Two new species of Chaetomidium (Sordariales)

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Abstract: Two new species of *Chaetomidium*, *C. galaicum* isolated from a granite rock sample collected in Galicia (Spain), and *C. triangulare* isolated from a soil sample collected on Salta province (Argentina), are described and illustrated. The former species has a cephalothecoid ascomatal wall with flexuous hairs, subglobose to broadly fusiform asci, and large fusiform ascospores, with a terminal germ pore. *Chaetomidium triangulare* is characterised by non-cephalothecoid, glabrous ascomata, clavate asci and triangular ascospores in upper view, with a terminal germ pore surrounded by a dark area. A key for the accepted species of *Chaetomidium* is provided.

Taxonomic novelties: *Chaetomidium galaicum* Stchigel & Guarro sp. nov., *Chaetomidium triangulare* Stchigel & Guarro sp. nov.

Key words: Ascomycota, Chaetomidium, Chaetomium, rock, soil, Sordariales.

INTRODUCTION

The genus Chaetomidium (Zopf) Sacc. (Chaetomiaceae, Sordariales) has been considered as the non-ostiolate counterpart of Chaetomium Kunze (von Arx et al. 1988). Currently, Chaetomidium comprises nine species, and it is characterised by non-ostiolate, globose ascomata, sometimes with a cephalothecoid peridium (four species), evenly hairy, 8-spored, clavate asci, and limoniform to broadly fusiform, often bilaterally flattened ascospores. Morphologically related genera, with also non-ostiolate ascomata, are Boothiella Lodhi & J.H. Mirza (characterised by 4spored, cylindrical asci, and broadly obovate or nearly spherical ascospores) and Thielavia Zopf (with a peridium of textura epidermoidea, and fusiform, ellipsoidal, slightly clavate or ovoid, brown ascospores, with a terminal or lateral, distinctive germ pore) (von Arx et al. 1988, Stchigel et al. 2002). Other morphologically related genera are Corynascella Arx & Hodges, with obovate to broadly clavate asci, and ascospores with a thickened wall arround the germ pores (one or two); Corynascus Arx, producing ascospores with two germ pores (one at each end) and a Myceliophthora Oorschot anamorph; and Melanocarpus Arx, with a pseudoparenchymatous peridium of several layers of angular or irregular cells, obovate or cylindrical-saccate asci, and opaque, ovate or broadly ellipsoidal and bilaterally flattened ascospores, with a distinct germ pore, and Chrysonilia-like anamorph (von Arx et al. 1988, Guarro et al. 1996, Stchigel et al. 2000).

Recently, during a survey of soil fungi and fungusaltered stones in different regions of Spain and South America, respectively, two interesting and undescribed fungi belonging to *Chaetomidium* were found, which are described in the present study.

MATERIALS AND METHODS

Granitic samples were collected near As Maus de Salas, in the Baixa Limia-Serra do Xurés natural park, Ourense province, Galicia, Spain. This area is very rich in megalithic monuments of 4000–6000 years old (Neolithic period). The altitude is arround 900 m, and the weather is wet and cold, with an annual precipitation to 1500 mm and an average annual temperature of 11 °C. The vegetation contains species of both Eurosiberian and Mediterranean origin.

In Argentina soil samples were collected near Tafí del Valle, Tucumán province. The terrain is sandy, and the vegetation is mainly composed of xerophilic plants (*Aphylloclados spartioides* Wedd., *Baccharis boliviensis* (Wedd.) Cabrera, *Bougainvillea spinosa* (Cav.) Heimerl, *Bulnesia schickendantzii* Hieron. ex Griseb., *Caesalpinia trichocarpa* Griseb., *Cassia crassiramea* Benth., *Cercidium andicola* Griseb., *Chuquiraga erinacea* D. Don, *Gochnatia glutinosa* D. Don, *Lycium* spp., *Proustia cuneifolia* D. Don, etc.), including a large diversity of cacti (*Trichocereus pasacanus* (F.A.C. Weber) Britton & Rose, *Opuntia* spp., *Parodia* spp.) (Cabrera 1994).



Figs 1–5. *Chaetomidium galaicum* (CBS 113678). 1. Ascomata. 2. Peridial wall. 3. Ascus with mature ascospores. 4-5. Ascospores. Scale bars: $1 = 200 \ \mu\text{m}$, $2 = 50 \ \mu\text{m}$, $3-5 = 20 \ \mu\text{m}$.

The altitude is 1976 m, and the weather is dry and hot, with an annual precipitation below to 200 mm and an average annual temperature of 22 °C.

