Mycologia, 104(4), 2012, pp. 962–973. DOI: 10.3852/12-003

Kabatiella bupleuri sp. nov. (Dothideales), a pleomorphic epiphyte and endophyte of the Mediterranean plant *Bupleurum gibraltarium* (Apiaceae)

Gerald F. Bills[1]
Victor González Menéndez
Gonzalo Platas

Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Avda. del Conocimiento 3, Parque Tecnológico de Ciencias de la Salud, E-18100 Armilla, Granada, Spain

Abstract: *Bupleurum gibraltarium* is an evergreen shrub endemic to southern Spain and northern Algeria and Morocco. We have collected and cultured an undescribed *Kabatiella* species that is consistently associated with the flower rachises and leaves of *B. gibraltarium* in the province of Granada. The fungus forms melanized acervuli on overwintered flower rachises. It also can be isolated from yeast-like conidial masses that emerge from senescing leaves and from surface-disinfected healthy leaves. Like other *Kabatiella* species, the fungus forms blastic falcate to lunate conidia simultaneously from the apex of conidiogenous cells in acervuli. In culture, melanized single-septate conidia form blastically from the vegetative hyphae that accumulate in yeast-like masses. These conidia germinate by budding to form secondary yeast-like cells or directly as hyphae. In culture, the fungus behaves like, and could be confused with, *Aureobasidium* and *Hormonema* species. We describe the growth of this species in agar media and its phylogenetic position based on the analyses of nuclear ribosomal RNA gene sequences. This new species is a sister species of the morphologically similar clover pathogen, *K. caulivora*.

Key words: *Aureobasidium*, black yeasts, endophytic fungi, meristematic fungi

Introduction

One of earth's biodiversity hotspots lies in the Baetic and Rif mountain complex of southern Iberian Peninsula and northern Morocco (Médail and Quezél 1997, Comes 2004). The area is estimated to be the home of 3800–4000 plant species or subspecies living in the southern Iberian Peninsula, of which more than 400 species are presumed endemic. (Rivas-Martínez et al. 1991, 1997; Giménez et al. 2004). Extrapolating from what is still unknown about novel fungus plant-host relationships in other areas of high plant endeminisms, a wealth of unknown fungi are expected to be discovered in southern Spain and Portugal and northwestern Africa. Partial checklists of the Ascomycota of Spain (Unamuno 1941; Ortega and Aguilera 1987; Checa 1997a, b, 1998; Checa and Barr 1999; Farr and Rossman 2011) corroborate that much of the fungal flora associated with native and endemic plants of Andalucía remain unknown. Other examples also support the hypothesis that the region harbors many unknown fungi; for example, novel beneficial endomycorrhizal fungus-host relationships have been observed from the rhizosphere of endemic plants in the Sierra de Baza and Sierra Nevada (Palenzuela et al. 2008, Palenzuela et al. 2010).

Bupleurum gibraltarium Lam. (adelfilla de Gibraltar, clujía, cuchilleja, revientabuey) is an evergreen shrub endemic to southern Spain and northern Algeria and Morocco. It grows in rocky areas, rock outcrops, cliffs and dry stream beds, usually in calcareous areas, but occasionally also found with slates or schists. The composition of the plant's essential oils, other volatile compounds and saikosaponins from root extracts has been characterized (Ashour and Wink 2011). Rural inhabitants have used it for folk remedies, and the anti-inflammatory activity and the in vitro antimicrobial activity of its essential oils and extracts have been investigated. Seventeen species and subspecies of *Bupleurum* are known in the Iberian Peninsula (Neves 2003), of which fungi have been recorded only on *B. fruticescens*, *B. praealatum*, *B. rigidum* and *B. spinosum* (Farr and Rossman 2011).

We have collected and cultured an undescribed *Kabatiella* species that consistently is associated with the flower stems and leaves of *B. gibraltarium* in the province of Granada. The fungus forms melanized acervuli on overwintered flower rachises. It also can be isolated from yeast-like conidial masses that emerge from senescing leaves and from healthy surface-disinfected leaves. Like other *Kabatiella* species, the fungus forms blastic falcate to lunate conidia from the apex of conidiogenous cells in acervuli. In culture, melanized aseptate or single septate conidia form blastically and often synchronously from the vegetative hyphae that accumulate in yeast-like masses. On some media the vegetative hyphae disarticulate directly. In culture the fungus behaves like and could be confused with *Aureobasidium* and *Hormonema* anamorphs of

Submitted 3 Jan 2012; accepted for publication 25 Jan 2012.
[1] Corresponding author. E-mail: gerald.bills@medinaandalucia.es

TABLE I. Strains *Kabatiella bupleuri* and related fungi sequenced for this study

Species	Strain numbers	Substratum	Location	GenBank accessions
Kabatiella bupleuri	F277039, CBS 131302	Dead flower rachises, *B. gibraltarium*	Upper parking, Presa de Quentar, Granada, Spain	JN886788
Kabatiella bupleuri	F277640	Dead flower rachises, *B. gibraltarium*	Rock cliffs, Near Fornes, Granada, Spain	JN886789
Kabatiella bupleuri	F277641	Dead flower rachises, *B. gibraltarium*	Cañada Real de Jayena, Granada, Spain	JN886790
Kabatiella bupleuri	F277979, CBS 131303	Dead flower rachises, *B. gibraltarium*	Presa de Quentar, Carretera de presa, Granada, Spain	JN886793
Kabatiella bupleuri	F278240, CBS 131304 Ex-holotype	Dead flower rachises, *B. gibraltarium*, HOLOTYPE	Embalse de Canales, Pinos Genil, Granada, Spain	JN886792
Kabatiella bupleuri	F278263	Endophyte of B. *gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886799
Kabatiella bupleuri	F278264	Endophyte of *B. gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886800
Kabatiella bupleuri	F278265	Endophyte of *B. gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886801
Kabatiella harpospora (Bres. & Sacc.) Arx	F121513, CBS 122914	Surface-disinfected stems and leaves of *Viscum album*	Robledo de Chavela, Madrid, Spain	JN886794
Selenophoma juncea (Mont.) Arx	F277101, CBS 131305	Dead leaves of *Spartium juncea*	Fuente de Hervidero, Granada, Spain	JN886791
Dothichiza sp.	F277643	Dead leaves of *Retama sphaerocarpa*	Pino Genil, Granada, Spain	JN886795
Aureobasidium pullulans (De Bary) G. Arnaud ex Cif., Ribaldi & Corte	F278259	Endophyte of *B. gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886796
Aureobasidium pullulans	F278260	Endophyte of *B. gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886797
Aureobasidium pullulans	F278261	Endophyte of *B. gibraltarium*	Embalse de Canales, Pinos Genil, Granada, Spain	JN886798

