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ORIGINAL ARTICLE

A new lineage of mazaediate fungi in the Eurotiomycetes: Cryptocaliciomycetidae *subclass. nov.*, based on the new species *Cryptocalicium blascoi* and the revision of the ascoma evolution

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Abstract
The class Eurotiomycetes (Ascomycota, Pezizomycotina) comprises important fungi used for medical, agricultural, industrial and scientific purposes. Eurotiomycetes is a morphologically and ecologically diverse monophyletic group. Within the Eurotiomycetes, different ascoma morphologies are found including cleistothecia and perithecia but also apothecia or stromatic forms. Mazaediate representatives (with a distinct structure in which loose masses of ascospores accumulate to be passively disseminated) have evolved independently several times. Here we describe a new mazaediate species belonging to the Eurotiomycetes. The multigene phylogeny produced (7 gene regions: nuLSU, nuSSU, 5.8S nuITS, mtSSU, *RPB1*, *RPB2* and *MCM7*) placed the new species in a lineage sister to Eurotiomycetidae. Based on the evolutionary relationships and morphology, a new subclass, a new order, family and genus are described to place the new species: *Cryptocalicium blascoi*. This calicioid species occurs on the inner side of loose bark strips of *Cupressaceae* (*Cupressus*, *Juniperus*). Morphologically, *C. blascoi* is characterized by having minute apothecioid stalked ascomata producing mazaedia, clavate bitunicate asci with hemiamyloid reaction, presence of hamathecium and an apothecial external surface with dark violet granules that becomes turquoise green in KOH. The ancestral state reconstruction analyses support a common ancestor with open ascomata for all deep nodes in Eurotiomycetes and the evolution of closed ascomata (cleistothecioid in Eurotiomycetidae and perithecioid in Chaetothyriomycetidae) from apothecioid ancestors. The appropriateness of the description of a new subclass for this fungus is also discussed.

Keywords Ascomycota · Cryptocaliciales ord. nov. · Cryptocaliciaceae fam. nov. · Ascoma evolution · Calicioid · Hemiamyloid asci · Spain

Introduction

Eurotiomycetes (Pezizomycotina, Ascomycota) is one of the most diverse groups of fungi concerning morphology and ecology (Geiser et al. 2006). Trophic modes in Eurotiomycetes are varied: saprotrophic, biotrophic, lichen mycobionts, ectomycorrhizal and endophytes (Chen et al. 2015). Different ascoma types (e.g. apothecioid, perithecioid, cleistothecioid, mazaediate) are found in the group (Jaklitsch et al. 2016). Eurotiomycetes includes many species important to human health, industry and basic research (e.g. Alexopoulous et al. 1996; Geiser et al. 2006). The great majority of human pathogenic Pezizomycotina are members of Eurotiomycetes and particularly from Eurotiales, Onygenales and Chaetothyriales (Alexopoulous et al. 1996; Jaklitsch et al. 2016).

Phylogenetic studies have contributed to unravel the evolutionary relationships within the class (e.g. Geiser et al. 2006; Wood et al. 2016; Réblová et al. 2017) and have been used to re-classify taxa accordingly. Recently, a new order and two subclasses have been described (Chen et al. 2015; Gueidan et al. 2014; Wood et al. 2016; Réblová et al. 2017) in the

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Eurotiomycetes. Current problems in the classification of families and orders within this class are the high amount of unknown or undescribed species (Chen et al. 2015), the high presence of species only known from their anamorph (Gibas et al. 2002a; Stchigel et al. 2013), un-assigned paraphyletic taxa (Gueidan et al. 2014) or limited availability of sequence data (Quan et al. 2020). Currently, Eurotiomycetes is generally accepted to comprise 5 subclasses: Chaetothyriomycetidae, Coryneliomycetidae, Eurotiomycetidae, Mycocaliciomycetidae and Sclerococcomycetidae (Dowell 2001; Geiser et al. 2006; Hibbett et al. 2007; Wood et al. 2016; Réblová et al. 2017).

Eurotiomycetidae encompasses three morphologically diverse orders: Arachnomycetales, Eurotiales and Onygenales. Eriksson (1999) classified these orders in the Eurotiomycetes, but they were instead treated as Plectomycetes by Geiser and LoBuglio (2001). Members of Eurotiomycetidae produce different ascoma types including cleistothecia, ascostromata or gymnothecia (Currah 1985, Gibas et al. 2002a, Hibbett et al. 2007, Jaklitsch et al. 2016). In Eurotiomycetidae, asci are usually evanescent, sometimes bitunicate, scattered throughout the ascoma without hamathecium elements and with eight ascospores (Hibbett et al. 2007). They include mostly saprophytic species, and some produce toxic and economically important secondary metabolites, food fermentation enzymes, or are used as genetic models, as *Aspergillus nidulans* (Jaklitsch et al. 2016). Animal-associated fungi are also known (pathogens of vertebrates in Onygenales, Jaklitsch et al. 2016). Chaetothyriomycetidae includes the common black yeast fungi, some of which are pathogens of humans and animals, but also contains lichenized groups and endophytes (Gueidan et al. 2014; Chen et al. 2015). Within this subclass, members of Verrucariales, Chaetothyriales and Pyrenulales are characterized by having perithecial ascomata and bitunicate or secondarily prototunicate asci with a dehiscence ranging from fissitunicate to evanescent and presence of hamathecium structure, producing pseudoparaphyses or periphysoids (Barr 1983). Phaeomoniellales, sister to the clade including Verrucariales and Chaetothyriales, comprises mainly endophytes and plant pathogens with perithecial ascomata when sexual states are present (Chen et al. 2015). Mycocaliciomycetidae includes non-lichenized members of the families Mycocaliciaceae and Sphinctrinaceae (Mycocaliciales, Tibell and Wedin 2000). They produce stalked or sessile apothecial ascomata, and spore dispersal is active or passive when the ascomata develop a mazaedium. Asci are unitunicate, cylindrical, mostly with a distinctly thickened apex and with 8 spores. Members of Mycocaliciomycetidae are parasites or commensals on lichenized or saprotrophic fungi. The new subclass Coryneliomycetidae was introduced by Wood et al. (2016) for the Coryneliaceae. Most members of this group form pseudothecial mazaedial ascomata, with initially bitunicate asci in which the outer wall disintegrates (prototunicate) and the ascospores finally accumulate as a dry mass at the ascoma beak (Johnston and Minter 1989; Geiser et al. 2006); but not all are mazaediate (Garrido-Benavent and Pérez-Ortega 2015). They are mostly biotrophic on leaves and stems, especially on *Podocarpaceae* in the southern hemisphere with some northern temperate species that occur on conifers (Garrido-Benavent and Pérez-Ortega 2015). Although the *Sclerococcum-Dactylospora* lineage was recovered as a distinct lineage in the broad multigene phylogenies by Schoch et al. (2009) and Chen et al. (2015), Sclerococcomycetidae was first formally recognized by Réblová et al. (2017) including in it the single order Sclerococcales. Later, *Dactylospora* was merged with *Sclerococcum* based on the close phylogenetic relationship of their type species (Diederich et al. 2018; Olariaga et al. 2019). Members of this subclass are characterized by apothecial ascomata with unitunicate, non-amyloid asci, covered with an amyloid or hemiamyloid gelatinous cap. The hamathecium consists of persistent pseudoparaphyses. They are non-lichenized, terrestrial, marine, bryophytic, corticolous, lignicolous, lichenicolous or are associated with beetles as a part of their intestinal microbiota. Olariaga et al. (2019) also pointed out the existence of several dematiaceous phialidic fungi within this clade, several strains isolated from digestive tracts of beetles and a new aquatic hyphomycete. Thus, more hyphomycetes are likely to belong to the Sclerococcomycetidae.

Most higher-level Ascomycota systematics hypotheses are based on ascoma type (Nannfeldt 1932; Luttrell 1955; Henssen and Jahns 1974) and ascus morphology and dehiscence (Luttrell 1951; Eriksson 1981; Hafellner 1984). Ancestral character state reconstruction analyses can provide valuable insights to understand the evolution (Schmitt et al. 2009a; Joy et al. 2016) of groups that are prone to shifts in ecological and morphological character states as the Eurotiomycetes (Geiser et al. 2006; Schoch et al. 2009). Regarding ascomata, ancestral state reconstruction analyses support the ancestor of Ascomycota as producing apothecia and show multiple transitions from apothecioid to perithecioid and cleistothecioid ascomata (Schoch et al. 2009), as Nannfeldt (1932) previously hypothesized. In the Eurotiomycetes, the ancestor has been reconstructed as most probably having apothecia with independent origins of perithecioid ascomata in Chaetothyriomycetidae and Eurotiomycetidae (Eurotiomycetes) and cleistothecioid ascomata in Eurotiales and Onygenales (Schoch et al. 2009). Regarding mazaediate ascomata, ancestral state reconstruction analyses showed a high degree of parallel evolution with multiple independent gains of the mazaedium in the Ascomycota (Prieto et al. 2012). Concerning asci, the ancestor of the Eurotiomycetidae and Chaeothyriomycetidae is reconstructed as producing fissitunicate asci; however, the ancestral state of the most basal node of the Eurotiomycetes is not resolved

(Schoch et al. 2009). This suggests the origin of eurotialean prototunicate, deliquescent asci from a fissitunicate ancestor (Geiser et al. 2006). However, new discoveries of novel lineages or the inclusion of new taxa in phylogenies is likely to change our understanding of the evolutionary history of this labile group of fungi concerning ascoma morphology.

