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RESEARCH ARTICLE

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Phylogenetic circumscription of *Arthrographis* (*Eremomycetaceae*, *Dothideomycetes*)

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Key words

arthroconidial fungi
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Eremomyces
phylogeny
taxonomy

Abstract Numerous members of *Ascomycota* and *Basidiomycota* produce only poorly differentiated arthroconidial asexual morphs in culture. These arthroconidial fungi are grouped in genera where the asexual-sexual connections and their taxonomic circumscription are poorly known. In the present study we explored the phylogenetic relationships of two of these ascomycetous genera, *Arthrographis* and *Arthropsis*. Analysis of D1/D2 sequences of all species of both genera revealed that both are polyphyletic, with species being accommodated in different orders and classes. Because genetic variability was detected among reference strains and fresh isolates resembling the genus *Arthrographis*, we carried out a detailed phenotypic and phylogenetic analysis based on sequence data of the ITS region, actin and chitin synthase genes. Based on these results, four new species are recognised, namely *Arthrographis chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora. Arthrographis chlamydospora* is distinguished by its cerebriform colonies, branched conidiophores, cuboid arthroconidia and terminal or intercalary globose to subglobose chlamydospores. *Arthrographis curvata* produced both sexual and asexual morphs, and is characterised by navicular ascospores and dimorphic conidia, namely cylindrical arthroconidia and curved, cashew-nut-shaped conidia formed laterally on vegetative hyphae. *Arthrographis globosa* produced membranous colonies, but is mainly characterised by doliiform to globose arthroconidia. *Arthrographis longispora* also produces membranous colonies, but has poorly differentiated conidiophores and long arthroconidia. Morphological variants are described for *A. kalrae* and our results also revealed that *Eremomyces langeronii* and *A. kalrae*, traditionally considered the sexual and asexual morphs of the same species, are not conspecific.

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INTRODUCTION

The arthroconidial genus *Arthrographis* was proposed by Cochet (1939) with *A. langeronii* as the type species, but it was invalid because it lacked a Latin diagnosis, which was at that time still required as prerequisite by the International Code of Botanical Nomenclature. Sigler & Carmichael (1976) subsequently validated the genus name based on *Oidiodendron kalrae*. In addition to the type species, *A. kalrae*, the genus currently includes three other taxa, *A. alba*, *A. lignicola* and *A. pinicola* (Sigler & Carmichael 1976, 1983, Sigler et al. 1990, Gené et al. 1996). Other species previously included in the genus were *A. cuboidea* and *A. sulphurea*. While the former species was transferred to the genus *Scytalidum* (Kang et al. 2010), *A. sulphurea* was considered a possible synonym of *Pachysolen tannophilus* (*Saccharomycetes*) (von Arx 1985).

Apart from *A. kalrae*, which was traditionally associated with the sexual morph *Eremomyces langeronii*, other ascomycetes have been described with unnamed *Arthrographi*s morphs, i.e., *Leucothecium coprophilum*, *L. emdenii* and *Faurelina indica* (von Arx & Samson 1973, von Arx 1978, von Arx et al. 1981, Malloch & Sigler 1988, Valldosera et al. 1991).

*Arthrographi*s species have been isolated from air, compost, marine sediments, soil, wood and, occasionally, from opportunistic infections in humans (de Hoog et al. 2011). Morphologically, they are recognised by a slow growth rate and by the presence of 1-celled, hyaline, smooth-walled, cylindrical arthroconidia released schizolytically from dendritic conidiophores (Sigler & Carmichael 1976). A particular feature of *A. kalrae* is the presence of a trichosporiella-like synasexual morph characterised by solitary, globose to subglobose conidia, which grow laterally and sessile on undifferentiated vegetative hyphae (Sigler & Carmichael 1983). Recently, a phylogenetic study based on sequences analysis of SSU, ITS and *RPB2*, revealed the polyphyly of *Arthrographis* (Kang et al. 2010).

Another arthroconidial genus morphologically similar to *Arthrographis* is *Arthropsis.* The genus comprises four species, i.e., *Arthropsis cirrhata*, *A. hispanica*, *A. microsperma* and *A. truncata* (Sigler et al. 1982, Sigler & Carmichael 1983, van Oorschot & de Hoog 1984, Ulfig et al. 1995). These fungi are usually reported from plant material, but *A. hispanica*, which was only known from marine sediments, has recently been isolated from clinical specimens (Giraldo et al. 2013). *Arthropsis* shows pigmented or non-pigmented arthroconidia, joined by adjacent connectives, released rhexolytically from undifferentiated conidiophores and occasionally has a *Humicola* synasexual morph (Sigler et al. 1982, van Oorschot & de Hoog 1984). Van Oorschot & de Hoog (1984) questioned the distinction between *Arthrographis* and *Arthropsis*, and suggested transferring *Arthrographis* species, excluding the type species *A. kalrae*, to the genus *Arthropsis.* Other authors, however, rejected this proposal (Malloch & Sigler 1988, Sigler et al. 1990).

In the present study we compared the D1/D2 sequences of the available types of *Arthrographis* and *Arthropsis* spp. with those of taxa retrieved from GenBank to clarify their taxonomy, and to determine their phylogenetic relationships. By combining morphological observations with multilocus DNA sequence

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analysis, several novel cryptic species of *Arthrographis* were delineated, which are newly described in this study.

MATERIALS AND METHODS

Isolates

The fungal isolates and DNA sequences included in the study are shown in Table 1. Twenty-six clinical *Arthrographis* isolates were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC), the majority previously identified as *A. kalrae* by Giraldo et al. (2013). Because these isolates varied in morphology and their DNA sequence data, all isolates were re-examined in the present study. The type strains from the new species described here were deposited in the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Phenotypic studies

Isolates were studied following the criteria of Sigler & Carmichael (1976, 1983) and Ulfig et al. (1995). Morphological features were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain), 2 % malt extract agar (MEA; BD Difco™, Franklin Lakes, NJ, USA), potato carrot agar (PCA; potatoes, 20 g; carrot, 20 g; agar, 20 g; distilled water to final volume of 1 000 mL) and oatmeal agar (OA; filtered oat flakes after 1 h of simmering, 30 g; agar, 20 g; distilled water to final volume of 1 000 mL). Cultures were incubated at 25 °C in the dark for 4 wk. Colony diameters were measured after 14 d of incubation and rated according to the colour charts of Kornerup & Wanscher (1978). Microscopic features were examined and measured in either 85 % lactic acid or lactophenol cotton blue under a light microscope Olympus CH-2 (Olympus Corporation, Tokyo, Japan). Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski differential interference.

The ability of the fungi to grow at 15, 20, 25, 30, 35, 37, 40, 42 and 45 °C was determined on PDA. To determine the resistance to cycloheximide, isolates were transferred to Petri dishes containing PCA supplemented with chloramphenicol (200 mg/L) and cycloheximide at a final concentration of 2 g/L, and incubated at 25 °C for 2 wk. All tests were performed in duplicate. To evaluate the ability of isolates to convert to the yeast phase, a portion from a fresh culture on PDA was transferred to tubes with Brain Heart Infusion broth (BHI; Becton Dickinson & Company, Franklin Lakes, NJ, USA) and incubated at 37 °C for 2 wk. Subsequently, several transfers to BHI broth were performed.