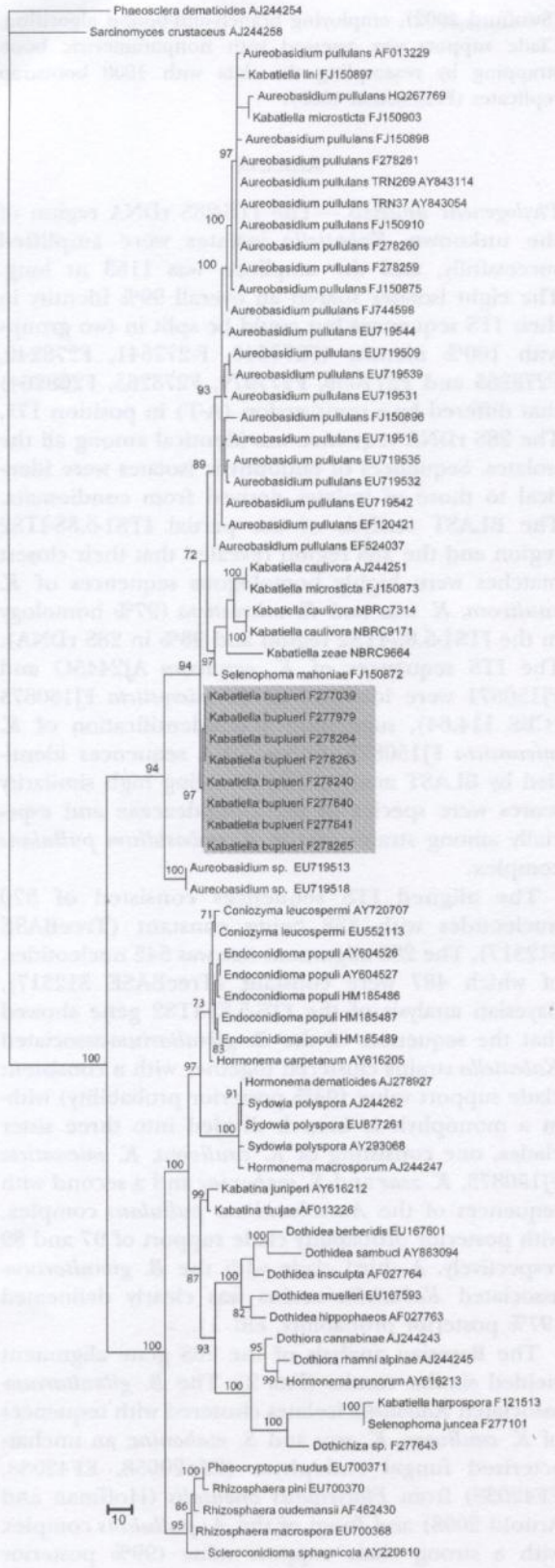
other dothideaceous fungi. We describe the field specimens, the growth of this species on agar media and its phylogenetic position based on the analyses of nuclear ribosomal RNA gene sequences. This new species is related to and is morphologically similar to *Kabatiella caulivora*, the fungus causing northern anthracnose of clover (*Trifolium* spp.).

MATERIALS AND METHODS

Isolation, cultures and morphology.—Fungi were isolated from conidia developing from acervuli on dead flower rachises, from new conidia developing from acervuli incubated on malt-yeast extract agar or from conidial pustules formed on senescent leaves. Conidia were separated manually or with a Skerman micromanipulator on cornmeal agar (CMA, Sigma-Aldrich) supplemented with 50 µg/mL streptomycin sulphate and oxytetracycline. Isolates were cultured in three different media, 2% malt agar (MEA), CMA, Czapek-Dox agar (CDA), to study their macro- and microscopic characteristics. Colony diameter, texture, pigmentation, margin appearance, exudates and colors were recorded after 3 wk at 22 C. Microscopic features were evaluated by observing structures mounted in 5% KOH or lactophenol and photographed. Strains designated with a format (e.g. F121513) were preserved as frozen conidia and mycelia in 10% glycerol at −80 C and maintained in the culture collection of Fundación MEDINA (www.medinaandalucia.es), unless designated otherwise.

To determine whether the fungus caused endophytic infections in healthy leaves five plants were collected near the Embalse de Canales, Granada (TABLE I). Two healthy leaves were removed from each plant and cut into pieces approximately 5 mm². Leaf pieces were surface-disinfected serially by passage through 95% ethanol (30 s), 25% household bleach (1 min), 95% ethanol (30 s), and 10 pieces from each leaf were aseptically transferred to a Petri dish with CMA supplemented with streptomycin sulfate and oxytetracycline (50 µg/mL). After 7 d and up to 21 d at 22 C, leaf fragments were observed for the emergence of the typical submerged filaments bearing lunate, melanized holoblastic conidia.

DNA extraction, PCR amplification and DNA sequencing.—Genomic DNA was extracted from aerial mycelia of strains



grown on malt-yeast extract agar (Bills et al. 1999). DNA fragments containing the ITS1-5.8S-ITS2 and the initial 600 nucleotides of the 28S gene were amplified with the 18S3 (5′–GATGCCCTTAGATGTTCTGGGG–3′) and NL4 (O'Donnell 1993) primers. PCR amplifications followed standard procedures (5 min at 93 C, then 40 cycles of 30 s at 93 C, 30 s at 53 C and 2 min at 72 C) using Taq DNA polymerase (QBiogene Inc.) following the manufacturer's recommended procedures. Amplification products (0.1 µg/mL) were sequenced with the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) following manufacturer's recommendations. Each PCR product was sequenced bidirectionally with the same primers used for PCR reactions. Partial sequences obtained during sequencing reactions were assembled with GeneStudio software (GeneStudio Inc., Georgia). The sequences of the complete ITS1-5.8S-ITS2-28S region or independent ITS and 28S rDNA sequences were compared with GenBank or the NITE Biological Resource Center (http://www.nbrc.nite.go.jp/) databases using the BLAST application.

Determination of genus and species depended on sequences from work on the *Aureobasidium* complex (Yurlova et al. 1999, Zalar et al. 2008), and published sequence accession numbers were used to label phylogenetic trees (FIGS. 1, 2, SUPPLEMENTAL DATA). In addition to the *Kabatiella* isolates from *B. gibraltarium*, a few sequences from other dothideaceous fungi isolated from plants collected in Granada were included (TABLE I). A preliminary sequence set was aligned with GeneStudio software and visually adjusted with GeneDoc 2.5 software (Nicholas and Deerfield 1997). All new sequences from this work were deposited in GenBank (TABLE I).

Phylogenetic analysis.—Species and genus groups were tested with Bayesian analysis employing the Markov chain Monte Carlo (MCMC) approach using MrBayes 3.01 (Ronquist and Huelsenbeck 2003). To improve mixing of the chain four incrementally heated simultaneous Monte Carlo Markov chains were run over 2 000 000 generations. Hierarchical likelihood ratio tests with MrModeltest 2.2 (Nylander 2004) calculated the Akaike information criterion (AIC) values of the nucleotide substitution models. The models selected by AIC for the alignments were GTR + I + G (28S) and SYM + I + G (ITS), allowing for both analysis, six classes of substitution types, a portion of invariant alignment positions and mean substitution rates varying across the remaining positions according to a gamma distribution. Priors used for the MCMC process were a Dirichlet distribution for substitution rates and nucleotide frequencies and a uniform prior for the rate parameter of the gamma distribution. Both analyses used the sampling

←

FIG. 1. Phylogenetic tree of *Kabatiella bupleuri* (gray box) and related species of the Dothideales generated by Bayesian analysis of ITS rRNA partial sequences. *Phaeosclera dematioides* was designated the outgroup. Clade probability values are indicated at the branches. Bar = 10 changes.