The study of a new mazaediate calicioid species suggested a phylogenetic placement within the Eurotiomycetes. Thus, the main aims of this work were to describe this new taxon, to explore its phylogenetic relationships and to analyse the evolution of the ascoma type in the Eurotiomycetes.

Material and methods

Taxon sampling

Preliminar Blast searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) suggested a placement of the new species within the Eurotiomycetes. Thus, in order to place the new species in the Eurotiomycetes, a larger sample of representatives of all subclasses and orders downloaded from GenBank was assembled (Table 1).

Morphological study

The macro- and microscopic description was made from fresh material and was completed by observing material soaked in water or in KOH 5%. Structures were measured from fresh material or from rehydrated material in H_2O. Only mature spores discharged from asci were measured. Spore statistics were calculated for each collection based on measures of 25 spores. Abbreviations of statistics referring to ascospores are L_m = mean length, W_m = mean width and $Q_m = L_m/W_m$. Mounting reagents used were water, Congo red in sodium dodecyl sulphate, Lugol's solution (IKI) and KOH 5%. Material is deposited in ARAN-Fungi, MAF herbaria (Thiers 2014), as well as in J. Etayo's private herbarium.

Culture observations

A dikaryotic culture of *Cryptocalicium blascoi* was obtained by depositing ascospores of a mazaedium on 2% malt extract agar (MEA; 2% malt extract, 2% agar-agar). The plate was sealed with laboratory film and incubated at room temperature. The culture was deposited at the Spanish Type Culture Collection (Spain, CECT). The identity of the culture was confirmed by comparing its nuITS region and sequences obtained from ascomata (Table 1).

Extraction, PCR and sequencing

DNA was extracted using DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The nuITS was amplified employing ITS1F and ITS4 primers (White et al. 1990; Gardes and Bruns 1993). The nuSSU region was amplified using NS1 (White et al. 1990), NS21, nuSSU-1203-3′ (NS23 reverse) and NSU24 (Gargas and Taylor 1992). We amplified nuLSU region with LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990) primers. The mtSSU region was amplified with mtSSU1 and mtSSU3R (Zoller et al. 1999). We used the primers *MCM7*-709for and *MCM7*-1348rev (Schmitt et al. 2009b) for amplification of the *MCM7* region. The protein coding *RPB1* was amplified using the primers *RPB1*-Af and *RPB1*-Cr (Stiller and Hall 1997) and the *RPB2* with *RPB2*-5F and *RPB2*-7cR (Liu et al. 1999). PCR amplifications were performed using Illustra™ Hot Start Mix RTG PCR beads (GE Healthcare, UK) in a 25 μl volume, containing 3 μl of diluted genomic DNA, 0.5-1.5 μl (10 μM) of each primer and distilled water. Amplifications were performed using the following programme: initial denaturation at 95 °C for 15 min, followed by 35–40 cycles of 95 °C for 45 s, 54–56 °C for 50 s, 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. PCR products were subsequently purified using the enzymatic method Exo-sap-IT (USB Corporation, Santa Clara, CA, USA). The purified PCR products were sequenced at Macrogen Europe service (www.macrogen.com), using the same amplification primers. Sequences were assembled and edited using Sequencher v. 4.10.1. (Genes Codes Corporation, Ann Arbor) and deposited in GenBank (Table 1).

Alignments and phylogenetic analyses

Sequences were aligned manually using AliView v.1.26 (Larsson 2014) and translated to amino acids in protein coding loci. Ambiguous regions (sensu Lutzoni et al. 2000) and introns were delimited manually and excluded from phylogenetic analyses. We also used MAFFT v. 7 (Katoh and Standley 2013) to align automatically and Gblocks 0.91b (Castresana 2000) to identify ambiguous regions. Since the maximum likelihood results were very similar between MAFFT-Gblocks and manually constructed matrices, the last ones were used for analyses. The combined alignment is available at TreeBASE (S28198). Individual gene regions were analysed using maximum likelihood-based inference (ML) in RAxML ver. 8.2.12 (Stamatakis 2014) with a GTRGAMMA model for tree inference and rapid bootstrapping with a GTRCAT model. Gene-tree incongruence was checked by comparing maximum likelihood bootstrap values (ML-BS) among individual gene trees. Clades were considered in conflict when a supported clade (bootstrap support >70%) for one marker was contradicted with

Table 1 Specimens used for analysis with GenBank accession numbers. Entries for newly obtained sequences (in boldface) include voucher numbers. *Data not included in the phylogenetic analyses