DNA extraction, amplification and sequencing

Isolates were grown on yeast extract sucrose agar (YES; yeast extract, 20 g; sucrose, 150 g; agar, 20 g; distilled water to final volume of 1 000 mL) for 10 d at 25 °C and DNA extracted using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The DNA was quantified using NanoDrop 3000 (ThermoScientific, Asheville, NC, USA). The internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S rDNA were amplified with the primer pairs ITS5/ITS4, NL1/NL4b and LR0R/LR5 (Vilgalys & Hester 1990, White et al. 1990, O'Donnell 1993). A portion of the actin gene (*ACT1*) was amplified using the primer set Act1/Act4 (Voigt & Wöstemeyer 2000) and a chitin synthase gene (*CHS1*) using the primers CHS-79F/CHS-354R (Carbone & Kohn 1999). PCR products were purified and sequenced at Macrogen Europe (Amsterdam, The Netherlands). The program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate. In addition, numerous D1/D2 sequences, corresponding to different classes, orders and families of ascomycetes retrieved from GenBank or NITE/NRBC databases were included in the phylogenetic study (Table 1). Most of these sequences were published by different authors (Sugiyama et al. 1999, Sugiyama & Mikawa 2001, Untereiner et al. 2002, Reeb et al. 2004, Xi et al. 2004, Murata et al. 2005, Wang et al. 2005, Wedin et al. 2005, Kodsueb et al. 2006, Réblová & Seifert 2007, Tsui et al. 2007, Gueidan et al. 2008, Boehm et al. 2009, Sugiyama et al. 2002, Boonmee et al. 2011, Pettersson et al. 2011, Réblová et al. 2011, Giraldo et al. 2013). The selection of these sequences was based on the results of a BLAST search using the D1/D2 and ITS sequences from each of the ex-type strains of the different species of *Arthrographis* and *Arthropsis*.

Phylogenetic analysis

Sequences were aligned using Clustal X v. 1.8 (Thompson et al. 1997) with default parameters, followed by manual adjustments with a text editor. The phylogenetic relationship between *Arthrographis* and *Arthropsis* species with other genera was determined through the analysis of D1/D2 sequences. Since genetic and also morphological variability was detected among isolates of *Arthrographis*, a multi-locus sequence analysis was carried out to confirm the results obtained from D1/D2 data. This analysis included a fragment of the *ACT1* gene, the *CHS1* gene and the ITS region. Phylogenetic analyses were performed with MEGA v. 5.05 (Tamura et al. 2011), using the Maximum Composite Likelihood (ML). The selection of the best nucleotide substitution model (Tamura-Nei with Gamma distribution) was made using the model selection analysis under MEGA v. 5.05. Gaps or missing data were treated as partial deletion with a site coverage cut-off of 95 % and Nearest-Neighbour-Interchange (NNI) used as Heuristic method. The internal branch support was assessed by a search of 1 000 bootstrapped sets of data. DNA sequence data were deposited in GenBank (Table 1), the alignment and trees in TreeBASE (http://www.treebase.org) and taxonomic novelties in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

RESULTS

Phylogenetic analyses

The D1/D2 phylogenetic tree that included the ex-type strains of the different species of *Arthrographis* and *Arthropsis* and representative members of different fungal classes and orders revealed that both genera are polyphyletic (Fig. 1). The type species of *Arthrographis*, *A. kalrae*, was included in a well-supported clade within the *Dothideomycetes* (85 % bootstrap, bs), forming together a highly supported subclade (99 % bs) with *Rhexothecium globosum*, *Eremomyces bilateralis*, *E. langeronii* and four unidentified species of *Arthrographis*. While the ex-type strain of *Arthrographis lignicola* was related to different genera of the *Lecanoromycetes*, such as *Sarea* and *Pycnora*, the ex-type strains of *Arthrographis pinicola* and *A. alba* were associated with the *Eurotiomycetes* (99 % bs). *Arthrographis pinicola* and *Eremascus albus* (*Eremascaceae*) formed a well-supported clade (93 % bs), while *A. alba* and *Leucothecium emdenii* formed a well-supported clade (99 % bs) within the *Onygenales*.

The type species of *Arthropsis*, *A. truncata*, clustered with *Porosphaerella borinquensis* and *Coniochaeta velutina* (99 % bs), both members of *Sordariomycetes*. *Arthropsis cirrhata* and *A. hispanica* were accommodated within the *Onygenales*. The only available reference strain of *A. microsperma* grouped with different members of the *Helotiales* (94 % bs).

The multilocus sequence analysis was carried out with the ex-type strains of *Arthrographis kalrae* (CBS 693.77) and *Eremomyces langeronii* (CBS 203.78), 12 isolates identified as *A. kalrae* and five isolates identified as an *Arthrographis* sp. Due

Table 1 Strains included in this study.