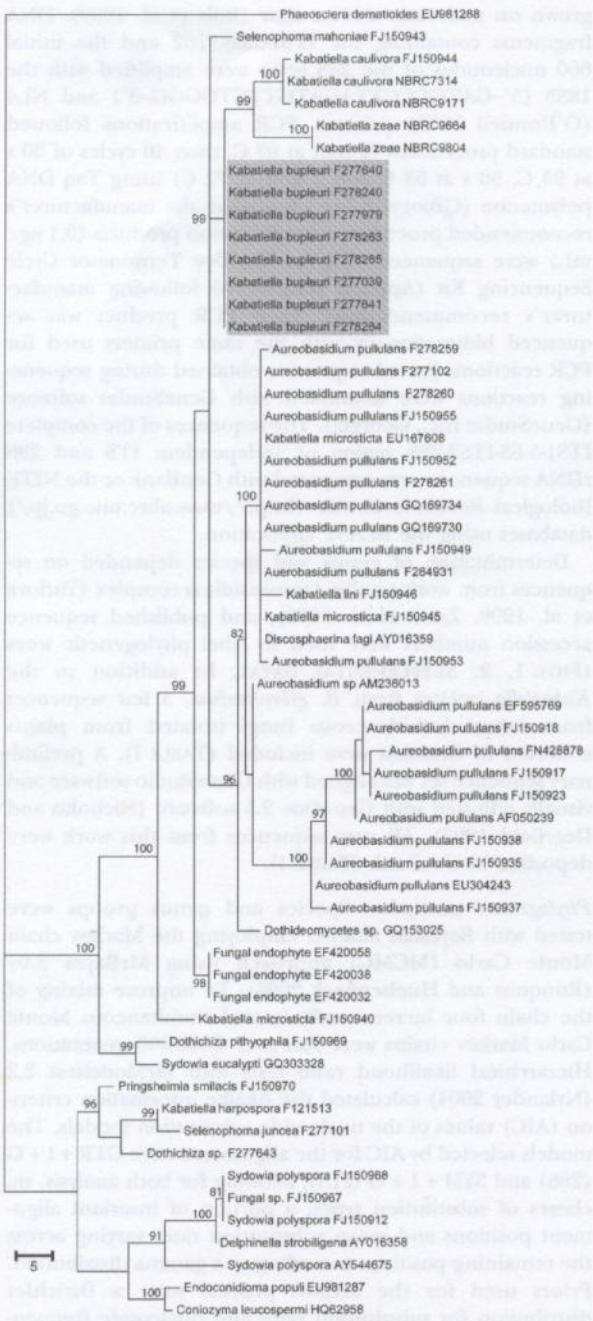


FIG. 2. Phylogenetic tree of *Kabatiella bupleuri* (gray box) and related species of the Dothideales generated by Bayesian analysis of 28S rRNA partial sequences. *Phaeosclera dematioides* was designated the outgroup. Clade probability values are indicated at the branches. Bar = 5 changes.

frequency of 100 to store trees, with the 1000 first trees discarded to estimate a majority rule consensus tree.

The ITS and 28S datasets also were analyzed by unweighted maximum parsimony (MP) with PAUP 4.0b10 (Swofford 2002), employing branch-and-bound algorithm. Clade support was assessed with nonparamertric bootstrapping by resampling the data with 1000 bootstrap replicates (Felsenstein 1985).

RESULTS

Phylogenetic analysis.—The ITS-28S rDNA region of the unknown *Kabatiella* isolates were amplified successfully, and the amplicon was 1163 nt long. The eight isolates shared an overall 99% identity in their ITS sequences but could be split in two groups with 100% identity (F277640, F-277641, F278240, F278265 and F277039, F277979, F278263, F268264) that differed by a transversion (A-T) in position 171. The 28S rDNA sequence was identical among all the isolates. Sequences of endophytic isolates were identical to those of isolates derived from condiomata. The BLAST analysis of the partial ITS1-5.8S-ITS2 region and the 28S region revealed that their closest matches were highly homologous sequences of *K. caulivora*, *K. zeae* and *K. microsticta* (97% homology in the ITS1-5.8S-ITS2 region and 98% in 28S rDNA). The ITS sequences of *K. caulivora* AJ2445O and FJ150871 were identical to *K. microsticta* FJ150873 (CBS 114.64), suggesting a misidentification of *K. microsticta* FJ150873. Other DNA sequences identified by BLAST analysis and revealing high similarity scores were species of the Dothideaceae and especially among strains of the *Aureobasidium pullulans* complex.

The aligned ITS sequences consisted of 570 nucleotides with 338 being constant (TreeBASE S12317). The 28S alignment size was 542 nucleotides, of which 487 were constant (TreeBASE S12317). Bayesian analysis of the ITS-5.8S-ITS2 gene showed that the sequences of the *B. gibraltarium*-associated *Kabatiella* strains clustered together with a consistent clade support value (94% posterior probability) within a monophyletic branch divided into three sister clades, one consisting of *K. caulivora*, *K. microsticta* FJ150873, *K. zeae* and *S. mahoniae* and a second with sequences of the *Aureobasidium pullulans* complex, with posterior probability clade support of 97 and 89 respectively. A third clade with the *B. gibraltarium*-associated *Kabatiella* strains was clearly delineated (97% posterior probability, FIG. 1).

The Bayesian analysis of the 28S gene alignment yielded similar results (FIG. 2). The *B. gibraltarium*-associated *Kabatiella* isolates clustered with sequences of *K. caulivora*, *K. zeae* and *S. mahoniae*, an uncharacterized fungal endophyte (EF420058, EF42038, EF42038) from *Platycladus orientalis* (Hoffman and Arnold 2008) and fungi of the *A. pullulans* complex with a strong clade support value (99% posterior

probability). Their closest neighbors were *K. caulivora* and *K. zeae* (98% identity) being clustered with a 99% posterior probability (FIG. 2).

MP analyses of the 28S and ITS-5.8S-ITS2 genes yielded trees with very similar topology to those of the Bayesian analysis (SUPPLEMENTAL DATA, SUPPLEMENTARY FIGS. 1, 2), and once again branches corresponding to the *A. pullulans* complex, the *K. caulivora-K. zeae* group and the *B. gibraltarium* isolates were evident. MP analysis of the 28S region (SUPPLEMENTARY FIG. 1) delineated an *Aureobasidium-Kabatiella* clade (87% bootstrap support), while the *B. gibraltarum*-associated *Kabatiella* appeared as a statistically unsupported sister clade to a distinct clade including *K. caulivora-K. zeae* (82% bootstrap support). MP analysis of the ITS-5.8S-ITS2 (SUPPLEMENTARY FIG. 2) clearly delineated a clade with the *B. gibraltarum*-associated *Kabatiella* strains (86% bootstrap support) and distinct branches separating *K. zeae* and *K. caulivora* from each other and from the *A. pullulans* complex.