Taxon name	nrLSU	nrSSU	nrITS	mtSSU	RPB1	RPB2	MCM7
Anthracothecium australiense	FJ358271[1]	FJ358339[1]	-	FJ225773[1]	FJ358403[1]	-	-
Arachniotus littoralis	FJ358272[1]	FJ358340[1]	AJ315833[d]	FJ225784[1]	FJ358404[1]	-	-
Arachnomyces glareosus	FJ358273[1]	FJ358341[1]	AY624316[2]	FJ225785[1]	FJ358405[1]	-	-
Arachnomyces minimus	FJ358274[1]	FJ358342[1]	AY123783[3]	FJ225786[1]	-	-	-
Arthroderma ciferrii	EF413625[4]	EF413624[4]	AJ877217[5]	FJ225787[1]	-	EF413626[4]	-
Arthroderma curreyi	AY176726[6]	AJ315165[d]	AJ877223[d]	-	-	-	-
Ascosphaera apis	FJ358275[1]	X69849[7]	FJ172292[1]	-	FJ358406[1]	-	-
Ascosphaera larvis	FJ358277[1]	-	U68330[8]	FJ225788[1]	JX401215[9]	JX401210[9]	-
Aspergillus fumigatus	AY660917[10]	AB008401[11]	JX944178[d]	-	XM_747744[12]	XM_741647[12]	XM_750254[12]
Baglietoa baldensis	EF643786[4]	EF689823[4]	EF3695229[4]	FJ225666[1]	EF689744[4]	-	-
Byssochlamys nivea	AY176750[6]	M83256[13]	FJ389935[1]	L14500[14]	FJ358408[1]	AF107794[15]	-
Caliciopsis orientalis	DQ470987[16]	DQ471039[16]	-	FJ190654[1]	DQ471185[16]	DQ470939[16]	-
Caliciopsis pinea	DQ678097[16]	DQ678043[16]	-	FJ190653[1]	-	EF411067[4]	-
Capronia fungicola	FJ358224[1]	FJ358292[1]	AF050246[17]	FJ225722[1]	FJ358356[1]	-	-
Capronia munkii	EF413604[4]	EF413603[4]	-	FJ225723[1]	EF413605[4]	EF413606[4]	-
Capronia parasitica	FJ358225[1]	FJ358293[1]	AF050252[17]	-	FJ358357[1]	-	JN993259[d]
Capronia pilosella	AF279378[18]	U42473[19]	AF050255[17]	AY584697[20]	JN989447[d]	JQ027717[d]	JN993263[d]
Ceramothyrium carniolicum	EF413628[4]	AF346418[21]	-	AF346423[21]	FJ358364[1]	EF413630[4]	KC455265[22]
Chaenotheca furfuracea	JX000087[23]	JX000068[23]	JX000101[23]	JX000121[23]	JX000137[23]	-	JX000158[23]
Chaenotheca trichialis	JX000085[23]	JX000069[23]	JX000102[23]	JX000120[23]	JX000136[23]	-	JX000159[23]
Chaenothecopsis savonica	AY796000[24]	U86691[25]	AY795868[24]	-	-	-	-
Cladophialophora boppii	FJ358233[1]	AJ232946[d]	JN882312[d]	FJ225732[1]	FJ358365[1]	-	-
Cladophialophora carrionii	FJ358234[1]	AY554285[d]	EU137266[26]	FJ225733[1]	FJ358366[1]	-	-
Cladophialophora minourae	FJ358235[1]	FJ358303[1]	AY251087[51]	FJ225734[1]	FJ358367[1]	-	-
Cladophialophora modesta	FJ358236[1]	FJ358304[1]	GU225939[d]	FJ225735[1]	FJ358368[1]	-	-
Clavascidium lacinulatum	EF469158[d]	EF689847[50]	EF469155[d]	FJ225689[1]	EF689765[50]	-	-
Coccidioides immitis	AAEC00000000[27]	AAEC00000000[27]	EF186787[d]	L14536[d]	AAEC00000000[27]	AAEC00000000[27]	-
Corynelia uberata	-	JQ663845[d]	JF811343[d]	-	-	-	-
***Cryptocalicium blascoi* (Holotype)**	**MW999967**	**-**	**MW999967**	**MZ004861**	**MZ020968**	**MZ020967**	**MZ020966**
***Cryptocalicium blascoi* (Etayo 31798)**	**MW999951**	**MW999950**	**MW999969**	**MZ004862**	**-**	**-**	**-**
Cryptocalicium blascoi* (culture ex Holotype)	**-**	**-**	**MW999968**	**-**	**-**	**-**	**-**
Cryptocalicium blascoi* (Etayo 30875)	**-**	**-**	**MW999969**	**-**	**-**	**-**	**-**
Ctenomyces serratus	FJ358282[1]	FJ358347[1]	AJ877222[5]	FJ225789[1]	-	-	-
Cyphellophora europaea	NG_067264[1]	NG_062768[1]	KF928467[28]	-	FJ358380[1]	-	
Cyphellophora laciniata	EF413619[4]	EF413618[4]	EU035416[29]	FJ225737[1]	FJ358370[1]	-	-
Dermatocarpon miniatum	EF469160[4]	EF689834[4]	DQ782837[30]	AY584616[20]	EF689752[4]	DQ782863[30]	-
Dolabra nepheliae	GU332516[31]	-	GU345749[31]	GU332518[31]	GU332520[31]	-	-
Eremascus albus	AY004345[32]	M83258[13]	U18359[d]	-	FJ358410[1]	-	-
Eremicella nidulans	AF454167[10]	U77377[d]	-	-	XM_653321[33]	XM_677297[33]	BN001301[34]
Exophiala bergeri	FJ358240[1]	FJ358308[1]	EF551462[d]	FJ225738[1]	FJ358371[1]	-	-
Exophiala castellanii	FJ358241[1]	X78480[35]	GU225940[d]	FJ225739[1]	FJ358372[1]	-	-
Exophiala dermatitidis	DQ823100[30]	DQ823107[30]	DQ826738[30]	FJ225740[1]	DQ840555[30]	DQ840562[30]	-
Exophiala jeanselmei	FJ358242[1]	FJ358310[1]	JN625228[d]	-	FJ358373[1]	AF107796[15]	-
Exophiala lecanii-corni	FJ358243[1]	FJ358311[1]	AY857528[36]	FJ225741[1]	FJ358374[1]	-	-
Exophiala nigra	FJ358244[1]	X91896[d]	EF551550[37]	FJ225742[1]	FJ358375[1]	-	-
Exophiala oligosperma	FJ358245[1]	AY554287[d]	JN625230[d]	FJ225743[1]	FJ358376[1]	-	-
Exophiala pisciphila	DQ823101[30]	DQ823108[30]	JF747131[38]	FJ225744[1]	DQ840556[30]	DQ840563[30]	-
Exophiala salmonis	EF413609[4]	EF413608[4]	JF747139[38]	FJ225745[1]	EF413610[4]	EF413611[4]	-
Exophiala xenobiotica	FJ358246[1]	FJ358314[1]	JN625227[d]	FJ225746[1]	FJ358377[1]	-	-
Fonsecaea monophora	FJ358247[1]	FJ358315[1]	EU938579[39]	FJ225747[1]	FJ358378[1]	-	-
Granulopyrenis seawardii	EF411062[4]	EF411059[4]	-	-	-	EF411065[4]	-
Gymnoascus reessii	FJ358284[1]	FJ358349[1]	JF922021[40]	FJ225790[1]	FJ358411[1]	-	-
Histoplasma capsulatum	GCF000149585[41]	GCF000149586[41]	AF322382[d]	-	GCF000149585[41]	GCF000149586[41]	-
Knufia perforans	FJ358237 [1]	FJ358305[1]	AJ244230[d]	FJ225736[1]	-	-	-
Leiothecium ellipsoideum	FJ358285[1]	FJ358350[1]	-	FJ225779[1]	FJ358412[1]	JN121541[42]	-
Leucothecium emdenii	FJ358286[1]	FJ358351[1]	-	FJ225791[1]	FJ358413[1]	-	-
Mycocalicium polyporaeum	AY789362[43]	AY789361[43]	AY789363[43]	-	-	-	-
Neophaeococcomyces catenatus	-	FJ358316[1]	JN040512[44]	FJ225749[1]	FJ358379[1]	-	-

Table 1 (continued)

Taxon name	nrLSU	nrSSU	nrITS	mtSSU	RPB1	RPB2	MCM7
Onygena corvina	FJ358287[1]	AB075364[45]	-	FJ225792[1]	FJ358414[1]	-	-
Peltula umbilicata	AF356689[46]	AF356688[46]	DQ832333[30]	-	-	AY641046[47]	-
Penicillium freii	JN939314[48]	JN939217[48]	JN942735[48]	-	JN985108[48]	JN985395[48]	JN993462[48]
Penicillium limosum	EF411064[4]	EF411061[4]	GU981568[49]	-	-	EF411068[4]	-
Penicillium javanicum	EF413621[4]	EF413620[4]	GU981613[49]	FJ2257781[1]	JN121651[42]	EF413622[4]	-
Phaeomoniella chlamydospora	AF353609[52]	-	AF197973[53]	-	-	-	-
Phaeomoniella prunicola	GQ154615[54]	GQ154636[54]	GQ154592[54]	-	-	-	-
Pseudotulostoma japonica	AB161193[d]	AB161195[55]	-	-	-	-	-
Pyrenula aspistea	EF411063[4]	EF411060[4]	JQ927450[56]	-	-	EF411066[4]	-
Pyrgillus javanicus	DQ823103[30]	DQ823110[30]	DQ826741[30]	FJ225774[1]	DQ842010[30]	DQ842009[30]	-
Sclerococcum mangrovei	FJ176890[d]	FJ176836[d]	-	KJ766383[57]	KJ766849[57]	FJ238375[d]	
Sclerococcum parasiticum	MK759892[58]	MK759888[58]	-	MK759899[58]	-	-	
Pseudosclerococcum golindoi	MK759890[58]	MK759887[58]	MK759885[58]	MK759904[58]	-	-	
Sphinctrina turbinata	EF413632[4]	EF413631[4]	AY795876[24]	FJ713611[d]	-	EF413633[4]	-
Spiromastix warcupii	DQ782909[30]	DQ782882[30]	DQ782848[30]	FJ225794[1]	EF413613[4]	DQ782870[30]	-
Staurothele areolata	EF643772[50]	EF689856[50]	JQ927448[56]	FJ225699[1]	EF689775[50]	-	-
Stenocybe pullatula	AY796008[24]	U86692[25]	AY795878[24]	-	-	-	-
Thermoascus crustaceus	FJ358289[1]	GU733371[d]	JF412002[59]	FJ225781[1]	JN121591[42]	JF417417[42]	-
Trichocoma paradoxa	FJ358290[1]	FJ358354[1]	JN899398[60]	FJ225782[1]	JN121718[42]	JN121550[42]	-
Wahlenbergiella striatula	EF643810[50]	EF689882[50]	-	FJ225721[1]	EF689810[50]	-	-
Xeromyces bisporus	FJ358291[1]	FJ358355[1]	JF922074[40]	FJ225783[1]	FJ358416[1]	JN121466[42]	-