Species	Strains[1]	Origin	Previous identification	GenBank accession no.[2]			
				28S rDNA	ITS	*ACT1*	*CHS1*
Acarospora smaragdula	–	Unknown, Sweden	*Acarospora smaragdula*	AY853354	–	–	–
Ajellomyces dermatitidis	ATCC 18187 T	Human, unknown	*Ajellomyces dermatitidis*	AY176704	–	–	–
Amauroascus albicans	NRRL 5141 T	Soil, Honduras	*Amauroascus albicans*	–	–	–	–
Apinisia graminicola	CBS 156.77	Skin lesion in dog, USA	*Apinisia graminicola*	AB040696	–	–	–
	CBS 721.68 T	Grass, United Kingdom		AY176709	–	–	–
Aquaphila albicans	BCC 3520	On wooden test block (*Acacia oblonga*), Thailand	*Aquaphila albicans*	DQ341102	–	–	–
	BCC 3543	On wooden test block (*Dipterocarpus alatus*), Thailand		DQ341101	–	–	–
Arachnomyces minimus	CBS 324.70 T	Decayed wood, Canada	*Arachnomyces minimus*	FJ358274	–	–	–
Arachnotheca glomerata	CBS 348.71 T	Unknown, Central African Republic	*Arachnotheca glomerata*	AB075352	–	–	–
Arthroderma cajetanum	UAMH 2937	Single ascospore isolate from a gymnothecium on soil, unknown	*Arthroderma cajetanum*	AB075326	–	–	–
Arthroderma ciferrii	CBS 272.66 T	Soil, USA	*Arthroderma ciferrii*	AB040681	–	–	–
Arthrographis alba	CBS 370.92 T	Marine sediments, Spain	*Arthrographis alba*	**HG004546**	AB213434	–	–
Arthrographis arxii	CBS 203.78 T	Dung of herbivore, India	*Eremomyces langeronii*	AB213426	GQ272638	**HG316563**	**HG316582**
Arthrographis chlamydospora	UTHSC 06-1053 T	Urine, USA	*Arthrographis* sp. III	**HG004543**	**HG004554**	**HG316560**	**HG316580**
Arthrographis curvata	FMR 4032	Marine sediments, Spain	*Arthrographis* sp. I	**HG004539**	**HG004557**	**HG316557**	**HG316577**
	UTHSC 11-1163 T	Nails, USA	*Arthrographis* sp. I	**HG004542**	**HG004556**	**HG316558**	**HG316578**
Arthrographis globosa	UTHSC 11-757 T	Bronchial wash, USA	*Arthrographis* sp. IV	**HG004541**	**HG004553**	**HG316561**	**HG316581**
Arthrographis kalrae	CBS 693.77 T	Sputum, India	*Arthrographis kalrae*	AB116544	AB116536	**HG316544**	**HG316564**
	UTHSC 01-2742	Artificial pulmonary valve, USA		–	**HG004570**	–	–
	UTHSC 04-2580	Blood, USA		–	**HG004569**	**HG316545**	**HG316565**
	UTHSC 04-3423	Toe nail, USA		–	**HG004568**	**HG316546**	**HG316566**
	UTHSC 05-17	Blood, USA		–	**HG004567**	**HG316547**	**HG316567**
	UTHSC 06-982	Pleural fluid, USA		–	**HG004571**	–	–
	UTHSC 06-3158	Toe nail, USA		–	**HG004572**	–	–
	UTHSC 07-2450	Eye, USA		–	**HG004566**	**HG316548**	**HG316568**
	UTHSC 08-527	Lung tissue, USA		–	**HG004573**	–	–
	UTHSC 08-786	Lung biopsy, USA		–	**HG004565**	**HG316549**	**HG316569**
	UTHSC 08-1699	Bronchial wash, USA		–	**HG004574**	–	–
	UTHSC 08-1804	Nails, USA		–	**HG004564**	**HG316550**	**HG316570**
	UTHSC 08-2107	Leg, USA		–	–	–	–
	UTHSC 08-3547	Sputum , USA		–	**HG004575**	–	–
	UTHSC 09-141	Lung biopsy, USA		–	**HG004563**	**HG316551**	**HG316571**
	UTHSC 09-2903	Bronchial wash, USA		–	**HG004576**	–	–
	UTHSC 10-1652	Cornea, USA		–	**HG004562**	**HG316552**	**HG316572**
	UTHSC 10-1719	Cornea, USA		–	**HG004577**	–	–
	UTHSC 10-2021	Catheter tip, USA		–	**HG004561**	**HG316553**	**HG316573**
	UTHSC 10-2583	Urine, USA		–	**HG004560**	**HG316554**	**HG316574**
	UTHSC 10-2729	Nasal sinus, USA		–	**HG004559**	**HG316555**	**HG316575**
Arthrographis kalrae	UTHSC 11-1256	Bronchial wash, USA		–	**HG004558**	**HG316556**	**HG316576**
	UTHSC 11-302	Eye, USA		–	–	–	–
Arthrographis lignicola	CBS 689.83 T	Gymnosperm wood chips and bark, Canada	*Arthrographis lignicola*	**HG004547**	–	–	–
Arthrographis longispora	UTHSC 05-3220 T	Foot, USA	*Arthrographis* sp. II	**HG004540**	**HG004555**	**HG316559**	**HG316579**
Arthrographis pinicola	CBS 653.89 T	Gallery of *Ips latidens* in *Pinus contorta*, Canada	*Arthrographis pinicola*	**HG004548**	–	–	–
Arthropsis cirrhata	CBS 628.83 T	Wall, The Netherlands	*Arthropsis cirrhata*	**HG004549**	–	–	–
Arthropsis hispanica	CBS 351.92 T	Bottom of water deposit, Spain	*Arthropsis hispanica*	HE965759	–	–	–
	UTHSC 09-3174	Bronchial wash, USA		HE965757	–	–	–

Arthropsis microsperma	UAMH 4290	Grass, England	*Arthropsis microsperma*	**HG004551**	–	–	–
Arthropsis truncata	CBS 584.82 ^{T}	Leaf litter, Perú	*Arthropsis truncata*	**HG004550**	–	–	–
Chalara longipes	NBRC 100564	Decaying fir needles, Japan	*Chalara longipes*	–	–	–	–
Chlamydotubeufia huaikangplaensis	–	–	*Chlamydotubeufia huaikangplaensis*	JN865198	–	–	–
Coniochaeta velutina	UAMH 10912	Ex gametophytes of *Hylcomium splendens*, Canada	*Coniochaeta velutina*	EU999180	–	–	–
Ctenomyces serratus	CBS 187.61 ^{T}	Soil, Australia	*Ctenomyces serratus*	AB040683	–	–	–
Eremascus albus	CBS 975.69	Unknown, USA	*Eremascus albus*	FJ358283	–	–	–
Eremomyces bilateralis	CBS 781.70 ^{T}	Dung of pack rat, USA	*Eremomyces bilateralis*	**HG004545**	–	**HG316562**	–
Eurotium herbariorum	CBS 516.65	Unpainted board, USA	*Eurotium herbariorum*	JF922029	–	–	–
Faurelina indica	CBS 126.78	Dung of cow, India	*Faurelina indica*	GU180654	–	–	–
	CBS 301.78	Dung of goat, India		GU180653	–	–	–
Geomyces pannorum	UAMH 10473	Ex biofilm on soil, United Kingdom	*Geomyces pannorum*	GU951697	–	–	–
Gymnascella aurantiaca	CBS 655.71 ^{T}	Clay soil, USA	*Gymnascella aurantiaca*	AB040684	–	–	–
Gymnascella hyalinospora	CBS 548.72 ^{T}	Dung of Guinea pig, India	*Gymnascella hyalinospora*	AB040687		–	–
Gymnoascoideus petalosporus	UAMH 3593	*Tinea pedis*, human, USA	*Gymnoascoideus petalosporus*	AB359428		–	–
Gymnoascus reesii	CBS 410.72 ^{T}	Soil, USA	*Gymnoascus reesii*	JF922021	–	–	–
Helicomyces macrofilamentosus	HKUCC 10235	–	*Helicomyces macrofilamentosus*	AY849942	–	–	–
Hyalodendriella betulae	CBS 261.82	*Alnus glutinosa*, The Netherlands	*Hyalodendriella betulae*	EU040232	–	–	–
Hysterographium fraxini	CBS 109.43	Unknown, Switzerland	*Hysterographium fraxini*	FJ161171	–	–	–
	CBS 242.34	Unknown, Canada		FJ161189	–	–	–
Lambertella brunneola	NBRC 6894	*Aucuba japonica*, Japan	*Lambertella brunneola*	–	–	–	–
Leucothecium emdenii	CBS 576.73 ^{T}	Agricultural soil, The Netherlands	*Leucothecium emdenii*	FJ358286	–	–	–
Malbranchea aurantiaca	CBS 127.77 ^{T}	Culture contaminant, USA	*Malbranchea aurantiaca*	AB040704	–	–	–
Malbranchea cinnamomea	CBS 960.72	Unknown, France	*Malbranchea cinnamomea*	JF922020	–	–	–
Mallochia reticulata	CBS 392.61 ^{T}	Rhizosphere of *Musa sapientum*, Honduras	*Mallochia reticulata*	AB075320	–	–	–
Monascus lunisporas	CBS 113675	Soil, Brazil	*Monascus lunisporas*	JF922026	–	–	–
Monascus ruber	FRR 2447 ^{T}	Soil, India	*Monascus ruber*	JF922025	–	–	–
Onygena corvina	JCM 9546	Decaying bone, Japan	*Onygena corvina*	AB075355	–	–	–
Onygena equina	CBS 947.70	Cow hoof, Germany	*Onygena equina*	AB075356	–	–	–
Ostreichnion curtisii	CBS 198.34	On *Quercus* sp., USA	*Ostreichnion curtisii*	FJ161176	–	–	–
Polytolypa hystricis	UAMH 7299	Ex porcupine dung, Canada	*Polytolypa hystricis*	AY176718	–	–	–
Porosphaerella borinquensis	ICMP 15117	Wood, New Zealand	*Porosphaerella borinquensis*	EF063573	–	–	–
Pseudoarachniotus trochleosporus	CBS 591.71	Soil, USA	*Pseudoarachniotus trochleosporus*	AB075344	–	–	–
Pycnora xanthococca	–	Unknown, Sweden	*Pycnora xanthococca*	AY853388	–	–	–
Rhexothecium globosum	CBS 955.73 ^{T}	Desert soil, Egypt	*Rhexothecium globosum*	**HG004544**	–	–	–
Rutstroemia cuniculi	NBRC 9671	Dung of rabbit, England	*Rutstroemia cuniculi*	–	–	–	–
Rutstroemia paludosa	NBRC 9672	On *Symplocarpus foetidus*, USA	*Rutstroemia paludosa*	–	–	–	–
Sarcoleotia globosa	–	–	*Sarcoleotia globosa*	AY789409	–	–	–
Sarea resinae	–	–	*Sarea resinae*	AY640965	–	–	–
Scleromitrula shiraiana	NBRC 30255	On *Morus bombycis*, unknown	*Scleromitrula shiraiana*	–	–	–	–
Scytalidium cuboideum	UAMH 7144	Ex lingula specimen, USA	*Scytalidium cuboideum*	AB213427	–	–	–
	UAMH 8435	Bronchial washing, USA		AB213428	–	–	–
Shanorella spirotricha	CBS 304.56	Dung of rabbit, USA	*Shanorella spirotricha*	FJ358288	–	–	–
Stromatinia gladioli	NBRC 7169	–	*Stromatinia gladioli*	–	–	–	–
Trichophyton ajelloi var. *ajelloi*	–	–	*Trichophyton ajelloi* var. *ajelloi*	AB075329	–	–	–