These results clearly delineate this set of epiphytic and endophytic strains from *B. gibraltarium* as a unique clade and an unknown taxon. But the analyses also suggest a taxonomic dilemma as to whether the *B. gibraltarum*-associated *Kabatiella* strains are congeneric with *A. pullulans* or whether these strains along with *K. caulivora* and *K. zeae* represent a sister genus of *Aureobasidium*. For the reasons explained below, we believe it was best to describe these strains as a new species in *Kabatiella*.

TAXONOMY

Kabatiella bupleuri Bills sp. nov. FIGS. 3–5
MycoBank MB563713.

A fungus morphologically similar to *K. caulivora* (Kirchn.) Karak. inhabits the leaves, stems and flowers of *B. gibraltarium* in Spain, forms persistent melanized acervuli on dead flower rachises and umbels, conidial pustules on senescent leaves, and causes symptomless endophytic infections. Conidiogenous cells holoblastic, formed from the inner cells of the acervulus wall. Conidia hyaline, smooth, aseptate, falcate, lunate, sigmoid. Conidia germinating by simultaneous elongation into short septate filaments and apical or lateral secondary synchronous budding into lunate to ellipsoidal yeast-like cells. In culture, hyphae predominately submerged in the age. Hyphae giving rise holoblastic conidia from undifferentiated loci on vegetative cells. Conidia elliptical, reniform or lunate, aseptate or one-septate in age, often constricted at the septum, hyaline to olivaceous black, 10–20 µm long, 4–8 µm wide, solitary, in small groups or accumulating in yeast-like masses.

Holotype: SPAIN. GRANADA: Pinos Genil, Embalse de Canales, GDA 58364, F278240, ex-holotype culture, CBS 131304, from dead flower rachises of *Bupleurum gibraltarium*.

Etymology: Referring to the genus of the plant host.

Acervular conidiomata on host: Forming during the winter as flower rachises senesce and persisting through the spring and summer. Originating subepidermally, probably from endophytic infections, becoming erumpent, irregular to ovoid, scattered to dense, often longitudinally confluent, granular, dull black, mostly 100–200(–400) µm diam, ostiole absent, opening by irregular splitting and erosion of epidermis, exposing a conidogenous layer that is a translucent when humid and imbibed with water. Acervular wall composed of tightly packed isodiametric to cylindrical, thick-walled cells, hyaline when young, becoming yellowish or reddish brown and finally black. Conidiophores absent. Conidiogenous cells formed from the inner cells of acervulus wall, thick-walled, indeterminate, discreet, hyaline to pale brown, ampulliform to cylindrical, 5–12 µm diam, holoblastic, giving rise to conidia from broad, flat apical surfaces or occasionally from a narrow restriction. Conidia mostly 15–20 µm long, 4–6 µm wide. *Acervular conidia* hyaline, smooth, eguttulate, aseptate, falcate, lunate, sigmoid to slightly curved, apex acute, base tapered and truncate with a faint scar, (12–)15–20(–24) µm long, 4–6 µm wide at midpoint. Conidia germinating by simultaneous elongation into short septate filaments and apical or lateral secondary synchronous budding into lunate to ellipsoidal yeast-like cells. Acervular conidiomata or conidial masses on leaves originating at or below the epidermis, first appearing a thin yeast-like layer, later forming moist, translucent pustules, drying white to cream and finally brown to black.

Colonies on MEA 35–40 mm diam (FIG. 5A), with margin submerged, feathery, fibrous to coarsely fimbriate, usually forming well defined, adpressed to submerged sinuous or tortuous radial strands, sometimes slightly furrowed, slightly raised and granular toward the center, consisting predominantly of submerged, radially extending hyphae, sometimes accumulating moist to granular masses of melanized yeast-like cells, and melanized and hyaline falcate conidia toward colony center, shiny to moist during the first few weeks, aerial mycelium absent to scant, but abundant submerged to adpressed filamentous non-sporulating, hyaline to pale pink mycelium may emerge at the surface or from distal regions of hyphal strand after prolonged incubation (>1 mo), or a hyaline, non-sporulating mycelium may form during spontaneous sectoring of older colonies. Initially mycelium melanized and producing and accumulating abundant melanized conidia and yeast-like cells on submerged hyphae, as hyphal strands extend downward

