
***Penicillium aureocephalum* anam. sp. nov.**

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Penicillium aureocephalum sp. nov. found in Catalonia (NE Spain) on fallen leaves of *Quercus suber* and *Cistus salviifolius* is described as a new species belonging in the subgenus *Biverticillium*. The synnematosous conidiomata formed in nature look like stalked Myxomycota sporangia and do not suggest its *Penicillium* identity, which only microscopic examination makes evident. A wide combination of features present in particular members of ser. *Corimigenum* and other series distinguish this fungus from species so far described.

Key words: mitosporic fungi, new species, taxonomy.

Introduction

On 5 December 1999, fallen leaves of *Quercus suber* L. (*Fagaceae*) and *Cistus salviifolius* L. (*Cistaceae*) showing fructifications resembling Myxomycota sporangia were collected by X. Llimona. Microscopic examination revealed the synnematosous fungal nature of these structures. The peculiar characteristics of the fungus collected lead us to describe it as a new mitosporic species.

Material and methods

Collection site. NE Spain, Catalonia, Province Girona, Selva de Mar, Cap de Creus National Park (UTM 31T EG 1586). A sunny clearing of a cork oak (*Quercus suber*) forest with dispersed shrubs of rockroses (*Cistus salviifolius*), at 150 m alt., facing the south, protected from the winds, with temperate winters and rainfall above 600 mm.

Isolations. Yellow synnematosous structures produced in nature fallen leaves of cork oak were directly plated on malt extract agar (MEA) as formulated by Pitt (1979). Pure colonies were obtained by transferring to new MEA plates: i) small pieces of marginal non-sporulating subsurface hyphae from the original

cultures; ii) solitary yellow mycelial knots formed at the colony margins. Monoconidial cultures were obtained by successive dilutions from the green sporulating parts of the original colony.

Nutrient media. Czapek Yeast Autolysate agar (CYA), Malt Extract agar (MEA), and Glycerol Nitrate agar (G 25 N) as recommended by Pitt (1979); also Potato Dextrose agar (PDA) and Czapek's agar (CA), used by Raper and Fennell (1948), Raper and Thom (1949), Ramírez (1982) and many others.

Culture conditions. Parallel sets of colonies were cultivated at 25 C and natural light conditions, and at 5 C and 37 C in the dark. Five replicates were performed for each condition.

Growth data. Colony diam. were measured on the 3rd, 7th, 14th and 20th d, and the development of vegetative and reproductive structures was followed on microscopic slides over two months.

Colour identification. The colours of the colonies and microscopic structures were registered according to the colour code-names and numbers (#) given by Séguy (1936).

Physico-chemical properties. The following data were recorded: a) reaction of synnemata to 2% KOH; b) reaction of the yellow amorphous granules encrusted on the hyphae to the following reagents: lactophenol, HNO₃, NaOCl, and FeCl₃; c) fluorescence under short wave (254 nm), and long wave (365 nm) UV light.

Microscopic studies. The morphology of colonies and fungal structures was studied and illustrated under light (LM), differential interference contrast (DIC), and scanning electron (SEM) microscopy. For the latter the material was air dried, coated with gold and observed with a Hitachi 2300 SEM.

Additional material examined for comparison. *Penicillium coalescens* Quintanilla, Spain, Valladolid Province, Montemayor de Pinilla, from a lyophilised type culture, CECT-2764T; and exsiccatum IMI - 27467(T).

Results

The orderly, characteristically biverticillate terminal penicilli suggested classification in *Penicillium* subgenus *Biverticillium* Dierckx. Because of its several distinguishing features it is here described as a new species.

***Penicillium aureocephalum* Munt.-Cvet., Hoyo and Gómez-Bolea
anam. sp. nov.**

(Figs. 1-10)

Etymology: Ad "aurea", golden yellow + "cephalum", head (referring to the appearance and colour of synnemata in nature).

Synnemata in natura globoso-capitata, simplicia; stipites ca. 400 µm alti, basi 150-160 µm lati, sub capitulo 270-280 µm lati; *capitulum* globosum, citrinum vel aureum, ca. 400 µm diam., *hyphae steriles* furcatae, glomerulis aureo-citrinis incrustatae; conidiophora hyphis sterilibus circumdata; cellulae conidiogenae biverticillatae penicillatae ad apicem conidiophorum locatae; metulae 8-11 × 2-3 µm; *phialides* cylindraceae, 5-7 in quoque metula, 8-9 × 2-2.5 µm; *conidia* catenata, ovoidea-ellipsoidea, apice rotundato, base truncata cum cicatrice minuti subrotundi pori 0.6-0.7 µm diam. simile, parietibus crassis et verrucosis, 0-septata, (3-) 3.5 (-4) × 2-2.5 (-3) µm.

