Unravelling the diversity of European *Caliciopsis* (*Coryneliaceae*, Ascomycota): *Caliciopsis valentina* sp. nov. and *C. beckhausii* comb. nov., with a worldwide key to *Caliciopsis*.

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Abstract

The new species *Caliciopsis valentina* from the eastern Iberian Peninsula is characterized morphological, anatomical and molecularly. The occurrence of *C. subcorticalis* (Cooke & Ellis) Fitzp. in Europe is discussed. Based on the revision of fresh and herbarium specimens we propose the new combination *Caliciopsis beckhausii* with a neotype selected for this taxon. New molecular data (nrITS and nuLSU) are used in combination of available sequences to build a preliminary phylogenetic hypothesis for this genus. We point some previously overlooked colour-reaction tests as relevant for the systematics of the group. Finally, an updated key for all known *Caliciopsis* species is provided.

Keywords

Introduction

Recent research on the evolution of the mazaedium, a type of passively spore-dispersion structure, confirmed that the genus *Caliciopsis* Peck (*Coryneliales*, Ascomycota) belongs to the *Eurotiomycetes* (Prieto et al. 2013). This was previously suggested in several comprehensive phylogenetic studies (Geiser et al. 2006; Spatafora et al. 2006). The sole family included in the order, *Coryneliaceae* (Lumbsch and Huhndorf 2010), comprises seven additional genera showing all of them bitunicate asci but *Coryneliopsis* (Johnston and Minter 1989; Geiser et al. 2006).

Caliciopsis is characterized by the presence of stalked, black perithecia, absence of paraphyses, evanescent and long pedicellate asci with brown, one-celled ascospores. Most species seem to be bound to a specific vascular plant host, either as saprobes or parasites. Some of them grow on overgrown tissues produced by cankers, or even on wasp galls as *C. quercina* Marm. (Marmolejo 1999). The role of parasitic species on the fitness of their hosts has been long overlooked, but Ramsfield et al. (2008) recently assessed the effect of *C. arceuthobii* (Peck) M.E. Barr as a biological control agent for *Arceuthobium americanum*, the lodgepole pine dwarf mistletoe.

The genus is known from temperate regions (North and South America, and Eurasia) and the tropics (Fitzpatrick 1920, 1942; Benny 1985; Rikkinen 2000; Pratibha et al. 2010). So far, twenty eight species are known worldwide but information regarding their distribution ranges is scarce for most of them. On the other hand, a phylogenetic hypothesis of the evolutionary relationships within the genus is still missing due to the lack of fresh specimens (Pratibha et al. 2010).

So far, only four species have currently been reported in Europe: *C. tiliae* Arnaud, *C. nigra* (Schrad.) Fitzp., *C. subcorticalis* (Cooke & Ellis) Fitzp. and *C. ventricosa* (Ach.) Tibell (= *C. pinea* Peck, see Tibell 1987). The latter three species show an amphiatlantic distribution and grow on different plant hosts from the same genus (Fitzpatrick 1920, 1942). No European revision of the genus is available. The few accessible citations (Germany, France, Finland, Spain, Switzerland, Italy, Yugoslavia) were mostly done one century ago (Fitzpatrick 1920, 1942; Benny et al. 1985) . In the Iberian Peninsula, only *C. nigra* had been reported so far growing on *Juniperus* galls mainly in the

Mediterranean region (Vila et al. 2004). However, during a recent work focused on epiphytic lichens growing on bark of holm oak in eastern Spain (Garrido-Benavent et al. 2013), two enigmatic *Caliciopsis* species were found growing on this broad-leaved tree. One of them apparently represents a new species and the other is morphologically similar to *C. subcorticalis*.

Thus, the present study has the following aims: 1) to characterize and to describe a new species of *Caliciopsis*, 2) to confirm the occurrence of *C. subcorticalis sensu* Fitzpatrick in Europe, 3) to re-evaluate the usefulness of several traits for the taxonomy of this genus, and 4) to present an updated key to all known species worldwide.

In summary, we hope to improve through these goals the current knowledge of this genus in Europe and more specifically in the Mediterranean area.

Materials and Methods

Morphological studies

All sampled specimens were examined using a Leica S8APO dissecting microscope fitted with a Leica EC3 image capture system. Slide preparations were observed in a Zeiss Axioplan 2 microscope fitted with 'Nomarski' differential interference contrast and photographs were taken with a Zeiss AxioCam digital camera. Microscopic observations and measurements were made using material mounted in H_2O by means of the Zeiss Axiovision 4.8 image analyser system. 10% KOH was used both for tissue dissociation and examination, and for testing possible colour reactions of cellular elements. NO_3H, commercial bleach (NaOCl) and Lugol were also used for the latter purpose. Ascospore measurements number (n) is given in the description of each species. At least, 20 spores per individual were measured for each sampling locality. The average is followed by its standard deviation, and the maximum and minimum values are given in parentheses. The length/width ratio (Q) gives minimum and maximum values, average and its standard deviation.

The types of the novel taxa are deposited in MA (isotypes in M, H). Herbarium acronyms follow Thiers (2014). Author citations follow Mycobank (http://www.mycobank.org/).

DNA extraction, PCR amplification, and DNA sequencing

Due to the tiny size of the fruiting bodies, about 10-15 fresh ascomata were sampled from the same bark portion. Their bases were removed with the help of a sterilized razor blade and a needle. The remnants were then further inspected under both the dissecting and light microscopes in order to avoid contamination by other fungi. Genomic DNA isolation was performed by means of a modified version of the CTAB method (Cubero et al. 1999). Internal transcribed spacer (ITS) and partial nuLSU rDNA regions were selected for amplification, using primers ITS1F (Gardes and Bruns 1993), ITS1LM (Myllys et al. 1999) and ITS4 (White et al. 1990) for ITS, and LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990) for nuLSU. PCR reactions were performed in a total volume of twenty-five microlitres, containing 2.5 µl of reaction buffer (Biotools®), 5 µl of dNTPs (1 mM), 1.25 µl of each primer (10 µM), 1 µl of $MgCl_2$ (50 mM) and 0.5 U of DNA polymerase (Biotools®); final volume was reached by adding distilled water. The following reaction conditions were used for both DNA regions: initial denaturation for 5 minutes at 95 ºC, followed by thirty-five cycles of 30 seconds at 95 ºC, 1 minute at 52 ºC (ITS1F-ITS4, LR0R-LR5) or 56 ºC (ITS1LM-ITS4), and 1 minute and 30 seconds at 72 ºC; the protocol concluded with a final extension step of 10 minutes at 72 ºC.

PCR products were purified and cleaned using the UltraClean® PCR Clean-Up Kit (MOBIO Laboratories, Inc.). Both complementary DNA strands were sequenced at MACROGEN EUROPE (The Netherlands). New sequences were generated for *Caliciopsis valentina* sp. nov., *C. beckhausii* comb. nov. and *C. nigra* and deposited in Genbank. Accession numbers are reported in Table 1.