[d] Direct submission, [1] Gueidan et al. 2008,[2] Gibas et al. 2004, [3] Gibas et al. 2002b, [4] Geiser et al. 2006, [5] Brasch and Graser 2005, [6] Untereiner et al. 2002, [7] Wilmotte et al. 1993, [8] Anderson et al. 1998, [9] Klinger et al. 2013, [10] Hinrikson et al. 2005, [11] Nikkuni et al. 1998, [12] Nierman et al. 2005, [13] Berbee and Taylor 1992b, [14] LoBuglio et al. 1993, [15] Liu et al. 1999, [16] Spatafora et al. 2006[17] Untereiner and Naveau 1999, [18] Bhattacharya et al. 2000, [19] Berbee 1996, [20] Lutzoni et al. 2004, [21] Lindemuth et al. 2001, [22] Reblova et al. 2013, [23] Prieto et al. 2013, [24] Tibell and Vinuesa 2005, [25] Wedin and Tibell 1997, [26] de Hoog et al. 2007, [27] Neafsey et al. 2010, [28] Attili-Angelis et al. 2014, [29] Crous et al. 2007, [30] James et al. 2006, [31] Rossman et al. 2010, [32] Lumbsch et al. 2000, [33] Galagan et al. 2005, [34] Wortman et al. 2009, [35] Haase et al. 1995, [36] Prenafeta-Boldu et al. 2006, [37] Zeng and De Hoog 2008, [38] de Hoog et al. 2011, [39] Najafzadeh et al. 2009, [40] Pettersson et al. 2011, [41] Sharpton et al. 2009, [42] Houbraken and Samson 2011, [43] Wang et al. 2005[44] Tsuneda et al. 2011, [45] Sugiyama et al. 2002, [46] Lutzoni et al. 2001, [47] Reeb et al. 2004, [48] Schoch et al. 2012, [49] Houbraken et al. 2011, [50] Gueidan et al. 2007, [51] Braun et al. 2003, [52] Weber et al. 2002, [53] Groenewald et al. 2001, [54] Damm et al. 2010, [55] Masuya and Asai 2004, [56] Weerakoon et al. 2012, [57] Miadlikowska et al. 2014, [58] Olariaga et al. 2019, [59] Morgenstern et al. 2012, [60] Samson et al. 2011

significant support by another. Because no supported nodes were in conflict, the data were concatenated. Best fitting substitution models and partitioning scheme for the concatenated 7-locus alignment were inferred by using a greedy algorithm using the AICc in PartitionFinder v. 2.1.1 (Guindon et al. 2010; Lanfear et al. 2017). Based on previous phylogenies (e.g. Prieto et al. 2019), a species of Lichinomycetes (*Peltula umbilicata*) was used as outgroup. The maximum likelihood analysis was conducted in RAxML ver. 8.2.12 (Stamatakis 2014) using the partitioning scheme suggested by PartitionFinder v.2.1.1 and a GTRGAMMA model. Rapid bootstrapping was run with the GTRCAT model. We also carried out a Bayesian analysis using MrBayes v.3.2.7a (Ronquist et al. 2012). The analyses consisted of four parallel runs of Metropolis-coupled Markov chain Monte Carlo for 50 M generations, starting from a random tree, and sampling one tree every 1000 generations from the posterior distribution. A burn-in sample of 100,000 trees was discarded. To estimate branch lengths and posterior probabilities (PPs), a 50% majority rule consensus tree was computed with the remaining 100,004 trees using the SUMT command of MrBayes. Chains were considered to have converged when average standard deviation of split frequencies across runs dropped below 0.01. Maximum likelihood and Bayesian analyses were run on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The resulting trees were edited in Figtree ver 1.3.1. (Rambaut 2010).

Evolution/ancestral state reconstruction for ascoma morphology

Although a high diversity of ascoma types is found in the Eurotiomycetes (Alexopoulous et al. 1996; Jaklitsch et al. 2016), states were coded as open (apothecioid), operculate (perithecioid) or closed (cleistothecioid) ascomata. A fourth character state was coded as absent when asci are not produced in a sporocarp or sexual morphs are unknown.

We inferred ancestral states and traced the evolution of ascoma morphology, using different methodologies and the last 5000 trees that resulted from each run from the Bayesian analysis of the concatenated data set (20000 trees in total). Maximum likelihood and parsimony ancestral state reconstructions were performed in Mesquite 2.75 (Maddison and Maddison 2019) with the ML model Mk1 and equal

probability for any particular character change. To account for topological uncertainty, we used the "trace character over trees" option that summarizes the ASR over a series of trees. We reconstructed the ancestral states using the Bayesian approach described by Pagel et al. (2004) and Pagel and Meade (2006), implemented in BayesTraits v. 3.02 (www.evolution.rdg.ac.uk). For this purpose, we used the same posterior tree sample as for the maximum likelihood and parsimony ancestral state reconstructions, with an exponential prior with the mean drawn from a uniform hyperprior on the 0–10 interval. The MCMC was ran for 100M generations, sampling every 1000 generations. The first 10M generations were discarded as burn-in.

Results

Based on the AICc, Partition Finder selected 9 partitions: nuLSU, nuSSU, mtSSU, 1st and 2nd positions of *RPB1*, 1st and 2nd position of *MCM7* and *RPB2*, 3rd position of *RPB1*, 3rd position of *RPB2*, all with a GTR+I+G model, 5.8S nuITS with a SYM+I+G model and the 3rd position of *MCM7* with an HKY+G model. The best maximum likelihood tree with bootstrap support and posterior probabilities from the two analyses (maximum likelihood and Bayesian) is provided in Fig. 1, together with the results from the Bayesian ancestral state reconstruction analysis. Main supported clades obtained agree with previous studies (Gueidan et al. 2014; Chen et al. 2015; Réblová et al. 2017). Contrary to the results by Wood et al. (2016), the position of Coryneliomycetidae is statistically supported. The Eurotiomycetes is highly supported as containing the Mycocaliciomycetidae, Sclerococcomycetidae and Chaetothyriomycetidae. The new species forms a supported clade sister to the Eurotiomycetidae, forming a supported clade together with Coryneliomycetidae. The ancestral state reconstruction analyses suggest that the ancestor of Eurotiomycetes has open ascomata (100% for ML and parsimony; open: 0.991, operculate: 0.006 and closed: 0.02 in Bayesian), the same as the ancestor of the basal node Chaetothyriomycetidae-Sclerococcomycetidae-Eurotiomycetidae-Coryneliomycetidae-*Cryptocalicium* (100% for ML and parsimony; open: 0.898, operculate: 0.083 and closed: 0.018 in Bayesian), while the ancestor of the Chaetothyriomycetidae is recovered as having operculate ascomata (100% for ML and parsimony; open: 0.002, operculate: 0.996 and closed: 0.0002 in Bayesian), and the one of Eurotiomycetidae as producing closed ascomata (100% for ML and parsimony; open: 0.0005, operculate: 0.00004 and closed: 0.999 in Bayesian). The state of the ancestor of Eurotiomycetidae-Coryneliomycetidae and *Cryptocalicium* is not resolved in parsimony and ML (2.31% open ascomata for parsimony, 30.50% in ML) but is resolved as having open ascomata in the Bayesian: 0.845 (operculate: 0.07, and closed: 0.07). The common ancestor of Chaetothyriomycetidae-Eurotiomycetidae-Coryneliomycetidae and *Cryptocalicium* is reconstructed as having open ascomata in the Bayesian: 0.763 (operculate: 0.2 and closed: 0.03 in the Bayesian), with no states in the parsimony, and 60.59% open ascomata in the ML. The clade including Eurotiomycetidae and *Cryptocalicium* is also reconstructed as producing open ascomata (open ascomata: 0.795, operculate: 0.008 and closed: 0.195 in Bayesian, but 11% open ascomata for parsimony, 57.13% open ascomata in ML).

Taxonomy

Cryptocaliciomycetidae M. Prieto, Etayo and Olariaga, *subclass nov.*

Mycobank: MB 837506.

Ascomata apothecioid, stalked, producing a mazaedium. Hymenium with septate, sterile protruding elements. *Asci* clavate and with a long pedicel, bitunicate with evanescent walls. *Ascospores* globose to subglobose, simple, pale brown, thick-walled, passively released.

Cryptocaliciales M. Prieto, Etayo and Olariaga, *ord. nov.*

Mycobank: MB 837505.

Ascomata apothecioid, stalked, producing a mazaedium. Hymenium with septate, sterile protruding elements. *Asci* clavate and with a long pedicel, bitunicate with evanescent walls, amyloid after a KOH + IKI treatment. *Ascospores* globose to subglobose, simple, pale brown, thick-walled, passively released.

Cryptocaliciaceae Etayo, Olariaga and M. Prieto, *fam. nov.*

Mycobank: MB 837504.

Family of saprotrophic inoperculate ascomycetes. *Ascomata* apothecioid, stalked, producing a mazaedium. Hymenium with septate, sterile protruding elements. *Asci* clavate and with a long pedicel, bitunicate with evanescent walls, amyloid after a KOH + IKI treatment. *Ascospores* globose to subglobose, simple, pale brown, thick-walled, passively released.

Type: *Cryptocalicium* Etayo, Olariaga and M. Prieto

Cryptocalicium Etayo, Olariaga and M. Prieto, *gen. nov.*

Mycobank: MB 837503.

Etymology: referring to the calicioid ascomata that occur hidden under the bark.

