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Fungal olecranon bursitis in an immunocompetent patient by Knoxdaviesia dimorphospora sp. nov.: case report and review
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Abstract:	Bursitis is a common medical condition that can occur either with or without infection. We present a case of fungal olecranon bursitis in an immunocompetent individual caused by the new species Knoxdaviesia dimorphospora. It is a dematiaceous filamentous fungus characterized by the production of two different conidia: hyaline and cylindrical, which rise up from phialidic conidiogenous cells located in the upper part of differentiated and unbranched conidiophores, and pale brown and ellipsoidal conidia produced by phialidic conidiogenous cells which are born directly on hyphae. In addition to its morphological peculiarities, the novelty of the fungus was confirmed by sequence analysis of the internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S of the nuclear rRNA gene. The fungal infection was confirmed by cytological examination and repeated cultures. The infection was resolved by surgical debridement and drainage, and the patient presented a complete functional recovery three months later. The in vitro antifungal susceptibility to this new human opportunist is provided, terbinafine being the drug with the most potent activity.

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Fungal olecranon bursitis in an immunocompetent patient by *Knoxdaviesia dimorphospora* sp. nov.: case report and review

Running title: Olecranon bursitis by *Knoxdaviesia dimorphospora* sp. nov.

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ABSTRACT

Bursitis is a common medical condition that can occur either with or without infection. We present a case of fungal olecranon bursitis in an immunocompetent individual caused by the new species *Knoxdaviesia dimorphospora*. It is a dematiaceous filamentous fungus characterized by the production of two different conidia: hyaline and cylindrical, which rise up from

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phialidic conidiogenous cells located in the upper part of differentiated and unbranched conidiophores, and pale brown and ellipsoidal conidia produced by phialidic conidiogenous cells which are born directly on hyphae. In addition to its morphological peculiarities, the novelty of the fungus was confirmed by sequence analysis of the internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S of the nuclear rRNA gene. The fungal infection was confirmed by cytological examination and repeated cultures. The infection was resolved by surgical debridement and drainage, and the patient presented a complete functional recovery three months later. The *in vitro* antifungal susceptibility to this new human opportunist is provided, terbinafine being the drug with the most potent activity.

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INTRODUCTION

Bursitis is a common medical condition that can occur either with or without infection [1]. Septic bursitis is a frequent cause of musculoskeletal pain and often prompts orthopedic consultation. It is almost always preceded by some kind of trauma, and the olecranon and the prepatellar bursa are the primary sites involved [2]. The most frequent cause of bursal infection are bacteria, with *Staphylococcus aureus* and *S. epidermidis* being the most common, although species of *Streptococcus* or other bacteria have also been reported [1, 3]. Fungal bursitis is rare and can affect both immunocompromised and immunocompetent individuals [4]. Most reported cases cite *Candida* species as the cause [5], although cases associated to different filamentous fungi, such as *Pleurostomophora, Purpureocillium* or *Sporothrix* can also be found in the literature [4]. The present report describes a case of septic olecranon bursitis in an immunocompetent individual caused by a novel species in the genus *Knoxdaviesia* and presents a review of bursal infections by filamentous fungi.

CASE REPORT

A 46-year-old gardener attended the emergency room due to a left olecranon bursitis. The patient reported no prior medical incident of interest or any history of trauma. Although no bursal aspiration was carried out for an accurate diagnosis, bacterial olecranon bursitis was suspected and empirical treatment was started with oral amoxicillin-clavulanic acid (875 mg / 125 mg) every 8 hours for one week. Although the patient remained afebrile and hemodynamically stable, there was no clinical improvement. On physical examination, he presented tumefaction, fluctuation, erythema, olecranon

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bursitis pain edema and fluctuation in the left forearm. Additionally, a leukocytosis stood out (11.94 x10^3/μl) with high C-reactive protein (CRP) of 5.6 mg/dl. Due to the bad evolution of the infection, a surgical debridement was performed and a great deal of purulent material was obtained, which was sent for both cytological and microbiological analysis. The lesion was washed with abundant saline solution containing rifampicin and a Penrose drain was placed for 48 hours. A new treatment was started with a higher dose of amoxicillin and clavulanic acid (1000 mg/62.5 mg every 8 hours) for one week, while waiting for the laboratory results. However, the patient's condition improved considerably 24 hours after debridement, with a decrease in the erythema and the fluctuation. On analysis, a decrease in the leukocytosis (7.61 x10^3/μl) as well as the CRP 2.8 mg/dl was proven. Three months later, every inflammatory parameter (leukocytes 8.14 x10^3/μl and CRP 0.2 mg/dl) was normal, and the patient was completely recovered.