Dark-coloured spots on the surface of granitic stones, that suggested biodeterioration, were removed using a disposable sterile scalpel, placed in sterilised polyethylene bags, closed by rubber band, and stored at room temperature. Soil material was collected mainly from the A horizon, placed into sterilised plastic bags, closed and stored in a refrigerator at 4-7 °C.

Approximately 1 g of rock spot or soil was suspended in 5 mL of 5 % v/v acetic acid, shaken vigorously for 5 min and left for further 5 min. The layer of acetic acid was decanted, the residual solid was resuspended in 9 mL of sterilised water, and the suspensions were plated in a Petri dish. Potato-carrot agar with 30 mg/L chloramphenicol (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L tap water) was poured over the suspension and mixed with it. Cultures were incubated at 15 and 25 °C under 12 h of darkness, alternating with 12 h of cool white fluorescent light (Stchigel et al. 2001). The fungi growing on this medium were transferred to oatmeal agar (OA; 30 g oatmeal, 15 g agar, 1 L water), PCA and potatodextrose agar (PDA; Difco) at 5, 15, 25 and at 35 °C under 12 h of darkness, alternating with 12 h of cool white fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984) (M. = Methuen). Fungal structures were mounted and measured in lactophenol and lactic acid.

RESULTS AND DISCUSSION

Chaetomidium galaicum Stchigel & Guarro, **sp. nov.** MycoBank MB500061. Figs 1–10.

Etymology: The epithet *galaicum* refers to the geographic province from where the samples were collected (Galicia).

Mycelium ex hyphis hyalinis vel subhyalinis, septatis, ramosis, levibus, 1–5 μ m latis compositum. Coloniae in "PCA" expansae, planae, hyalinae; reversum album-flavescens. Ascomata superficialia, non-ostiolata, pilosa, atrobrunnea, globosa, 100–300 μ m diam. Pili 1–5 per ascoma, flexuosi, septati, verrucosi et crassi-tunicati, brunnei, ad 250 μ m longi, 6–10 μ m lati ad basim. Peridium cephalothecoideum ex 1–2 stratis, textura angulari compositum, atrobrunneum. Asci 8-spori, subglobosi vel late fusiformes, 25–35 × 12–19 μ m. Paraphyses nullae. Ascosporae unicellulares, fusiformes, 14–19 × 5–7 μ m, laeves, foramine germinali singulari apicali praeditae. Anamorphosis absens.

Mycelium composed of hyaline to sub-hyaline, septate, branched, anastomosing, smooth-walled 1-5 µm wide hyphae. Colonies on PCA growing quickly, attaining more than 80 mm diam in 14 d at 25 °C, flat, white, sulcate, with hyaline exudate; reverse yellowish white (M. 4A2). Ascomata formed abundantly after 14 days, superficial, scattered, non-ostiolate, hairy, dark brown, globose, 100-300 µm diam. Hairs 1-5 per ascoma, flexuose, septate, verrucose- and thickwalled, brown, up to 250 µm long, 6-10 µm wide at the base. Peridium cephalothecoid, 1-2-layered, 5-10 um thick, of textura angularis, dark brown, composed of polygonal plates up to 50 µm diam; external cells prismatic, measuring 5-25 µm diam. Asci 8-spored, subglobose to broadly fusiform, $25-35 \times 12-19 \mu m$, without differentiated apical structures, evanescent.

Paraphyses absent. *Ascospores* 1-celled, brown, fusiform, 14–19 × 5–7 μ m, smooth-walled, with a terminal germ pore, up to 1 μ m diam. *Anamorph* unknown. *Colonies* on PDA at 25 °C attaining more than 80 mm diam in 14 d, velvety to cottony, white, sulcate, without production of ascomata; reverse pale yellow (M. 4A2). *Colonies* on OA at 25 °C attaining 58–63 mm diam in 14 d, flat, white; reverse white. *Colonies* growing on OA, PCA and PDA at 15 °C, similar to those growing at 25 °C, but with a diam of 50–53 mm on OA, 30–32 mm on PCA, and 63–67 mm on PDA. No growth at 5 °C and 35 °C.

Typus: **Spain**, Galicia, Ourense province, Serra do Xurés natural park, from a black spot on granite rock sample, 10 Nov. 2001, coll. by V. Jato and A.M. Stchigel, isol. A.M. Stchigel, IMI 392312 **holotypus**; cultures ex-type CBS 113678 = FMR 8192.



Figs 6–10. *Chaetomidium galaicum* (CBS 113678). 6. Ascoma. 7. Detail of peridial wall. 8. Hair. 9. Ascus with mature ascospores. 10. Ascospores. Scale bars: $6 = 100 \mu m$, $7-10 = 20 \mu m$.

