

# On a new species of *Chaetomidium*, *C. vicugnae*, with a cephalothecoid peridium and its relationships with *Chaetomiaceae* (*Sordariales*)

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*Ascomycete.org*, 10 (2) : 86–96

Mise en ligne le 22/04/2018

doi 10.25664/ART-0231



**Abstract:** A sample of vicuña dung from a Chilean coastal desert was submitted to the attention of the author, who at first sight noticed the presence of different pyrenomycetes. Several hairy cleistothecia particularly caught his attention and were subjected to a morphological study that proved them to belong to a new species of *Chaetomidium*. After mentioning the main features of *Sordariales* and *Chaetomiaceae*, the author describes in detail the macro- and microscopic characters of the new species *Chaetomidium vicugnae* and compares it with all the other *Chaetomidium* spp. with a cephalothecoid peridium. The extensive discussion focuses on the characterization and relationships of the genus *Chaetomidium* and *Chaetomidium vicugnae* within the complex family *Chaetomiaceae*. All collections of the related species are recorded and dung is regarded as the preferential substrate. Keys are provided to sexual morph genera of *Chaetomiaceae* and to *Chaetomidium* species with a cephalothecoid peridium.

**Keywords:** Ascomycota, coprophily, germination, homoplasy, morphology, peridial frame, systematics.

## Introduction

My studies on coprophilous ascomycetes (DOVERI, 2004, 2011) allowed me to meet with several representatives of *Sordariales* Charad. ex D. Hawksw. & O.E. Erikss., an order identifiable with the so called “pyrenomycetes” *s.str.*, i.e. fungi with an ascohymenial ontogeny resulting in dark, membranous to coriaceous, perithecial or cleistothecial ascomata with true walls. *Sordariales* are also characterised by a usually paraphysate centrum, non-amyloid asci often with an apical apparatus, usually dark-pigmented, one- to poly-celled ascospores with germ pore(s), sometimes with gelatinous equipment, and a saprobic behaviour as decomposers of dung, vegetable debris and soil (KIRK *et al.*, 2008). The order, which formerly included over ten families (ERIKSSON & HAWKSWORTH, 1998), was redefined based on phylogenetic analyses (HUHNDORF *et al.*, 2004) and reduced to three families — the monophyletic *Sordariaceae* G. Winter, the paraphyletic *Chaetomiaceae* G. Winter and the *Lasiosphaeriaceae* Nannf.

*Chaetomiaceae* are characterised by dark, often hairy or setulose, non-stromatic perithecia or cleistothecia, with a pseudoparenchymatous, sometimes cephalothecoid peridium, fasciculate, ephemeral asci lacking an apical apparatus, and one-celled, pigmented ascospores with one or two (rarely more) germ pores, without a gelatinous perisporium, often extruded as a sticky cirrus. Their asexual morph, when present, is hyphomycetous (ARX *et al.*, 1988; CANNON & KIRK, 2007). Most are cellulolytic saprobes (KIRK *et al.*, 2008) with a high economic significance as contaminants of cellulose-containing materials, some behave as occasional opportunistic pathogens to humans and animals (YEGHEN *et al.*, 1996; SCHULZE *et al.*, 1997; DE HOOG *et al.*, 2000; SIGLER, 2002; SERENA *et al.*, 2003; AHMED *et al.*, 2016).

I recently had my first opportunity to analyse a sample of vicuña (*Vicugna vicugna*) droppings from the Andean highlands of Chile and to observe on them the growth of several pyrenomycetes. Some hairy cleistothecia particularly caught my attention. They were soon suspected to be representatives of *Chaetomiaceae* and, after a morphological study, proved to be a new species of *Chaetomidium* (Zopf) Sacc.

The rarity and plenty of pyrenomycetes occurring on this sample of vicuña dung impressed me so much that I wished to learn more about the animal, its communities and habit. Vicuñas are wild camelids living in the Andean highlands of Chile, Argentina, Bolivia and Peru, between 3000 and 5000 m a.s.l. Families of 5–10 females, their pups and one adult male defecate in the same spots, which appear as dark circular areas of about one meter in diameter, surrounded by herbaceous vegetation.

The aim of this work is to describe a new species of *Chaetomidium* and define it within the complex family *Chaetomiaceae*, emphasising

the importance of a future systematic study of vicuña dung for a better knowledge of the generic relationships in this family.

## Materials and methods

A sample of vicuña dung was collected in the Atacama desert (Chile) and a few days later brought to Italy which, after a first observation, was placed in a non-sterilised damp chamber, following the methods suggested by DOVERI (2004). Cultured material, incubated at room temperature (18–25°C) under natural light, but not exposed to direct sunlight, was examined on alternate days under a stereomicroscope. When first observed all cleistothecia of the new *Chaetomidium* had already developed in their natural state. No development of new specimens was noticed in the next forty days. Slides for microscopic examinations were mounted in water, Congo red, cotton blue in lactic acid, methyl blue, and an iodide solution. Fifty spores from five specimens were measured in water.

Abbreviations: MCVE = herbarium of Civic Museum of Venice (Italy).

## Taxonomy

*Chaetomidium vicugnae* Doveri, *sp. nov.* — Mycobank 824904 Figs 1–12

**Holotype:** On dung of vicuña (*Vicugna vicugna*) at Miscanti lake, Atacama desert, San Pedro de Atacama, Chile, 23°44'6"S 67°46'8"W, alt. 4.200 m, 15 Feb. 2015, coll. M.T. Seu, Herb. MCVE 29549.

**Etymology:** “*vicugnae*” = as growing on vicuña dung.

**Cleistothecia** gregarious, sometimes crowded, superficial, globose, 250–350 µm diam., dark brown, membranous, hairy. **Peridium** two-layered, dehiscing by splitting: endostratum a *textura angularis* of pale brown, thin-walled, polygonal cells, 7–17 × 6–12 µm; exostratum a *textura cephalothecoidea* of cylindrical, wavy, thick-walled, dark brown cells, 2–3 µm diam., in frontal view appearing radiate from a central, translucent hole and forming poorly defined plates. **Hairs** widespread, hyaline to very pale brown, often undulate, septate, thin-walled, unbranched, encrusted, superficial, with a slightly enlarged base, usually roundish at the apex, up to 3.5 µm diam., 60–200 µm long, but possibly longer as brittle and hardly measurable. **Paraphyses** not observed. **Asci** ephemeral, fasciculate, unitunicate, non-amyloid, 8-spored, broadly clavate, long-stalked, rounded at the apex, lacking an apical apparatus, 90–120 × 20–27 µm. **Ascospores** irregularly biseriolate, hyaline and non-dextrinoid in the early stages, yellowish to greyish later, containing several droplets or 1–2 larger guttulae, dark greyish brown and opaque at maturity, (21–) 23–27 (–32) × (9–) 10–12 (–13) µm, lacking a gelatinous peris-