Sequence alignment and phylogenetic analysis

Electropherograms were checked, trimmed and assembled using SeqmanII v.5.07© (Dnastar Inc.). Alignments were carried out using Muscle v3.6 (Edgar 2004) and were manually examined in Bioedit v.7.0.9 (Hall 1999). Gblocks 0.91b (Castresana 2000) was used to remove ambiguously aligned regions, using the least restrictive options for removing gaps and allowing for positions with a gap in less than 50% of the sequences. jModelTest v.2.1.4 (Darriba et al. 2012) was used to select the best nucleotide

substitution model for subsequent analyses using the Akaike Information Criterion (AIC, Akaike 1974). The GTR+G model was selected for both regions. Bayesian analyses were those implemented in Mr. Bayes v3.2 (Ronquist et al. 2012). Partitions were used for the combined ITS + nuLSU analysis. Settings included two parallel runs with eight-chain runs over 10M generations. Sampling was performed after every 200th step; the first 10% of saved data was discarded as 'burnin'. Convergence of chains and ESS values were checked in Tracer v.1.6 (available at http://tree.bio.ed.ac.uk/software/tracer/). Phylogenetic trees were visualized in FigTree v. 1.3.1 and Adobe Illustrator CS2® was used for artwork. Maximum likelihood analyses were performed using PHYML v.3.0 (Guindon et al. 2010; Gascuel, 2003) through the web platform ATGC (http://atgc.lirmm.fr/phyml/); a non-parametric bootstrap analysis (1000 replicates) was carried out in order to assess branch support (Felsenstein 1985). *Hamigera avellanea* (Thom & Turesson) Stolk & Samson and *Spiromastix tentaculata* Guarro, Gené & De Vroey were used as outgroups.

Further material examined

Samples collected by the authors as well as several specimens loaned from the Botanische Staatssammlung München (M) and the University of Helsinki (H) were analysed morphologically and anatomically, and for assessing putative colour reactions of several fruiting body structures. The material studied is listed below:

CALICIOPSIS CALICIOIDES (Ellis & Everh.) Fitzp. FINLAND, Etelä-Häme (Ta), Lammi, Evo, 0.5 km NNW of Vähä Ruuhijärvi, grid 27° E: 6792:396, on solitary *Populus tremula* tree left in recent cut-over area, T. Ahti & P.A. Esseen, 22.05.1996, H-6030732. U.S.A: Washington, Pend Oreille county, edge of Middle Creek near its confluence with the Pend Oreille River, on bark of *Populus* sp., labelled as *Caliciopsis ellisii* Sacc., H.K. Goree, 30.07.1969, H-7023176.

CALICIOPSIS MAXIMA (Berk. & M.A. Curtis) Höhn. BRAZIL, Rio de Janeiro state, Teresiópolis, Serra Geral, on *Polypodium* sori, labelled as *Capnodiella maxima* (B. et C.) Sacc., Rick S. J., 1.01.1909, Rehm: Ascomycetes (M-0223081); Serra de Itatiaia, Mont Serrat, c. 900 m asl., on *Polypodium crassifolium*, labelled as *Sorica maxima* (Berk. & M.A. Curtis) Giesenh., P. Dusén, 22.07.1902, M-0223082 & H-7023177; Paraná state, Serra do Mar, Itupava, P. Dusén, 17.09.1908, Vestergren-*Micromycetes rariores selecti*, M-0223083.

CALICIOPSIS NIGRA (Schrad.) Fitzp. FRANCE, Var region, Massif de la Ste. Baume, Hotellerie, Wäldchen, on *Juniperus oxycedrus*, labelled as *Phaeostoma juniperina* (Ellis & Everh.) Arx & E. Müll., S.K. Bose & E. Müller, 5.06.1959, H-7023165. SPAIN, province of Castelló, c. 3 km S of Herbés, close to Mas de Andreu, 40º42'N, 0º01'E, c. 1000 m asl., forest dominated by *Pinus halepensis*, *Quercus ilex* and *Juniperus oxycedrus*, on living and dead branches of several shrubs of *Juniperus oxycedrus*, associated with galls, V. Calatayud, 10.11.1998; Vistabella del Maestrat, Parc Natural de Penyagolosa, near Sant Joan de Penyagolosa monastery, 40º15'07.44" N, 0º20'52.5" W, 1270 m asl., on galls forming on twigs of *Juniperus communis*, in a forest of *Pinus sylvestris*, leg. I. Garrido-Benavent 306 & N. Cháfer, 11.10.2013, MAXXXXX; near Masia Collet, 40º16'46.19" N, 0º19'03.84" W, 1285 m asl., on *Juniperus communis* galls in a forest dominated by *Quercus ilex* subsp. *rotundifolia*, *Q. pyrenaica* and *Pinus sylvestris*, leg. I. Garrido-Benavent 305 & N. Cháfer, 11.10.2013, MAXXXXX.

CALICIOPSIS SUBCORTICALIS (Cooke & Ellis) Fitzp. U.S.A, New Jersey, Newfield, parasitic on old *Dichaena strumosa* on *Quercus coccinea*, labelled as *Hypsotheca subcorticalis* C. & E., Ellis & Everhart, April 1888, specimen no. 2123, M-0223095.

CALICIOPSIS VENTRICOSA (Ach.) Tibell *(= C. PINEA* Peck). CANADA, Ontario province, Petawawa forest Exp. Sta., on *Pinus strobus*, leg. C.G.Riley, det. H.S. Jackson, 16.09.1947, H-7023175. GERMANY, Bavaria state, Schöngeising, labelled as *Cyphelium stenocyboides* Nyl., leg. Lederer?, September 1894, M-0223089. U.S.A: North Carolina, Mount Pisgah, on *Pinus virginiana*, leg. G.G. Hedgcock, det. W.W.Diehl, 24.06.1928, H-7023164; on *Pinus pungens*, leg. G.G. Hedgcock, det. W.W.Diehl, 24.06.1928, H-7023187; on *Pinus pungens*, G.G. Hedgcock, 06.1908, M-0223086.

Results

Our study has revealed that the two enigmatic *Caliciopsis* species found in the Iberian Peninsula represent a new species, *C. valentina*, and a species similar to *C. subcorticalis* but with distinctive traits that relate it to an apparently different, forgotten species described in central Europe.

Phylogenetic analysis

Available sequences of *Caliciopsis* in public databases are scarce (we could only retrieve ITS sequences for 2 species and nuLSU ones for 3) and specimens found in herbaria are too old for successful DNA analyses. In this study we have newly sequenced the new taxon *C. valentina*, *C. beckhausii* and *C. nigra*. Fig. 1 shows the results corresponding to the Bayesian and ML analyses (only Bayesian topologies are shown, ML topologies were almost identical) for the single ITS and nuLSU as well as for the combined dataset. ITS and nuLSU trees mostly differed in the phylogenetic position of *C. beckhausii* regarding *C. valentina*. In the ITS tree, *C. beckhausii* come up in the same politomy as *C. valentina* but with a longer branch (both species differ in 9 positions in the ITS alignment). On the other hand, the nuLSU shows a closer relation of *C. beckhausii* with *C. indica* than with *C. valentina*, however neither by the Bayesian nor the ML analyses support this topology. The combined ITS + nuLSU tree does not offer any further insight into the relationships within the genus *Caliciopsis*.

Colour reaction tests

All species but *C. beckhausii* showed a strikingly, powerful NO_3H reaction of the entire ascomata which turned bright reddish orange. On the other hand, the addition of KOH generally produced a green-turquoise reaction at the beak fringe level, while the reaction location varied in *C. calicioides*, and was not detected in *C. maxima*. All specimens of *C. calicioides* tested produced additionally a brownish fluid expelled mainly from the upper half of the ascomata.

Taxonomy

Caliciopsis beckhausii (Körb.) Garrido-Benavent & Pérez-Ortega, **comb. nov.**
(Fig. 2)
MycoBank no.: MB XXXXX; Genbank: XXXXXXX-XXXXXX

Basionym: *Coniocybe beckhausii* Körb., Parerga Lichenologica pag. 301 (1865). Typus missing. (See *Taxonomical and nomenclatural notes* below).

Neotypus of *Coniocybe beckhausii* (hic designatus): Spain, Burgos Province, San Juan del Monte, road from San Juan del Monte to Vadocondes, 41º39'10" N, 3º32'26"

W, 835 m asl., in a mixed forest of *Quercus ilex subsp. rotundifolia* with *Pinus pinaster* and *Juniperus oxycedrus*, on the trunk of holm oaks, inside its crevices and over the surface, leg. S. Pérez-Ortega & M.A. Pérez Muriel, 28.09.13, Neotypus (MAXXXXX).