Microbiological and cytological analysis

Two different debridement fluid samples were cultured, following the recommendations of the Spanish Society of Infectious Diseases and Clinical Microbiology [6]. From both samples, numerous colonies of a melanized filamentous fungus with stachybotrys-like morphology were obtained on Sabouraud dextrose agar (SDA; BBL, Baltimore, USA) after 72 h of incubation at 25 and 37 ºC. Cultures for bacteria were negative in both samples. For cytological examination, the fluid material was stained with Papanicolaou, Giemsa, periodic acid-Schiff and Grocott's silver methenamine stain and

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revealed the presence of filamentous fungal elements, consisting of septate and pigmentated hyphae, with abundant inflammatory cells (Figure 1).

Mycological assessment

Two isolates were selected from the SDA cultures and submitted to the Mycology Unit of the Universitat Rovira i Virgili (Reus, Spain) for identification, characterization and *in vitro* antifungal susceptibility testing. The accession numbers in culture collection were FMR 15026 and FMR 15027.

Preliminary cultures of the two isolates on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) at 25 ⁰C produced fast growing, cottony colonies with white mycelia that became greenish black after seven days of incubation in darkness. The two isolates showed similar microscopic features and they were initially identified as *Knoxdaviesia* sp. For further characterization, in addition to PDA, the isolates were studied on malt extract agar (MEA; Difco Laboratories, Baltimore, USA), potato carrot agar (PCA; 20 g potatoes, 20 g carrot, 20 g agar, 1000 mL distilled water) and oatmeal agar (OA; 30 g filtered oat flakes after 1 h simmering, 20 g agar, 1000 mL distilled water), incubated at 25⁰C in the dark. Their ability to grow at 10, 15, 25, 30, 37, 40 and 45 ⁰C was tested on PDA. Color notations used in colony descriptions were from Kornerup and Wanscher [7]. Microscopic features could be observed by making direct wet mounts with 85% lactic acid and Shear's solution. Scanning electron microscope (SEM) photographs were also taken using a Jeol JSM- 6400 (Tokyo, Japan) with techniques described previously [8].

For molecular identification, genomic DNA was extracted from colonies growing on PCA for 7-14 days at 25 ⁰C, and DNA extraction was made using

the FastDNA® kit protocol (MP Biomedicals, Solon, OH), according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S nuclear rDNA (LSU) were amplified with the primer pair ITS5/LR5 [9, 10]. Sequencing was carried out in both directions using the same PCR primers at Macrogen Europe (Amsterdam, the Netherlands). Consensus sequences were obtained using SeqMan version 7.0.0 (DNAStar Lasergene, Madison, WI).

BLAST sequence homology searches revealed that the LSU sequences (approximately 781 bp) from our isolates showed a 98% similarity with sequences of two *Knoxdaviesia* species, i.e. *K. cecropiae* (accession no. KM495392) and *K. capensis* (labeled as *Gondwanamyces capensis* accession no. EU552136 and EU552135; 100% query coverage). For assertive identification, phylogeny was performed using ITS and LSU sequence data. Sequences from each locus were aligned with MEGA v 6.0 software [11], using the CLUSTALW algorithm [12], refined with MUSCLE [13], and visually adjusted on the same software platform. The phylogenetic study was made with individual and combined genes using maximum-likelihood (ML) in MEGA v. 6.0. For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference; support for internal branches was assessed by 1,000 ML sets of data. A bootstrap support (bs) ≥ 70 was considered significant. We included sequences of species of closely related genera, i.e. *Custingophora* and *Ceratocystis* [14]. The phylogenetic analysis (Figure 2) showed that the case isolates were located in the clade representative of the genus *Knoxdaviesia*, forming a fully supported branch, distant from the other *Knoxdaviesia* species included in the analysis. Since the isolates represented a

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novel lineage and showed morphological features different from the nine species described in the genus [14], they are proposed below as a new species of *Knoxdaviesia,* named *K. dimorphospora.*

DNA sequences from FMR 15026 obtained in this study were deposited in GenBank under accession numbers LT671628 for ITS, and LT671629 for LSU.