**Plate 1** – *Chaetomidium vicugnae*  
1. Hairy cleistothecia on dung. Scale bar: = 300  $\mu$ m.

porium, narrowly ellipsoid to clearly fusiform, sometimes naviculate ( $Q = 2.08\text{--}3.20$ ; average  $Q = 2.43$ ), smooth, thick-walled, often somewhat inequilateral, with attenuated, more rarely slightly apiculate ends, sometimes containing a De Bary bubble in iodide solution, with two large, up to 2  $\mu$ m diam., eccentric germ pores, one at each end, often with 1–4 additional small germ pores which are indifferently disposed at some level, fast germinating, even in the hyaline stages, so becoming pedunculate, clavate, or laterally knobby. **Asexual morph** not observed.

## Discussion

### Circumscription of genera within *Chaetomiaceae*

LUMBSCH & HUHDORF (2010) accepted thirteen genera of *Chaetomiaceae*, seven with perithecial ascomata: *Achaetomium* J.N. Rai, J.P. Tewari & Mukerji, *Bommerella* Marchal, *Chaetomiopsis* Moustafa & Abdul-Wahid, *Chaetomium* Kunze, *Farrowia* Hawksw., *Guanomyces* M.C. Gonzáles, Hanlin & Ulloa and *Subramaniula* Arx; six with cleistothecial ascomata: *Boothiella* Lodhi & J.H. Mirza, *Chaetomidium* (Zopf) Sacc., *Corynascus* Arx, *Corynascella* Arx & Hodges, *Emilmueleria* Arx and *Thielavia* Zopf.

***Achaetomium*** is characterised by a hyphal peridium, usually of *textura intricata*, covered with pale delicate hairs, cylindrical asci, and spherical to ellipsoid or limoniform, very dark ascospores with a germ pore. It was accommodated in *Chaetomiaceae* based on morphological (RAI *et al.*, 1964; MUKERJI & SAXENA, 1975; CANNON, 1986; ARX *et al.*, 1984, 1988) and phylogenetic or comparative studies (LEE & HANLIN, 1999; RODRIGUEZ *et al.*, 2004; WANG *et al.*, 2016a).

***Bommerella*** is morphologically very similar to *Chaetomium*, but distinct in having an association of setulose perithecial hairs, a cephalothecoid peridium, dextrinoid, triangular ascospores, and a scopulariopsis-like asexual morph. ARX *et al.* (1984, 1986) regarded it as a possibly independent genus from *Chaetomium* and KIRK *et al.*

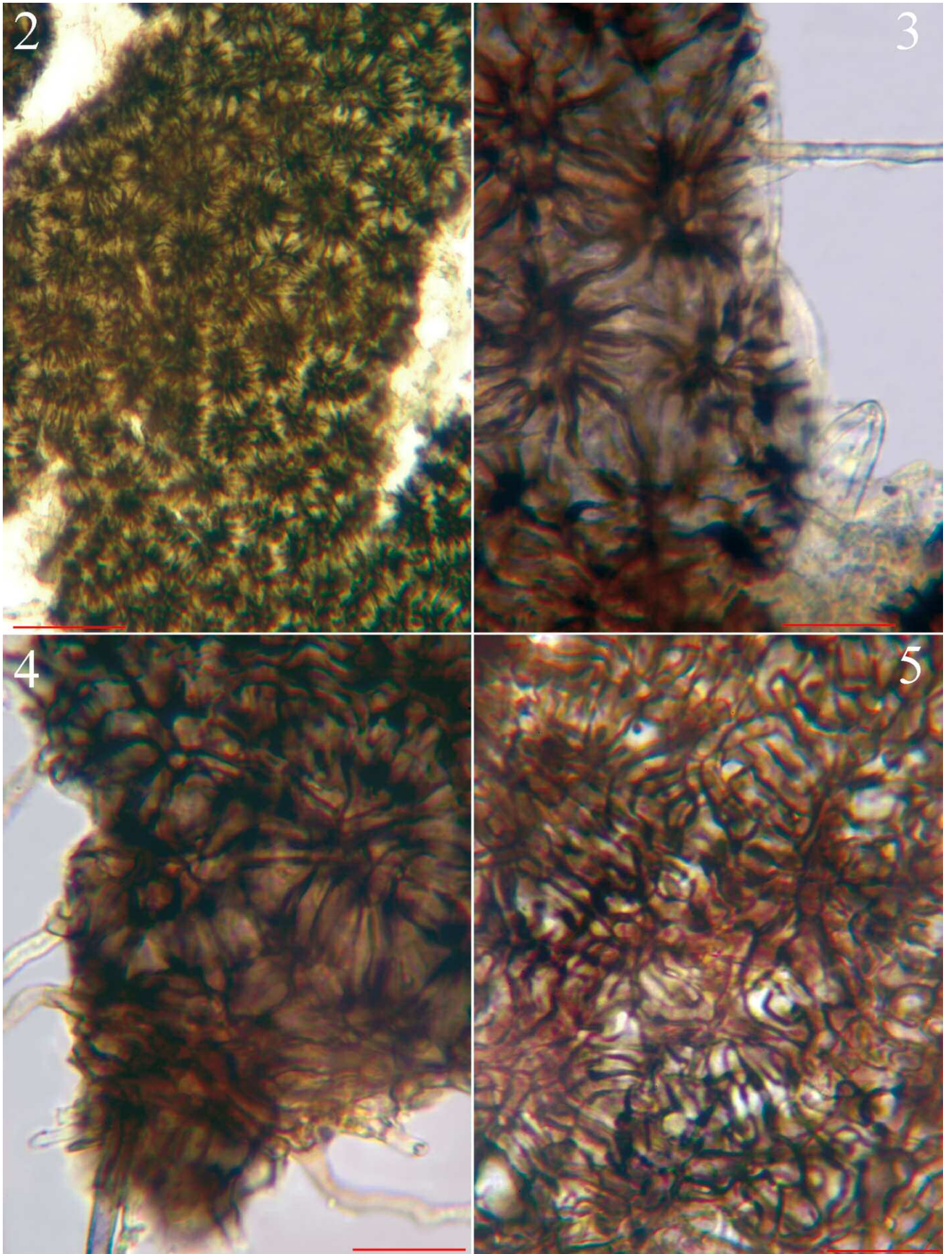
(2008) as an independent genus. Although DNA sequences of *Bommerella* were deposited in GenBank (under *Chaetomium trigonosporum*), they have not been utilised for phylogenetic studies.