Stromata not seen. Ascomata gregarious to forming dense groups, individuals mainly isolated, black, straight or curved, not branched, (0.5)0.9±0.2(1.5) mm high, basal stalk (50)75±10(100) µm broad, slightly swollen at the point of attachment to the substrate (70)100±15(145) µm diam. Ascigerous swelling median, usually tending to subterminal, rarely laterally collapsed, (125)190±30(260) µm high and (75)110±20(160) µm diam. Upper part attenuated and sharp-pointed in young ascomata, at maturity developing into a distinct beak (150)285±70(450) µm long and (30)50±10(75) µm wide, ending in a flattened tip. This can sometimes develop in a non-urceolate mazaedium. Mazaedium hyphal hairs 2–3 diam., septate, branched, tips undifferentiated to capitate, even truncate, hyaline, with incrustations of violet pigment seen under light microscope which turn green-turquoise in KOH.

Asci clavate, bitunicate, evanescent, eight-spored, 45–65 µm long, stalk 1–4 µm diameter, sporiferous part (p. sp.) 15–21×7–10 µm. Ascospores mainly globose to subglobose, but ellipsoidal ones are also in high proportion in some individuals, rarely slightly angular, at first hyalines and becoming brown when mature, turning greyish in KOH, smooth, (5.4)7.4±1(11)×(4.8)6.6±0.9(10) µm, Q= (1)1.1±0.1(1.7) (n= 230), with a widened wall up to 1.2 µm.

Conidiomata pycnidium-like, solitary to caespitose, sometimes a few growing associated to the fruiting body basis, very variable in morphology, ranging from obpyriform (more frequent) with an ostiolar papillae more definite in young specimens to subglobose, ovoid or digitiform, often collapsed when dried, black, 100–250×65–150(250) µm in size. Conidiogenesis phialidic, acropleurogenous; terminal conidiogenous cells ampulliform. Conidia subcylindrical to allantoid, non-septate, hyaline, (3.5)4.2±0.3(4.7)×(1.2)1.5±0.2(1.7) µm.

Chemical tests: Beak fringe turning strongly green-turquoise in KOH. Entire ascomata and mass of extruded spores showing a weaker, paler reddish-orange reaction to NO_3H, compared to *C. valentina*. NaOCl test negative. Lugol (I) test negative.

Habitat: On *Quercus ilex* subsp. *rotundifolia*, mainly growing on the sides of bark crevices and also on the surface, intermixed with lichen from the families *Lecanoraceae*, *Teloschistaceae* and *Physciaceae*.

Additional material studied: SPAIN, Valencia province, Vall d'Albaida region, Bocairent, Partida del Cirer, El Pinatell, behind Càmping de Mariola, 38º45'11.34" N, 0º33'01.87" W, 907 m asl., on *Quercus ilex* subsp. *rotundifolia* trunk bark (same microhabitat for all specimens listed below), leg. I. Garrido-Benavent 301 & N. Cháfer, 14.09.2013, (MAXXXXX); Castelló province, Vistabella del Maestrat, near Masia de Vicent Canales, 40º17'23.36" N, 0º16'27.66" W, 1055 m asl., leg. I. Garrido-Benavent 302 & N. Cháfer, 11.10.2013, (MAXXXXX); near Masia Collet, 40º16'46.19" N, 0º19'03.84" W, 1285 m asl., leg. I. Garrido-Benavent 303 & N. Cháfer, 11.10.2013, (MAXXXX); Teruel province, Calamocha, road from Calamocha to Luco de Jiloca, 40°57'47.8'' N, 1°18'24.1'' W, 875 m asl., leg. I. Garrido-Benavent 304, 17.3.2014, (MAXXXXX). GERMANY, Baden-Wurtemberg state, Heidelberg, in oak branches, labelled as *Caliciopsis ephemera* (Zwackh) Rehm, v. Zwackh, 1864, H-9503818 (type!), M-0223080, M-0086521.

Distribution: The species is so far known from Heidelberg (Germany) and from the four localities in the Iberian Peninsula cited here (Fig. 3) in the upper meso-mediterranean to supra-mediterranean belts (Rivas-Martínez 1981; Costa 1982). It seems to prefer localities with a rather continental climate but a more thorough survey is needed in order to reveal the current distribution of this fungus across the western Mediterranean area.

Remarks: Apparently, stromata may develop deeply beneath the bark surface not forming the small black cushion detected in other species as *C. valentina*, *C. pseudotsugae* and *C. orientalis* (Funk 1963). This may represent an extreme reduction of larger stromata mainly produced by canker-inducing species such as *C. ventricosa* (Funk 1963).

Caliciopsis beckhausii ascomata often lack the fuzzy, powdery appearance observed in other species due to spore extrusion. These fruiting bodies rather tend to have smooth or flattened tips (Fig. 2a–c). This could be explained by intrinsic factors as lower spore discharge rates, or extrinsic ones as, for example, higher rainfall rates derived from the mesic climate that could rapidly wash the discharged spores off.

On the other hand, subtle variations in the ascigerous swelling position, similar to that observed in *C. beckhausii*, has also been documented in many other species such *as C. calicioides*, *C. nigra* (Fitzpatrick 1942) and *C. pseudotsugae* (Funk 1963), thereby being

a common phenomenon in the genus that may be environmentally induced (Rikkinen & Poinar 2000).

The closest relative species seems to be *C. subcorticalis* regarding ascomata morphology and ecology. However, ascospore size range clearly exceeds that of *C. subcorticalis*, showing in fact some of the largest ascospores found in *Caliciopsis*. Differences from *C. beckhausii* to *C. subcorticalis* and *C. valentina* are summarized in Table 2. *Caliciopsis tiliae* has always a subterminal ascigerous swelling and more elliptical to subfusiform spores. Other species, such as *C. ventricosa* and *C. orientalis* show smaller ascospores and different host range. *Caliciopsis clavata* (Lév.) Fitzp. often produce ascigerous swellings up to 275 µm in diameter, which has not been observed in the new species. *Caliciopsis quercina* is a taxon so far known from Nuevo León, Mexico, growing on wasp galls on living twigs of *Quercus canbyi* (Marmolejo 1999). Apart from the ecology, it differs by its smaller fruiting bodies not reaching 1 mm in height and having submedian ascigerous swelling.

Taxonomical and nomenclatural notes: The taxonomy of this group of species is rather puzzling (Fitzpatrick 1920). So far, *Caliciopsis. subcorticalis sensu* Fitzpatrick (1920, 1942) included North American and European specimens due to his acceptance of Rehm's statement that the collections he studied from the both continents were identical (Rehm 1896). Those collections were: Zwackh, Lich. exs. 477, labelled as *Calicium ephemerum* Zwakch (European), and Ellis & Everhart, N. Am. Fungi no. 2123, labelled as *Hypsotheca subcorticalis* (Cooke & Ellis) Ellis & Everh. (North American). We have thoroughly studied the same specimens and have concluded that they are rather different in several ascomata and microscopic traits. Rikkinen (2000) also pointed out that both collections were "far from being identical". Thus, North American specimens show fruiting bodies with a rather acute beak, not forming a distinct mazaedium (see also Figs. 8–9 corresponding to the holotype of *C. subcorticalis* in Fitzpatrick (1942)) whereas the European specimens often develop a flattened tip, sometimes mazaediate (Fig. 2). Additionally, we noticed a conflict in spore size values, probably due to the spore maturity stage or to the use of different microscopes by each author. In the protologue of *Hypsotheca subcorticalis* (Ellis and Everhart 1885), the author gave a value of 3–3.5 µm of diameter for the spores; Fitzpatrick (1920, 1942) rose it to 4–5×3–4 after examining the specimen no. 2123; Rehm (1896) also gave values c. 5 µm breadth in spores from the same specimen. On the other hand,

ascospores measurements made by us in the same specimen turned to be a bit larger (4.5)6.2±0.6(7.3)×(3.4)4.5±0.6(5.9) µm (n= 70). However, European specimens (including Zwackh Lich. exs. 477, from H and M and those from the Iberian Peninsula) show slightly but consistent larger values, especially in diameter, (5.4)7.4±1.1(11)×(5)6.6±0.9(10). See Table 2 for further morphological and anatomical differences.