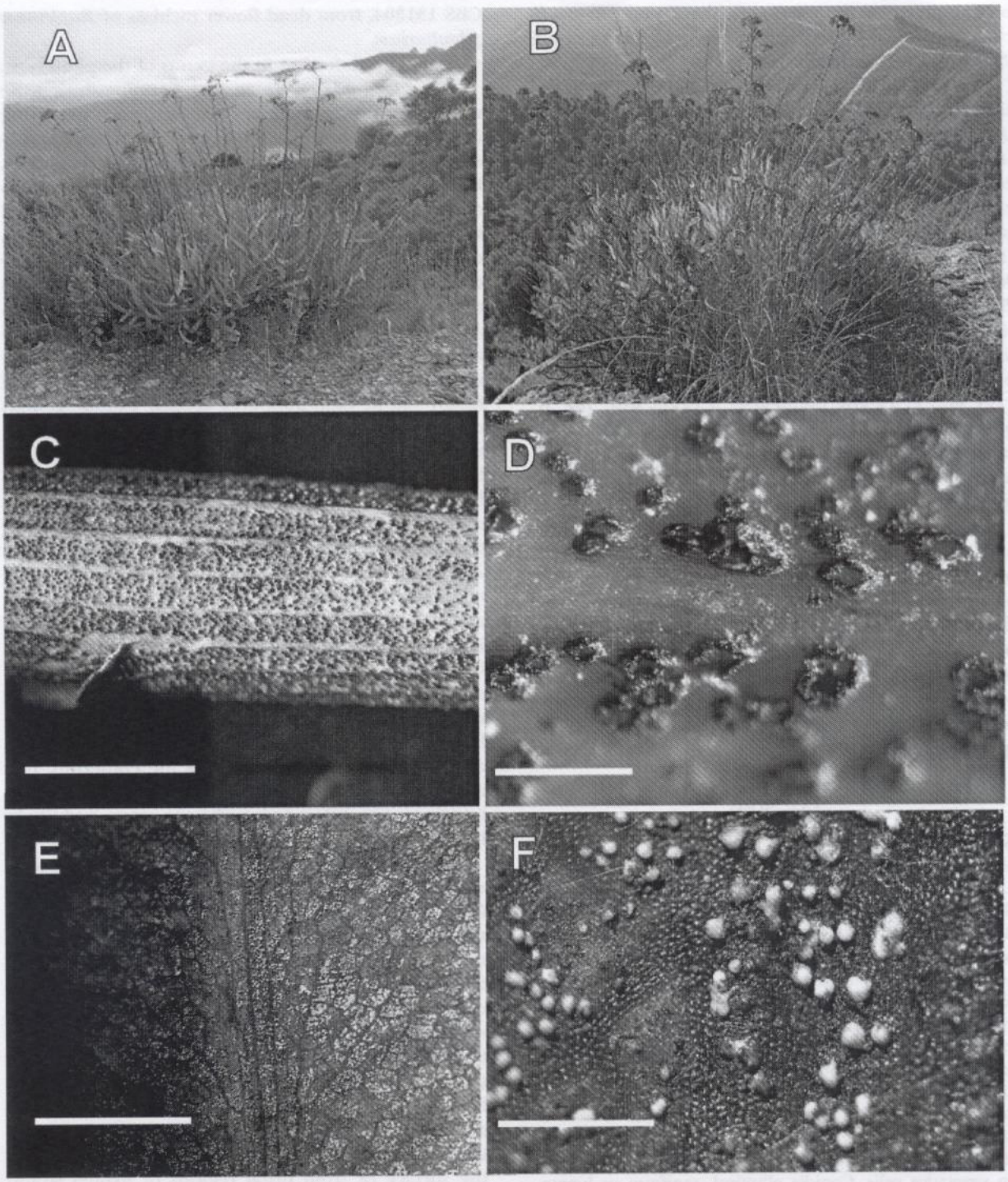


FIG. 3. *Bupleurum gibralatarum* and *Kabatiella bupleuri*. A. *B. gibraltarium*. Habit. Mirador de la Carretera de Cabra Montesa, Granada, Spain. Jan 2011. Note senescent flower rachises. B. *B. gibraltarium*. Mesa de Fornes, Granada, Spain. Habit. Jul 2009. Note blooming flower rachises. C. *K. bupleuri*. Acervular conidiomata on dead flower rachis (F277640). Bar = 5 mm. D. *K. bupleuri*. Acervular conidiomata on dead flower rachis (F278240). Bar = 500 µm. E. *K. bupleuri*. Conidial pustules on senescent leaves (F277640). Bar = 500 µm. F. *K. bupleuri*. Conidial pustules on senescent leaves F278240). Bar = 500 µm.

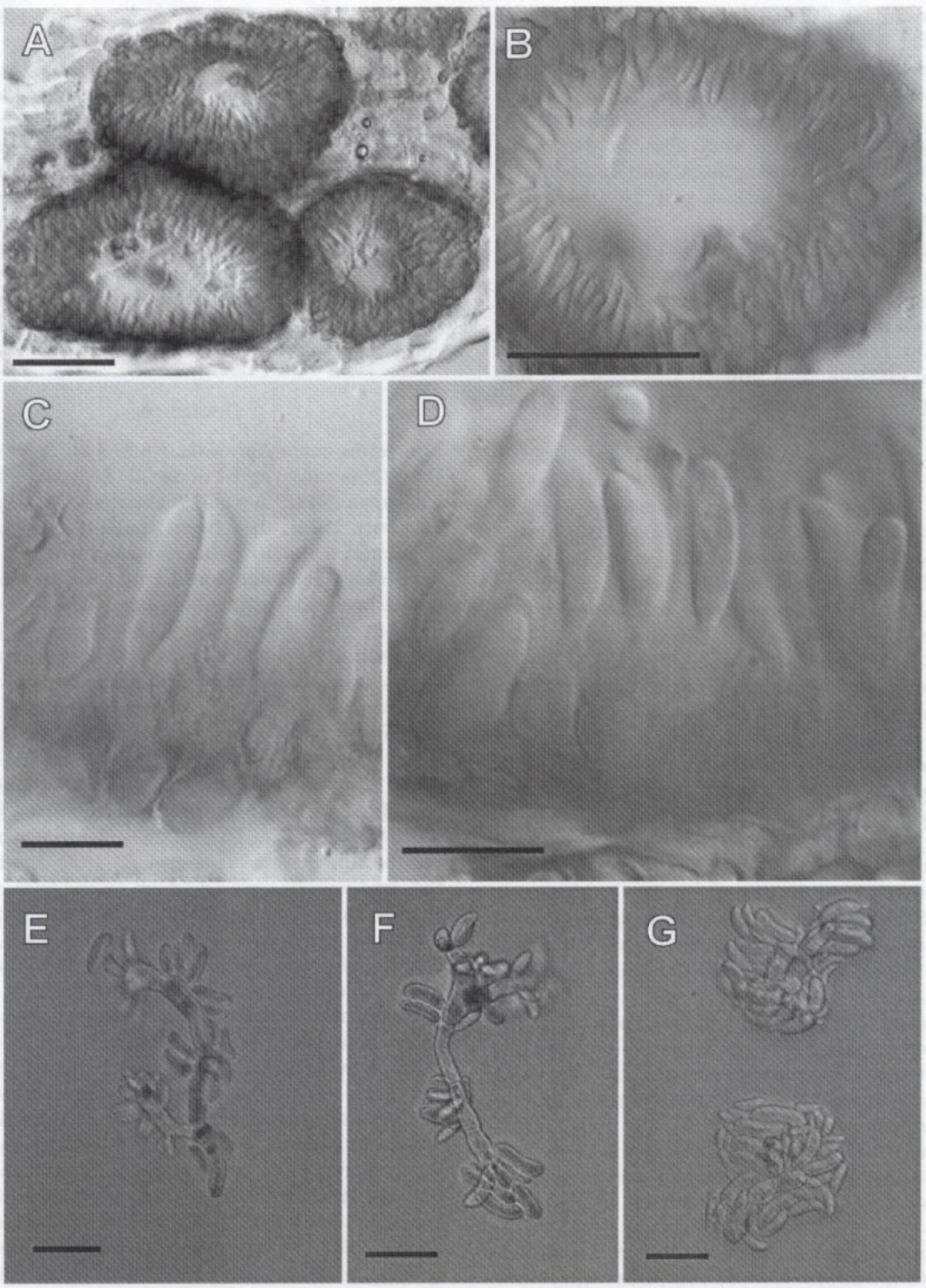


FIG. 4. Micromorphology of *K. bupleuri*. A. Conidiomata transverse section (F278240). Bar = 100 µm. B. Condiomata transverse section (F278240). Bar = 100 µm. C. Conidiogenous cells and conidia (F278240). Bar = 10 µm. D. Conidiogenous cells and conidia (F278240). Bar = 20 µm. E. Germinating conidia from conidiomata. Bar = 10 µm. F. Germinating conidia from conidiomata. Bar = 20 µm. G. Germinating conidia from conidiomata (F278240). Bar = 20 µm.

and outward sporulation gradually diminishes or ceases, finally with filamentous hyphae developing at extremes of older colonies. In some strains, scattered to gregarious incomplete acervuli formed in >4 wk at the agar surface, usually with masses of moist conidia. From pale olive-brown to dark olive, or dark olive-brown, eventually becoming black, with distal ends or sectors hyaline to pale pinkish gray. No growth was observed at 37 C.