[1] ATCC: American Type Culture Collection, Manassas, VA, USA; BCC: Biotec Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; FFR: culture collection of CSIRO, Australia; FMR: Faculty of Medicine Reus, Spain; HKUCC, Hong Kong University Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; ICMP: International Collection of Microorganisms, Landcare Research, Auckland, New Zealand; JCM: Japanese collection of microorganism; NBRC: NITE Biological Resource Center, Japan; UAMH: University of Alberta Microfungus Collection and Herbarium; Edmonton, Canada; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX, USA; ^{T} = Ex-type strain.

[2] Accession numbers of sequences newly determined in this study are indicated in **bold**. ITS: internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; *ACT1*: partial actin gene; *CHS1*: chitin synthase gene.

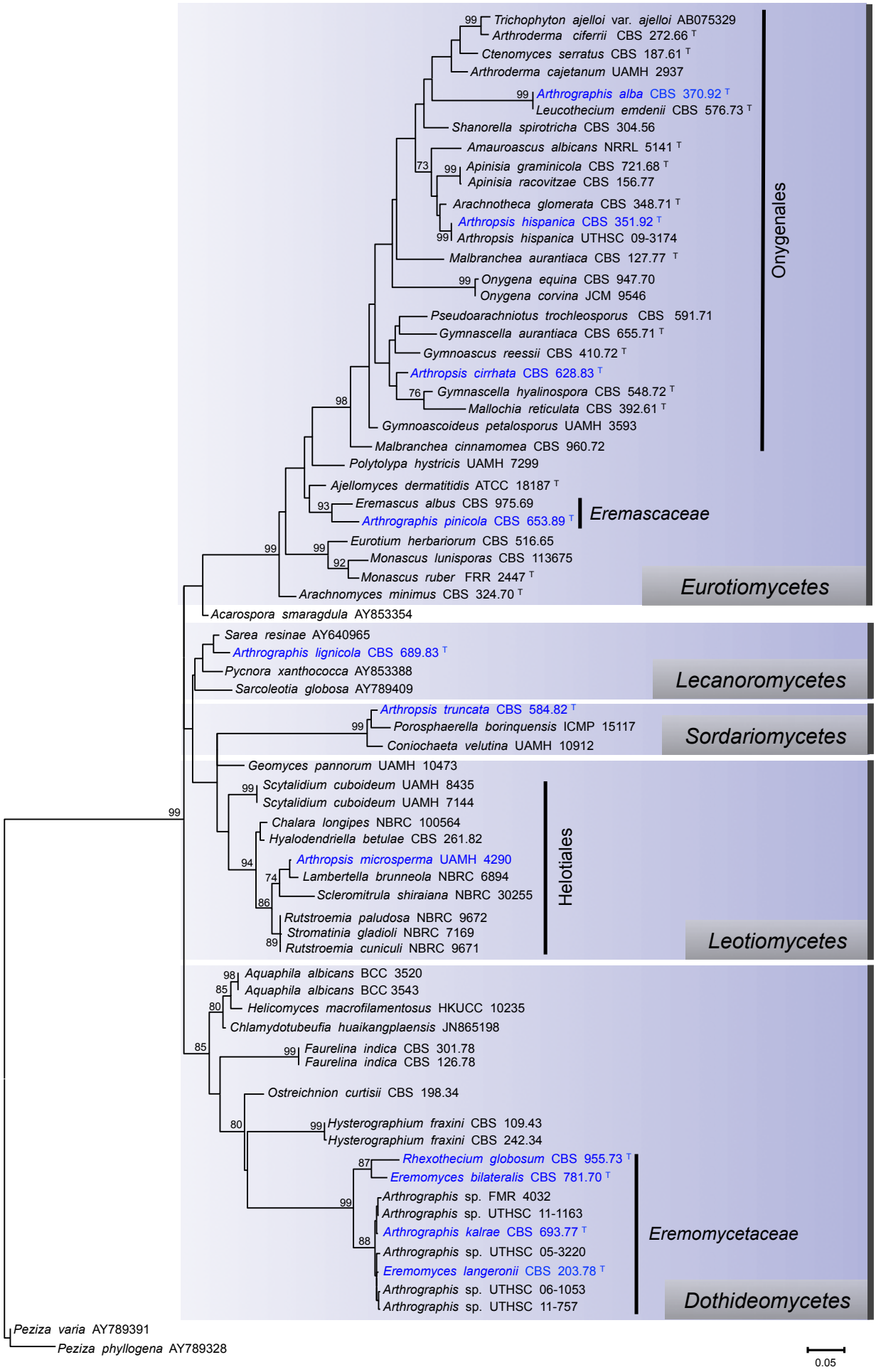

Fig. 1 Maximum-likelihood (ML) tree constructed with sequences of the D1/D2 domains of the 28S rRNA gene. Bootstrap support values above 70 % are indicated at the nodes. The phylogenetic tree was rooted to *Peziza varia* and *Peziza phyllogena*. ^{T} = Ex-type strain.