The monotypic ***Chaetomiopsis*** was accepted in *Chaetomiaceae* by KIRK *et al.* (2008) and LUMBSCH & HUHDORF (2010) on the basis of morphological features, which were earlier compared with *Chaetomium* (MOUSTAFA & ABDUL-WAHID, 1990) in their protologue. Molecular data are not available for this genus, characterised by densely hairy, dark perithecia with a peridium of *textura angularis*, clavate asci, and brown, prominently pitted, ellipsoidal to irregular ascospores, rounded at both ends, flattened at one side, with two eccentric or lateral germ pores.

***Chaetomium***, the type genus of the family, was subjected to extensive morphological (BAINIER, 1909; CHIVERS, 1915; AMES, 1963; SETH, 1970; CANNON, 1986; ARX *et al.*, 1986) and molecular studies (LEE & HANLIN, 1999; UNTEREINER *et al.*, 2001; ZHANG *et al.*, 2006; KRUYIS *et al.*, 2015). The ARX *et al.* (1986) monograph forms the basis for the modern classification of the genus, which appears extremely heterogeneous. A broad conception of *Chaetomium* includes membranous, hairy or setose, ostiolate ascomata with several types of peridial frame, clavate, fusiform, or cylindrical asci in fascicles, pigmented, one-celled ascospores often extruded as a sticky cirrhous, variable in shape but usually limoniform, ovoidal or fusiform, exceptionally dextrinoid, with one, two or more germ pores, and several types of asexual morphs (ARX *et al.*, 1986; GUARRO *et al.*, 2012; AHMED *et al.*, 2016).

Morphological studies combined with phylogenetic inferences based on six loci (WANG *et al.*, 2016b) were used to define the limits of the so called “*Chaetomium globosum*-complex”, a monophyletic group of thirty-six species that also included *Chaetomidium fimeti* (Fuckel) Zopf, the type species of the genus *Chaetomidium*. Linked phylogenetic analyses (WANG *et al.*, 2016a) led them to recognise another thirteen monophyletic clades in *Chaetomiaceae* and to





**Plate 2** – *Chaetomidium vicugnae*  
2–5. Details of cephalothecoid exoperidium. Scale bars: 2 = 25  $\mu$ m; 3–5 = 10  $\mu$ m.





**Plate 3** – *Chaetomidium vicugnae*  
6. Immature clavate asci. 7. Hairs. Scale bar = 20 µm.

confirm the taxonomic concept and monophyly of the *Chaetomium globosum*-complex. This complex is now regarded as a distinct lineage representing *Chaetomium s.str.* (WANG *et al.*, 2016a). Some known genera of *Chaetomiaceae* have been supported by this study (WANG *et al.*, 2016a) and new genera erected to accommodate species of *Chaetomium s.l.* forming distinct lineages outside the *Chaetomium globosum*-complex. The new taxonomic concept of *Chaetomium s.str.* has been enlarged to include species with both ostiolate and non-ostiolate (some taxa previously regarded as *Chaetomidium*) ascomata, but only those with hypha-like hairs, peridia with a *textura intricata* or *epidermoidea*, rarely *angularis*, clavate or fusiform asci, limoniform, globose or rarely irregular, bilaterally flattened ascospores and, when present, an acremonium-like asexual morph (WANG *et al.*, 2016a)

*Farrowia* was erected by HAWKSWORTH (1975) to accommodate a group of chaetomium-like species with a usually long neck formed of elongated cells or fused setae, limoniform, biapiculate ascospores, and a botryotrichum-like (humicola-like according to WANG *et al.*, 2016a) asexual morph. It was accepted as an independent genus also by UDAGAWA & MUROI (1981) but not by ARX *et al.* (1986). A more recent phylogenetic study (UNTEREINER *et al.*, 2001) provided evidence that *Farrowia* is too close to *Chaetomium* to be accepted as a separate genus.

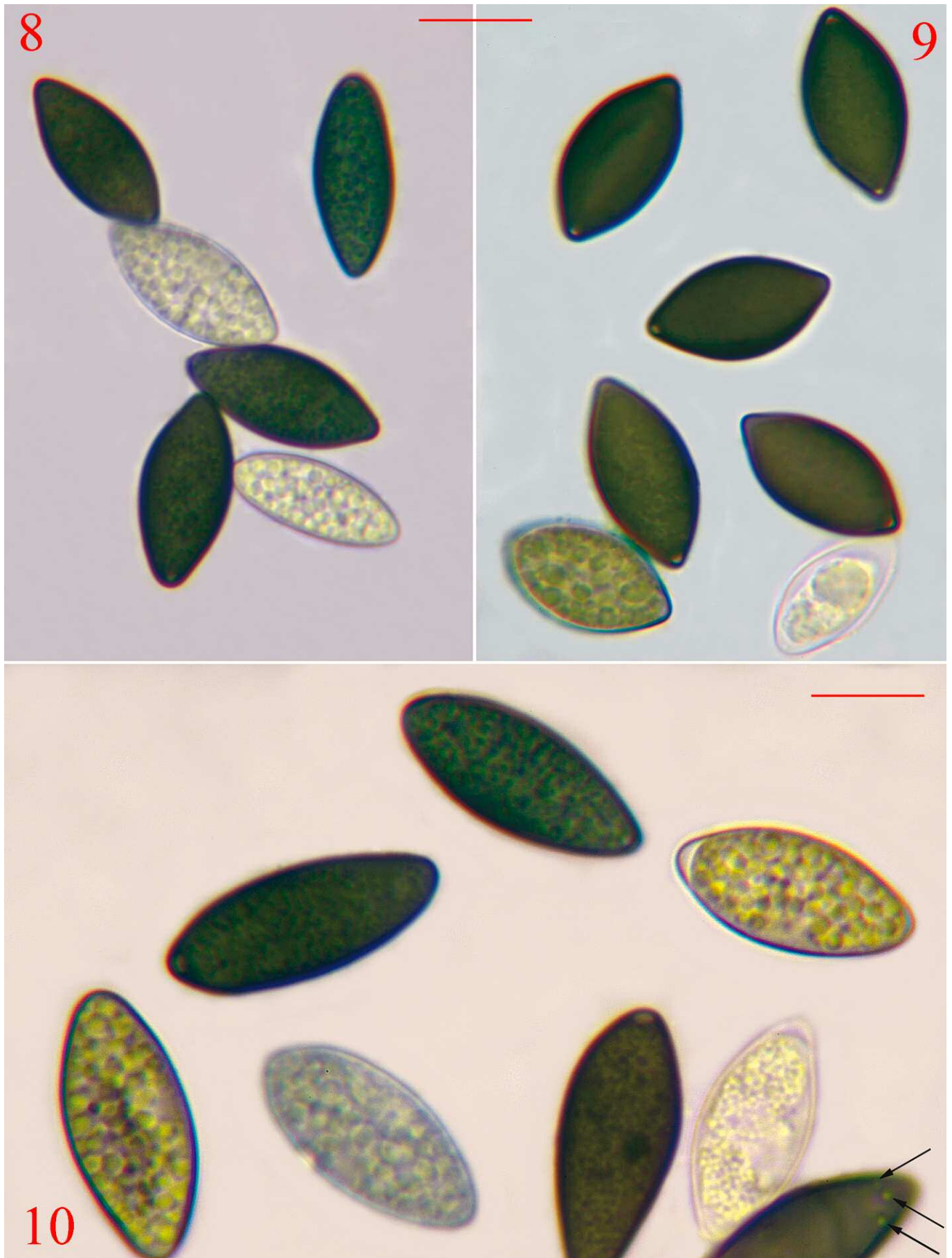
The monotypic *Guanomyces*, accommodated in *Chaetomiaceae* on the basis of comparative morphological and molecular studies (GONZÁLES *et al.*, 2000; STCHIGEL *et al.*, 2006), is characterised by brown, hairy, long-necked perithecia with single glandular hairs at the neck sides and a tuft of apical hairs, peridium of *textura angularis*, 8-spo-