Antifungal susceptibility

The *in vitro* antifungal susceptibility te st was carried out according to the CLSI M38-A2 standard [15]. The drugs tested were amphotericin B (AMB), anidulafungin (AFG), caspofungin (CFG), micafungin (MFG), 5-fluorocytosine (5FC), itraconazole (ITC), posaconazole (PSC), voriconazole (VRC) and terbinafine (TRB). The minimal effective concentration (MEC), defined as the lowest drug concentration at which short, stubby, highly branched hyphae were observed, was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration to exhibit 100% inhibition of any visible growth for ITC, PSC, and VRC or 50% and 80% reduction in growth for 5FC, FLC and TRB, respectively. *Candida krusei* ATCC 6258 was used as quality control. Terbinafine showed the most potent activity against *K. dimorphospora*, with a MIC ≤0.03 µg/ml, followed by AMB, VRC and PSC with MIC values of 1.0 µg/ml. Among echinocandins, CFG and MFG was also highly effective *in vitro*, with MECs of 0.125 µg/ml and 0.25 µg/ml, respectively. AFG, ITC and 5FC were the least active *in vitro* against the case isolates, with a MEC of 8 µg/ml, and MICs of 4 µg/ml and >16 µg/ml, respectively.

Taxonomy

Knoxdaviesia dimorphospora Guevara-Suarez, Pujol, Llauradó & Gené, sp. nov. (Figure 3). MycoBank accesion number MB821526.

Etymology. Name referred to the presence of two types of conidia.

Typus. Spain, Catalonia, Reus, from a human olecranon bursitis, Jun. 2016, M. Llauradó & I. Pujol (holotype CBS H-23142; cultures ex-type CBS 142753, FMR 15026).

Micromorphology (on PCA at 25ºC). *Mycelium* partly superficial and partly immersed, composed of branched, septate, hyaline to subhyaline, smooth-walled, 1 to 2 μm wide hyphae. *Conidiophores* solitary, macronematous, erect, unbranched, 80 to 123 μm long (up to 210 μm on OA), arising from rhizoid foot cells, and terminating in an appressed whorl of 10 to 15 phialidic conidiogenus cells; stipe septate, cylindrical, 3.5 to 6 μm wide, often sinuous in the upper part, pale brown to brown, smooth-walled. *Phialides* terminal, cylindrical, 9 to 10(12) by 2 to 3.5 μm, with a minute collarette, pale brown to brown, smooth-walled. *Conidia* aseptate, cylindrical, 7 to 10(12) by 2 to 3 μm, with a rounded apex and truncate base, hyaline to subhyaline, smooth-walled, aggregated in slimy masses. A second asexual morph is present in all culture media tested, consisting of groups of clavate phialides, 3 to 4 by 2.5 to 3 μm, usually growing laterally on the hyphae or on short stalked cells, up to 6 μm long and to 3 μm wide; conidia ellipsoidal, 8 to 14 by 3 to 5 μm, subhyaline to pale brown, smooth- and thick-walled. Sexual morph not observed.

Culture characteristics (in darkness, at 25 °C after 7 d). Colonies attaining 30 to 35 mm diam on MEA, 38 to 42 mm diam on PDA and PCA, and

60 to 78 mm diam on OA. On PCA, colonies flat, cottony, greenish grey (26F2) with aerial mycelium white to grey (26D1), margin entire; reverse near black (25F4).

Cardinal temperatures for growth. Optimum 30 to 37 ºC, minimum 15 ºC, maximum 40 ºC.

Differential diagnosis. *Knoxdaviesia dimorphospora* can be distinguished from the other species of the genus by the production of a second phialidic asexual morph and by its profuse growth at 37 °C (61 to 68 mm diam on PDA).