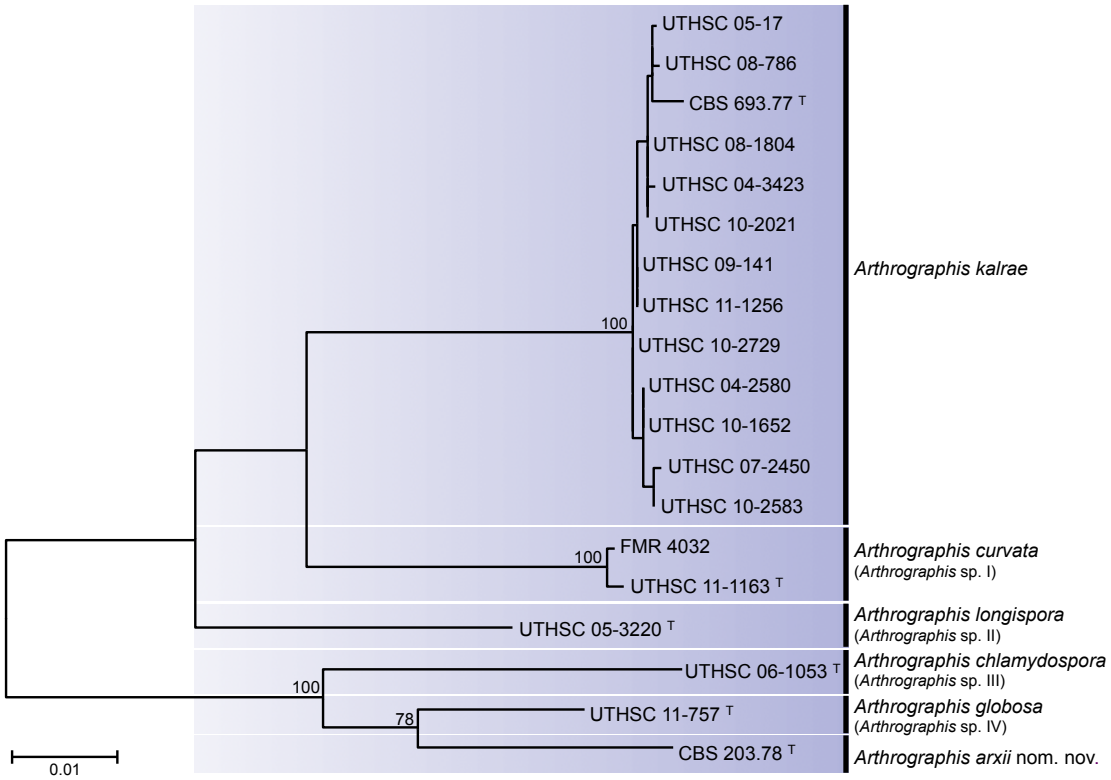

Fig. 2 Maximum-likelihood (ML) tree obtained from the combined DNA sequence data from three loci (ITS, *ACT1*, *CHS1*). Bootstrap support values above 70 % are indicated at the nodes. ^{T} = Ex-type strain.

to the low intra-specific variability detected in the ITS sequences among the 22 isolates identified as *A. kalrae* (98.5–100 % similarity), we selected 12 isolates that represented the most characteristic morphological variants observed.

With the primers used, we were able to amplify and sequence 300–350 bp, 450–500 bp and 750–820 bp of the *CHS1* gene, the ITS region and *ACT1* gene, respectively. The topology of the combined ML tree was similar to trees based on individual genes (data not shown). The combined tree included 1 544 bp and showed four main lineages (Fig. 2). The largest lineage was represented by a clade with 12 clinical strains of *A. kalrae*, including the ex-type strain. Sequences within the clade were practically identical, showing similarities of 98.5–100 % for each over the three loci. The second lineage (*Arthrographis* sp. I, 100 % bs) included one strain from marine sediments (FMR 4032) and another from nails (UTHSC 11-1163), with an intra-specific similarity of 99–100 %. The third lineage (*Arthrographis* sp. II) comprised only one strain (UTHSC 05-3220) from clinical origin (foot). Finally, the fourth lineage comprised a clade with three strains separated from each other by a considerable genetic distance. They were the clinical strains UTHSC 06-1053 (*Arthrographis* sp. III) and UTHSC 11-757 (*Arthrographis* sp. IV) and CBS 203.78, the ex-type strain of *E. langeronii* from herbivore dung. The latter two strains formed a well-supported subclade and showed genetic similarities that ranged from 93.8 % for ITS region to 96–96.7 % for *CHS1* and *ACT1* genes. Surprisingly, the ex-type strains of *A. kalrae* and *E. langeronii* were located in two different clades, showing genetic similarities of 94.1 % for ITS region and 92.8 % and 88.1 % for *ACT1* and *CHS1* genes, respectively.

Phenotypic studies

Most of the strains included in the *A. kalrae* clade (Fig. 2) showed the typical phenotypic characters described for the species; i.e., colonies at 25 °C with slow to moderate growth (up to 10–21 mm diam after 10 d on PDA), flat to slightly folded, initially beige and moist with a yeast-like appearance, becoming tan or yellowish and powdery to granular (Fig. 3a–f); conidiophores hyaline and usually branched (Fig. 3g); conidiogenous hyphae hyaline, simple or branched; arthroconidia 1-celled, hyaline, smooth-walled, cylindrical with truncate ends, 2.5–9 × 1–2 µm. All strains formed a trichosporiella-like synasexual morph with sessile, globose to subglobose, hyaline, thin and smooth-walled conidia, 2–4 × 2–3 µm (Fig. 3h). Several strains showed some atypical characters not previously described for this species. The strains UTHSC 02-1022, UTHSC 06-982, UTHSC 07-2450, UTHSC 08-1804, UTHSC 08-2107, UTHSC 10-1652, UTHSC 10-2583 and UTHSC 11-1256 produced intercalary or terminal chlamydospores with smooth or slightly rugose walls. While in most of these isolates the chlamydospores were hyaline to subhyaline, those of strain UTHSC 11-1256 turned brown on PDA and OA (Fig. 3i, j) giving a dark pigmentation to the colony. The UTHSC 05-17 strain showed a predominance of small conidiophores (up to 70 µm long) composed of a terminal whorl of numerous short chains of clavate or cylindrical arthroconidia with rounded ends (Fig. 3k, l); in old cultures (12 wk) this isolate developed immature ascomata submerged in the agar of all media tested. These ascomata were spherical, non-ostiolate, 37–70 µm diam, with a dark brown, pseudoparenchymatous peridium of *textura angularis*, surrounded by brown hyphae (Fig. 3m).