Considering that the correct name for the North American species should be *C. subcorticalis*, the conflict arises when trying to find the older, legitimate name for the European specimens. On the one hand, according to all references cited here and to Mycobank, Zwackh described the European collections as *Calicium ephemerum* in Die Lichenen Heidelbergs: 81 (1883). Shockingly, we have not found neither a description nor illustrations of this species there. Instead, there is a reference to *Coniocybe beckhausii* Körb. and to *Stilbum rugosum* Fr. Fitzpatrick rejected the synonymy of the later taxon with *C. subcorticalis* because its name was never found in any of the writings of Fries (see Fitzpatrick 1920). On the other hand, we have also noticed that the specimens of *Calicium ephemerum* loaned from H and M (including the type) were firstly labelled as *Coniocybe beckhausii*. Rehm (1896) transferred *Calicium ephemerum* to *Caliciopsis ephemera* (Zwackh) Rehm, making the only available description of this taxon so far, and included *Coniocybe beckhausii* as a synonym. Therefore, as the original publication of *Coniocybe beckhausii* (Parerga Lichenologica: 301, 1865) antedates that of *C. ephemera*, the European species should be named as *Caliciopsis beckhausii*. Fitzpatrick (1920) had already rejected the synonymy of *Coniocybe beckhausii* to *C. subcorticalis* because Körber (1865) had failed to find asci. We have performed a thorough and fruitless search in most of the major European herbaria in order to find the type specimen of *Coniocybe beckhausii*. Moreover, the original description made by Körber didn't include an illustration.

In conclusion, despite the fact that *Caliciopsis ephemera* has an available type (Zw. Lich. exs. 477), the name must be considered illegitimate if accepting that the only original reference to *Calicium ephemerum* was supposedly that of Die Lichenen Heidelbergs, p. 81 (1883). Thus, given that *Coniocybe beckhausii* type is missing, we propose the new combination *Caliciopsis beckhausii* and establish a neotype based in the recent collections from Spain. Besides the macro and microscopic descriptions, nrITS and nLSU sequences are also provided.

Caliciopsis valentina Garrido-Benavent & Pérez-Ortega, **sp. nov.**

(Fig. 4)

Diagnosis: Ascomata gregarious, black, straight to curved, sometimes branched, up to 5 mm high and 90–150 µm wide at the basis level; ascigerous swelling subterminal, 150–240 µm high and 100–160 µm broad; upper portion (beak) up to 180 µm long. Asci clavate, eight-spored, evanescent. Ascospores from subglobose to ellipsoid, brown when mature, mean size 7×5.7 µm, Qm= 1.2, greyish in KOH. Conidiomata obpyriform to subglobose, black, 120–170×95–135 µm. Conidia subcylindrical to allantoid, hyaline, non-septate, 4.5×1.4 µm.

Holotype: SPAIN, Valencia province, Vall d'Albaida region, Quatretonda, Pla dels Arenals, 38º57'34.84" N, 0º22'25.87" W, 367 m asl., on the trunk of *Quercus ilex* subsp. *rotundifolia*, deep inside its crevices and through the fringes of bark patches, leg. I. Garrido-Benavent 290, 29.09.2013. Holotype (MAXXXXX), Isotypes (M, H).

Etimology: *valentina* derives from "Valencia", a region from the Eastern Iberian Peninsula.

Stromata perceptible as scattered, minute, more or less rounded black spots usually developing into a single fructification, either ascomata or conidiomata. Ascomata scattered to gregarious or forming dense groups, individuals being often isolated while in some cases two or a few more can grow united by a single basis, black, straight or curved, (0.6)0.9±0.2(5) mm high and (60)80±10(110) µm wide just below the ascigerous swelling, sometimes branched, slightly widening towards the basis, which has (90)110±15(150) µm diam. Ascigerous swelling (loculus) subterminal, laterally collapsed in dried specimens, (150)190±20(240) µm high and (100)130±15(160) µm diam. The upper portion tapering towards a reddish-brown urceolate mazaedium and forming a short beak of (70)130±25(180) µm long and (80)95±10(115) µm wide in its narrower portion. Hyphal hairs present at the upper part of the mazaedium 2–4 µm diam., septate, usually branched, tips not differentiated, hyaline and slightly coarse when observed under light microscope due to incrustations of violet pigment that turn green-turquoise in KOH.

Asci developing within a fasciculate hymenium, clavate, bitunicate, evanescent, eight-spored, 50–65 µm long, stalk about 1.2 µm wide, sporiferous part 14–20×5–7 µm and 20–35×6–10 µm when containing mature spores. Ascospores variable in shape, from subglobose to definitely ellipsoidal, frequently angular, smooth, at first hyaline but

becoming brown when mature, turning greyish in KOH, (5.3)7±0.8(9.6)×(4)5.7±0.7(8) µm, Q= (1)1.2±0.2(1.9) (n= 220); ascospore wall up to 1.4 µm.

Conidiomata pycnidium-like, from solitary to in small groups when growing among ascomata, or forming crowded groups in samples with few or no ascomata, obpyriform to subglobose with a defined apical ostiolar papillae, often sessile, sometimes laterally collapsed when dried, black, 120–170×95–135 µm in size. Conidiogenesis phialidic, acrogenous; conidiogenous cells narrowly ampulliform. Conidia subcylindrical to allantoid, non-septate, hyaline, (4)4.5±0.3(5)×(1)1.4±0.2(1.6) µm.

Chemical tests: Hyphal tissue of the beak fringe showing a strong green-turquoise KOH reaction. Entire ascomata and mass of extruded spores showing a strong reddish-orange reaction to NO$_3$H. NaOCl test negative. Lugol (I) test negative.

Habitat: On *Quercus ilex* subsp. *rotundifolia* (*Fagaceae*), usually growing deep inside the crevices of the trunk bark, also below it, and being difficult to observe to the naked eye. Only in rare cases it has been found atop the bark surface, intermixed with lichen thalli such as *Lecanora hybocarpa*, *L. horiza*, *L. carpinea*, *Lecidella elaeochroma*, *Caloplaca pollinii*, *Physcia adscendens*, *Physciella chloantha*, *Schismatomma picconianum*, *Flavoparmelia soredians* and *Xanthoria parietina*.

