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

Gerald F. Bills1
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 gibraltarum 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 racemes and leaves of B. gibraltarum in the province of Granada. The fungus forms melanized acervuli on overwintered flower racemes. 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. pullulans.

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 Quézel 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 endemisms, 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 gibraltarum Lam. (adelfilla de Gibraltar, chujia, cuchileja, 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. frutescens, B. praecalatum, 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. gibraltarum in the province of Granada. The fungus forms melanized acervuli on overwintered flower racemes. 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
Table I. Strains Kabatiella bupleuri and related fungi sequenced for this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain numbers</th>
<th>Substratum</th>
<th>Location</th>
<th>GenBank accessions</th>
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<tr>
<td>Kabatiella bupleuri</td>
<td>F277099, CBS 131302</td>
<td>Dead flower rachises, B. gibraltarium</td>
<td>Upper parking, Presa de Quentar, Granada, Spain</td>
<td>JN886788</td>
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<td>Kabatiella bupleuri</td>
<td>F277640</td>
<td>Dead flower rachises, B. gibraltarium</td>
<td>Rock cliffs, Near Fornes, Granada, Spain</td>
<td>JN886789</td>
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<td>F277641</td>
<td>Dead flower rachises, B. gibraltarium</td>
<td>Cañada Real de Javina, Granada, Spain</td>
<td>JN886790</td>
</tr>
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<td>Kabatiella bupleuri</td>
<td>F277979, CBS 131303</td>
<td>Dead flower rachises, B. gibraltarium</td>
<td>Presa de Quentar, Carretera de presa, Granada, Spain</td>
<td>JN886793</td>
</tr>
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<td>Kabatiella bupleuri</td>
<td>F278240, CBS 131304</td>
<td>Dead flower rachises, B. gibraltarium, HOLOTYPE</td>
<td>Embalse de Canales, Pinos Genil, Granada, Spain</td>
<td>JN886792</td>
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<tr>
<td>Kabatiella bupleuri</td>
<td>F278263</td>
<td>Endophyte of B. gibraltarium</td>
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<td>JN886801</td>
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<tr>
<td>Kabatiella harpophora</td>
<td>F121513, CBS 122914</td>
<td>Surface-disinfected stems and leaves of Viscum album</td>
<td>Robledo de Chavela, Madrid, Spain</td>
<td>JN886794</td>
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<td>(Bres. &amp; Sacc.) Arx</td>
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<td>Selenophoma juncea</td>
<td>F277101, CBS 131305</td>
<td>Dead leaves of Spartium junceae</td>
<td>Fuente de Herádero, Granada, Spain</td>
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<td>Pino Genil, Granada, Spain</td>
<td>JN886795</td>
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<td>Dothichiza sp.</td>
<td>F277643</td>
<td>Dead leaves of Hetamia sphaerocarpa</td>
<td>Embalse de Canales, Pinos Genil, Granada, Spain</td>
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<td>Embalse de Canales, Pinos Genil, Granada, Spain</td>
<td>JN886797</td>
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<td>(De Bary) G. Arnaud ex Gil, Ribaldí &amp; Corte</td>
<td>F278260</td>
<td>Endophyte of B. gibraltarium</td>
<td>Embalse de Canales, Pinos Genil, Granada, Spain</td>
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</table>

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 caudivora, 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 racemes 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 Sermak 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 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.
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 20,000,000 generations. Hierarchical likelihood ratio tests with MrModest 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 (2S8) 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 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 183S (5’-GATGCGCTTAGATGTCTGCGGG-3’) and NL4 (O’Donnell 1983) primers. PCR amplifications followed standard 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 and 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 Aurobasidium complex (Yurlova et al. 1999, Zalai 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 R. geotrichum, a few sequences from other dothideomycetous fungi isolated from plants collected in Granada were included (Table 1). A preliminary sequence set was aligned with GeneStudio software and visually adjusted with GeneDoc 2.5 software (Nicholas and Decker 1997). All new sequences from this work were deposited in GenBank (Table 1).

Phylogenetic tree of Kabatiella buxlinii (gray box) and related species of the Dothideales generated by Bayesian analysis of ITS rDNA partial sequences. Phaeosolenia dematioides was designated the outgroup. Cline probability values are indicated at the branches. Bar = 10 changes.

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Fig. 1. Phylogenetic tree of Kabatiella buxlinii (gray box) and related species of the Dothideales generated by Bayesian analysis of ITS rDNA partial sequences. Phaeosolenia dematioides was designated the outgroup. Cline probability values are indicated at the branches. Bar = 10 changes.
Fig. 2. Phylogenetic tree of *Kabatiella buxelli* (gray box) and related species of the Dothideaceae generated by Bayesian analysis of 28S rDNA partial sequences. *Phaeosclera densataIDES* 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 nonparametric 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 F277099, 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 condidomata. The BLAST analysis of the partial ITS-1-5.8S-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* APJ24450 and FJ150871 were identical to *K. microsticta* FJ150873 (CBS 11464), suggesting a misidentification of *K. microsticta* FJ150873. Other DNA sequences identified by BLAST analysis and revealing 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. gibraltarum*-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. gibraltarum*-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. gibraltarum*-associated *Kabatiella* isolates clustered with sequences of *K. caulivora*, *K. zeae* and *S. mahoniae*, an uncharacterized fungal endophyte (E420058, 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. cauli-
vora* and *K. zea* (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, Supple-
mental Figs. 1, 2), and once again branches corre-
spending to the *A. pullulans* complex, the *K.
cauliwora-K. zea* group and the *B. gibrallarium*
isolates were evident. MP analysis of the 28S region
(Supplemental Fig. 1) delineated an *Aurobasidiurn-
Kabatiella* clade (87% bootstrap support), while the
*B. gibrallarium*-associated *Kabatiella* appeared as
a statistically unsupported sister clade to a distinct clade
including *K. cauliwora-K. zea* (82% bootstrap sup-
port). MP analysis of the ITS-5.8S-ITS2 (Supplement-
ary Fig. 2) clearly delineated a clade with the *B.
gibrallarium*-associated *Kabatiella* strains (86% boo-
strap support) and distinct branches separating *K.
zea* and *K. cauliwora* from each other and from the *A.
pullulans* complex.