Notes: The morphologically closest species to *C. galaicum* are *C. khodense* Cano, Guarro & El Shafie (1993) and *C. megasporum* Doveri, Guarro, Cacialli & Caroti (1998). All have a cephalothecoid peridium and ellipsoidal to fusiform ascospores.



Figs 11–13. *Chaetomidium triangulare* (CBS 113677). 11. Ascoma. 12. Ascospores. 13. Young asci. Scale bars: $11 = 50 \mu m$, $12, 13 = 10 \mu m$.

However, *C. galaicum* differs from *C. khodense* in number (1–5 per ascoma *vs.* more than 5) and in the length (up to 250 μ m *vs.* up to 1000 μ m) of the ascomatal hairs, in the size of the ascospores (14–19 × 5–7 μ m *vs.* 11–13 × 6.5–7 μ m), and the position of the germ pore (terminal *vs.* subapical); from *C. megasporum*, it differs in the ornamentation (smooth in *C. galaicum* and coarsely warted in *C. megasporum*) and the branching pattern (unbranched in *C. galaicum* and dichotomously branched *C. megasporum*) of the ascomatal hairs, and in the smaller size of the ascospores (19–21.5 × 11–13 μ m in *C. megasporum*).

Chaetomidium triangulare Stchigel & Guarro, **sp. nov**. MycoBank MB500062. Figs 11–16.

Etymology: The epithet *triangulare* refers to the triangular form of the ascospores.

Mycelium ex hyphis hyalinis vel dilute brunneis, septatis, ramosis, laevibus, 1–5 μ m latis compositum. Coloniae in "PCA" restrictae, pallidae vel atro-brunneae; reversum pallidum vel atro-brunneum. Ascomata superficialia, non-ostiolata, glabra, atrobrunnea, globosa, 100–200 μ m diam. Peridium ex 2–3 stratis, textura angulari compositum. Asci 8-spori, clavati, 35–40 × 12–15 μ m. Paraphyses nullae. Ascosporae unicellulares, brunneae pallidae vel brunneae, 218

triangulares e latere visae ellipsoideae, $9-11 \times 7-8 \times 5-6$ µm, laeves, foramine germinali singulari apicali praeditae. Anamorphosis absens.

Mycelium composed of hyaline to pale brown, septate, branched, anastomosing, smooth-walled 1-5 µm wide hyphae. Colonies on PCA growing slowly, attaining 14-19 mm diam in 14 d at 25 °C, flat, pale to dark brown (M. 6D5 to 6F5), slightly granulose due to the production of ascomata, exudate hyaline, soluble pigment pale orange; reverse pale to dark brown (M. 6D5 to 6F5). Ascomata appearing after 14 days, superficial (rarely immersed), scattered, non-ostiolate, glabrous, dark brown, globose, 100-200 µm diam. Peridium 2-3 layered, 3-5 µm thick, of textura angu*laris*, brown; external cells polygonal, 7–15 µm diam. Asci 8-spored, fasciculate, clavate, $35-40 \times 12-15$ μm, stipitate (stipe approximatelly 10 μm long), without differentiated apical structures, evanescent. Paraphyses absent. Ascospores 1-celled, pale brown to brown, triangular in upper view and ellipsoidal in lateral view, $9-11 \times 7-8 \times 5-6$ µm, smooth-walled, with a terminal germ pore surrounded by a dark area, up to 1 µm diam. Anamorph unknown. Colonies on PDA at 25 °C attaining 20-23 mm diam in 14 d, velvety to fasciculate, yellowish white (M. 4A2), without production of ascomata, soluble pigment light orange; reverse pale yellow (M. 4A3). Colonies on OA at 25 °C attaining 15-18 mm diam in 14 d, flat, pale yellow to greyish yellow (M. 4A3 to 4B3), without production of ascomata, soluble pigment pale orange; reverse pale yellow to greyish yellow (M. 4A3 to 4B3). Colonies growing on PDA and OA at 15 °C, similar to those growing at 25 °C, but with a diameter of 8 mm. No growth at 5 °C and 35 °C.

Typus: **Argentina**, Salta province, Tafí del Valle, from a soil sample, 22 May 2000, coll. and isol. A.M. Stchigel, IMI 392313 **holotypus**; cultures ex-type CBS 113677 = FMR 7545.



Figs 14–16. *Chaetomidium triangulare* (CBS 113677). 14. Ascoma. 15. Ascus with mature ascospores. 16. Ascospores. Scale bars: $14 = 50 \ \mu\text{m}$, $15, 16 = 10 \ \mu\text{m}$.