Arthrographis sp. I (FMR 4032 and UTHSC 11-1163) (Fig. 5a–j) showed similar morphological characteristics to those of the *A. kalrae* clade, but differed in the following features: the colonies on MEA 2 % were orange-yellow (4B8) and showed a very slow growth (6–7 mm diam in 14 d) (Fig. 5a); in addition to the trichosporiella-like synasexual morph (Fig. 5e), both strains produced on PDA at 25 °C and BHI at 37 °C curved and cashew-nut-shaped sessile conidia formed laterally on undifferentiated hyphae (Fig. 5f, g); and the strain UTHSC 11-1163 produced superficial spherical ascomata with evanescent asci and navicular ascospores (Fig. 5h–j).

The lineage representing *Arthrographis* sp. II (UTHSC 05-3220), produced membranous colonies in all the media tested (Fig. 7a, b),

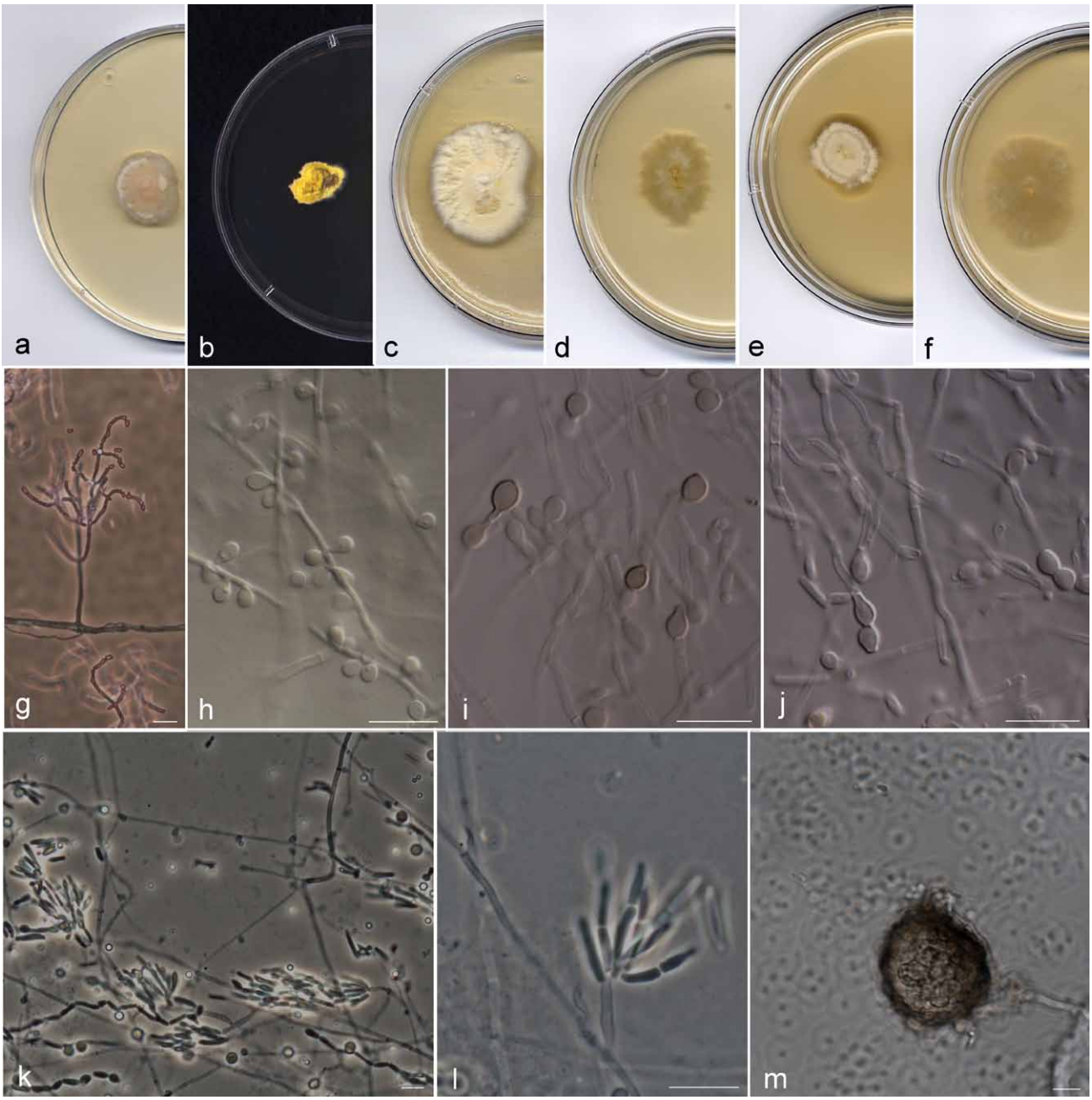

Fig. 3 *Arthrographis kalrae*. a–c. Colonies on PDA after 21 d at 25 °C; d–f. colonies on MEA 2 % at 25 °C after 21 d; g. branched conidiophores; h. lateral sessile conidia; i, j. pigmented chlamydospores and hyaline arthroconidia; k, l. whorls of short arthroconidial chains; m. sterile ascoma (a, d. CBS 693.77; b, e. UTHSC 09-141; c, f–h, k–m. UTHSC 05-17; i, j. UTHSC 11-1256). — Scale bars = 10 µm.

conidiophores poorly differentiated (Fig. 7c, d) and arthroconidia longer (5–10(–13) µm) than those of the members of *A. kalrae* clade (Fig. 7e, f). In this strain, as in *Arthrographis* sp. III and *Arthrographis* sp. IV, the production of a trichosporiella-like synasexual morph was not observed.

Arthrographis sp. III displayed umbonate, cerebriform and velvety colonies on PDA (Fig. 4a), branched conidiophores (Fig. 4c, d), cylindrical, cubic and doliiform arthroconidia (Fig. 4e–g) and terminal or intercalary globose chlamydospores (Fig. 4h).

Finally, the most representative morphological characters observed in *Arthrographis* sp. IV were the production of membranous colonies (Fig. 6a, b), poorly differentiated conidiophores (Fig. 6c) and doliiform, ellipsoidal, slightly fusiform or globose arthroconidia (Fig. 6d, e).