DISCUSSION

Septic bursitis caused by filamentous fungi are rarely described, and most of the cases have been associated to *S. schenckii.* Wang et al. [16] reported a case of bursal sporotrichosis, and found in the literature eight other cases caused by that species. However, since Wang's review, no more case of sporotrichal bursitis have been published. In our review, we found 13 cases of bursal infection caused by filamentous fungi different to *S. schenckii* between 1979–2016 (Table 1). Apart from this latter fungus, no genus or species have been found associated to this type of infection, although *Pleurosthomophora risardsidae* and *Purpureocillium lilacinum* are reported in three and two cases, respectively [17 - 21]. These two species, as well as *Aspergillus terreus*, *Exophiala oligosperma* and *Fonsecaea pedrosoi* have been described previously as human opportunists that can cause both superficial and deep infections [2, 4, 22, 23], wherears *Anthopsis deltoidea* and *Phomopsis bougainvilleicola* are only known from the cases of septic bursitis [24, 25]. It is of note, however, that we found 3 out of 13 cases in which the causal agent has

not been identified at the genus or species level [22, 26 - 28]. Therefore, the species diversity of filamentous fungi found is a handicap for establishing the epidemiology, and the proper diagnosis and therapy of this type of infection. In the present study, we describe the first case of human infection caused by a species of *Knoxdaviesia*, in which cytological examination and culturing have been crucial for confirming the infection. However, a detailed phenotypic and molecular characterization has also been required to identify a new fungus, *K. dimorphospora*, as human opportunist.

Knoxdaviesia is a genus that currently comprises nine species and it is classified in the order Microascales [14], a group of fungi which includes other relevant human pathogens in the genera *Lomentospora*, *Microascus*, *Scedosporium* or *Scopulariopsis* [4, 29]. Morphologically, *Knoxdaviesia* is clearly distinguished from all them by its large and unbranched conidiophores, usually arising from rhizoids and bearing terminally a whorl of phialidic conidiogenous cells that produce hyaline, one-celled, cylindrical to allantoid conidia, arranged in slimy heads [14]. In addition, some of its species produce the sexual morph, which is characterized by perithecial ascomata with long necks, very similar to those produced by the genus *Ophiostoma* [14, 30]. For this reason, *Knoxdaviesia* are often defined as ophiostomatoid fungi, but phylogenetically they belong to different sordariomycetous orders. *Ophiostoma* is included in the Ophiostomatales and *Knoxdaviesia* in the Microascales, as mention before [14]. Our new species does not produce ascomata, however it shows the typical asexual structures of the genus with cylindrical conidia and also produces a second type of asexual morph (synanamorph) with ellipsoidal and pale brown conidia. Although the synanamorph production is reported for

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the first time in *Knoxdaviesia*, it is a relatively common feature in species of other microascalean genera such as *Scedosporium*, *Petriella* or *Wardomyces* [29, 31]*,* however the role in the nature of the synanamorph is poorly known. Another relevant feature of *K. dimorphospora* that it shares with other important human pathogenic species of this group of fungi, such as *L. prolificans* or *S. apiospermum*, is the ability to grow at 40 °C. On the contrary, the other two species of *Knoxdaviesia*, i.e. *K. capensis* and *K. proteae*, from which this feature has been evaluated show a maximum temperature for growth at 30ºC [32]. Our sequence analysis of the ITS barcode and LSU region of the rDNA has been useful for determining phylogenetic relationships and for establishing species boundaries among *K. dimorphospora* and the currently accepted species of *Knoxdaviesia*, except for distinguishing between *K. capensis* and *K. wingfieldii* (Figure 1). In fact, the proposal of the latter species by Roets & Dreyer was based mainly on its morphological differences from *K. capensis*, since their ITS sequences were very similar (99%) [33].

Olecranon bursitis is the most common and clinically relevant of the superficial bursae due to its predisposition to inflammation and infection given its locations [1, 34]. In fact, the elbow is the most frequent location among the reviewed cases, as it was in our patient, and the infection is usually acquired after traumatic inoculation of the fungus into the skin and subcutaneous tissue (Table 1). Although our patient did not report any direct trauma to the affected area, considering the gardener profession, he had probably become infected by pricking from contaminated plant material. It is of note that most *Knoxdaviesia* species inhabit different trees [14, 32, 35, 36], and despite *K. dimorphospora*

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being described from a human lesion, its natural habitat is probably the same as its counterparts.