Ascomata apothecioid, stalked, producing a mazaedium. *Hymenium* with septate, sterile protruding elements. *Asci* clavate and with a long pedicel, bitunicate with evanescent walls, amyloid after a KOH + IKI treatment. *Ascospores* globose to subglobose, simple, pale brown, thick-walled, passively released. *Stalk* corticate. Outermost layer of the stipe composed of 1–2 rows of cylindrical hyphae, aseptate, dark reddish brown, tightly adhered. Stalk medulla composed of cylindrical hyphae, hyaline. *Pigment* granules on the external surface of

ascomata, dark violet in water, partly dissolving and turning turquoise green in KOH.

Type: *Cryptocalicium blascoi* Etayo, Olariaga and M. Prieto

Cryptocalicium blascoi Etayo, Olariaga and M. Prieto, sp. nov.

MycoBank: 837519.

Holotypus: Spain, Madrid, Hoyo de Manzanares, Parque de la Cabilda, 40.626832 -3.897757, on the inner side of loose bark strips of *Juniperus oxycedrus*, 15 May 2020, leg. I. Olariaga, ARAN-Fungi 14723. Ex-type culture: CECT 21190.

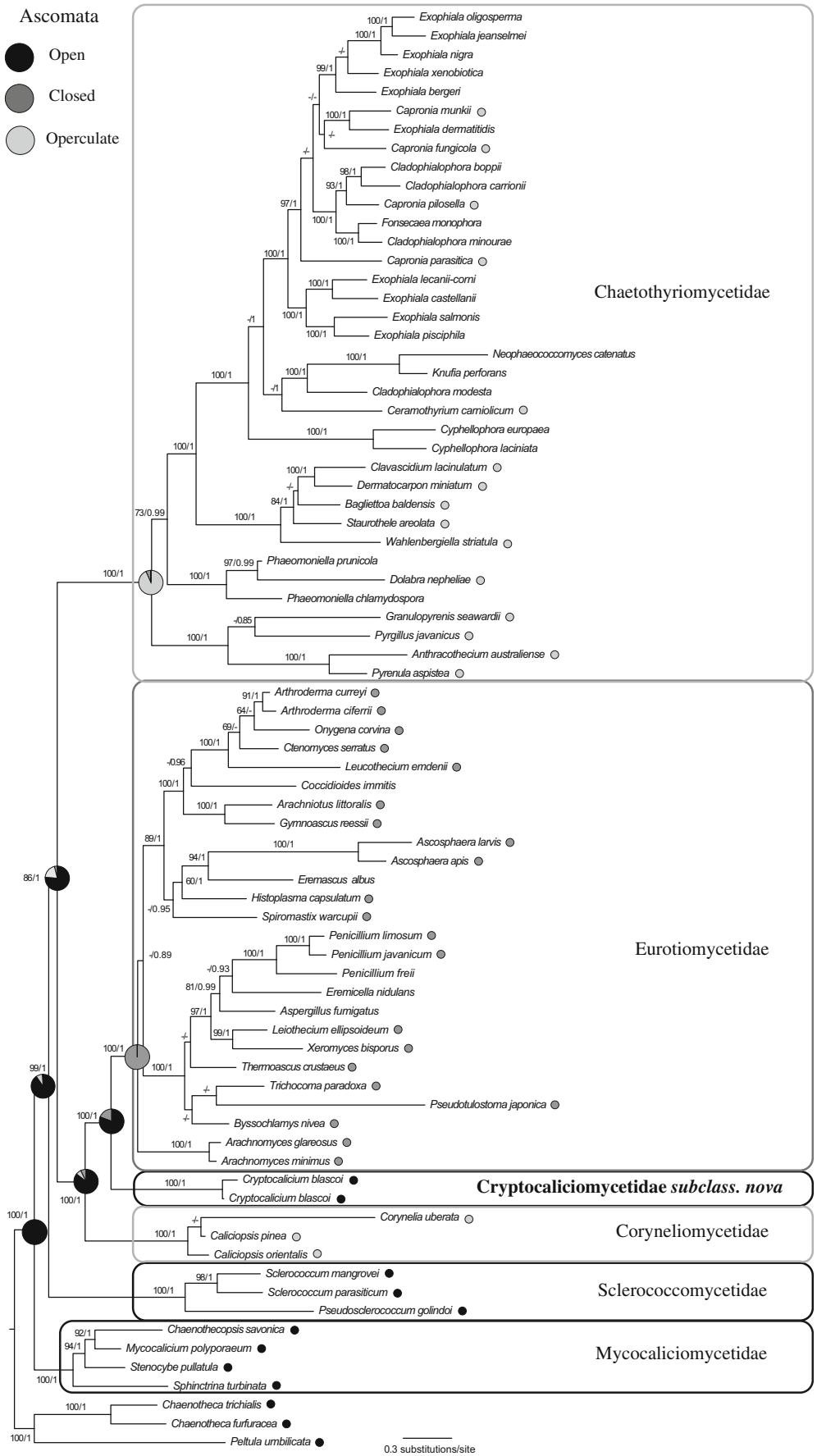
Etymology: named after Javier Blasco Zumeta, outstanding Aragonese naturalist, who showed to us the first locality where this species was found and who provided us with further material of it.

Ascomata saprobic on the inner side of loose bark strips (Fig. 1A–B) still attached to the tree, solitary or gregarious, 0.15–0.36 mm high (mazaedium excluded) (Fig. 2 C–H), Fig. 3A). *Capitulum* urniform to hemispherical, 0.05–0.10 × 0.10–0.15 mm, dark violaceous brown when wet, black and shiny when dehydrated, monocephalic, with an ochre disc. *Mazaedium* well developed, light ochre (4A6) to greyish green (1D2), spores that keep together. *Stalk* cylindrical, sometimes with a broader base, straight, seldom bent, smooth, black, shiny, 0.10–0.26 × 0.02–0.04 mm.

Ascospores (Fig. 3B) globose to subglobose, rarely ellipsoid, hyaline inside asci, pale brown when mature, with homogeneous content, with two minute refractive bodies in Congo Red (Fig. 3C), smooth when viewed in a light microscope, (3)3.3–4(4.7) × 2.8–3.5 (4.0) μm (L_m=3.7–3.9, W_m=3.2–3.3, Q=1.08–1.25(1.36), Q_m=1.15–1.19; n=25), slightly thick-walled (0.2–0.4 μm). *Asci* clavate, bitunicate, initially thick-walled (wall 1 μm), then thin-walled, evanescent, 8-spored, 20–27 μm long; pedicel 1 μm diam.; sporiferous part 10–16 × 5–7 μm (Fig. 3D–H). *Hamathecium* composed of regularly scattered sterile elements, protruding 10–30 μm over mature asci, cylindrical, obtuse, with 2–4 septa, hyaline to very pale brown, smooth or slightly rough, 32–40 × 1.5–2 μm (Fig. 3I). *Subhymenium* poorly differentiated from the medullary excipulum, composed of a *textura angularis-globulosa* with more or less isodiametrical hyphae, pale brown in water, green in IKI (Fig. 3J) and amyloid in KOH+IKI (Fig. 3K). *Stalk* corticate (Fig. 3 L–M). Outermost layer of the stipe composed of 1–2 rows of parallel-arranged hyphae, cylindrical, aseptate, slightly thick-walled, dark reddish brown, tightly adhered, unchanged in KOH, 2.7–3.5 μm broad. Stalk medulla composed of parallel-arranged hyphae, cylindrical, septate, hyaline, thick-walled (0.2–0.4), 1.2–1.6 μm broad. *Ectal excipulum* poorly differentiated from the medullary excipulum, composed of periclinal-arranged hyphae, of *textura prismatica* in surface view (Fig. 3N), with cells progressively shorter towards the margin of the capitulum, pale brown, thick-walled (0.5–1 μm), 3.5–5.5 μm broad, covered with crusts of dark reddish brown pigment (Fig. 3O), unchanged in KOH. *Medullary excipulum* 15–20 μm thick, composed of a *textura angularis-globulosa* with more or less isodiametrical hyphae, hyaline to very pale brown, thin-walled, 2–4 μm broad, non-gelatinized. *Pigment* granules present on the ectal excipulum (Fig. 3O–P), surface of the stipe and the subhymenium, dark violet, opaque, partly dissolving and turning turquoise green in KOH (Fig. 3Q).