Teleomorph: Non visa.

Holotypus: NE Hispania, Catalonia, Prov. Girona, Selva de Mar, Cap de Creus National Park (UTM 31T EG 1586), 150 m alt. BCC Myc. MC 473, Xavier Llimona lectus, die 5, mensis decembris, anno 1999.

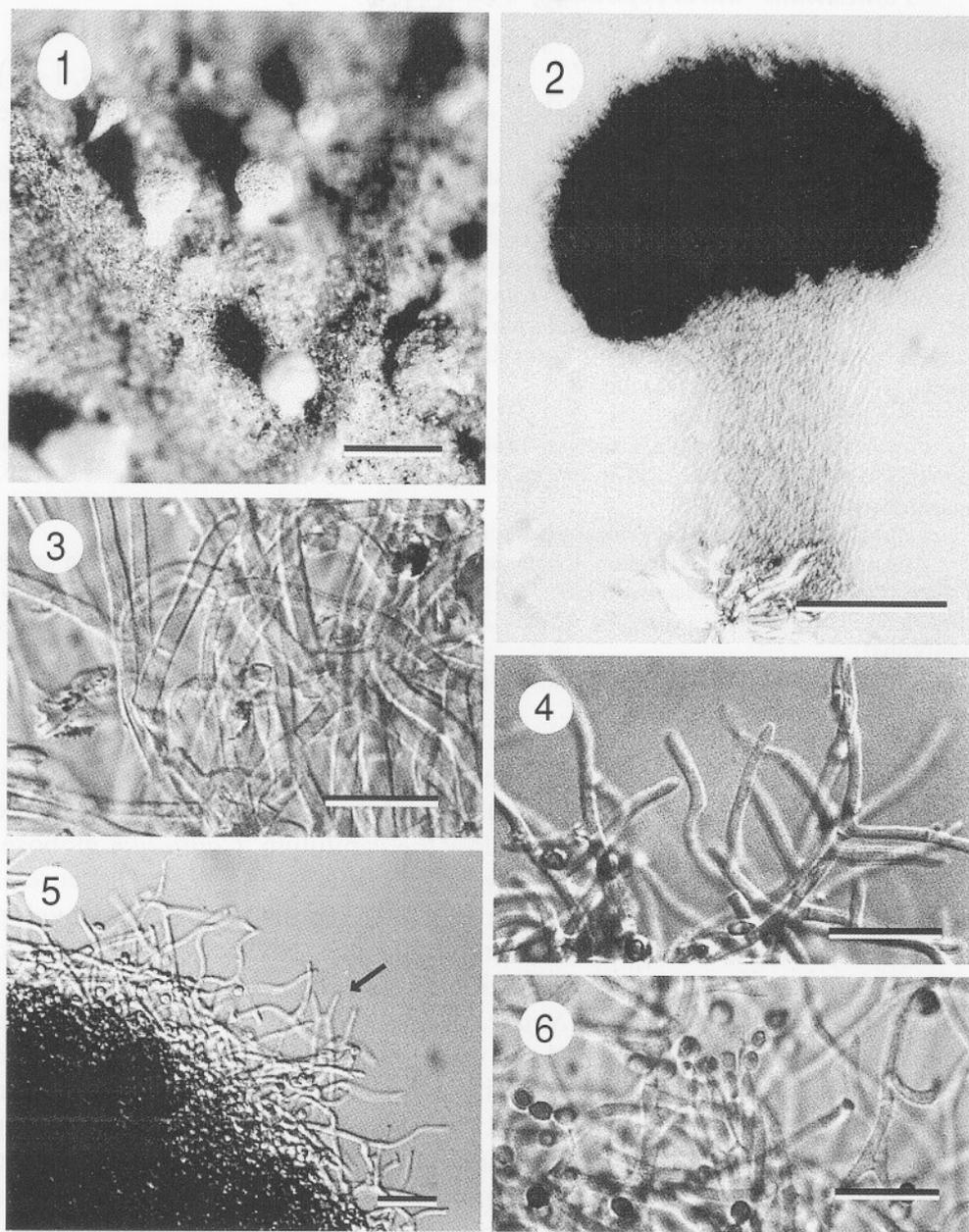
Substratum: Supra foliis emortuis *Quercus suber* ad solum.