Additional examined material: SPAIN, Valencia province, Vall d'Albaida region, Quatretonda, Pla de Pastor, 38º55'41.44" N, 0º26'00.27" W, 166 m asl., on *Quercus ilex* subsp. *rotundifolia* trunk bark (same microhabitat for all specimens listed below), leg. I. Garrido-Benavent 294, 2.04.2012, MAXXXX; costera del Cap del Bou, barranc de Les Fontetes, 38º56'45.48" N, 0º23'29.09" W, 218 m asl., leg. I. Garrido-Benavent 295, 22.12.2013, MAXXXX; Llutxent, Pla de Triola, 38º58'26.94" N, 0º18'23.3" W, 607 m asl., leg. I. Garrido-Benavent 296, 11.04.2012, MAXXXXX; Bellús, El Salt, 38º56'36.5" N, 0º29'43.7" W, 141 m asl., leg. I. Garrido-Benavent 292, 18.04.2012, MAXXXXX; Vallada, Pla del Campillo, near Casa de Juan Garrido, 38º52'47.46" N, 0º40'31.64" W, 585 m asl., leg. I. Garrido-Benavent 291, 1.05.2012, MAXXXX; Bèlgida, El Veto, barranc de Rendaguanya, 38º52'20.08" N, 0º28'42.07" W, 216 m asl., leg. I. Garrido-Benavent 297, 23.04.2012, MAXXXXX; Beniatjar, El Canari, barranc de Benicadell, 38º51'57.42" N, 0º25'25.72" W, 265 m asl., leg. I. Garrido-Benavent 299, 24.04.2012, MAXXXXX; Ontinyent, near Finca Santa Rosa, barranc del Rei, 38º49'21.9" N, 0º38'27.43" W, 405 m asl., leg. I. Garrido-Benavent 298, 4.05.2012, MAXXXXX; Bocairent, Partida del Cirer, El Pinatell, near Càmping de Mariola,

38º45'11.26" N, 0º32'48.4" W, 900 m asl., leg. I. Garrido-Benavent 293 & N. Cháfer, 29.12.2013, MAXXXXX.

Distribution: This new species is so far known from the Vall d'Albaida region (Valencia, Spain), in the most eastern area of the Iberian Peninsula, which configures a natural valley oriented west to east with an extension of 722 km^2 and surrounded by mountains reaching up to 1104 m (Fig. 3). The climate is termomediterranean to upper mesomediterranean with dry to subhumid range ombroclime (Costa 1982), average annual temperatures between 15.5 ºC and 17 ºC and rainfall between 450 mm and 750 mm, and the potential natural vegetation is the holm oak forest (*Rubio longifoliae–Querceto rotundifoliae*) (Conca and García 1994; Benavent-Alberola 1996).

Remarks: *Caliciopsis valentina* is a rather variable species. Usually, ascomata growing below bark fragments are not fertile and develop longer and more branched fruiting bodies, which are strongly curved towards the bark surface, what may point to the existence of phototropism, a fact also suggested for *C. orientalis* by Funk (1963). The nearer to the surface the ascomata start growing, the earlier they develop the ascigerous swelling and produce spores, so that they become shorter in length. Rikkinen and Poinar (2000) stated that insects and other invertebrates may constitute the main spore dispersion vectors, as occur in other mazaediate fungi. However, the growing patterns of the new species within bark crevices may also point to a water role in spore dispersion (Funk 1963).

The mass of extruded ascospores confers a powdery, fuzzy appearance to the mazaedium of *C. valentina* (Fig. 4a,c), as remarked by other authors for other *Caliciopsis* species (Fitzpatrick 1920, 1942; Funk 1963).

Laterally-produced conidiomata have been observed on long black ascomata-like structures lacking the characteristic ascigerous swelling, as in the case of the samples collected in one locality (Els Surars, Llutxent) where only this type of fructification was found. This kind of structures is also produced by *C. ventricosa* and *C. orientalis* (Funk 1963). It has been also found a stalked, fusoid pycnidia producing identical conidia in one of the samples.

Benny et al. (1985) proposed that the fruiting body inner tissue of *Caliciopsis* corresponds to *textura intricata* and that this could be a distinctive trait of this genus within the order *Coryneliales*. The ascomata and conidiomata walls of *C. valentina* also have the same tissue type in side view, with deep brown, elongated hyphae densely packed and irregularly arranged. On the other hand, hyphae of the stromata walls are

reduced in length and form a tissue better resembling that of *textura angularis*. As noticed by Funk (1963), the subsequent differentiation of the fruiting body leads to an elongated, more or less parallel hyphal growth. El Surars (Llutxent) locality is also interesting by the fructifications having a slightly different wall tissue with cells becoming shorter and more polygonal. Further genetic studies have not been yet conducted in order to understand such discrepancy.

Large ascospores, subterminal ascigerous swelling and restricted distribution and host range are the key traits defining *C. valentina*. Similar species regarding ecological and microscopic traits are *C. subcorticalis* and *C. tiliae*, respectively. As stated before, the former is a poorly known species with few collections that has smaller spores and a median to subterminal loculus (Table 2), whereas *C. tiliae*, which has only been found growing on *Tilia*, produces ellipsoidal to subfusiform spores and smaller conidia. Differences shown in the discussion of *C. beckhausii* in regard to *C. ventricosa*, *C. orientalis*, *C. quercina* and *C. clavata* are also valid for *C. valentina*. Finally, *C. toonae* Rikkinen and *C. rhoina* Rikkinen (Rikkinen 1999) are restricted to East Asia where they grow on *Meliaceae* and *Anacardiaceae* host tree species and also have smaller spores than *C. valentina*.

The phylogenetic tree has shown that *C. valentina* and *C. beckhausii* are closely related species (Fig. 1). However, they differ from each other in the more slender habit of the later, and the position of the ascigerous swelling, always subterminal in the former (Table 2). Other features that are apparently constant in each species and that let establish differences between them are: spore wall thickness, which is greater in *C. valentina* than *C. beckhausii*, the slightly larger spore size and its more rounded shape in *C. beckhausii* that can explicitly be corroborated by the Q ratio range values, and the conidia size, being slightly longer in *C. valentina*. *Caliciopsis beckhausii* shows an acropleurogenous conidiogenesis whereas conidia are apparently produced acrogenously in *C. valentina* (Table 2). Besides, the NO_3H reddish-orange reaction is clearly weaker in *C. beckhausii* than in *C. valentina*, and it has also been corroborated both in recently collected and herbarium material.

DISCUSSION

Several ascospore, ascomata and habitat traits are regarded as useful for distinguishing among *Caliciopsis* species (Fitzpatrick 1942, Funk 1963, Rikkinen

2000). Table 2 summarizes the differences in those traits between *C. valentina*, *C. beckhausii* and *C. subcorticalis*. On the other hand, the distribution of the first two species shows clear bioclimatic patterns. *Caliciopsis valentina* is mostly found at lower altitudes in more or less xeric localities and *C. beckhausii* seems to replace the former in higher and mesic to humid habitats. However, both species have been found cohabiting in the Serra de Mariola, a higher, mesic locality. Arnaud (1930) noticed that most tropical species of *Coryneliaceae* possess deep hymenia (therefore lower loculus position) and suggested this being an adaptive trait for species with slow-maturation spores growing in moist habitats. The current distribution of *C. valentina* (subterminal loculus) and *C. beckhausii* (median to subterminal loculus) agrees with this hypothesis despite no further attempts have been performed to assess its physiological and ecological importance.

On the other hand, the wide ascospore size range detected both in *C. valentina* and *C. beckhausii* constitutes a phenomenon that Funk (1963) had also detected in other species such as *C. pseudotsugae*.

Finally, a major sampling effort is needed due to the fact that molecular data are so far available for seven out of twenty eight *Caliciopsis* species. However, the new sequences obtained in this study for three species of *Caliciopsis* contribute to some extent to the reconstruction of the evolutionary history of this genus (Fig. 1).