red, long-stalked, clavate asci, hyaline, slightly aculeate, ellipsoidal ascospores without germ pores, and no asexual morph (GONZÁLES *et al.*, 2000).

*Subramaniula* was placed in *Chaetomiaceae* based on morphological studies (CANNON, 1986; ARX *et al.*, 1988). More recently a few gene sequences of the genus have been deposited (AHMED *et al.*, 2016). *Subramaniula s.str.* is characterised by smooth, urniform ascomata with a broad ostiole surrounded by a collarette, a pale peridium of *textura angularis* to *epidermoidea*, 8-spored, subglobose or broadly clavate asci, broadly fusiform or dacryoid to irregular, brown ascospores with a germ pore, and an unknown or papulospore-like asexual morph (GUARRO *et al.*, 2012; AHMED *et al.*, 2016). Combined morphological and molecular studies (WANG *et al.*, 2016b) expanded its generic concept to include some chaetomium-like species forming a monophyletic lineage with *Subramaniula* spp.

The monotypic *Boothiella* has pale, smooth cleistothecia with a peridium of *textura angularis*, 4-spored, cylindrical asci, and dark-pigmented, subspherical to broadly ovate ascospores with a basal germ pore. It was placed in *Chaetomiaceae* on the basis of morphological studies (LODHI & MIRZA, 1962; ARX & MAHMOOD, 1968; MUKERJI & SAXENA, 1975; ARX *et al.*, 1984, 1988). Gene sequences of this genus are not deposited.

*Chaetomidium* is a heterogeneous genus encompassing species with usually hairy or setose cleistothecia, peridium of a *textura angularis* or *cephalothecoidea*, clavate or sometimes ovoidal (4)-8-spored asci, ovoidal, limoniform, fusiform, triangular ascospores with a germ pore, without an asexual morph or with an acremonium-like



**Plate 4** – *Chaetomidium vicugnae*

8–10. Ascospores in different stages (arrows highlighting eccentric or lateral germ pores). Scale bars: 8–9 = 20  $\mu$ m; 10 = 10  $\mu$ m.