Material examined: NE Spain, Catalonia, Girona, Selva de Mar, 150 m, on fallen leaves of *Cistus salviifolius*, 5 December 1999, X. Llimona, BCC.Myc. [MC 473/C, **syntypus**]; on fallen leaves of *Quercus suber*, 24 March 2000, Xavier Llimona, BCC.Myc. [MC 521, **topotypus**].

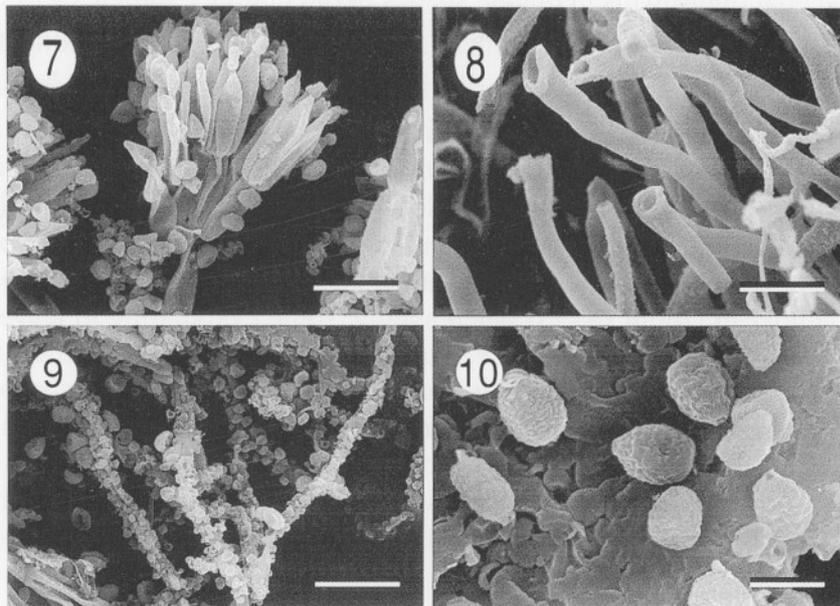
Isotypus a living culture and herbarium specimens in CBS 102801.

Colonies in nature

Conidiomata resembling stalked Myxomycota sporangia (Fig. 1), consisting of synnemata with a stipe ca. 400 µm high, 150-160 µm wide at the base, expanding up to 270-280 µm below the capitulum. *Capitulum* globose, ca. 400 µm diam. (Fig. 1), lemon yellow or yellow under low power because of the yellow granules that coat the upper part of the sterile hyphae that envelop the conidiophores and surpass them forming the cap, olivaceous when seen in microscope squash mounts because of the conidial mass concealed in the fertile region of the synnemata (Fig. 2). *Stipes* composed of hyphae *textura intricata* at the base, then erect, sinuous, 2-4 µm wide, multiseptate, individually hyaline or yellowish, smooth (Figs. 3, 8), terminating as sterile hyphae; sterile hyphae sinuous or curved, much branched or forked (Figs. 4, 5), frequently anastomosing, distally encrusted with amorphous yellow granules up to 1.7 µm diam. (Fig. 9), septate, 1.5-3 µm wide. *Conidiophores* surrounded by sterile hyphae and terminating in well-differentiated penicillate structures (Fig. 6) characteristically of the biverticillate type (Fig. 7); *metulae* slightly divergent, 8-11 × 2-3 µm; *phialides* cylindroid, 5-7 per metula, 8-9 µm in length, 2-2.5(-3) µm at the widest part, near the apex; *conidia* ovoid-ellipsoidal, apex rounded, base truncate with a detachment scar resembling a pore 0.6-0.7 µm diam. (Figs. 6, 10), slightly verrucose, 0-septate, (3-)3.5(-4) × 2-2.5(-3) µm;



Figs. 1-6. *Penicillium aureocephalum*. 1. Synnematosus conidiomata scattered on fallen leaves of *Quercus suber*. 2. Squash mount view of a synnema from nature. 3. Hyphae from the stipe. 4. DIC micrograph of forked sterile hyphae from a MEA colony. 5. Sterile hyphae overgrowing the conidial mass of the cap; the arrow shows an anastomosis. 6. Penicillate structures inside the synnema. Bars: 1 = 600 μm ; 2 = 150 μm ; 3, 4, 6 = 20 μm ; 5 = 40 μm).