Remarks on *Caliciopsis* systematics

During this work we realized that previous works on *Caliciopsis* have never used chemical tests (see Fitzpatrick 1920, 1942; Funk, 1963; Benny et al 1985). Therefore, we loaned some *Caliciopsis* specimens housed in several European herbaria in order to check for chemical reactions (see studied specimens). The powerful reddish orange NO_3H reaction of the ascomata is common in other Ascomycota fungi with melanin-like compounds in their walls. On the other hand, some violet-coloured crystal-like incrustations, that irregularly cover both the outermost hyphae of the beak fringe and the hyphal hairs (Fig. 2g; 4f, g) are presumed to react to KOH. The presence or absence of these crystals is a character also overlooked by previous authors and whose taxonomical value should be investigated in further studies. Despite the existence of hyphal hairs was reported before by Fitzpatrick (1920) and Funk (1963), no attempt to study the variability of this character through the entire genus has ever been performed. From the

specimens studied here, we conclude that there is little variation in morphology and branching pattern, but some variability is found in hyphal width among species (e.g. *C. valentina* and *C. beckhausii* with respect to *C. maxima*). Nevertheless, it would be fair to make a first comparative study of both characters from freshly collected material before suggesting any systematic relevance for them.

Key to worldwide *Caliciopsis* species

1 On fern sori (e.g. *Polypodium*), usually undergoing repeated apical proliferation **C. maxima**.

1*On spermatophytes (seed plants) 2

2 On gymnosperms, mostly conifers 3

2* On angiosperms 10

3 Ascigerous swelling basal; ascomata crowded, on gall-like structures formed on *Cupressus* twigs **C. nigra**

3* Ascigerous swelling not basal 4

4 Ascigerous swelling submedian to median 5

4* Ascigerous swelling subterminal to terminal 6

5 Ascigerous swelling 200–280 µm wide; ascomata up to 1 mm high; spores 5.6–7 µm long; on *Araucaria* **C. brevipes**

5* Ascigerous swelling 55–110 µm wide; ascomata smaller; spores up to 3.6 µm long; on *Podocarpus* **C. podocarpi**

6 Ascomata height usually exceeding 1 mm 7

6* Ascomata height up to 1 mm 9

7 Spores ellipsoidal to subglobose, 2–4 µm wide; ascomata up to 3 mm high; ascigerous swelling up to 250 µm wide; on diverse members of the *Pinaceae* **C. pseudotsugae**

7* Spores mainly ellipsoidal, 3–4.5 µm wide 8

8 Ascomata up to 2 mm high, solitary or gregarious; ascigerous swelling always subterminal, 150–200 µm in diameter; species known only on *Tsuga canadensis* **C. orientalis**

8* Ascomata up to 1.5 mm high, developing on a single stroma; ascigerous swelling often terminal, urceolate-like, 125–175 µm in diameter; on several members of the *Pinaceae* **C. ventricosa** *(= C. pinea)*

9 Spores globose to ovoid, 4.5–5.5 µm wide; ascigerous swelling sometimes terminal, 180–260×80–140 µm; mainly on *Cupressaceae* species **C. cochlearis**

9* Spores subglobose to globose, 3-3.5 µm wide; ascigerous swelling always subterminal, 150–175×110 µm; species known only on *Chamaecyparis* **C. thujina**

10 Ascomata small, developing on pistillate flowers of several *Arceuthobium* species; spores globose, 4 µm wide **C. arceuthobii**

10* Ascomata larger; on other hosts 11

11 Ascigerous swelling basal, 225×100–125 µm; ascomata up to 1.5 mm high; spores globose to subellipsoidal **C. arrhiza**

11* Ascigerous swelling not basal 12

12 Ascigerous swelling submedian to median 13

12* Ascigerous swelling subterminal to terminal, occasionally median 20

13 On wasps galls on twigs of *Quercus canbyi* **C. quercina**

13* On other hosts, not on insect galls 14

14 Ascomata height not exceeding 1 mm; ascigerous swelling median, rarely submedian 15

14* Ascomata height often exceeding 1 mm; ascigerous swelling often submedian 17

15 Spores 5–6 µm wide; ascomata up to 0.9 mm high; ascigerous swelling on average 300×150 µm; on *Elytranthe* **C. elytranthicola**

15* Spores up to 4.7 µm wide; ascomata not exceeding 0.4 mm high 16

16 On *Struthanthus*; spores regularly subglobose to globose **C. struthanthi**

16* On leaves of *Xanthostemon,*often producing necrotic lesions; spores rather variable in shape, from globose to occasionally ellipsoidal **C. xanthostemonis**

17 Ascospores subfusiform to ellipsoid or oval, 6–8×3.5–5 µm; ascomata height up to 2 mm; on *Populus* **C. calicioides**

17* Ascospores subglobose to globose; on other hosts 18

18 Ascomata large, 1.5-4.5 mm; ascigerous swelling 200–300×140–250 µm; spores 3.5–5 µm wide; on *Toona* and *Choerospondias* **C. toonae**

18* Ascomata and ascigerous swelling considerably smaller; spores wider 19

19 Ascomata undergoing repeated apical proliferation after dehiscence; ascigerous swelling 110–115 μm wide; spores globose, 5.5–7.8 μm wide; on *Rapanea* **C. rapaneae**

19* Ascomata not proliferating after dehiscence; ascigerous swelling 60–100 μm wide; spores subglobose to globose, 3.5–8 μm wide; on other species of the *Myrsinaceae* **C. confusa**

20 Spores ellipsoidal to subfusiform, 7.5–10×4–5 μm; species known only on *Tilia cordata* **C. tiliae**

20* Spores mainly globose to subglobose, rarely ellipsoidal 21

21 On *Quercus spp*. bark; some spores, at least, ellipsoidal 22

21* On other hosts; spores never ellipsoidal 24

22 Ascigerous swelling always subterminal; ascomata stout, on average 0.9 mm high, but sometimes as high as 5 mm; spores on average 7×5.7 μm; species known only on *Quercus ilex* subsp. *rotundifolia* from eastern Spain **C. valentina**

22* Ascigerous swelling mainly median, rarely subterminal; spores more variable in shape; ascomata slender, up to 3 mm 23

23 Spores on average 7.4×6.6 μm; ascomata on average 0.9 mm high; Europe *C*. **beckhausii**

23* Spores on average 4–5×3–4 μm; ascomata up to 3 mm high; North America **C. subcorticalis**

24 Spores ≤ 5 mm wide 25

24* Spores broader, up to 10 μm 28

25 Ascomata 0.2–0.3 mm high, with a clavate/urceolate habitus; ascigerous swelling 70–100×45–70 μm; species known only on *Garcinia indica* **C. indica**

25* Ascomata and ascigerous swelling on average larger; on other hosts 26

26 Ascomata with a clavate/urceolate habitus; ascigerous swelling 230–330 μm long; spores 3.6–4.2×3.1–3.6 μm **C. veillonii**

26* Ascomata with a smaller, mainly subterminal ascigerous swelling, thus not showing such an urceolate habitus 27

27 Spores 1.8–3.1 μm wide; ascomata 0.5–0.6 mm high; ascigerous swelling 60–85 μm wide; species known only on *Myrtus emarginata* **C. myrticola**

27* Spores 4–5 µm wide; ascomata 0.7–1.4 mm high; ascigerous swelling 120–130 µm wide; species known only on *Rhus chinensis and Toona sinensis* **C. rhoina**

28 Ascomata subterminal to terminal, with a clavate/urceolate habitus, up to 1.1 mm high; spores 8–10 µm wide; on *Drymis* **C. clavata**

28* Ascomata subterminal to median, up to 2.7 mm high; spores 5.5–7 µm wide; on *Symplocos* **C. symploci**

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Fig. 1 Bayesian phylogram obtained for the ITS, nuLSU and the combined analyses ITS + nucLSU. Branches showing strong support (Posterior Probabilities ≥ 0.95) are in bold. Numbers refer to the extraction codes cited in Tab. 1