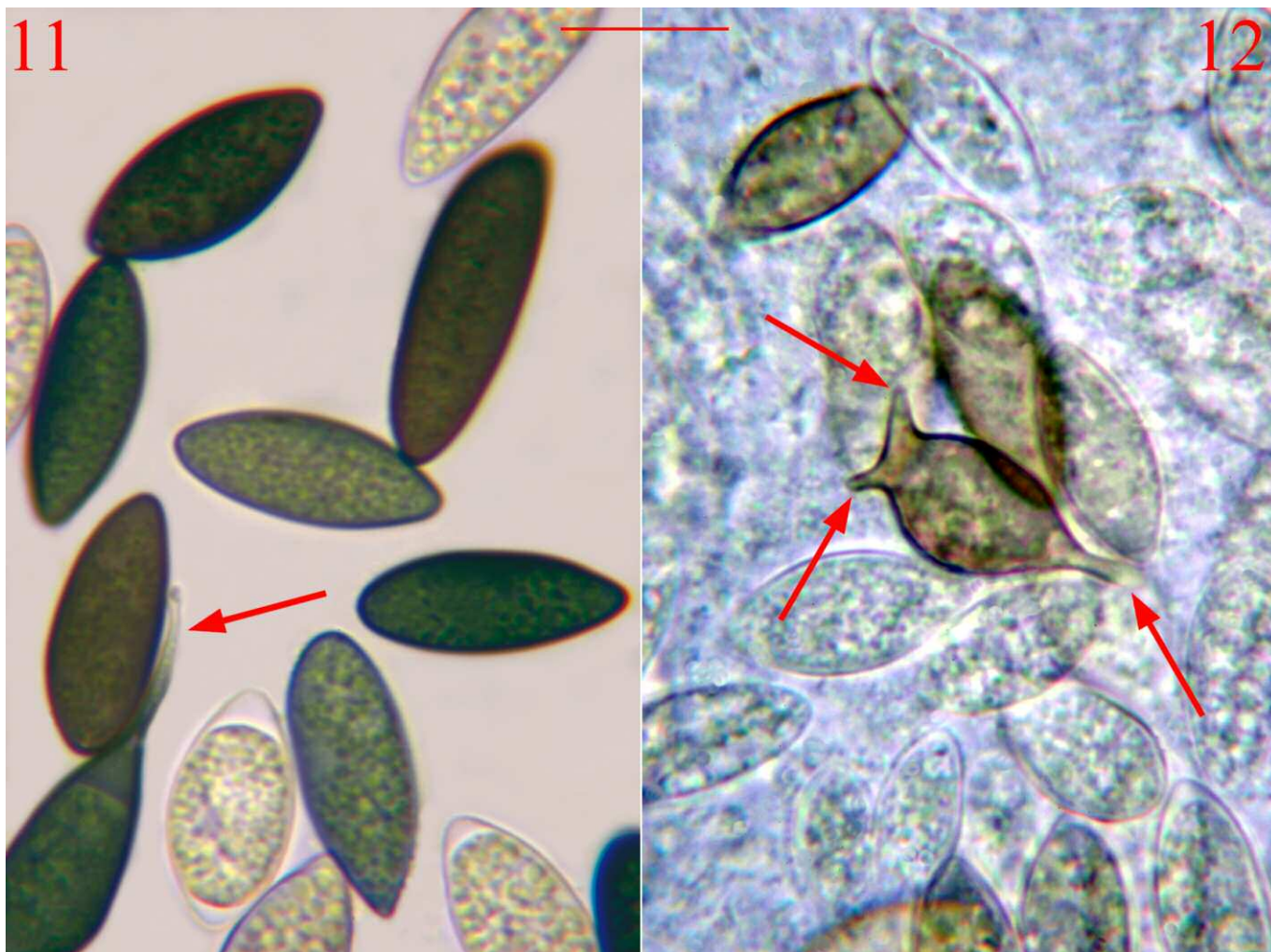


Plate 5 – *Chaetomidium vicugnae*

11–12. Germinating ascospores (arrows). 12. Ascospore germinating from three pores. Scale bar = 15 µm.

or botryotrichum-like asexual morph (GUARRO *et al.*, 2012). It was located in CHAETOMIACEAE after morphological (ARX, 1975; MUKERJI & SAXENA, 1975; ARX *et al.*, 1984, 1988) and comparative studies (SILVA & HANLIN, 1996), but phylogenetic analyses based on LSU,  $\beta$ -tubulin and rpb2 sequence data (GREIF *et al.*, 2009) proved *Chaetomidium* to be polyphyletic, with the majority of its species scattered among the Chaetomiaceae and one species with an uncertain position in Lasiosphaeriaceae Nannf.

*Corynascus* was placed in Chaetomiaceae after morphological (ARX, 1975; ARX *et al.*, 1988) and comparative molecular studies (STCHIGEL *et al.*, 2000; KRUYIS *et al.*, 2015). Further phylogenetic studies (BRINK *et al.*, 2012) proved that *Myceliophthora* Costantin and *Corynascus* cluster together in Chaetomiaceae without an obvious separation and suggested that all *Corynascus* spp. should be recombined under the older name *Myceliophthora*. A comparative, phenotypic and phylogenetic study on *Myceliophthora* (MARÍN-FELIX *et al.*, 2015) split it into four strongly supported clades and restricted it to only the type species *Myceliophthora lutea* Costantin. Two new genera with a myceliophthora-like asexual morph, *Crassicarpon* Y. Marín, Stchigel, Guarro & Cano and *Thermothelomyces* Y. Marín, Stchigel, Guarro & Cano, were consequently erected and *Corynascus* was re-established. After this study the concept of *Corynascus* must be restricted to homothallic, mesophilic species with a peridium of *textura epidermoidea* formed of reticulate or verrucose cells, ellipsoidal, broadly fusiform, or irregularly shaped, dark ascospores with two terminal germ pores, and a myceliophthora-like asexual morph.

However, the taxonomic concept of *Corynascus* has evolved after phylogenetic analyses (WANG *et al.*, 2016a) revealed that *Myceliophthora lutea* and the species of *Corynascus* with *Myceliophthora*

asexual morphs cluster in a strongly supported clade, suggesting again that *Corynascus* be synonymized with *Myceliophthora*.

*Corynascella* is characterised by pale cleistothecia covered with dark appendages, a peridium of *textura intricata* to loosely *epidermoidea*, ovoidal or rarely broadly clavate, 8-spored asci, dark, ellipsoidal, fusiform or reniform ascospores with a thickened wall around the (1) 2 (4) germ pores, and an unknown asexual morph. It was placed in Chaetomiaceae according to morphological (ARX, 1975; ARX *et al.*, 1988) and phylogenetic studies (BRINK *et al.*, 2012; WANG *et al.*, 2016a). GUARRO *et al.* (1997) also accommodated in this genus *Corynascella arabica*, which shows morphological features intermediate between *Corynascella* (broadly ellipsoidal, thick-walled ascospores with a germ pore at each end) and the group of *Chaetomidium* spp. with a cephalothecoid peridium (dark cleistothecia, clavate asci, peridial plates of radiating cells).

The monotypic, coprophilic *Emilmuelleria* has black cleistothecia with tufts of pale, spirally coiled hairs, a thick peridium of *textura angularis*, 8-spored, long-stalked, clavate asci, dextrinoid, greyish, ellipsoidal ascospores with an apical germ pore, and an unknown asexual morph. Morphological (ARX, 1985; ARX *et al.*, 1988) and phylogenetic (SILVA & HANLIN, 1996) studies suggested the placement of this genus in Chaetomiaceae. Combined morphological and phylogenetic studies (WANG *et al.*, 2016a) confirmed its position in Chaetomiaceae but at the same time suggested its synonymy under the expanded genus *Botryotrichum* Sacc. & Marchal, which also includes some chaetomium-like ostiolate species.

*Thielavia* was subjected to numerous morphological (BOOTH, 1961; MALLOCH & CAIN, 1973; ARX, 1975; ARX *et al.*, 1984, 1988) and phylogenetic (LEE & HANLIN, 1999; STCHIGEL *et al.*, 2002; CAI *et al.*, 2006a) studies, which certainly proved that it belongs to *Chaetomiaceae* and represents a well-supported monophyletic lineage (WANG *et al.*, 2016a). The genus encompasses more than twenty species characterised by usually glabrous or setose cleistothecia with a *textura epidermoidea* peridium, 8-spored, ellipsoidal or saccate, rarely cylindrical asci, thin-walled, ellipsoid-fusiform, ovoidal or even subclavate, brown ascospores with a germ pore, and an occasional presence of chrysosporium-like aleurioconidia (STCHIGEL *et al.*, 2003).

*Melanocarpus* was erected by ARX (1975), who regarded it as related to *Thielavia*. Later, it was described and discussed by ARX *et al.* (1988), who compared it with *Boothiella*, and monographed by GUARRO *et al.* (1996), who wished that molecular studies would better define the genus. LUMBSCH & HUHDORF (2010) placed *Melanocarpus* in *Sordariales incertae sedis*, although a comparative morphological and molecular study (STCHIGEL *et al.*, 2002) proved that it is related to *Thielavia* in *Chaetomiaceae*. Its placement in *Chaetomiaceae*, where it represents a monophyletic clade, was confirmed by WANG *et al.* (2016a). The genus includes mesophilic and thermophilic species with dark cleistothecial ascomata covered with short setae or simple hyphae, a peridium of *textura angularis* or rarely *epidermoidea*, 8-spored, subglobose or cylindrical-saccate asci, thick-walled, brown, often oblate or broadly ellipsoid to ovoid ascospores with a germ pore, and a chrysonilia-like asexual morph (GUARRO *et al.*, 2012).