These results clearly delineate this set of epiphytic
and endophytic strains from *B. gibrallarium* as a
unique clade and an unknown taxon. But the analyses
also suggest a taxonomic dilemma as to whether the
*B. gibrallarium*-associated *Kabatiella* strains are con-
generic with *A. pullulans* or whether these strains along
with *K. cauliwora* and *K. zea* represent a sister genus
of *Aurobasidium*. 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 MB5637313.

A fungus morphologically similar to *K. cauliwora*
(Kirchn.) Karak. inhabits the leaves, stems and flowers
of *B. gibrallarium* in Spain, forms persistent melanized
acervuli on dead flower rachises and umbels, conidial pus-
tules on senescent leaves, and causes symptomless endo-
phytic infections. Conidiogenous cells holoblastic, formed
from the inner cells of the acervulus wall. Conidia hyaline,
smooth, aseptate, falcate, lunate, sigmoid. Conidia germi-
nating by simultaneous elongation into short septate filaments
and apical or lateral secondary synchronous budding into
lunate to ellipsoidal yeast-like cells. In culture, hyphae
predominantly submerged in the age. Hyphae giving rise
holoblastic conidia from undifferentiated loci on vegetative
cells. Conidia elliptical, reniform or lunate, aseptate or one-aseptate 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
gibrallarium*.

**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 sub-
epidermally, 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 epider-
miss, exposing a conidigenous layer that is a
translucent when humid and imbied with water.
Acervular wall composed of tightly packed isodiamet-
tric to cylindrical, thick-walled cells, hyaline when
young, becoming yellowish reddish brown and
finally black. Conidiophores absent. Conidiogenous
cells formed from the inner cells of acervulus wall,
with-walled, indeterminate, discrete, 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 restric-
tion. Conidia mostly 15–20 µm long, 4–6 µm wide.
*Acervular conidia* hyaline, smooth, eguttate, asept-
ate, 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 septe 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, some-
times slightly furrowed, slightly raised and granular
toward the center, consisting predominantly of
submerged, radially extending hyphae, sometimes accu-
mulating moist to granular masses of melanized yeast-
like cells, and melanized and hyaline falcate conidia
budding toward colony center, shiny to moist during the first
few weeks, aerial mycelium absent to scant, but abun-
dant submerged to adpressed filamentous non-spor-
ulating, 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 sponta-
neous, segregating older colonies. Initially mycelium
meltanized and producing and attracting abundant
melanized conidia and yeast-like cells on sub-
merged hyphae, as hyphal strands extend downward

**Figs. 3–5**
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 fibrillate at the margin, becoming raised rugulose, cerebriform to mucoid, with a clear transition from membranaceous yeast-like conidial 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 hyphae.

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. gibraltarum. 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 58564 and ex-holotype culture F278240 = CBS 131804 and specimens and living cultures (Table 1).

Comments: Kabatiella bupleuri can coexist with A. pululans on the host plant (Table 1), 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 teleomorphic genus Discosphaerina Höhn. (Sivanesan 1984). Because some Kabatiella species may occur on decaying leaves the two genera were considered to form a tropic 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 bupliurei* (F278240) in culture. A. Growth on 2% malt agar, 14 d. B. Growth on Czapek Dox agar, 14 d. 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. G. Germinating
and our results mirrored those of that analyses. The boundaries between *Kabatiella* and *Aurobasidium* 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 *Aurobasidium* 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 *Aurobasidium*. Second, other strains (e.g. some strains of *K. microsticta*, *K. zea*, *K. caulivora*, K. *bupleuri*) appear to belong to sister clades of the *A. pullulans* complex. Strains of *K. zea* (Narita and Hiraizuka 1959) from maize in Japan (NRRC 9664, 9804) are close relatives of *K. bupleuri* (Figs. 1, 2), while the ITS sequence of a German strain of *K. zea* (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 sterigmata conidigenous 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 endophyte 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. gibraltarum* are extremely harsh and highly dissimilar from the humid habitats of clover or maize, the respective hosts of the *K. caulivora* and *K. zea*. 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 *Buplereum* species, and specifically on *B. gibraltarum*, 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 *Aurobasidium*, 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 bupleuri* 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 at 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. zea* 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.
and epiphytes (e.g., *Alternaria, Cladosporium* species, *Aurobasidium pullulans*) by initializing its colonization from internal infections. Therefore, its life cycle could be categorized as a class 3 endophyte (Rodriguez et al. 2009).

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**LITERATURE CITED**


----. 1997b. Annotated list of the Lophiostomataceae and Mytilinidaeae (Dostidiellales, Ascomycota) reported from the Iberian Peninsula and Balearic Islands. Mycotaxon 63:467–491.


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