Fig. 2 *Caliciopsis beckhausii*. Macroscopic and microscopic characters. **a-c** Habitus. **d-e** Young bitunicate asci (double layer indicated by an arrow). **f** Mature asci. **g** Hyphal hairs. **h** Ascospores. **i-j** Pycnidia. **k** Conidiogenous cells and conidia. *Scale bars* 0.5 mm (a-c), 10 μm (d-h, k), 0.1 mm (i-j)

Fig. 3 Distribution of *C. valentina* and *C. beckhausii* across the Iberian Peninsula

Fig. 4 *Caliciopsis valentina*. Macroscopic and microscopic characters. **a-b** Habitus. **c** Detail of mazaedium. **d** Young bitunicate asci (double layer indicated by arrows). **e** Mature asci. **f-g** Hyphal hairs. **h** Ascospores. **i** Pycnidia. **j**. Conidiogenous cells and conidia. *Scale bars* 0.5 mm (a-b), 0.1 mm (c, i), 10 μm (d-h, j)

Table1

Table 1 List of taxa used in this study, with collection numbers and Genbank accession numbers; newly produced sequences are in bold

Taxa	Locality and collection number	ITS	LSU
Caliciopsis beckhausii I162	San Juan del Monte, Burgos, SPAIN, leg. S. Pérez-Ortega & M. A. Pérez Muriel	XXXXXX X (neotype)	XXXXXX XX (neotype)
Caliciopsis calicioides	USA	JX968549	-
Caliciopsis indica 1	INDIA	GQ259981	GQ259980
Caliciopsis indica 2	INDIA	NR119752 (type)	
Caliciopsis valentina I164	Quatretonda, Valencia, SPAIN, leg. I. Garrido-Benavent 290	XXXXXX X (holotype)	XXXXXX XX (holotype)
Caliciopsis valentina I198	Bellús, Valencia, SPAIN, leg. I. Garrido-Benavent 292	XXXXXX X	-
Caliciopsis valentina I199	Bocairent, Valencia, SPAIN, leg. I. Garrido-Benavent 293 & N. Cháfer	XXXXXX X	XXXXXX X
Caliciopsis valentina I200	Vallada, Valencia, SPAIN, leg. I. Garrido-Benavent 291	XXXXXX X	XXXXXX X
Caliciopsis nigra I163	Vistabella del Maestrat, Castelló, SPAIN, leg. I. Garrido-Benavent 305 & N. Cháfer	-	XXXXXX XX
Caliciopsis orientalis	Unknown	-	DQ470987
Caliciopsis ventricosa (= *C. pinea*)	Unknown	-	DQ678097
Hamigera avellanea	USA	GU968675	AB000620
Spiromastix tentaculata	AFRICA	NR111162	AY176722

Table2

Table 2 Morphological and anatomical comparisons between *Caliciopsis valentina*, *C. beckhausii* and *C. subcorticalis*

	C. valentina	*C. beckhausii* *	*C. subcorticalis***
Ascomata height (µm)	(0.6)0.9±0.2(5)	(0.5)0.9±0.2(1.5)	Up to 3
Stalk width (µm)	(60)80±10(110)	(50)75±10(100)	80-125
Ascigerous swelling (µm)	Subterminal	Median to subterminal	Median to subterminal
Beak size (µm)	(70)130±25(180) × (80)95±10(115)	(150)285±70(450) × (30)50±10(75)	Up to 500 × 80-125
Swelling size (µm)	(150)190±20(240) × (100)130±15(160)	(125)190±30(260) × (75)110±20(160)	200-325 × 150-175
Spore shape	Subglobose to ellipsoidal	Globose, subglobose to ellipsoidal	Globose, oval to ellipsoidal (tending to ellipsoidal)
Spores size (µm)	(5.3)7±0.8(9.6) × (4)5.7±0.7(8)	(5.4)7.4±1.1(11) × (5)6.6±0.9(10)	4-5 x 3-4 [(4.5)6.2±0.6(7.3) × (3.4)4.5±0.6(5.9)]
Length/width ratio, Q	(1)1.2±0.2(1.9)	(1)1.1±0.1(1.7)	[(1)1.4±0.2(1.9)]
Conidiomata size (µm)	120-170 × 95-135	100-250 × 65-150(250)	100 broad
Conidiomata shape	Obpyriform to subglobose	Obpyriform to subglobose	Not mentioned
Conidia size (µm)	(4)4.5±0.3(5) × (1)1.4±0.2(1.6)	(3.5)4.2±0.3(4.7) × (1.2)1.5±0.2(1.7)	2.5-3.5 long [(2.8)3.6±0.3(3.9) × (1.1)1.3±0.2(1.6)]
KOH reaction of the beak fringe	+, strongly green-turquoise	+, strongly green-turquoise	+, strongly green-turquoise
NO3H reaction of ascomata and mass of extruded spores	+, strongly reddish-orange	+, weaker, paler reddish-orange	+, strongly reddish-orange

* Based on the recently collected specimens from the Iberian Peninsula
** Based also on Ellis and Everhart (1885), Fitzpatrick (1920, 1942) and Rikkinen (2000); in square brackets, values for the specimen no. 2123 obtained by us