MAHARACHCHIKUMBURA *et al.* (2015) provided an updated outline of the *Sordariomycetes* O.E. Erikss & Winka particularly based on molecular data published after LUMBSCH & HUHDORF (2010). They included in *Chaetomiaceae* some additional asexual morph genera and transferred *Zopfiella* G. Winter and the related *Diplogelasinospora* Cain from *Lasiosphaeriaceae* to *Chaetomiaceae* possibly misinterpreting the CAI *et al.* (2006b) phylogenetic analyses, which proved that the polyphyletic *Zopfiella* should be placed in *Lasiosphaeriaceae*.

### Historical survey of *Chaetomidium*

*Chaetomidium* was erected by ZOPF (1881) as subgenus of *Chaetomium* and regarded as an independent genus by SACCARDO (1882), to accommodate a single species, *Chaetomium fimeti*, with non-ostiolate ascomata and “wire-like” basal rhizoids.

BAINIER (1909), CHIVERS (1915), SKOLKO & GROVES (1953) and WHITESIDE (1962) accepted *Chaetomidium* and BAINIER (1909) also established two new taxa in this genus, *C. phyllactineum* and *C. magnum*, which MALLOCH & CAIN (1973) transferred to *Thielavia* and ARX *et al.* (1988) considered doubtful species.

CAIN (1961) established *Chaetomidium minutum*, but MALLOCH & CAIN (1973) transferred it to *Thielavia* and GUARRO *et al.* (1996, 2012) confirmed it in the latter genus.

SETH (1967) described *Chaetomidium subfimeti* as a new species from vegetable material, very close to *C. fimeti* but with somewhat smaller ascospores.

MALLOCH & CAIN (1973) did not accept *Chaetomidium* as a genus independent from *Thielavia*, stating that the presence of differentiated hairs in the former and glabrous cleistothecia in the latter are not enough to separate them at the genus rank, especially because intermediate forms are known. ARX (1975) did not attribute the same importance to the presence or absence of hairs, and recognised *Chaetomidium* as a genus independent from *Thielavia*, having pseudoparenchymatous rather than *textura epidermoidea* peridia and limoniform or laterally flattened ascospores. He accepted in *Chaetomidium* only species with clavate asci and ascospores with one germ pore, and first recognised in the genus one species with a cephalothecoid peridium, *C. cephalothecoides* (Malloch & Benny) Arx. Besides *C. fimeti* and *C. subfimeti*, he also accepted three other species with a peridium of *textura angularis*, i.e. *C. pilosum* (C. Booth & Sipton) Arx, *C. spirotrichum* (R.K. Benj.) Malloch & Cain and *C. tri-*

*chorobustum* Seth. *Chaetomidium spirotrichum* was later recombined in *Emilmuelleria spirotricha* (R.K. Benj.) Arx. ARX *et al.* (1988) confirmed *Chaetomidium* as a genus and recognised *C. heterotrichum* R.J. Mey. and *C. arxii* Benny, the latter with a cephalothecoid peridium.

DOVERI (2004) discussed *Chaetomidium* and provided a key to the species with a cephalothecoid peridium, including *C. khodense* Cano, Guarro & El Shafie (CANO *et al.*, 1993) and *C. megasporum* Doveri, Guarro, Cacialli & Caroti (DOVERI *et al.*, 1998), two taxa established after the ARX *et al.* (1988) monograph.

STCHIGEL *et al.* (2004) provided a key to all *Chaetomidium* spp. and described two new species: *Chaetomidium galaicum* Stchigel & Guarro, close to both *C. khodense* and *C. megasporum* for having fusiform ascospores and a cephalothecoid peridium, and *C. triangulare* Stchigel & Guarro, the first *Chaetomidium* sp. with smooth ascomata and triangular ascospores.

GREIF & CURRAH (2007) recombined *Aporoethelavia leptoderma* (C. Booth) Malloch & Cain in *Chaetomidium leptoderma* (C. Booth) Greif & Currah after proving by ultra-structural techniques that its cephalothecoid peridium has a development and dehiscence very similar to *C. arxii*.

GREIF *et al.* (2009) proved that *Chaetomidium* is polyphyletic and restricted the genus to *C. fimeti* and *C. subfimeti*. They also noticed a minimal difference of gene sequences between *C. leptoderma* and *C. galaicum* and regarded them as conspecific.

GUARRO *et al.* (2012) accepted 11 species of *Chaetomidium*, 5 with a cephalothecoid peridium, i.e. *C. arxii*, *C. cephalothecoides*, *C. khodense*, *C. leptoderma* and *C. megasporum*.

Based on GREIF *et al.* (2009) and new personal analyses of  $\beta$ -tubulin and rpb2 gene sequences, WANG *et al.* (2016b) showed that *Chaetomidium fimeti* and *C. subfimeti* cluster within the *Chaetomium globosum* species complex (*Chaetomium s.str.*). So, they rejected the genus name *Chaetomidium* and regarded it as synonym of *Chaetomium*.

### Characterization, relationships and ecology of *Chaetomidium vicugnae* and related species

*Chaetomidium vicugnae* is characterised by a cephalothecoid peridium, very pale peridial hairs, large, narrowly ellipsoid to fusiform ascospores with two large, subapical germ pores and sometimes with additional, smaller pores, and growth on dung. No other species of *Chaetomidium s.l.* shows an association of such features. The species with a cephalothecoid peridium particularly differ in having dark pigmented hairs and significantly smaller ascospores with a single germ pore. *C. arxii* further differs from *C. vicugnae* in that it has straight, usually smooth hairs and broadly ellipsoidal ascospores (BENNY, 1980) and *C. leptoderma* (compared to *C. arxii* by GREIF & CURRAH, 2007) is also distinguishable by its sparsely hairy perithecia and knob-like protrusions of the peridial cells. Unlike *C. vicugnae*, *C. cephalothecoides* and *C. megasporum* have ellipsoidal to broadly fusiform ascospores (average  $Q < 2$  versus 2.43) and almost smooth, sometimes hooked hairs (MALLOCH & BENNY, 1973), and hairs with end branches at right angles (DOVERI *et al.*, 1998) respectively.