Fungal bursitis is treated with oral or parenteral antifungals as well as drainage of infected bursal fluid; which is frequently carried out by needle aspiration. Bursectomy is indicated when there is no response to that treatment, when the bursal fluid cannot be drained or when there is infection of the surrounding soft tissue [1]. Our patient improved quickly after surgical debridement and drainage for 48 h, without receiving any antifungal therapy, probably due to his immunocompetent condition. Most of the cases listed in Table 1 occurred in patients over the age of 50 and about half of them were immunocompromised. The most common treatment reported is the combination of bursal aspiration and/or bursectomy with antifungal therapy, as it is also found in bursal infections by *Candida* species [5] or *S. schenckii* [16]. The drug used for the treatments varies, probably due to the empirical administration or to the result of the *in vitro* antifungal test. Since our patient was infected by a novel species belonging to a genus of Microascales never described, it precludes any type of comparison. However, it is a species susceptible to practically all drugs tested, contrary to the antifungal susceptibility shown by other microascalean fungi of clinical relevance such as *Microascus, Scedosporium, Scopulariopsis* or *Lomentospora*, which include species with reduced susceptibility or pan resistance to clinically available antifungal drugs [37 - 40].

In summary, the most outstanding facts of the cases reviewed on bursal infection by filamentous fungi is that: 1) coinfection with other microorganisms is not reported, 2) there is a considerable species diversity able to cause this type of infection, 3) the most frequent anatomical site affected was the olecranon

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followed by prepatellar bursae, and 4) practically all cases were resolved with bursal aspiration or bursectomy and antifungal therapy. In our case, both the presence of fungal elements as well as an important inflammatory reaction observed on the fluid material obtained from the bursa have been key to determining the role of the new fungus, *K. dimorphospora,* as an etiological agent of septic bursitis in an immunocompetent patient. Given the nature of the isolated fungus, we speculate that it might have been acquired by direct trauma with contaminated plant material.

Conflict of interest: All authors declare no conflict of interest.