Figure1
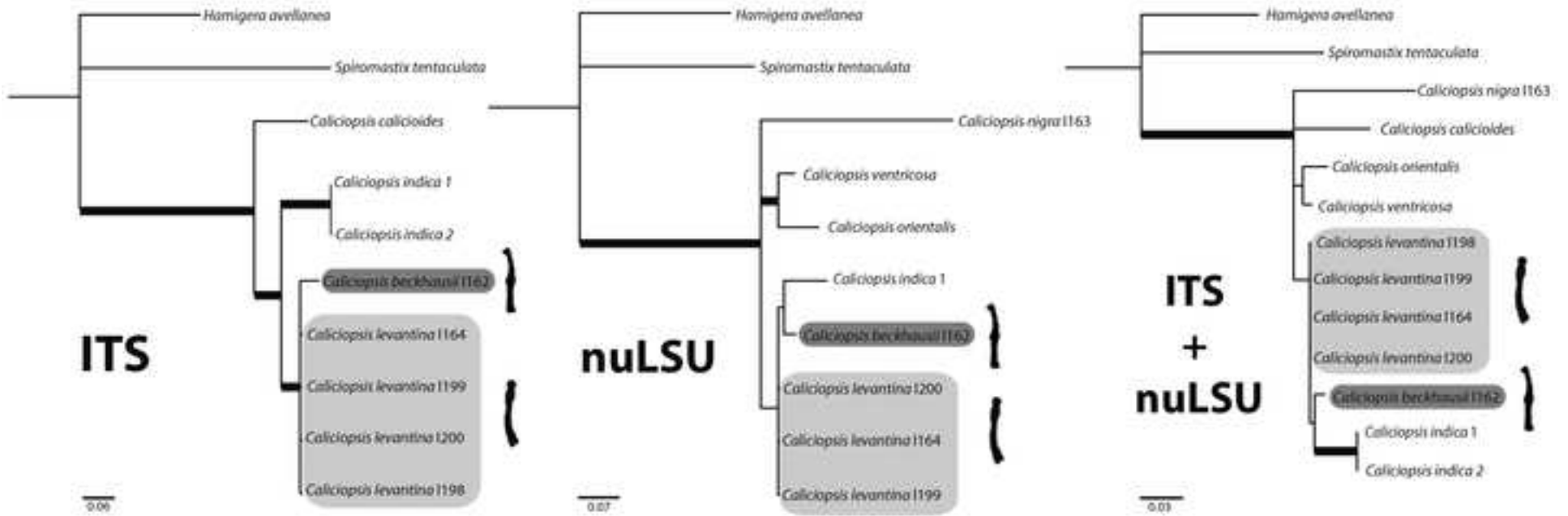
Figure2
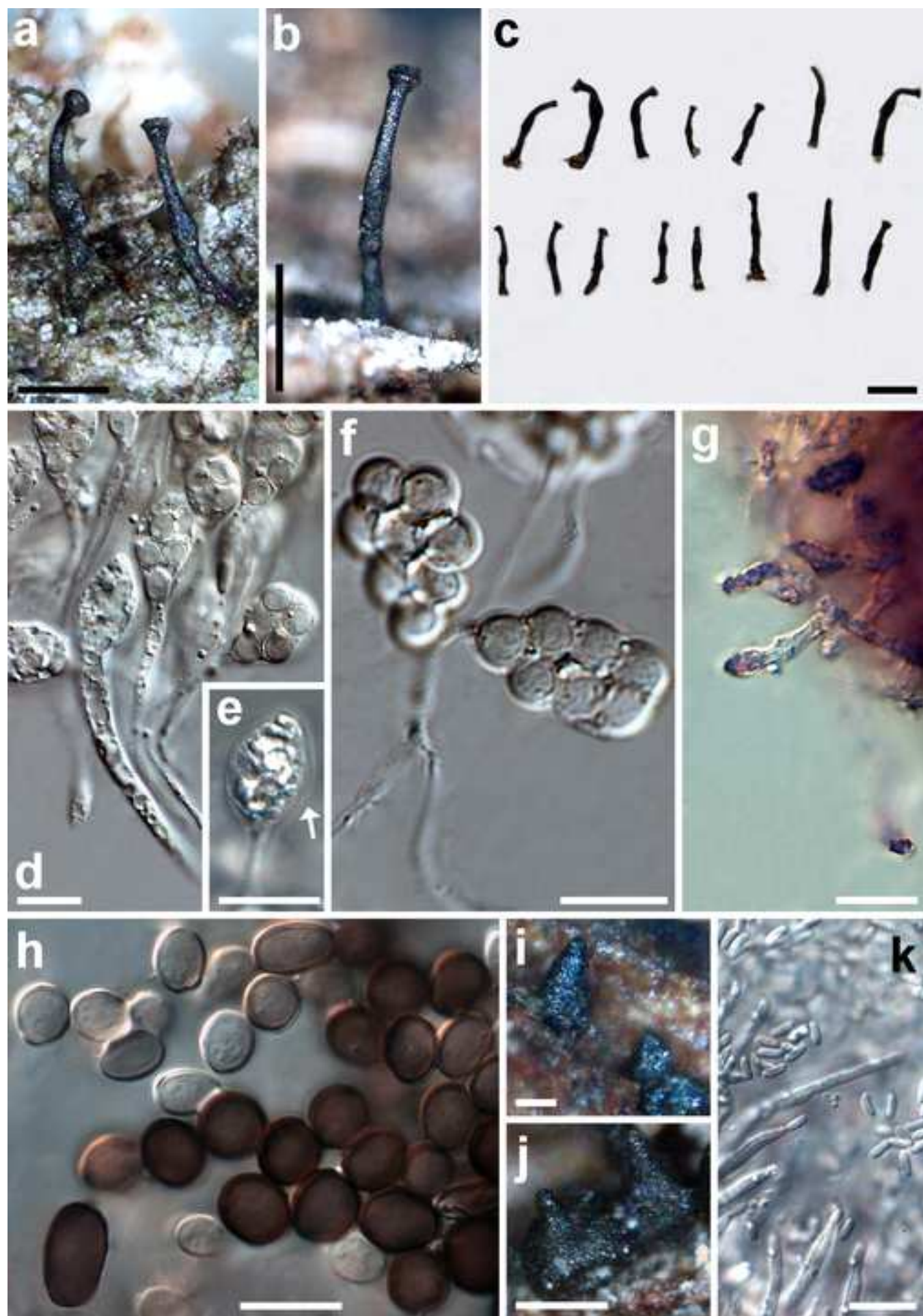Click here to download high resolution image
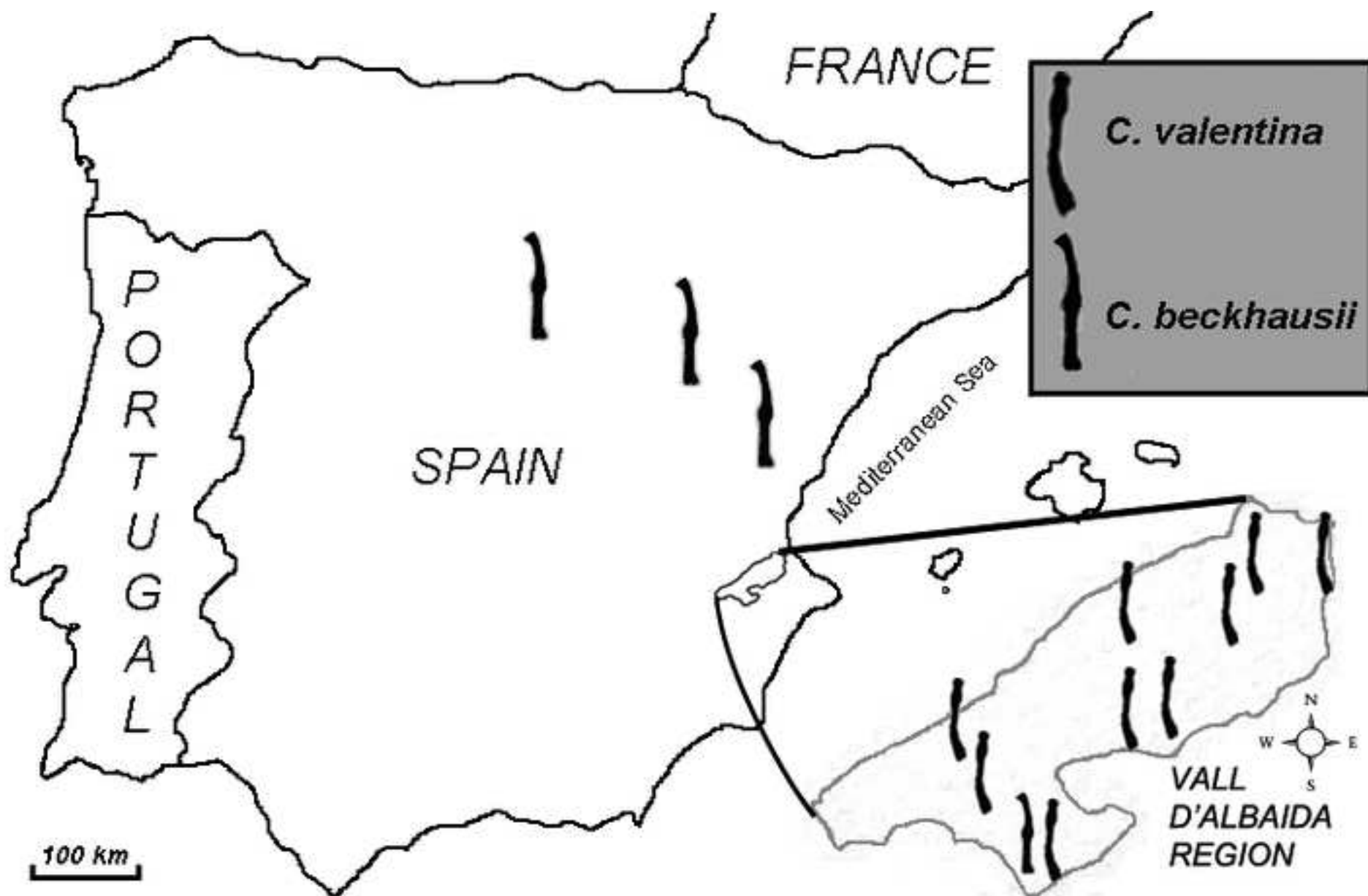
Figure3
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Figure4
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