Colonies on MEA 9–17 mm diam and 4–6 mm high after 90 days at 5 °C, superficial, effuse, convex to obtusely conical, initially even, later wrinkled-cerebriform, cauliflower-like, cream white, tomentose (Fig. 4A). Reverse pale brownish ochre. Margin lobed, distinct (Fig. 4B). Vegetative hyphae sometimes arranged in parallel, cylindrical, septate, sometimes constricted at septa, with sparse intercalary connections, hyaline, smooth, 1.5–2 μm broad (Fig. 4C). *Asexual morph* hyphomycete-like, observed only in culture. Conidiophores 30–48 μm long, covering the surface of cultures, basally branched, formed by 2–6(8) catenulate segments (Fig. 4D). Segments irregular in shape, usually cylindrical to subglobose, often triangular or angled, constricted at septa and connected to each other by 1–3, thin, 1 μm broad appendices, easily disarticulating in microscopic preparations, 5–9 × 3–6.5 μm (Fig. 4E). Conidiogenous cells cylindrical to ovoid, 5–12 × 1.5–4 μm, bearing one simple apical conidiogenous locus, 1 μm broad, to which immature conidia are attached. Conidia single, first pyriform then subglobose, slightly truncate at the basal end, thin-walled, smooth, hyaline, with small oily droplets, 2.3–3 μm (L_m = 2.6) (Fig. 4F).

Additional specimens examined: SPAIN. Ávila: El Barraco, south shore of Embalse del Burguillo, Peña del Roble, 40.409325 -4.567302, on bark strips of *Juniperus oxycedrus*, 3 October 2020, M. Prieto & I. Olariaga, ARAN-Fungi 14748. Burgos: Briongos de Cervera, espacio natural La Yecla y Sabinares de Arlanza, stand with large *J. thurifera* trees, 300 m ahead the town, 41.913889 -3.497222, 1095 m, on bark strips of *Juniperus thurifera,* 04 August 2019, J. Etayo 31925 (MAF). Santo Domingo de Silos, track from Carazo, espacio natural La Yecla y Sabinares de Arlanza, km 48, 1130 m, on bark strips of *J. thurifera*, 41.958333 -3.400000, 04 August 2019, J. Etayo 31836 (hb. Etayo). Madrid: Hoyo de Manzanares, Camino del Cementerio, 40.623941 -3.898383, 20 m from the crossing with M-618 road, on bark strips of *Cupressus sempervirens*, 4 June 2020, I. Olariaga, ARAN-Fungi 14725. Moralzarzal, La Navata, near the Arroyo de Peregrinos stream, 40.617972 -3.951778, on loose bark strips of *Juniperus oxycedrus*, 7 June 2020, M. Prieto and I. Olariaga, ARAN-Fungi 14724. Pedrezuela, El Tiradero, 40.740483 -3.637523, on bark of a large *J. oxycedrus* tree, 27 March 2021, M. Prieto & I. Olariaga, ARAN-Fungi 15628. Soto del Real, close to


Ascomata
Open
Closed
Operculate
Exophiala oligosperma
Exophiala jeanselmei
Exophiala nigra
Exophiala xenobiotica
Exophiala bergeri
Capronia munkii
Exophiala dermatitidis
Capronia fungicola
Cladophialophora boppii
Cladophialophora carrionii
Capronia pilosella
Fonsecaea monophora
Cladophialophora minourae
Capronia parasitica
Exophiala lecanii-corni
Exophiala castellanii
Exophiala salmonis
Exophiala pisciphila
Neophaeococcomyces catenatus
Knufia perforans
Cladophialophora modesta
Ceramothyrium carniolicum
Cyphellophora europaea
Cyphellophora laciniata
Clavascidium lacinulatum
Dermatocarpon miniatum
Bagliettoa baldensis
Staurothele areolata
Wahlenbergiella striatula
Phaeomoniella prunicola
Dolabra nepheliae
Phaeomoniella chlamydospora
Granulopyrenis seawardii
Pyrgillus javanicus
Anthracothecium australiense
Pyrenula aspistea
Chaetothyriomycetidae
Arthroderma curreyi
Arthroderma ciferrii
Onygena corvina
Ctenomyces serratus
Leucothecium emdenii
Coccidioides immitis
Arachniotus littoralis
Gymnoascus reessii
Ascosphaera larvis
Ascosphaera apis
Eremascus albus
Histoplasma capsulatum
Spiromastix warcupii
Penicillium limosum
Penicillium javanicum
Penicillium freii
Eremicella nidulans
Aspergillus fumigatus
Leiothecium ellipsoideum
Xeromyces bisporus
Thermoascus crustaeus
Trichocoma paradoxa
Pseudotulostoma japonica
Byssochlamys nivea
Arachnomyces glareosus
Arachnomyces minimus
Eurotiomycetidae
Cryptocalicium blascoi
Cryptocalicium blascoi
Cryptocaliciomycetidae subclass. nova
Corynelia uberata
Caliciopsis pinea
Caliciopsis orientalis
Coryneliomycetidae
Sclerococcum mangrovei
Sclerococcum parasiticum
Pseudosclerococcum golindoi
Sclerococcomycetidae
Chaenothecopsis savonica
Mycocalicium polyporaeum
Stenocybe pullatula
Sphinctrina turbinata
Mycocaliciomycetidae
Chaenotheca trichialis
Chaenotheca furfuracea
Peltula umbilicata
0.3 substitutions/site

◀ **Fig. 1** Best maximum likelihood tree obtained from RAxML based on a 7-locus data set (5.8S nuITS, nuLSU, nuSSU, mtSSU, *MCM7*, *RPB1* and *RPB2*). Nodes show bootstrap support (ML-BS) from maximum likelihood, and posterior probabilities (PP) obtained in the Bayesian analysis, ordered as ML-BS/PP. ML-BS below 60% and PP below 0.85 are not shown. Results from the ancestral state reconstruction with BayesTraits are shown in the studied nodes. Ascoma types are also indicated except for those species devoid of sporocarps or with unknown sexual morphs

Arroyo del Recuenco, 40.754794 -3.834830, on the underside of bark strips of *Juniperus oxycedrus*, on dried resin drops, 13 June 2020, M. Prieto and I. Olariaga, ARAN-Fungi 14726. Soria: Borobia, El Frontón, 41.646448 -1.913891, on bark strips of *Juniperus thurifera*, 17 October 2020, I. Olariaga, ARAN-Fungi 15627. Toledo: Almorox, near arroyo de Labros, 40.300343 -4.243062, on bark strips of *Juniperus oxycedrus*, 10 October 2020, M. Prieto and I. Olariaga, ARAN-Fungi 14747. Zaragoza: Pina de Ebro, Retuerta de Pina, on underside of bark strips of *Juniperus thurifera*, 9 October 2017, J. Blasco, Etayo 30875, 31798 (hb. Etayo).

Comments

Cryptocalicium blascoi is probably the tiniest known calicioid fungus. The unusual ecology on the underside of bark strips of Cupressaceae, the presence of a mazaedium, the clavate hemiamyloid asci, hamathecial filaments and the dark violet pigment granules that turn blue-green in KOH make *C. blascoi* unique. Our attempts to morphologically identify *C. blascoi* with the existing literature on calicioid fungi (e.g. Tibell 1999) failed, and molecular data confirmed that *C. blascoi* does not group in any lineage of known calicioid fungi. The clavate asci, with a pedicel, bitunicate and evanescent, are consistent with the phylogenetic position of *C. blascoi* between the Eurotiomycetidae and the Coryneliomycetidae, both having these characteristics as well. The hemiamyloid reaction of asci and the presence of hamathecial filaments, however, have not been cited for any species in those groups.

So far, *C. blascoi* has been found in areas with large ancient *Juniperus oxycedrus* and *J. thurifera* trees in continental Mediterranean areas. In central Spain (Ávila, Madrid, Soria, Toledo), *C. blascoi* has been detected in almost every large *J. oxycedrus* tree sampled in search for it. Although *C. blascoi* can be very hardly observed in nature even using a hand lens of 10–20 ×, it can be collected by gathering loose bark strips and by scrutinizing those under the dissecting scope. *Cryptocalicium blascoi* grows directly on the inner part of the bark and occurs often on blackened zones. We have observed several times that a few ascomata grew on solid resin drops. Our efforts to find *C. blascoi* in areas with higher precipitation rates or where large ancient Cupressaceae do not exist (Huesca, Navarre) failed so far. Thus, it is possible that