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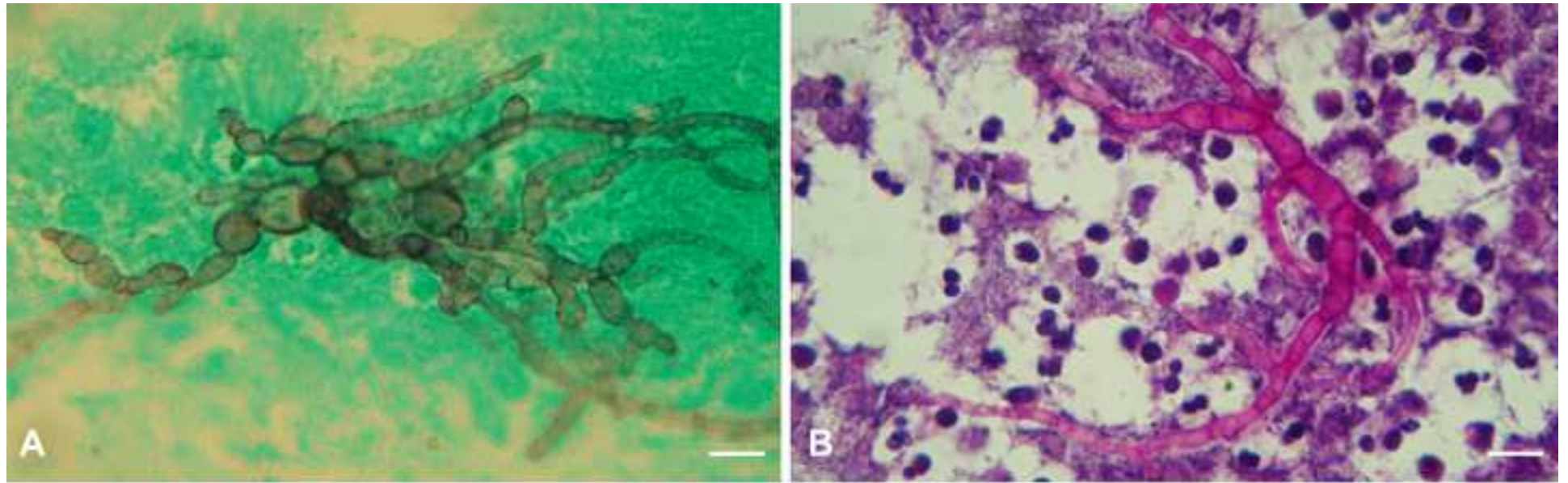
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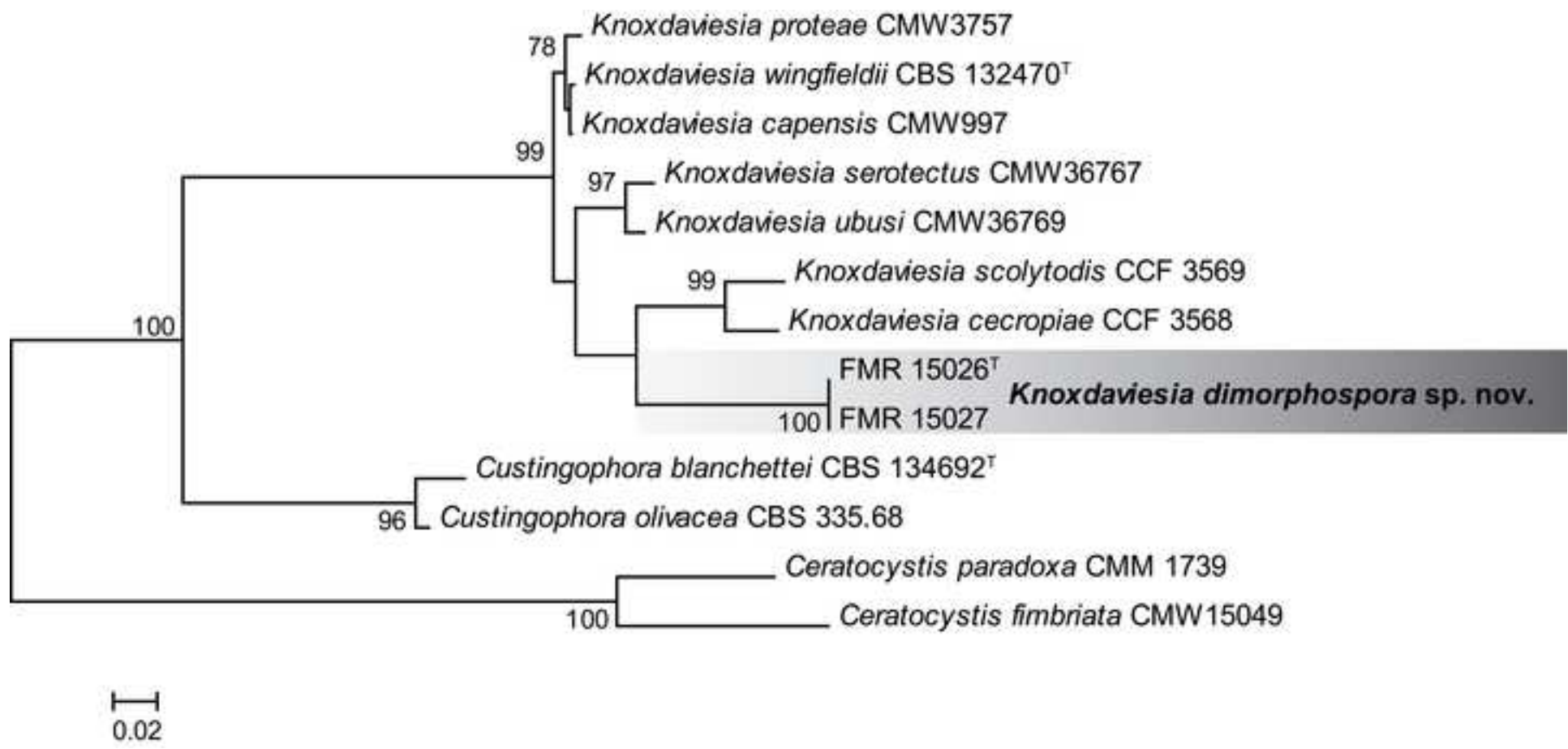
Figure 1. (A, B) *Knoxdaviesia dimorphospora* in debridement fluid samples from the olecranon bursa. (A) Grocott's silver methenamine stain revealing the presence of septate, branched and pigmented hyphae. (B) PAS stain showing hyphae in the midst of abundant inflammatory cells. Scale bars = 10 μm.