Figs. 7-10. SEM micrographs of *Penicillium aureocephalum*. **7.** Biverticillate penicillus. **8.** Hyphae of the synnema. **9.** Sinuous sterile hyphae incrustated with yellow granules. **10.** Conidia. (Bars: 7-9 = 10 μ m; 10 = 2.5 μ m).

conidial mass gathered in the cap amongst the sinuous furcate ends of sterile hyphae (Figs. 2, 5).

Colonies on artificial nutrient media

On CA, 7 d: growth not perceptible at 5 C, 25 C, or 37 C; 14 d: at 25 C very poor colonies, 10 mm diam., transparent, consisting of white, thin, submersed hyphae, conidial structures very scanty and ill-developed; reverse uncoloured.

On CYA, 7 d: growth neither at 5 C nor at 37 C; at 25 C, 9-10 mm diam., greenish grey, plane, thin, with poorly developed funicles, reverse in whitish shades. After 20 d, 40 mm diam., surface thin but uneven, brick red in colour (#186); exudate absent; reverse in the same colours as the surface; diffusible pigment intense cadmium yellow (#226).

On MEA, 7 d: at 5 C, 2 mm diam; at 37 C, 5 mm; at 25 C, 30-33 mm, plane, at first velutinous and powdery because of the heavy conidiogenesis, then lanose or funiculose, greyish green to dull green (#356), surface uneven because of the funicles and the abundant sterile hyphae, commonly zonate,

with mycelial outgrowths in orange shades in old cultures; funicles 4 μm wide; synnemata appearing irregularly after 15-20 d incubation or much longer, with stipes shorter than in nature; sterile hyphae abundant, sinuous, branching, forked (Figs. 5, 6), densely coated with amorphous granules 5-9 μm diam., mostly yellow, sometimes reddish, rarely almost hyaline, giving the colonies a peculiar multi-antennate aspect; conidiophores borne on surface mycelium or on funicles and then 60-80(-180) μm in length, otherwise as in nature; exudate limited or absent in some colonies, clear and abundant as minute guttules light to dark amber in colour (#153) when ageing, some evaporating to leave scant minute pits on the colony surface; margins white when young, then lemon yellow (#272), finally dull yellow in old colonies, coarsely granular or forming cushion-like hirsute yellow masses, surrounded by a 2-4 mm wide subhyaline margin of subsurface hyphae; reverse orange, dark orange, vivid brown red or vinaceous brown in the 10-15 mm wide central area, dull yellowish grey towards the margins; diffusible pigment orange red, very intense in old cultures, covering the whole plate.

On G 25 N, 7 d: growth neither at 5 C nor at 37 C; at 25 C, colonies 4-5 mm diam., attaining 20 mm in 20 d, very pale coloured in "Brun havane" (#131) and "Mastic" (#340) at the margins; reverse in paler shades; exudate and diffusible pigment absent.

Physico-chemical properties. Colour reaction to KOH, coral red; yellow granules, soluble in KOH and in lactophenol; negative reaction to HNO₃, NaOCl, and FeCl₃. Orange fluorescence under UV light.

Discussion

Penicillium aureocephalum exhibits a combination of characters that can be found in particular synnematosus species placed by Pitt (1979) in separate groups of subgenus *Biverticillium*. Colony features and growth rates with standard nutrient media and temperatures, coloured diffusible pigments, encrusted hyphae and penicillus elements can be mentioned among these characters. Their particular combination makes *P. aureocephalum* distinct from other taxa which have been systematized by Pitt (1979) or described later (Quintanilla, 1984; Samson *et al.*, 1989), i.e. *P. coalescens* Quintanilla, *P. dendriticum* Pitt, *P. duclauxii* Delacr., *P. marneffeii* Segretain, Capponi and Sureau, *P. palmae* Samson, Stolk and Frisvad, *P. panamense* Samson, Stolk and Frisvad, and *P. pseudostromaticum* Hodges, Warner and Rogerson. Relationships based on morphological characteristics and profiles of secondary

Table 1. Microscopic characters of selected biverticillate *Penicillium* spp.