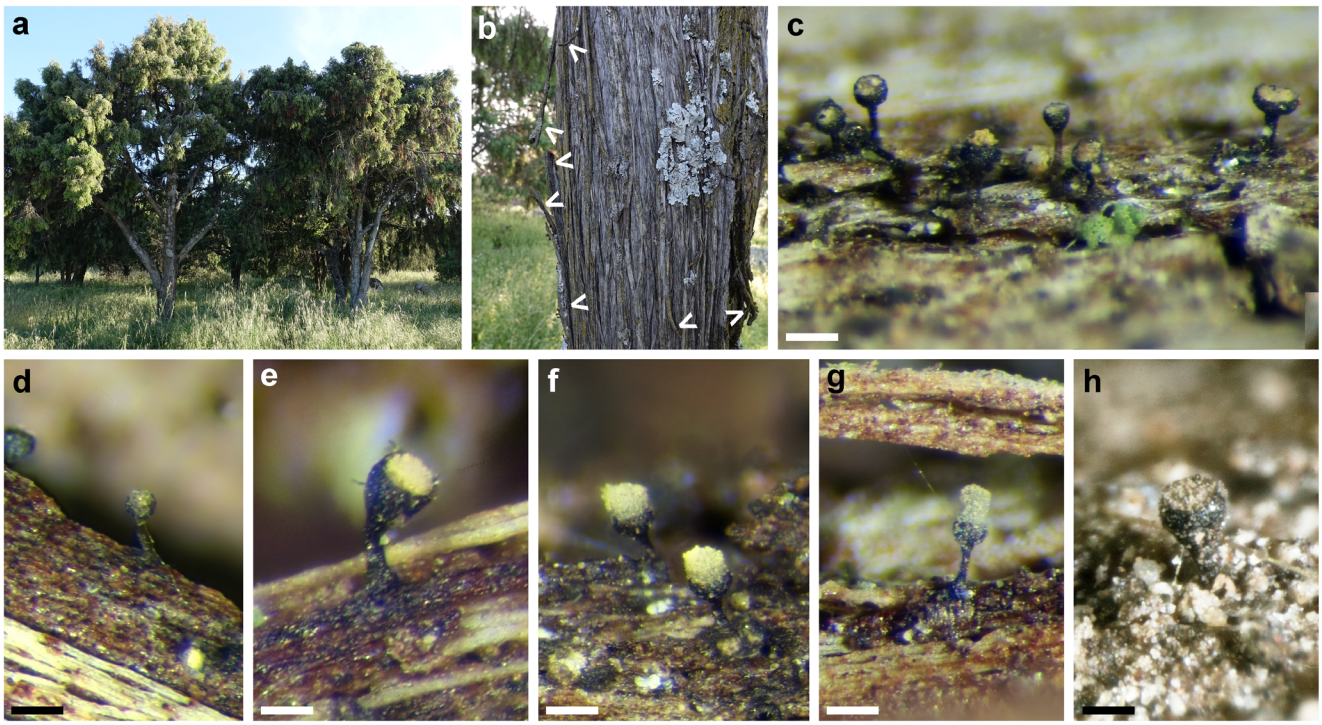
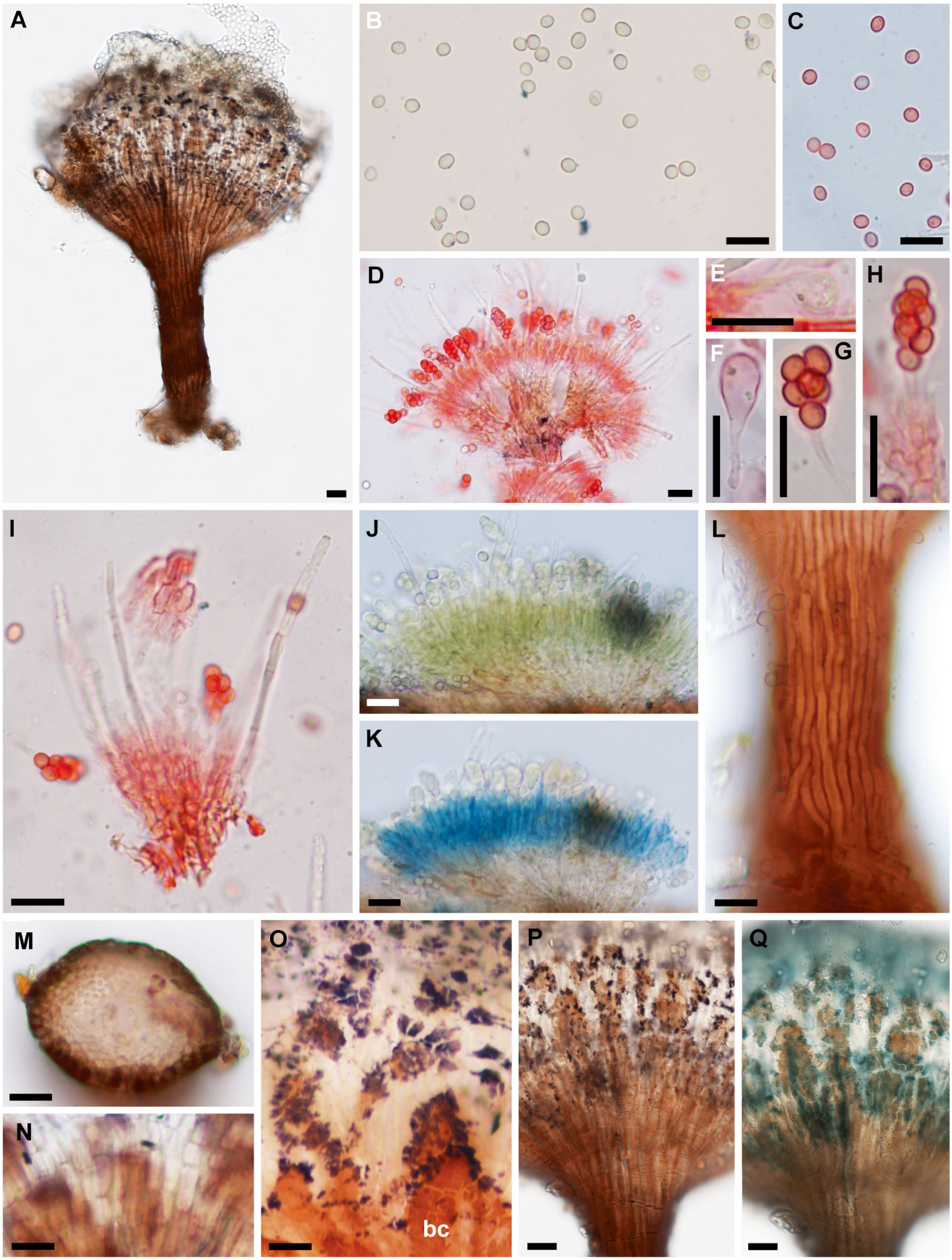


Fig. 2 *Cryptocalicium blascoi* (holotype). **A** Type locality. **B** *Juniperus oxycedrus* tree with loose bark strips. White arrows indicate suitable places for *C. blascoi*. **C** Gregarious ascomata. **D** Young ascoma with unexposed hymenium. **E** Older ascoma with exposed hymenium. **F**–**G** Ascomata with mazaedia. **H** Old ascoma without mazaedium (Etayo 31798). Scale bars = 100 μm. Photographs I. Olariaga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◀ **Fig. 3** Microscopic features of *Cryptocalicium blascoi* (holotype unless otherwise indicated). **A** Ascoma (Etayo 31798). **B** Ascospores in water. **C** Ascospores in CR. **D** Portion of hymenium showing asci and protruding sterile elements in SDS-CR. **E** Young thick-walled ascus in CR. **F** Older thin-walled ascus in CR. **G** Mature ascus without wall in CR. **H** Mature ascus attached to the subhymenium in CR. **I** Protruding sterile elements (CR). **J** Hymenium in IKI showing a green reaction. **K** The same hymenium portion in KOH+IKI, showing an amyloid reaction. **L** Stalk surface in water. **M** Stalk section in water. **N** Cells of the ectal excipulum in surface view in water. **O** Close-up of the ectal excipulum covered with crusts of dark reddish brown pigment and dark violet pigment granules in water. **P** Dark violet pigment granules in water (Etayo 31798). **Q** Partly dissolved and turquoise green pigment granules in KOH (Etayo 31798). Scale bars = 10 μm. Abbreviations: CR, SDS Congo Red; IKI, Lugol solution; KOH, Potassium hydroxide; bc, brown crust. Photographs I. Olariaga

C. blascoi is restricted to well-preserved stands of ancient *Juniperus* and *Cupressus*. Due to the minute size of *C. blascoi* and to the fact that one of the collections was made on *Cupressus sempervirens*, it is likely to have been overlooked and to have a considerably wider distribution.