I also compared *C. vicugnae* with *Chaetomidium biapiculatum* Lodha and *C. hyalotrichum* Taparia & Lodha, listed in Index Fungorum but appearing almost like ghost species as they were never mentioned in other works on *Chaetomidium* and never recombined or regarded as synonyms of other taxa. According to the protologue (LODHA, 1974) *Chaetomidium biapiculatum* has morphological features of a true *Chaetomidium*, close to the type species *C. fimeti*, but with straight peridial hairs and ascospores with two germ pores. It stands quite distant from *C. vicugnae* for having broadly ellipsoidal ascospores and a peridium of *textura angularis*. *Chaetomidium hyalotrichum* shares hyaline peridial hairs with *C. vicugnae* and ascospores with two germ pores (TAPARIA & LODHA, 1974) but, like *C. biapiculatum*, clearly differs from *C. vicugnae* in having a peridium of angular cells and broadly ellipsoidal ascospores.



### Key to sexual morph genera of *Chaetomiaceae*

- 1) Ascomata ostiolate ..... 2  
1\*) Ascomata non-ostiolate ..... 6
- 2) Ascomata urniform, smooth, with a broad ostiole, up to 150 µm diam. Ascospores often dacryoid or irregular in shape ..... ***Subramaniula s.str.***
- 2\*) Ascomata hairy or setose, with a much narrower ostiole. Ascospores different in shape ..... 3
- 3) Ascomata with a long neck covered with lateral glandular hairs and a tuft of apical hairs. Ascospores ellipsoidal with rounded ends, imperceptibly aculeate, without germ pores. .... ***Guanomyces***
- 3\*) Ascomata without a neck or when long-necked, then lacking glandular hairs. Ascospores smooth or differently ornamented, usually with germ pores ..... 4
- 4) Ascomata with dark, coarsely warted, dichotomously branched hairs. Peridium of *textura angularis*. Ascospores comparatively large, 18–24 × 14–16 µm, ellipsoidal to irregular, rounded at the ends, prominently pitted, with two germ pores ..... ***Chaetomiopsis***
- 4\*) Ascomata dark hairy to setulose or with a pale, hyphal covering. Ascospores smooth, usually different in shape, often with one germ pore, rarely two ..... 5
- 5) Ascomata obovate or ampulliform, covered with pale hyphal hairs. Peridium usually of *textura intricata*. Asci cylindrical. Ascospores spherical and slightly oblate or ellipsoid-limoniform. Asexual morph unknown ..... ***Achaetomium***
- 5\*) Ascomata hairy or setulose, sometimes long-necked. Peridium of *textura angularis*, *epidermoidea*, *intricata*, rarely *cephalothecoidea*. Asci clavate or cylindrical, exceptionally ovoidal or fusiform. Ascospores variously shaped, with one germ pore, exceptionally triangular, rarely spherical then usually with two germ pores. Asexual morph often present ..... ***Chaetomium s.l.*** (inclusive of *Bommerella* and *Farrowia*)
- 6) Peridium of *textura angularis*, sometimes *cephalothecoidea* (*epidermoidea* only in one species of *Melanocarpus*), translucent or opaque ..... 7
- 6\*) Peridium of *textura epidermoidea* or *intricata*, translucent ..... 11
- 7) Ascomata glabrous, with a peridium of *textura angularis*. Asci 4–6-spored ..... 8
- 7\*) Ascomata with a vestiture. Asci 8-spored (4-spored in one *Chaetomidium* sp.) ..... 9
- 8) Peridium pale and very thin. Asci 4-spored, cylindrical. Ascospores subspherical or broadly ovate, with one basal germ pore. Asexual morph unknown ..... ***Boothiella***
- 8\*) Peridium blackish and thick. Asci 4–6-spored, broadly clavate. Ascospores broadly ellipsoidal, with two germ pores, one at each end. Asexual morph myceliophthora-like. Thermophilic ..... ***Crassicarpon***
- 9) Ascomata very dark and opaque, hairy or covered with undifferentiated hyphae. Peridium of *textura angularis*. Asci subglobose or cylindrical-saccate. Ascospores subglobose to broadly ovoidal or ellipsoidal, uni- or bilaterally flattened, with one apical or lateral germ pore. Asexual morph chrysonilia-like. Meso- or thermophilic ..... ***Melanocarpus***
- 9\*) Ascomata hairy or setose. Asci usually clavate and stalked. Ascospores with one apical or subapical germ pore. Mesophilic ... 10
- 10) Ascomata very dark, with tufts of spirally coiled appendages and flexuous hairs. Peridium of *textura angularis*. Ascospores dextrinoid when young, ellipsoidal. Asexual morph unknown ..... ***Emilmuelleria*** (= *Botryotrichum p.p.*, s. WANG *et al.* 2016a)
- 10\*) Ascomata translucent, with a hairy or setose peridium of *textura angularis* or *cephalothecoidea*. Ascospores ovoidal, ellipsoidal-limoniform and bilaterally flattened or broadly fusiform, exceptionally triangular, not dextrinoid. Asexual morph acremonium-like, botryotrichum-like, or unknown ..... ***Chaetomidium s.l.***
- 11) Ascomata dark hairy or setulose. Peridium of *textura intricata*. Asci 8-spored, ovoidal or broadly clavate, stalked. Ascospores ellipsoidal, fusiform or reniform, usually unilaterally flattened, with a thickened wall around two or more, terminal germ pores. Asexual morph unknown ..... ***Corynascella***
- 11\*) Ascomata usually glabrous with a peridium of *textura epidermoidea*. Asci 8-spored or exceptionally 4-spored. Ascospores without a thickened wall around the terminal or lateral germ pores ..... 12
- 12) Peridial cells reticulate or verrucose. Ascospores with two germ pores, one at each end, ellipsoidal, broadly fusiform, or irregularly shaped. Asci ovoidal or subspherical. Asexual morph myceliophthora-like. Mesophilic ..... ***Corynascus*** s. MARIN-FELIX *et al.* 2015
- 12\*) Peridium without such features. Ascospores with one germ pore ..... 13
- 13) Ascospores ellipsoidal, fusiform, subclavate, sometimes broadly ovoidal and bilaterally flattened, with an apical or subapical germ pore. Asci ellipsoidal or saccate, rarely cylindrical. Asexual morph often unknown or chrysosporium-like. Mesophilic or thermophilic ..... ***Thielavia***
- 13\*) Ascospores ellipsoidal, with one apical germ pore. Asci ellipsoidal. Asexual morph myceliophthora-like. Thermophilic ..... ***Thermothelomyces***



Finally, we have compared *Chaetomidium vicugnae* with *Corynascella arabica* Guarro, Al-Saadoon, Gené & Abdullah, an atypical species of *Corynascella* with dark cleistothecia, short undifferentiated hairs, a cephalothecoid peridium, stalked, clavate asci, and ascospores lacking a thickened wall around the germ pores (GUARRO *et al.*, 1997). Most of these features make it similar to the group of *Chaetomidium* spp. with a cephalothecoid peridium, particularly to *Chaetomidium vicugnae* due to the presence of ascospores with two eccentric germ pores. But *Corynascella arabica* can be distinguished by its much shorter peridial hairs, smaller asci, and smaller, thick-walled, broadly ellipsoidal ascospores.