Notes: Chaetomidium triangulare differs from the other species of the genus by the absence of ascomatal hairs and by its triangular ascospores. Similar ascospores are present in other members of *Chae*-

tomiaceae such as Chaetomium microascoides Guarro and Chaetomium trigonosporum (Marchal) Chivers (von Arx et al. 1986). The former has ascospores of similar size as those of Chaetomidium triangulare, but it has ostiolate and beaked ascomata, with sparse, short, straight or reflexed, smooth-walled or punctulate hairs. Chaetomium trigonosporum differs in having narrower ascospores $(9-12 \times 5-7 \times 4-5 \mu m)$, an ascomatal wall composed of cells arranged in a petaloid pattern (cephalothecoid) with seta-like hairs, and a Scopulariopsis-like anamorph. As well as Chaetomidium triangulare, Pidoplitchkoviella terricola Kirilenko has non-ostiolate, glabrous ascomata, clavate asci, and triangular ascospores, but the latter differs in having a peridium of *textura epidermoidea* (*textura angularis* in *C. triangulare*) and smaller ascospores ($7-9 \times 4-5 \times 2.8-3.5 \mu$ m) without germ pores. *Microascus* Zukal (*Microascaceae*) also has similar ascospores (i.e. *M. inopitatus* Udagawa & Furuya, *M. trigonosporus* var. *macrosporus* G.F. Orr, and *M. trigonosporus* C.W. Emmons & B.O. Dodge var. *trigonosporus*), but differs by obovate to spherical asci, straw-coloured to reddish brown ascospores, and by the presence of *Scopulariopsis* Bainier or *Wardomycopsis* Udagawa & Furuya anamorphs.

Key to the species of Chaetomidium

| | Asci 4-spored |
|----------|---|
| 2. 2. | Ascomatal wall cephalothecoid |
| | Ascomatal hairs circinate at the apex |
| | Ascomatal hairs straight; ascospores obovate |
| | Ascomatal hairs coarsely warted, and dichotomously branched |
| | Ascospores measuring $11-13 \times 6.5-7 \ \mu m$ |
| | Ascomata glabrous; ascospores triangular in upper view |
| | Ascomatal hairs circinate at the tips, > 9 μ m wide at the base <i>C. trichorobustum</i> Seth (1968) Ascomatal hairs not circinate, < 9 μ m wide at the base |
| | Ascomatal hairs stiff, verruculose and pale colored <i>C. pilosum</i> (C. Booth & Shipton) Arx (1975) Ascomatal hairs of two tipes: long and smooth, and short and verruculose |
| | Ascospores $8-11 \times 7-8 \times 6-7 \mu m$ |

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REFERENCES

- Arx JA von, Figueras MJ, Guarro J (1988). Sordariaceous ascomycetes without ascospore ejaculation. *Beihefte zur Nova Hedwigia* **94**: 1–104.
- Arx JA von, Guarro J, Figueras MJ (1986). The ascomycete genus *Chaetomium*. *Beihefte zur Nova Hedwigia* 84: 1–162.
- Cabrera AL (1994). Enciclopedia Argentina de Agricultura y Jardinería. Tomo II. Fascículo 1. Regiones Fitogeográficas Argentinas. Editorial Acme SACI, Buenos Aires.

- Cano J, Guarro J, El Shafie AE (1993). A new *Chaetomidium* from Oman. *Mycotaxon* **49**: 399–403.
- Doveri F, Guarro J, Cacialli G, Caroti V (1998). Contribution to the study of fimicolous fungi. XXVII. A new *Chaetomidium* from Italy with cephalothecoid peridium. *Mycotaxon* **67**: 427–432.
- Guarro J, Abdullah SK, Al-Bader SM, Figueras MJ, Gené J (1996). The genus *Melanocarpus*. *Mycological Research* 100: 75–78.
- Kornerup A, Wanscher JH (1984). *Methuen handbook of colour*. 3rd ed. Erye Methuen, London.
- Stchigel AM, Cano J, MacCormack W, Guarro J (2001). Antarctomyces psychrotrophicus gen. et sp. nov, a new ascomycete from Antarctica. Mycological Research 105: 377–382.
- Stchigel AM, Figuera L, Cano J, Guarro, J (2002). New species of *Thielavia*, with a molecular study of representative species of the genus. *Mycological Research* 106: 975–983.
- Stchigel AM, Sagués M, Cano J, Guarro J (2000). Three new thermotolerant species of *Corynascus (Sordariales, Chaetomiaceae)* from soil, with a key to the known species. *Mycological Research* **104**: 879–887.