Colonies on CDA 14–20 mm (FIG. 5B), submerged and finely fimbriate at the margin, becoming raised rugulose, cerebriform to mucoid, with a clear transition from membranaceous yeast-like condial masses and at the center to submerged mycelia growth toward the margins, dark olive at the center, to dull grayish pink, pink to hyaline, sometimes with watery zones or sectors mixed with submerged hypae.

Colonies on CMA 31–40 mm diam (FIG. 5C), submerged to adpressed, extending as tortuous to feathery radial strains from the center, silky to shiny, moist, dark olive, hyaline to pale pinkish gray, finally olivaceous black to brownish black, with an accumulation of brownish black yeast-like cells at the center.

Sporulation in culture occurs on submerged or appressed hyphae (FIG. 5E, F). Hyphae initially consisting of isodiametric to short cylindrical, thick-walled cells, occasionally forming aggregates or thick-walled, melanized cells with transverse septae, often forming moniliform terminals in age or continuing to extend as hyaline septate mycelium. Conidiogenous cells absent or on short lateral undifferentiated hyphae. Conidia arising holoblastically from undifferentiated loci on vegetative hyphae, elliptical, reniform or lunate, aseptate or one-septate in age, smooth, often constricted at the septum, hyaline to olivaceous black, 10–20 µm long, 4–8 µm wide, solitary, in small groups or accumulating in yeast-like masses, occasionally arising as endoconidia in older vegetative hyphae.

Habitat: Inhabiting stems and leaves of *B. gibraltarium.* Conidiomata may be observed year-round on dead flower stems and sometimes on dead leaves and petioles. Sporulation is most abundant during late winter and spring rains.

Known distribution: Spain, observed in at least a dozen locations in the mountainous regions of Granada but likely to coincide with the range of the host.

Specimens examined: SPAIN, GRANADA: Embalse de Canales, dried holotype GDA 58364 and ex-holotype culture F278240 = CBS 131304 and specimens and living cultures (TABLE I).

Comments: Kabatiella bupleuri can coexist with *A. pullulans* on the host plant (TABLE I), however it is easily distinguished by its stronger tendency to form submerged mycelial strands deep into the agar, and its more melanized hyphae and formation of falcate conidia, especially melanized two-celled conidia (FIG. 5F). In addition to its host association, *K. bupleuri* can be distinguished from the corn eye spot fungus, *K. zeae*, by its relatively shorter and broader conidia and its consistent formation of dark two-celled conidia in newly isolated cultures; it also lacks the long clavate conidiophores formed on the host described for *K. zeae* and *K. microsticta.* On the host plant *K. bupleuri* can be distinguished from *K. caulivora* by its larger and more persistent acervuli and in agar culture by its two-celled melanized conidia.

DISCUSSION

The genus name *Kabatiella*, typified by *K. microsticta* Bubák, the causal agent of day lily (*Hemerocallis* spp.) leaf streak (Yoshikawa and Yokoyama 1987, Leahy and Schubert 1996), has been applied to melanized, filamentous, usually plant-associated fungi that produce slimy, yeast-like conidia that are formed basipetally in a non-synchronous manner from one or few loci on cells of undifferentiated vegetative hyphae (Hermanides-Nijhof 1977, von Arx 1981, Siefert et al. 2011). At least 33 species and combinations have been included in the genus (www.mycobank.org). The generic boundaries have been ambiguous, and the genus is likely to be heterogeneous and overlap with other dothideaceous genera. *Kabatiella* has been considered a synonym of *Aureobasidium* (Hermanides-Nijhof 1977). The rationale for the synonymy was that the genera are morphologically similar, but they traditionally were separated on the basis of their trophic strategies. *Kabatiella* species were regarded mainly as plant parasites, causing leaf spots, while *Aureobasidium* species were mainly epiphytes and saprobes (Hermanides-Nijhof 1977, Yurlova et al. 1999). Both genera have been connected to the teleomorph genus *Discosphaerina* Höhnel (Sivanesan 1984). Because some *Kabatiella* species may occur on decaying leaves the two genera were considered to form a trophic continuum and consequently were treated as synonyms. In contrast, von Arx (1970, 1981) recognized that generally *Kabatiella* species are plant parasites, have a restricted host range and produce intrastomatal hyphal bodies on which the erumpent conidiogenous cells arise and therefore regarded *Kabatiella* species as the parasitic, acervular counterparts of *Aureobasidium* species.

To test the monophyly between the *K. bupleuri*, other *Kabatiella* species and *Aureobasidium*, recent data from a phylogenetic reassessment of the *A. pullulans* complex (Zalar et al. 2008) were resampled,