Figure 2. Maximum-likelihood (ML) tree constructed with the combination of ITS (606 bp) and LSU (781 bp) sequence data available from the species of *Knoxdaviesia* genus. Bootstrap support values above 70% are indicated at the nodes. The phylogenetic tree was constructed with species of closely related genera: *Custingophora* and *Ceratocystis.* T: type strain. The new species proposed is shown in the dark box.

Figure 3. (A-N) *Knoxdaviesia dimorphospora*, ex-type FMR 15026. (A) Colonies growing on PCA, MEA, PDA and OA after 7 d at 25 °C. (B-E) Conidiophores, F-H. Foot cells with rhizoids, I.-J Conidia. (K-N) Conidiogenous cells and conidia of the synanamorph. Scale bars = 10 μm.

Knoxdaviesia proteae CMW3757
78
Knoxdaviesia wingfieldii CBS 132470[T]
Knoxdaviesia capensis CMW997
99
97 *Knoxdaviesia serotectus* CMW36767
Knoxdaviesia ubusi CMW36769
99 *Knoxdaviesia scolytodis* CCF 3569
Knoxdaviesia cecropiae CCF 3568
FMR 15026[T]
Knoxdaviesia dimorphospora sp. nov.
100 FMR 15027
Custingophora blanchettei CBS 134692[T]
96 *Custingophora olivacea* CBS 335.68
Ceratocystis paradoxa CMM 1739
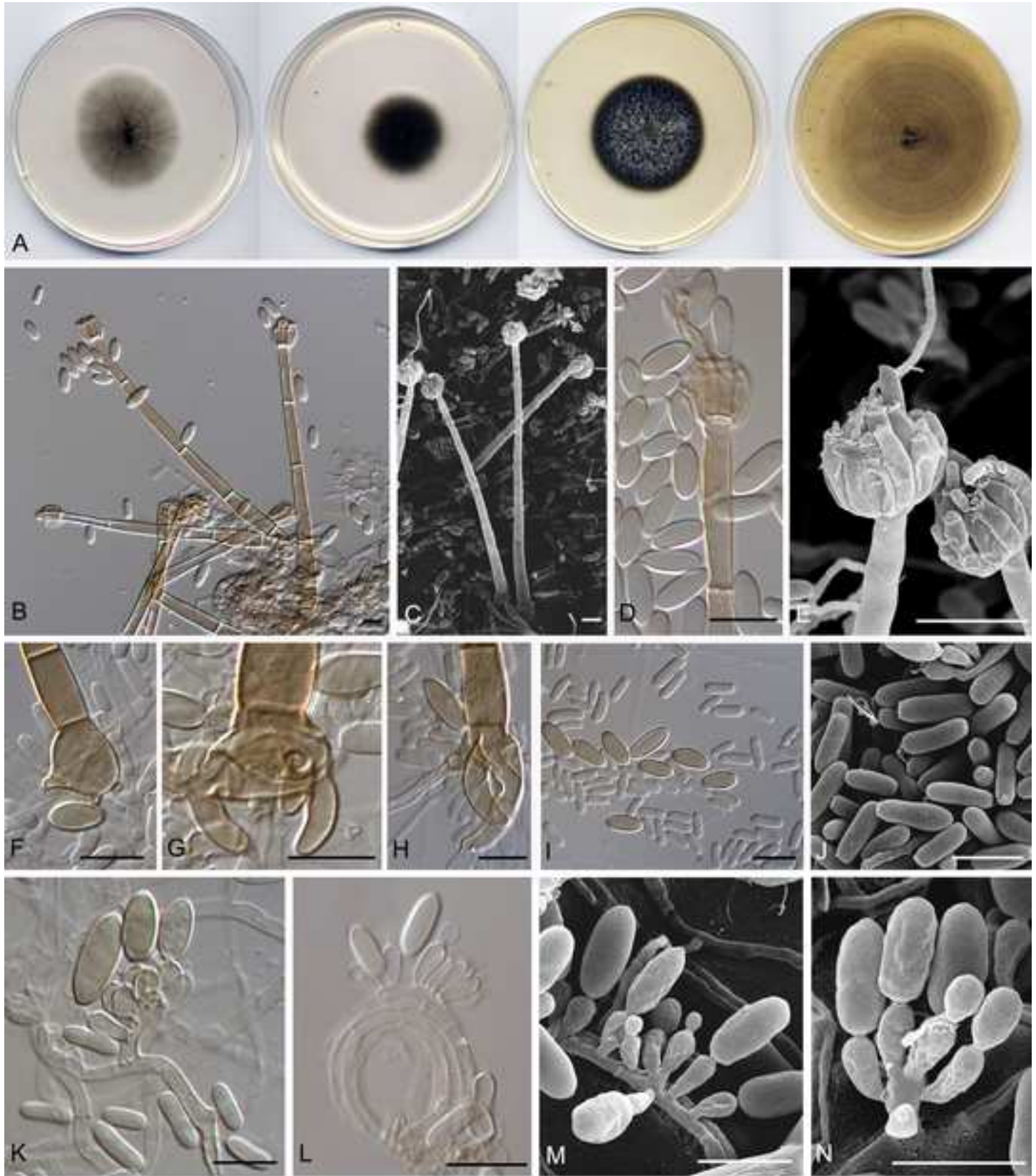100 *Ceratocystis fimbriata* CMW15049
100