All strains grouped in the clade of *A. kalrae* were able to grow at all the temperatures tested, attaining up to 30 mm diam at 40 °C and 5–15 mm at 45 °C on PDA after 14 d. *Arthrographis* sp. I and *Arthrographis* sp. III grew well at 37 °C (13–16 mm diam after 14 d), but at 40 °C the growth of both species was restricted (6–7 mm diam after 14 d). Conversely, *Arthrographis* sp. II and sp. IV were not able to grow at 37 °C. All isolates tolerated high doses of cycloheximide (2 g/L). Only isolates of *A. kalrae* were able to convert to a yeast phase, producing oval to ellipsoidal (2.5 × 4 µm) yeast-like budding cells at 37 °C after several transfers in BHI broth.

Taxonomy

On the basis of the morphological features observed, which correlated with the phylogenetic analysis, we concluded that *Arthrographis* spp. I–IV are different from the taxa currently accepted in this genus and are therefore described here as new. These species are named *A. chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora*. In addition, the new name *Arthrographis arxii* is proposed for the ascomycete *Eremomyces langeronii*.

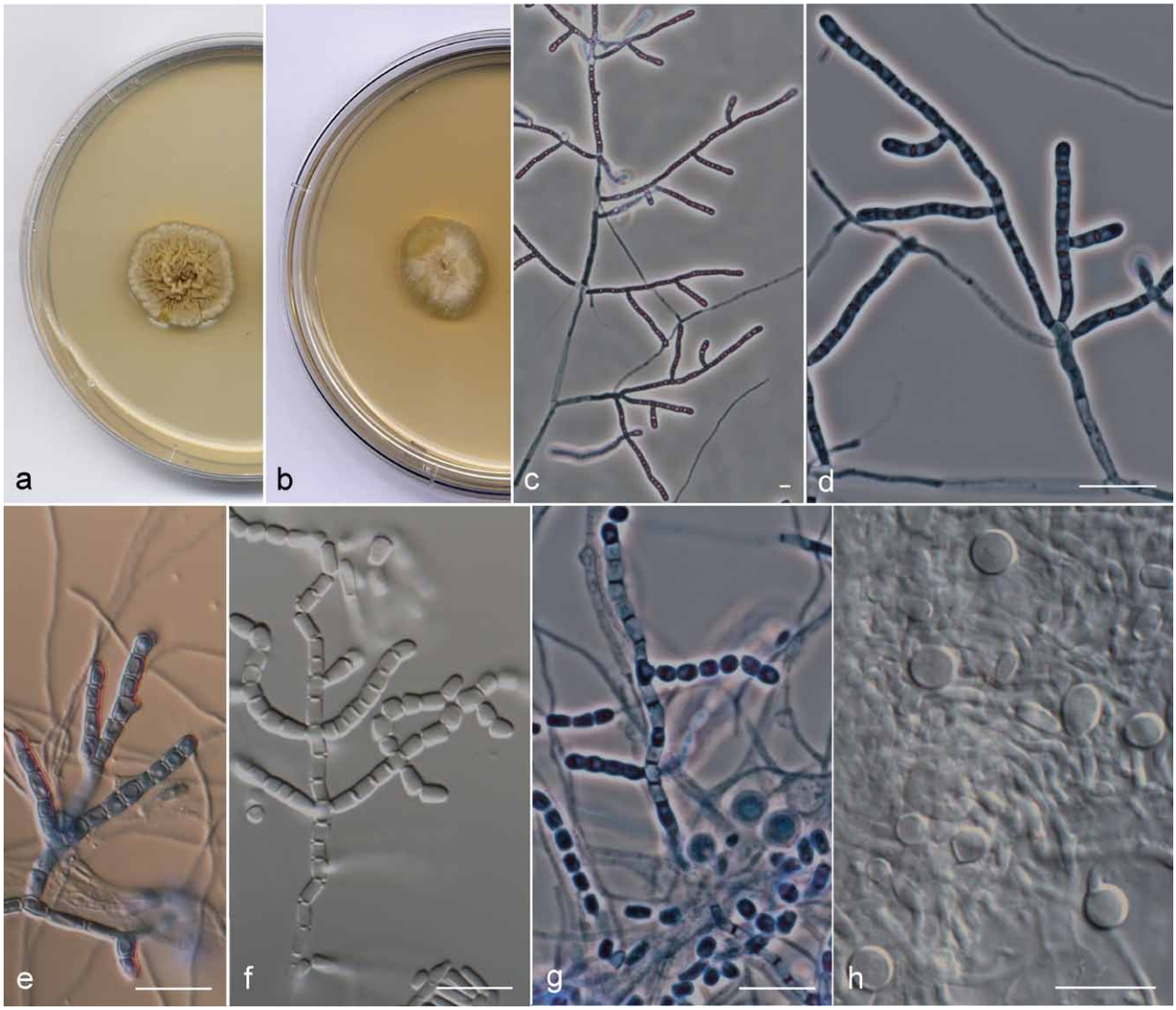

Fig. 4 *Arthrographis chlamydospora* UTHSC 06-1053. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c, d. branched conidiophores; e, f. conidiogenous hyphae fragmenting schizolytically; g. cylindrical and doliiform arthroconidia; h. chlamydospores. — Scale bars = 10 µm.

Arthrographis arxii Guarro, Giraldo, Gené & Cano, *nom. nov*. — MycoBank MB804634

Basionym. *Pithoascus langeronii* Arx, Persoonia 10: 24. 1978.
≡ *Pithoascina langeronii* (Arx) Valmaseda, T.A. Martínez & Barrasa, Canad. J. Bot. 65: 1805. 1987.
≡ *Eremomyces langeronii* (Arx) Malloch & Sigler, Canad. J. Bot. 66: 1931. 1988.

Etymology. The specific epithet is given in honour of the mycologist Josef Adolf von Arx (1922–1988), who actively published on this group of fungi.

Notes — Since our results demonstrated that *A. kalrae* and *E. langeronii* are not conspecific, and the name *A. langeronii* was occupied, a new name is proposed for *E. langeronii.*

Arthrographis chlamydospora Giraldo, Deanna A. Sutton, Gené & Madrid, *sp. nov*. — MycoBank MB804632; Fig. 4

Etymology. Referring to the presence of chlamydospores.

Colonies on PDA at 25 °C attaining 15–16 mm diam after 14 d, pale to greyish orange (5A–B3) with whitish margin, umbonate, cerebriform, velvety. On OA and PCA at 25 °C attaining 23–25 mm and 15–16 mm diam, respectively, after 14 d, orange-white (5A2), flat, powdery or granulose. On MEA 2 % at 25 °C attaining 14–15 mm diam in 14 d, orange-yellow (4B8), flat, radially striated, granulose. At 37 °C on PDA the colonies attaining 12–13 mm diam after 14 d, brownish orange (6C3–4), cerebriform, velvety. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* mostly repeatedly branched, erect, up to 350 µm long, hyaline, smooth-walled. *Conidiogenous hyphae* simple or laterally branched, 1.5–2.5 µm wide, thick-walled, forming septa basipetally to form arthroconidia released via schizolythic secession. *Arthroconidia* unicellular, cylindrical, cuboid or doliiform, straight, 3–6(–7) × 1.5–2.5 µm, hyaline to subhyaline, thick- and smooth-walled. *Chlamydospores* terminal or intercalary, solitary, unicellular, globose or subglobose, 5–6 × 5–6 µm, hyaline, rough- and thick-walled, strongly chromophilic. Sexual morph and trichosporiella-like synasexual morph not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 42 °C, minimum 15 °C. The fungus was unable to grow at 45 °C.