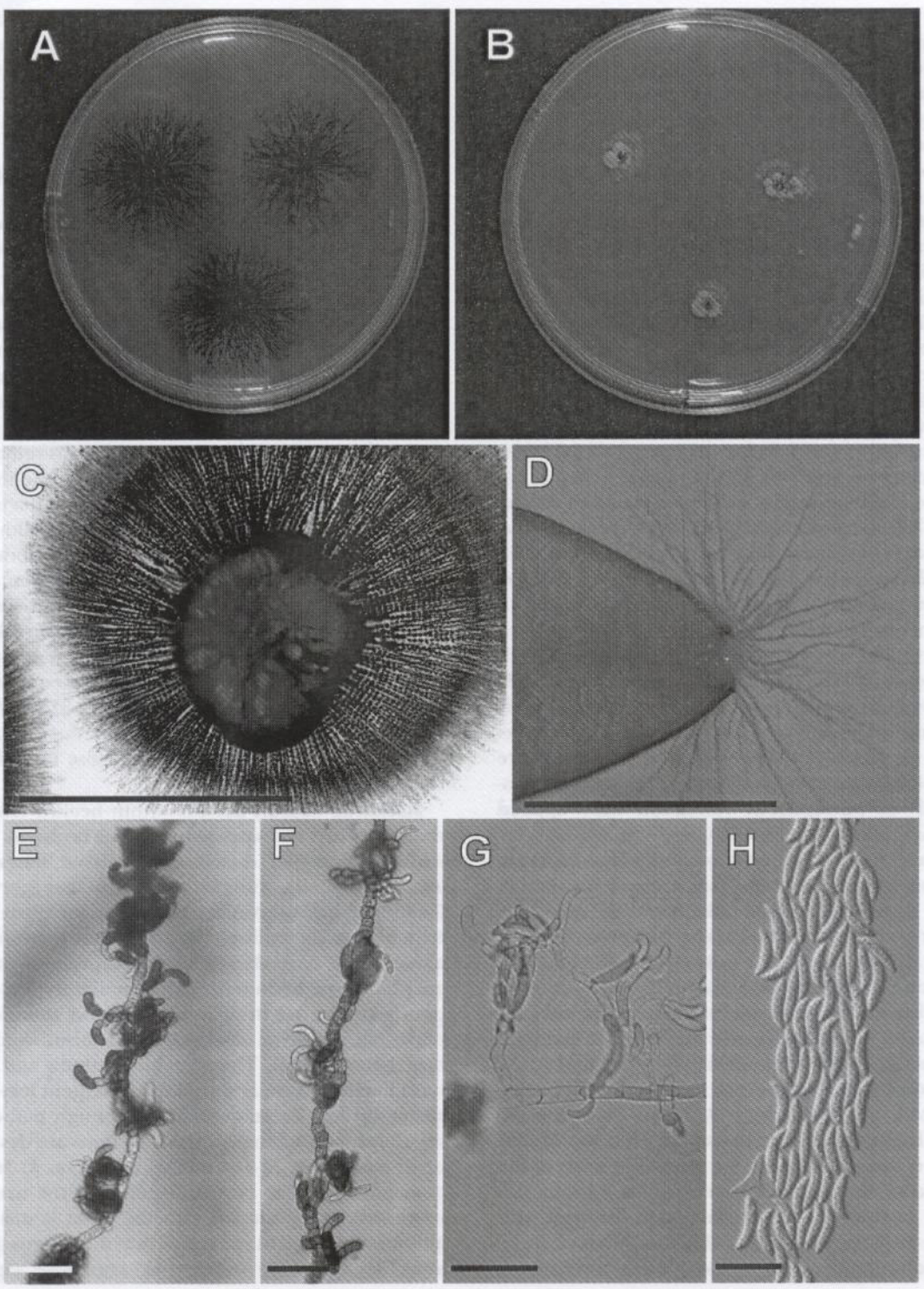


FIG. 5. *Kabatiella bupleuri* (F278240) in culture. A. Growth on 2% malt agar, 14 d, 22 C. B. Growth on Czapek Dox agar, 14 d, 22 C. C. Young colony on malt yeast extract agar initiated from conidia on host. Bar = 1 cm. D. Emergence of sporulating hyphae from surface sterilized leaf. Bar = 1 cm. E. Hyphae, conidiogenesis, and conidia. Bar = 10 μm. F. Hyphae, conidiogenesis and conidia. Bar = 10 μm. F. Hyphae, conidiogenesis and conidia. Bar = 10 μm. G. Germinating

and our results mirrored those of that analyses. The boundaries between *Kabatiella* and *Aureobasidium* remained ambiguous because relatively few sequences of *Kabatiella* species were available for analysis, and of those none were type strains. Analysis of these sequences leads to three outcomes and interpretations and results in a polyphyly. First, as described by the authors of the *Aureobasidium* reassessment (Zalar et al. 2008), some *Kabatiella* strains (e.g. *K. lini* and some strains identified as *K. microsticta*) appear to be conspecific with *A. pullulans*. Therefore, either they were misidentified or, if correctly identified, the implication would be that *Kabatiella* is synonymous with *Aureobasidium*. Second, other strains (e.g. some strains of *K. microsticta*, *K. zeae*, *K. caulivora*, *K. bupleuri*) appear to belong to sister clades of the *A. pullulans* complex. Strains of *K. zeae* (Narita and Hiratsuka 1959) from maize in Japan (NRBC 9664, 9804) are close relatives of *K. bupleuri* (FIGS. 1, 2), while the ITS sequence of a German strain of *K. zeae* (CBS 767.71) indicated affinities with the Coniochaetales (data not shown). Other species placed in *Kabatiella* are more distantly related. For example, we have isolated a dothideaceous endophyte of *Viscum album* (TABLE I) that synchronously produced falcate conidia from sterigmate conidiogenous cells and closely matched morphological descriptions of *K. harpospora*. Although this strain was phenotypically consistent with the morphological and trophic definition of *Kabatiella*, phylogenetically it was more closely allied with *Dothiora* (FIGS. 1, 2). Third, if similar levels of phylogenetic resolution were applied to defining *Kabatiella* species as were applied for delimiting species among ecologically disparate strains of *A. pullulans*, one might conclude that *K. bupleuri* is a specialized population of *K. caulivora*. However, our perception of the distinctness of *K. bupleuri* derives from its consistent association with aerial portions of this perennial host plant, its consistent absence on other plants at the same sites, its conspicuous, persistent, heavily melanized acervuli on flower rachises and its consistent two-celled melanized conidia on hyphae submerged in agar. Furthermore, the climate and habitat of *B. gibraltarium* are extremely harsh and highly dissimilar from the humid habitats of clover or maize, the respective hosts of the *K. caulivora* and *K. zeae*. Therefore, we hypothesize that *K. bupleuri* has coevolved with its host and adapted to its local climate, which is reflected in its genetic divergence. Because no species corresponds to collections on *Bupleurum* species, and specifically on *B. gibraltarium*, a new species has been proposed. Recent trends that recognize genetically divergent populations of ascomycetes as cryptic species would further justify this conclusion (Geiser et al. 1998, Matute et al. 2006, Alamouti et al. 2011).

Regardless of how these outcomes might be interpreted, a refined phylogenetic definition of the genus *Kabatiella* must await recollection along with analysis of more authentic strains of the described species and in particular the type species *K. microsticta*. For the time being, we have continued to recognize the morphological distinction between *Kabatiella* and *Aureobasidium*, which may be supported by a phylogenetic hiatus, while acknowledging that in the future synonymy or recognition of a new genus may be warranted.

Conidial germination in *K. bupleuri* was remarkably similar to the sequence of germination described for *K. caulivora* where in both organisms conidia initially started to germinate by apical budding, followed by elongation of the conidium into a short unbranched and eventually branched filaments (Sampson 1928). As the filament elongates, the budding process continued at hyphal apices or along the main filament axes. On nutrient rich medium (e.g. malt-yeast extract agar), elongation of initial filaments may be obscured by the accumulation of yeast-like cells. The morphological similarities between *K. bupleuri* and *K. caulivora* were consistent with the close homology of their ITS and LSU sequences.

Kabatiella bupeuri persists within healthy tissues of the host plant and apparently causes no harm. When surface-disinfected dissected leaf tissue senesces in agar culture, the fungus grew into the agar while forming abundant conidial masses on the plant tissue. Reliance on the endophytic isolates for identification alone would have skewed our perception of the fungal phenotype because its melanized acervuli develop poorly or not all in agar culture, and therefore its persistent epiphytic dispersal mechanism would not have been evident. The acervuli on stems and leaves most likely disperse and re-infect emergent leaves and stems during the rainy season. Although pathogenic relationships and endophytic infections are described in *K. microsticta*, *K. zeae* and *K. caulivora*, *K. bupleuri* does not appear to be pathogenic and the nature of its consistent association with the plant is unknown, although one might speculate that the fungus gains a saprobic advantage over other aggressive saprobes

conidia from conidiomata (F278240). Bar = 20 μm. G. Conidiogenesis cells from acervulus in agar culture (F277641). Bar = 20 μm. H. Conidia from growth of endophytic infection on surface-disinfected leaf. Bar = 20 μm.

and epiphytes (e.g. *Alternaria*, *Cladosporium* species, *Aureobasidium pullulans*) by initializing its colonization from internal infections. Therefore, its life cycle could be categorized as a class 3 endophyte (Rodriguez et al. 2009).