0.02

Table 1. Clinical cases of septic bursitis caused by filamentous fungi different to *Sporothrix schenckii*.[16]

Species involved	Age/sex	Infected bursa	Comorbidities	Other risk factors	Co-infection	Treatment	Reference
Anthopsis deltoidea	79/M	Right olecranon	Prostatic carcinoma	Corticosteroids	Negative	Bursectomy, flucytosine	[24]
Aspergillus terreus	72/M	Right olecranon	Non-insulin-dependent diabetic	Trauma	Negative	Bursectomy	[2]
Exophiala oligosperma	62/M	Left olecranon	Wegener's granulomatosis, immunosuppressive therapy	Corticosteroids, trauma	Negative	Aspiration of bursal fluid, amphotericin B	[23]
Fonsecaea pedrosoi	65/F	Left olecranon	None	Trauma	Negative	Aspiration of bursal fluid	[22]
Knoxdaviesia dimorphospora	46/M	Left olecranon	None	None	Negative	Surgically debrided, drainage	Current case
Phaeoacremonium sp.	54/F	Right olecranon	Myelodysplastic syndrome	Trauma	Negative	Bursectomy, itraconazole	[27]
Phomopsis bougainvilleicola	61/M	Right knee	Chronic renal disease	Corticosteroids, kidney transplant	Negative	Aspiration of bursal fluid, voriconazole	[25]
Purpureocillium lilacinum (as *Paecilomyces lilacinus*)	35/M	Right knee	None	None	Negative	Aspiration of bursal fluid, fluconazole, miconazole, ketoconazole.	[18]
Purpureocillium lilacinum	86/M	Left olecranon	Chronic lymphocytic leukemia	Secondary common variable immuno-deficiency with intravenous immunoglobulin	Negative	Aspiration of bursal fluid, fluconazole, ketoconazole, bursectomy	[20]
Pleurostomophora richardsiae (as *Phialophora richardsiae*)	79/M	Right shoulder	None	Trauma	Negative	Bursectomy, cephalexin	[17]
Pleurostomophora richardsiae	77/M	Right knee	None	None	Negative	Aspiration of bursal fluid, amphotericin B	[19]
Pleurostomophora richardsiae	54/M	Right prepatellar	None	Trauma	Negative	Patient refused treatment	[21]
Penicillium sp.	76/F	Right olecranon	Asthma, congestive heart failure, deep vein thrombosis, adenocarcinoma of the colon	Corticosteroids, trauma	Negative	Surgically debrided, ketakonazole	[26]
Dematiaceous mold (Unknown)	76/M	Left olecranon	None	None	Negative	Bursectomy, itraconazole	[28]

M=male; F=female