiogenous loci and hila (Bensch *et al.* 2010, 2012). Intercalary and secondary conidia are slightly more slender those reported by Bensch *et al.* (2010, 2012). However, the molecular identification of this species is carried out by sequencing of ITS rDNA region (unpublished data). This is the first report of this species from Iran.



Fig. 5. *Cladosporium pseudocladosporioides*: A. Conidiophore, B. Conidia, C. Branches, D. Ramoconidia and secondary ramoconidium (Bars: A = 50,  $B-D = 10 \mu m$ ).

Cladosporium sphaerospermum Penz., Michelia 2(8): 473 (1882)

Colonies on PDA reaching 6 cm diameter after 14 days, surface gray-olivaceous and reverse dark grayolivaceous, margins white, regular, aerial mycelium absent or sparse, growth flat with an elevated colony centre, sporulation profuse. Colonies on MA attaining 4 cm diameter, surface gray-olivaceous and reverse irongray to olivaceous-black, margins colorless or white, regular, radially furrowed, aerial mycelium almost absent, sporulation profuse.

On SNA plates mycelium immersed and superficial, hyphae sparingly branched, (1-)1.5-3 µm, rarely 4 µm wide, septate, often with chlamydospore-like structures, pale medium olivaceous, smooth to sometimes minutely vertuculose, sometimes irregular in outline due to swellings and constrictions, walls slightly thickened. Conidiophores solitary, arising terminally and laterally from hyphae, erect or ascending, straight, slightly attenuated to the apex, cylindrical-oblong, neither geniculate nor nodulose, unbranched or branched,  $(8-)23-120(-186) \times 2.5-3.5(-4.5) \mu m$ , up to four septa or occasionally pluriseptate, branches denticulate or short, up to 10 µm, septa darkened and somewhat thickened, pale medium to medium olivaceous-brown, smooth to minutely vertuculose. Conidiogenous cells integrated, terminal, occasionally intercalary, cylindrical, up to 32  $\mu$ m long, with 2-3(-4) apical scars, loci protuberant, denticulate, (<1-)1-1.5 µm diameter, thickened. Ramoconidia often formed, cylindrical,  $20-33(-47) \times 3 \mu m$ , up to two septa, base broadly truncate, 2-3 µm wide, slightly thickened. Conidia catenate, in branched chains, branching in all directions, with 4-5 conidia in the unbranched parts, small terminal conidia globose to subglobose, seldom ovoid, (2.5-)  $3-4(-4.5) \times (2.5-)3-3.5(4) \mu m$ , aseptate, minutely verruculose to verrucose, rounded or slightly narrower at both ends, intercalary conidia,  $(4-)6-8(-9) \times (2.5-)3-4$ um, aseptate, sometimes with one septate, attenuated towards apex and base, with up to 2(-3) apical hila,

subglobose, ovoid to ellipsoid. secondary ramoconidia ellipsoid to cylindrical,  $9-29(-33) \times (2.5-)3-4 \mu m$ , 0-2(-3)-septate, not constricted at septa, but septa somewhat darkened and thickened, pale to usually medium olivaceous-brown, smooth to minutely verruculose, with 4-5 pronounced, denticulate distal hila,  $1-1.5 \mu m$ diameter, thickened and darkened, microcyclic conidiogenesis not observed (Fig. 6).

Specimens examined: Iran: Guilan province, Rasht, on decaying parts of fruit of capsicum., 15 Aug. 2011, coll. A.R. Amirmijani (CC129); Kerman province, Jiroft, on fallen wood of *Phoenix dactylifera*, 01 April 2012, coll. A.R. Amirmijani (H1147); Guilan province, Praesh Kooh, on necrotic tissue and dead leaves of *Ficus carica*, 18 Aug. 2012, coll. A.R. Amirmijani (CC130).

Members of *Cladosporium sphaerospermum* complex are cosmopolitan occurring as secondary invaders on numerous plants, saprobic on dead leaves, stems, wood and some isolates are human pathogens (Zalar *et al.* 2007, Bensch *et al.* 2012).

Cladosporium sphaerospermum complex differs from C. cladosporioides s. lat. and other species of the Cladosporium s. str. by having globose to subglobose terminal conidia, obclavate, short rostrate, sometimes "alternarioid" conidia (Zalar et al. 2007, Bensch et al. 2012). C. halotolerans is also similar to this species but the conidiophores in C. sphaerospermum are often wider and usually branched and it has wider secondary ramoconidia. Morphology of the specimen examined with some differences agrees with description provided by Zalar et al. (2007) and Bensch et al. (2012). Based on Zalar et al. (2007) and Bensch et al. (2012), conidiogenous cells are short in length attaining 6-18 µm but in Iranian specimens were longer (up to 32 µm). In addition, unusual chlamydospore-like structures were observed, whereas such structures have not been reported in Zalar et al. (2007) or Bensch et al. (2012). Nevertheless, sequencing of ITS rDNA region confirmed morphological identification (unpublished data). This is the first report of this species from Iran.