ACKNOWLEDGMENTS

Portions of this work were supported the Junta de Andalucía Project in Scientific Excellence RNM-7987 Sustainable use of plants and their fungal parasites from arid regions of Andalucía for new molecules useful for antifungals and neuroprotectors. Marc Stadler, Sybren de Hoog and an anonymous reviewer made helpful suggestions on text.

LITERATURE CITED

Alamouti SM, Wang V, DiGuistini S, Six DL, Bohlmann J, Hamelin RC, Feau N, Breuil C. 2011. Gene genealogies reveal cryptic species and host preferences for the pine fungal pathogen *Grosmannia clavigera*. Mol Ecol 20: 2581–2602, doi:10.1111/j.1365-294X.2011.05109.x

Ashour ML, Wink M. 2011. Genus *Bupleurum*: a review of its phytochemistry, pharmacology and modes of action. J Pharm Pharmacol 63:305–321, doi:10.1111/j.2042-7158.2010.01170.x

Bills GF, Platas G, Peláez F, Masurekar P. 1999. Reclassification of a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. Mycol Res 103:179–192, doi:10.1017/S095375629800687X

Checa J. 1997a. Annotated list of the Hysteriaceae (Dothideales, Ascomycotina) reported from the Iberian Peninsula and Balearic islands. Mycotaxon 62:349–374.

———. 1997b. Annotated list of the Lophiostomataceae and Mytilinidiaceae (Dothideales, Ascomycotina) reported from the Iberian Peninsula and Balearic Islands. Mycotaxon 63:467–491.

———. 1998. Annotated list of the pleosporacean fungi and related genera reported from the Iberian Peninsula and Balearic Islands. Mycotaxon 68:205–249.

———, Barr ME. 1999. Pyrenomycetes sensu lato from Almeria (Spain). Crypt Mycol 20:79–90, doi:10.1016/S0181-1584(99)80010-4

Comes HP. 2004. The Mediterranean region—a hotspot for plant biogeographic research. New Phytol 164:11–14, doi:10.1111/j.1469-8137.2004.01194.x

Farr DF, Rossman AY. 2011. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved 30 May 2011 (/fungaldatabases/)

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791, doi:10.2307/2408678

Geiser DM, Pitt JI, Taylor JW. 1998. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc Natl Acad Sci USA 95:388–393, doi:10.1073/pnas.95.1.388

Giménez E, Melendo M, Valle F, Gómez-Mercado F, Cano E. 2004. Endemic flora biodiversity in the south of the Iberian Peninsula: altitudinal distribution, life forms and dispersal modes. Biodivers Conserv 13:2641–2660, doi:10.1007/s10531-004-2140-7

Hermanides-Nijhof EJ. 1977. *Aureobasidium* and allied genera. Stud Mycol 15:141–177.

Hoffman MT, Arnold AE. 2008. Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycol Res 112:331–344, doi:10.1016/j.mycres.2007.10.014

Leahy RM, Schubert TS. 1996. Daylily leaf streak. Fla Dept Agric Consum Serv Pl Path Cir 376:1–3.

Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, Bagagli E, Rauscher JT, Restrepo A, Morais F, Niño-Vega G, Taylor JW. 2006. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. Mol Biol Evol 23: 65–73, doi:10.1093/molbev/msj008

Médail F, Quezél P. 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean basin. Ann Mo Bot Gard 84:112–127, doi:10.2307/2399957

Narita T, Hiratsuka Y. 1959. Studies on *Kabatiella zeae* n. sp., the causal fungus of a new leaf spot disease of corn. Ann Phytopathol Soc Japan 24:147–153 (in Japanese)., doi:10.3186/jjphytopath.24.147

Neves S. 2003. *Bupleurum* L. In: Castroviejo S, Nieto G, Jury SK, Herrero A, eds. Flora Ibérica: plantas vasculares de la Península Ibérica e Islas Baleares. Vol. X. Madrid: Real Jardín Botánico, CSIC. p 240–265.

Nylander JA. 2004. MrModeltest 2. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala Univ. Distributed by the author.

O'Donnell K. 1993. *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW, eds. The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. Wallingford, UK: CAB International. p 225–233.

Ortega A, Aguilera A. 1987. Contribución al catálogo micológico de Andalucía I. Pezizales. Bol Soc Micol Madrid 11:223–240.

Palenzuela J, Barea JM, Ferrol N, Azcón-Aguilar C, Oehl F. 2010. *Entrophospora nevadensis*, a new arbuscular mycorrhizal fungus from Sierra Nevada National Park (southeastern Spain). Mycologia 102:624–632, doi:10.3852/09-145

———, Ferrol N, Boller T, Azcón-Aguilar C, Oehl F. 2008. *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). Mycologia 100:296–305, doi:10.3852/mycologia.100.2.296

Rivas-Martínez S, Asensi A, Díez-Garretas B, Molero-Mesa J, Valle F. 1997. Biogeographical synthesis of Andalusia (southern Spain). J Biogeogr 24:915–928, doi:10.1046/j.1365-2699.1997.00149.x

———, ———, Molero-Mesa J, Valle F. 1991. Endemismos vasculares de Andalucía. Rivasgodaya 6:5–76.

Rodriguez RJ, White JF Jr, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. New Phytol 182: 314–330, doi:10.1111/j.1469-8137.2009.02773.x

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models.

Bioinformatics 19:1572–1574, doi:10.1093/bioinformatics/btg180

Sampson K. 1928. Comparative studies of *Kabtiella caulivora* (Kirchn.) Karak. and *Colletotrichum trifolii* Bain and Essary, two fungi which cause red clover anthracnose. Trans Br Mycol Soc 13:103–142, doi:10.1016/S0007-1536(28)80009-X

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of Hyphomycetes, Utrecht, the Netherlands. 997 p.

Sivanesan A. 1984. The bitunicate Ascomycetes and their anamorphs. Vaduz, Liechtenstein: J. Cramer. 701 p.

Swofford DL. 2002. PAUP*4: phylogenetic analysis using parsimony analysis (*and other methods). Sunderland, Massachusetts: Sinauer Associates.

Unamuno LM. 1941. Enumeración y distribución geográfica de los Ascomicetos de la Península Ibérica y de las Islas Baleares. Mem Real Acad Cien Exac Fís Nat Madrid 8:1–403.

von Arx JA. 1970. A revision of the fungi classified as *Gloeosporium*. Biblioth Mycol 24:1–203.

———. 1981. The genera of fungi sporulating in pure culture. Vaduz, Liechtenstein: J. Cramer.

Yoshikawa M, Yokoyama T. 1987. Leaf blight of daylily caused by *Aureobasidium microstictum* (Bubák) W.B. Cooke. Ann Phytopathol Soc Japan 53:606–615, doi:10.3186/jjphytopath.53.606

Yurlova NA, de Hoog GS, Gerrits van den Ende AHG. 1999. Taxonomy of *Aureobasidium* and allied genera. Stud Mycol 43:63–69.

Zalar P, Gostincar C, de Hoog GS, Ursic V, Sudhadham M, Gunde-Cimerman N. 2008. Redefinition of *Aureobasidium pullulans* and its varieties. Stud Mycol 50:21–38, doi:10.3114/sim.2008.61.02