Coprophily is a well-known lifestyle of many *Chaetomidium* species (ARX *et al.*, 1988; GUARRO *et al.*, 2012), particularly of those with a cephalothecoid peridium (DOVERI, 2004; GUARRO *et al.*, 2012). Unfortunately, some of them are known only by single collections, i.e. *C. megasporum* from cattle dung (DOVERI *et al.*, 1998), *C. vicugnae* from vicuña droppings, and *C. leptoderma* from soil (BOOTH, 1961). *C. arxii* was twice collected from rodent dung (BENNY, 1980; LORENZO, 1993) and once recorded from *Eurygaster integriceps*, the insect agent of the wheat Sunn pest (ARZANLOU *et al.*, 2012). The distribution of *C. khodense* is restricted to the Sultanate of Oman, where it was found on cattle (CANO *et al.*, 1993), goat and camel dung (ELSHAFIE, 2005), while that of *C. cephalothecoides* extends to North America, where it was originally isolated from mouse dung (MALLOCH & BENNY, 1973), and Europe from sheep dung (VALLDOSERA & GUARRO, 1992; DOVERI, 2004; MOYNE & PETIT, 2006). *Corynascella arabica* was once isolated from donkey dung in Iraq (GUARRO *et al.*, 1997). At present, therefore, the scarce number of findings does not allow the substrate preference and tolerance of each species to be precisely defined.

## Conclusions

Cleistothecial ascomycetes, even belonging to unrelated orders and classes (STCHIGEL & GUARRO, 2007; LUMBSCH & HUHNDOF, 2010) are quite frequent on dung, where they apparently behave as saprobic decomposers (KIRK *et al.*, 2008). The cleistohymenial development represents an effective defence against several environmental threats, enabling the reproductive structures to be exposed only at full maturity. Several pyrenomyces with non-ostiolate ascomata show a cephalothecoid peridium, consisting of plates of elongated, radiating cells, separated by lines of weakness along which the peridial frame splits at maturity (GREIF & CURRAH, 2007). Fractures occur

by means of active forces from inside (MALLOCH & CAIN, 1970; GREIF *et al.*, 2004; GREIF & CURRAH, 2007) or by external forcings (BENNY *et al.*, 1980) resulting from the contact with arthropods or beetles, which also act as spore carriers and dispersers.

The cephalothecoid peridial structure is sporadically present in unrelated taxa, even belonging to different classes, such as in *Cephalotheca* Fuckel (*Dothideomycetes* O.E. Erikss. & Winka) or in *Cercophora* Fuckel and *Chaetomidium* (*Sordariomycetes*), proving that this feature is homoplastic, resulting from independent, parallel evolution in different lineages of ascomycetes (SUH & BLACKWELL, 1999; GREIF *et al.*, 2009).

The ascospores of *Chaetomidium vicugnae* are able to germinate even when still hyaline, proving that they reach reproductive maturity also at this stage. This striking phenomenon is typical of the genus *Cercophora* and sometimes occurs in *Podospora* Ces. sect. *Podospora* (LUNDQVIST, 1972), suggesting, perhaps, a close phylogenetic relationship with *Chaetomidium vicugnae*.

To my knowledge vicuña droppings have never been subjected to extensive studies or surveys on coprophilous ascomycetes. The sample of vicuña dung I examined is surprising because although numerous ascomata were observed, only four species were recorded. Three of these were cleistothecial, two with a complete or partial cephalothecoid peridium, i.e. *Chaetomidium vicugnae* and a *Chaetomium* species of the *murorum*-group respectively.

I think that further studies of fungal isolates from vicuña dung can improve the knowledge of phylogenetic relationships between genera and families of *Sordariales*.

## Acknowledgements

The author is grateful to Dan Mahoney for revising the text. He also thanks Maria Teresa Seu for providing him with the material described in this article.

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### Key to *Chaetomidium* and *Corynascella* species with a cephalothecoid peridium

- 1) Peridium covered with hyaline hairs. Ascospores with two terminal germ pores, sometimes with 1–4 additional smaller pores . 2
- 1\*) Peridium with dark pigmented hairs. Ascospores with one germ pore ..... 3
- 2) Peridium with very short hyphal hairs. Ascospores broadly ellipsoidal, thick-walled (0.5–0.8 µm), 9–11 × 6.5–7.5 µm ..... *Corynascella arabica*
- 2\*) Peridial hairs up to 200 µm long or more. Ascospores narrowly ellipsoidal to fusiform, often flattened on one side, thinner, sometimes with additional germ pores, 23–27 × 10–12 µm ..... *Chaetomidium vicugnae*
- 3) Peridial hairs straight, smooth. Ascospores broadly ellipsoidal or ovoidal, with an apical germ pore surrounded by a dark ring, 14–15 × 10.5–11.5 µm ..... *Chaetomidium arxii*
- 3\*) Peridial hairs flexuous. Ascospores different in shape ..... 4
- 4) Hairs sparse. Peridial cells with knob-like protrusions. Ascospores narrowly ellipsoid-fusiform, flattened on one side, 14–19 × 5–7.5 µm ..... *Chaetomidium leptoderma*
- 4\*) Hairs densely disposed. Peridial cells without protrusions. Ascospores ellipsoidal to broadly fusiform ..... 5
- 5) Hairs sometimes loosely coiled. Peridial cells irregularly disposed in plates. Ascospores with a subapical germ pore, 11–13 × 6.5–7 µm ..... *Chaetomidium khodense*
- 5\*) Hairs with different features. Peridial cells with a regular petaloid disposition. Ascospores with an apical germ pore ..... 6
- 6) Hairs almost smooth, sometimes uncinuate or circinate. Ascospores 12–15.5 × 8–10.5 µm. Asexual morph botryotrichum-like or staphylotrichum-like ..... *Chaetomidium cephalothecoides*
- 6\*) Hairs warted, often with end branches at right angles. Ascospores 19–21.5 × 11–13 µm. Asexual morph unknown ..... *Chaetomidium megasporum*

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