Discussion

The phylogenetic position of the new species constitutes a further example of the high ecological and morphological diversity of the Eurotiomycetes. Other members of Eurotiomycetes with mazaediate and stipitate open ascomata belong in the Mycocaliciomycetidae (Tibell and Wedin 2000), which mainly differ from *C. blascoi* in having cylindrical asci without iodine reactions. Within Coryneliomycetidae some species also have mazaediate ascomata, but in these, the spores accumulate in perithecial beaks that arise from a stromatic tissue (pseudothecia) (Garrido-Benavent and Pérez-Ortega 2015; Wood et al. 2016). Within both Eurotiomycetidae and Chaetothyriomycetidae, several genera such as *Onygena*, *Pseudotulostoma*, *Pyrgillus* or *Trichocoma* also include mazaediate—perithecioid or cleistothecioid—members. Ancestral state reconstruction analyses have shown that several independent gains of the mazaedium have occurred in Ascomycota and that this character is highly homoplastic (Prieto et al. 2012). Regarding ecology, many Eurotiomycetes colonize dead plant tissues and living animals (Jaklitsch et al. 2016; Quan et al. 2020). Eurotiomycetidae includes mostly saprophytic species occurring in various substrates like wood, compost, dung, decaying plant material or foodstuffs (Jaklitsch et al. 2016). Members of Coryneliomycetidae are mostly biotrophic on leaves and stems of Podocarpaceae (Garrido-Benavent and Pérez-Ortega 2015; Wood et al. 2016). Mycocaliciomycetidae includes saprophytic species on bark, wood or lichenicolous (Jaklitsch et al. 2016), whereas Sclerococcomycetidae comprises also corticolous and lignicolous species (Réblová et al. 2017). Thus, the ecology of *C. blascoi* is not surprising as some species of related lineages share similar trophic modes (i.e. saprophytic).

The sister relationship of Cryptocaliciomycetidae with the rest of Eurotiomycetidae and Coryneliomycetidae opens the possibility of including both Coryneliomycetidae and Cryptocaliciomycetidae within the Eurotiomycetidae or of describing a new subclass (Cryptocaliciomycetidae) following the same criteria as in Wood et al. (2016). These authors

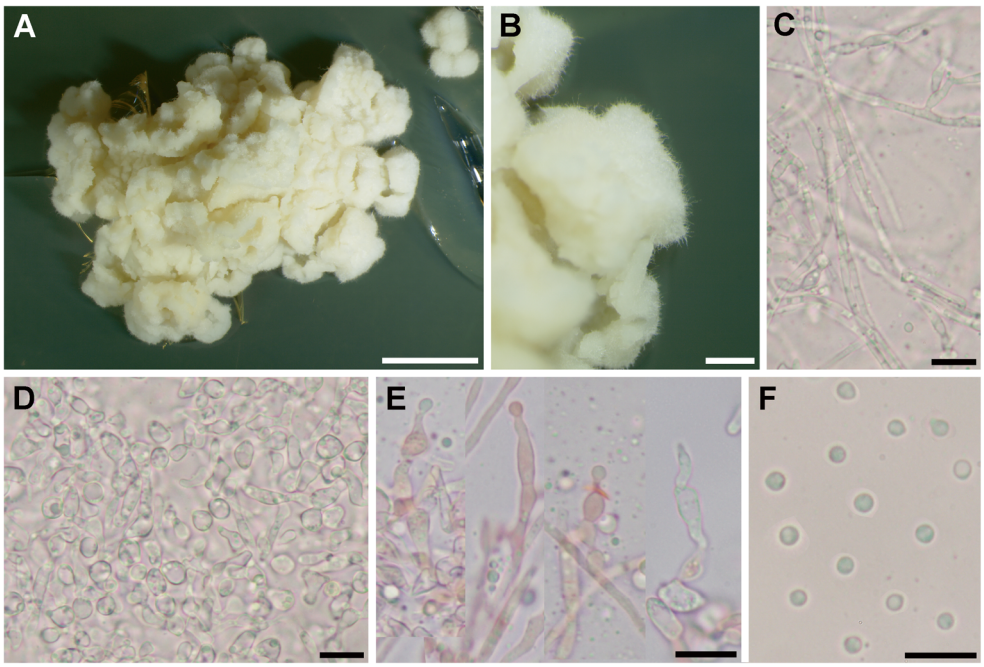

Fig. 4 Cultural characters of *Cryptocalicium blascoi* (ex-holotype culture CECT 21190). **A** Colony in MEA. **B** Colony margin and surface. **C** Vegetative hyphae in water. **D** Colony surface showing catenulate and branched conidiophores and some conidiogenous cells and conidia in water. **E** Conidiogenous cells with conidia in formation in CR. Scale bars = 5 mm (A), 1 mm (B) and 10 μm (C–F). Abbreviations: CR, SDS Congo Red

introduced Coryneliomycetidae to encompass the Coryneliaceae and argued its unique position in the Eurotiomycetes and its morphology. They underlined the presence of pseudothecial mazaedial ascomata containing initially double-walled asci, with the outer layer deliquescing as opposed to Eurotiomycetidae (usually with cleistothecia/gymnothecia and prototunicate asci). The newly described species *C. blascoi* differs also from members of Eurotiomycetidae in having apothecia and presence of hamathecium and hemiamyloid asci and furthermore represents a genetically distinct lineage.

Ascoma types including open (apothecioid) or closed (perithecioid, cleistothecioid) forms have traditionally been used as key paradigms for ascomycete classification (Schmitt et al. 2009a). Molecular phylogenies show that ascoma evolution is complex, with multiple phylogenetic origins, and that ascoma type is an inappropriate character to circumscribe classes (Schmitt et al. 2009a; Schmitt 2011). Stchigel and Guarro (2007) underlined the high diversity of ascoma types in the Eurotiales: true cleistothecia (e.g. *Chaetosartorya*, *Dichotomomyces*, *Eurotium*, *Hemisartorya*, *Neosartorya* and *Sclerocleista*); asci borne in hyphal masses or tufts (e.g. *Byssochlamys*, *Dendrosphaera*, *Sagenoma*, *Talaromyces* and *Trichocoma*); asci sitting on a stroma (e.g. *Eupenicillium*, *Hamigera*, *Hemicarpenteles*, *Neocarpenteles*, *Penicillliopsis*, *Thermoascus* and *Warcupiella*); cleistothecia produced in a stroma or surrounded by a mass of Hülle cells (e.g. *Cristaspora*, *Dichlaena*, *Emericella*, *Fennellia* and *Petromyces*) or naked asci without an ascoma (e.g. *Edyuillia*, syn. *Eurotium*). Moreover, in the case of Chaetothyriomycetidae, both ascolocular and ascohymenial developmental types of ascomata exist in Pyrenulales, Verrucariales and Chaetothyriales (Schmitt 2011). However, it is unknown how these ascoma types have evolved, and regardless of their ontogeny, these types may be considered as closed ascomata (perithecioid and cleistothecioid). Our results show that all these ascoma types have evolved from an open ascoma type and support the use of the ascoma gross morphology to define (together with additional characters) subclasses within Eurotiomycetes. Stchigel and Guarro (2007) also stated that taxonomic schemas based on a few morphological characters have proved to be unstable and suggested that an appropriate approach should reflect a natural classification following the evolutionary relationships between the considered organisms. Our ancestral state reconstruction analyses of the ascoma type and the differences with the rest of members of Eurotiomycetidae support the decision of describing new subclasses for both Coryneliomycetidae (Wood et al. 2016) and Cryptocaliciomycetidae, thus reflecting the natural evolution within Eurotiomycetes.

Concerning ascoma evolution, our results, as those by Schoch et al. (2009), support that the ancestor of Eurotiomycetes produced an open ascoma. It is shown here that this character is more stable in Chaetothyriomycetidae, Sclerococcomycetidae and Mycocaliciomycetidae than in the clade including Eurotiomycetidae, Coryneliomycetidae and Cryptocaliciomycetidae. Molecular data have shown that cleistothecia originated several times within the Sordariomycetes—a group producing predominantly perithecioid ascomata—through the loss of the ostiolar canal (Berbee and Taylor 1992a; Rehner and Samuels 1995; Suh and Blackwell 1999). Within the Eurotiomycetes, Schoch et al. (2009) also suggest that cleistothecia have evolved from perithecia. Our results disagree in this respect as it is here suggested that both perithecioid and cleistothecioid forms have arisen from apothecioid ancestors in the Eurotiomycetes. Within Lecanoromycetes (Schmitt et al. 2009a) and Leotiomycetes (Schoch et al. 2009), perithecia and cleistothecia have evolved independently several times from ancestors producing apothecia. All in all, it seems that the evolution of different types of ascomata shows different patterns in different groups of Ascomycota.

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Code availability Not applicable.

Author contribution MP, IO and JE designed the work; MP, IO and JE analysed the data; and MP, IO and JE contributed in writing the manuscript.

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Data Availability NCBI GenBank. TreeBASE.

Declarations

Conflict of interest The authors declare no competing interests.

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