Fig. 6. *Cladosporium sphaerospermum*: A. Conidiophore, B. Ramoconidia and Conidia, C. Secondary ramoconidium, D. Unusual chlamydospore-like structure in hyphae (Bars =  $10 \mu m$ ).

*Cladosporium tenuissimum* Cooke, Grevillea 6(40): 140 (1878)

Colonies on PDA attaining up to 9–9.5 cm diameter after 14 days, surface gray-olivaceous and reverse iron-gray to olivaceous-black, margins glabrous, white, aerial mycelium abundant, sporulation profuse. Colonies on MA attaining about 7.5 cm diameter after 14 days, surface greenish-olivaceous and reverse olivaceous-gray, margins narrow, glabrous and white, aerial mycelium abundant, sporulation profuse.

On SNA plates mycelium immersed and superficial, hyphae branched, 1-4.5(-5) µm wide, septate, sometimes constricted at septa, pale to medium brown and finally olivaceous-brown to brown at the base of conidiophores, smooth to sometimes minutely verruculose, walls unthickened or very slightly thickened, seldom forming ropes. Conidiophores solitary, arising terminally and laterally from hyphae; straight or slightly flexuous, cylindrical-oblong to almost filiform, often with a swelling at the apex (on SNA) and a few additional swellings on a lower level (on PDA), swellings quite distant from the apex and from each other; most conidiophores neither geniculate nor nodulose, unbranched or branched, branches sometimes

only as short denticle-like prolongations just below a septum, rarely long, conidiophores  $(40-)65-255(-340) \times$ (2.5-)3-4(-4.5) µm, 6-7-septate or sometimes pluriseptate, pale to medium brown or olivaceous-brown, smooth, sometimes slightly rough-walled at the base, walls somewhat thickened, sometimes slightly attenuated towards the apex. Conidiogenous cells integrated, terminal and intercalary, cylindrical-oblong, often swellings 4-5(-7)nodulose, μm wide. cells (7-)16-46(-50) µm long, loci often situated on swellings but not restricted to them, in terminal cells apex usually head-like uni- or multilaterally swollen with up to six pronounced, subdenticulate to denticulate loci crowded at the tip, loci 1-1.5(-2) µm diameter, thickened and darkened. Ramoconidia occasionally formed, subcylindrical or cylindrical-oblong,  $19-37(-40) \times$ 3-4(-4.5) µm, 0-1(-2)-septate, base broadly truncate, 2-3.5(-4) µm wide. Conidia catenate, in densely branched chains, up to five conidia in the terminal unbranched part of the chain, branching in all directions, small terminal conidia subglobose, obovoid, seldom globose,  $3-5.5(-6) \times (2-)2.5-3 \mu m$ , aseptate, apex broadly rounded, intercalary conidia ovoid, ellipsoid or subcylindrical,  $(4-)5-13(-18) \times (2-)3-4(-4.5) \mu m$ ,

0(-1)-septate, with 3-5(-6) distal hila, secondary ramoconidia ellipsoid, fusiform to subcylindrical or cylindrical,  $7-23(-32) \times 3-4(-4.5) \mu m$ , 0-1-septate, with up to six distal hila, sometimes with 1-2 hila at the basal end, pale brown or pale olivaceous-brown, smooth, walls unthickened or almost so, hila conspicuous, subdenticulate to denticulate,  $(0.5-)1-1.5 \mu m$  diameter, microcyclic conidiogenesis occasionally occurring (Fig. 7).

Specimens examined: Iran: Guilan province, Pirbazar, on lesions and leaf spots of *Fragaria ananassa* as a secondary invader, 09 Aug. 2011, coll. A.R. Amirmijani (H1148); Rasht, on dead parts of leaves of *Robinia* sp., 16 Aug. 2011, coll. A.R. Amirmijani (H1149); Pirbazar, on decaying parts of root of *Phaseolus vulgaris*, 18 Jun. 2012, coll. Z. Mehri (CC131); Rasht, on lesions and leaf spot of *Nerium oleander*, 06 Aug. 2012, coll. A.R. Amirmijani (H1140); Paresh Kooh, on leaves of *Citrus* sp. infected with sooty moulds as a secondary invader, 18 Aug. 2012, coll. A.R. Amirmijani (CC132).

According to Bensch et al. (2010) C. tenuissimum is a common saprobic hyphomycete that belongs to the С. cladosporioides complex. This species is distinguishable from other species of this complex by having long conidiophores with a head-like swelling on the apex. On PDA plates conidiophores of this species are very long and darker, forming several nodules. The formation of such swellings in the C. tenuissimum is similar to those of C. oxysporum but the latter species does not form such nodose conidiophores on PDA (Bensch et al. 2010, 2012). Morphology of the specimen examined agrees with description provided by Bensch et al. (2010, 2012). This is the first report of this species from Iran.



Fig. 7. *Cladosporium tenuissimum*: A. Conidiophore in PDA, B. Conidiophore in SNA, C. Conidia (Ramoconidium, Secondary ramoconidia, Terminal conidia), D. Head-like swollen (Bars = 10 µm).

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