<i>Penicillium</i> spp.	Synnemata	Metulae (μm)	Phialides (μm)
<i>P. aureocephalum</i>	Yellow, stipe sterile, 400 \times 160 μm , head globose, 400 μm , concealing conidia	Slightly to moderately divergent, 8-11 \times 2-3	Slightly to moderately divergent, cylindroid, 8-9 \times 2-2.5 (-3)
<i>P. coalescens</i>	Aspergilliform: stipe sterile, 1-1.5 mm tall; head globose, fertile, conidia not hidden.	Compressed, 8-11 \times 2.5-3	Lageniform distally subulate, slender, appressed, 9-12 \times 2-2.5
<i>P. dendriticum</i>	Up to 5 mm tall, conidiophores appressed, non branching	Almost parallel, 10-15 \times 2.5-3.5	Acerose, collula gradually tapering to the apex, 12-15 \times 2-2.2
<i>P. duclauxii</i>	Acicular, undetermined length, no defined fertile regions	Verticils closely packed, 10-12 \times 3.5-4	Acerose-lanceolate, 9-11 \times 2.5-3
<i>P. marneffei</i>	Funiculose growth, stipes 70-100 μm	7-11 (-25) \times 2.5-3	Ampulliform - acerose, 6-8 (-12) \times 2.8-3
<i>P. palmae</i>	Yellow-green, flattened, furcate, up to 8 mm tall, covered with conidioph.	Appressed, slightly inflated at the apex, 10-15 \times 2-2.5	Cylindrical-acerose, neck short, narrow, 10-14 \times 1.8-2.2
<i>P. panamense</i>	White, then yellow, finally green, 5-15 mm tall, stipe sterile, head club-shaped covered with conidioph.	Appressed, slightly inflated at the apex, 8-10 \times 2-2.5	Acerose, parallel, neck short narrow, 9-12 \times 2.2-2.5
<i>P. pseudostromaticum</i>	Stroma-like, 10 mm tall, \pm sterile	Cylindrical, 11-15 \times 2.5-3	Slender, lanceolate, 13-20 \times 2-4

metabolites have been discussed by Samson *et al.* (1989) for several species in this group.

According to Pitt's classification (1979), *Penicillium aureocephalum* belongs in section *Coremigenum* (Biourge) Pitt, series *Dendritica* Pitt, in which synnemata are produced after long incubation periods. But the habit of *P. aureocephalum* as it appears on *Quercus suber* fallen leaves in nature is one of its most notable features: the synnema appears yellow, with well defined middle-sized cylindrical stipe and a yellow globose cap that conceals the conidia.

Special attention was paid to *Penicillium coalescens*, a species that like *P. aureocephalum* is found in Spain. However, the ecological conditions are

Table 2. Colony diam. (mm) and coloured diffusible pigments (- = no diffusible pigments) of *Penicillium aureocephalum* and related biverticillate *Penicillium* species grown upon conventional nutrient media at standard temperatures, after 7 d.

Nutrient media	CYA			MEA			G25N		
	5 C	25 C	37 C	5 C	25 C	37 C	5 C	25 C	37 C
<i>P. aureocephalum</i>	0	9-10	0	2	30-33	5	0	3-6	0
	-	Becoming intense cadmium yellow	-	-	Becoming deep amber red	-	-	-	-
<i>P. coalescens</i>	0	35-40	2-7		45-47			3-6	
	-	-	Purple		-			Intense red violet	
<i>P. dendriticum</i>	0	8-25	0 (-5)		30-40			3-6	0 (-5)
	-	-	-		Sometimes yellow			-	-
<i>P. duclauxii</i>	0-3	15-20			15-25			0-10	
	-	Yellow brown			-			-, usually	
<i>P. marneffei</i>	0	28-32	2-5		28-30				
	-	-	-		Sometimes red				
<i>P. palmae</i>		25-33			22-32				
		-			-				
<i>P. panamense</i>		16-23			28-34				
		-			-				
<i>P. pseudostromaticum</i>	0	15-25 (-30)	0 (-5)		35-45			(2-)4-8	0
	-	Yellow amber	-		-			-	-

quite different. *Penicillium coalescens* was originally recovered from alkaline soil under *Pinus* sp. in the supramediterranean belt, whilst *P. aureocephalum* was found on *Quercus suber* fallen leaves on acid soil in the mesomediterranean belt (Rivas-Martínez, 1987).

Significant differences between these two species are presented in Tables 1 and 2. In *Penicillium aureocephalum* the yellow cushion-like hirsute structures dominating the margins of mature colonies on PDA or MEA are on first sight reminiscent of gymnothecial initials of the teleomorph genus *Talaromyces* C.R. Benj.; they were, however, constituted by aggregations of

sterile hyphae encrusted with amorphous yellow granules and gymnothecia were not seen even after several months.

According to Locquin (1984), as the responses of the hyphal surface grains to the reagents tested in this study were negative, the yellow colour of the globules cannot be attributed to carotenoid compounds.

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