Specimen examined. USA, Florida, from human urine, *D.A. Sutton* (holotype CBS H-21346, cultures ex-type CBS 135396, FMR 12129, UTHSC 06-1053).

Arthrographis curvata Giraldo, Gené, Deanna A. Sutton & Cano, *sp. nov*. — MycoBank MB804630; Fig. 5

Etymology. Referring to the presence of curved conidia.

Colonies on PDA at 25 °C attaining 17–19 mm diam in 14 d, pale to greyish orange (5A–B3) with whitish margin, umbonate at centre and flat toward the periphery, powdery. On OA and

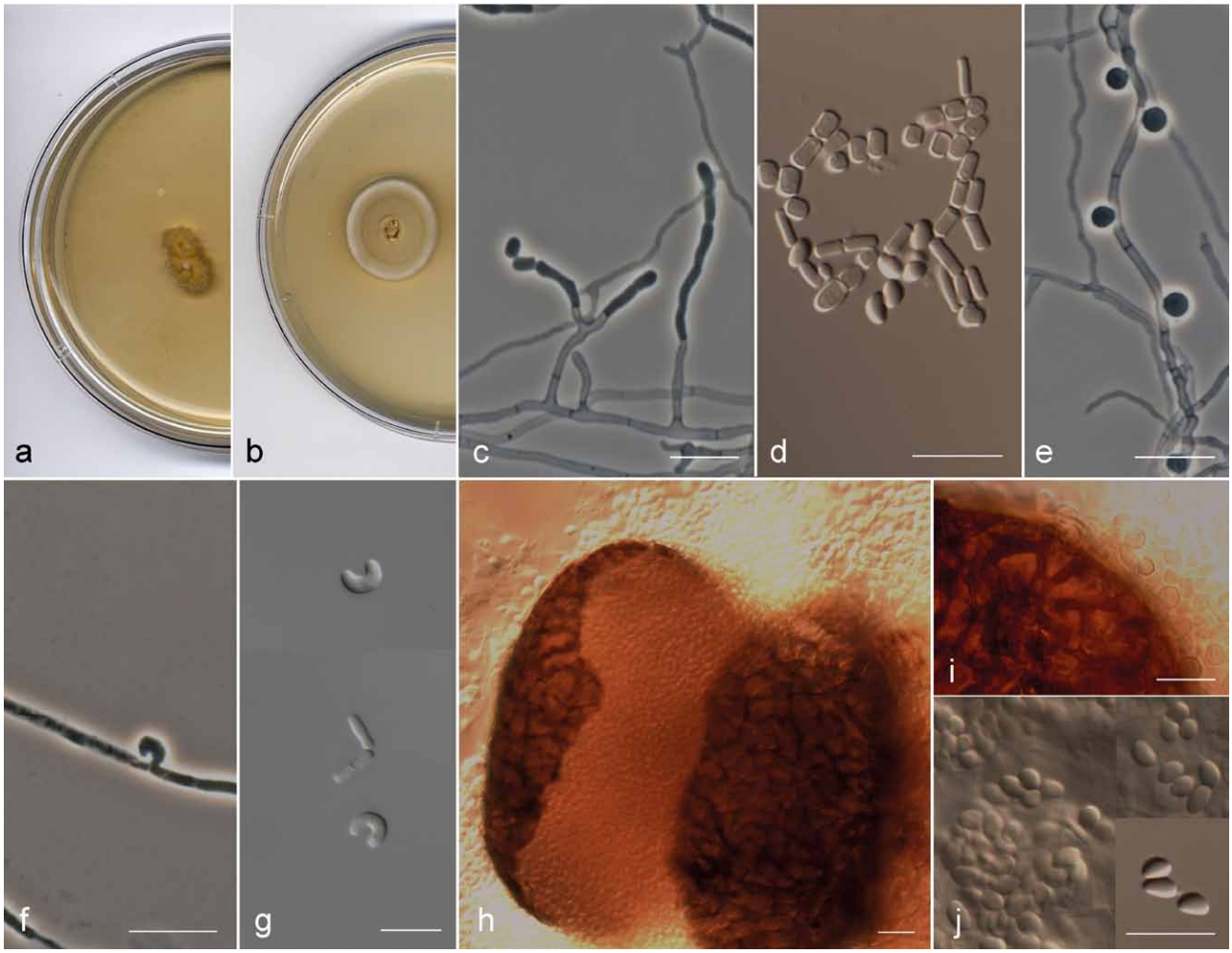

Fig. 5 *Arthrographis curvata*. a, b. Colonies on MEA 2 % and PDA, respectively, after 21 d at 25 °C. — c–g. Asexual morph: c. simple or poorly branched conidiophores; d. arthroconidia and ascospores; e. lateral sessile conidia. f, g. curved conidia produced in BHI at 37 °C. — h–j. Sexual morph: h. ascoma; i. dark brown hyphae on the peridium; j. ascospores (a–c, e, g. FMR 4032; d, f, h–j. UTHSC 11-1163). — Scale bars = 10 µm.

PCA at 25 °C attaining 19–20 mm and 24–26 mm diam, respectively, after 14 d, whitish, flat, dusty. On MEA 2 % at 25 °C attaining 6–7 mm diam in 14 d, orange-yellow (4B8), elevated, cerebriform, membranous. At 37 °C on PDA the colonies attaining 15–16 mm diam after 14 d, orange-grey (5B2), flat, powdery. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Ascomata* cleistothecial, superficial, spherical, brown, 52–132 µm diam, peridium pseudoparenchymatous with *textura angularis*, surrounded by dark brown, thick-walled hyphae. *Asci* evanescent, globose, thin-walled. *Ascospores* unicellular, navicular in lateral view, ellipsoidal in front view, thin- and smooth-walled, without germ pores, 2.8–3.8 × 1.4–2 µm, hyaline to pale brown in mass. *Conidiophores* poorly differentiated, erect, simple or slightly branched, up to 35 µm long, hyaline, smooth-walled. *Conidiogenous hyphae* simple or branched, 1–2 µm wide, thin-walled, forming septa basipetally to form arthroconidia released by schizolythic secession. *Arthroconidia* unicellular, cylindrical or short-cylindrical, straight or slightly curved, 3–4.5(–7) × 1–2 µm, hyaline to subhyaline, thin- and smooth-walled. *Synasexual morph* trichosporiella-like with conidia growing directly on undifferentiated hyphae, lateral, sessile, globose, 2–3 µm diam, hyaline and smooth-walled. On PDA and BHI at 25 °C and 37 °C, respectively, conidia were occasionally observed to be unicellular, curved, cashew-nut-shaped, hyaline and smooth-walled, 3.5–6 × 1.5–2 µm, growing solitary and sessile on vegetative hyphae.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 42 °C, minimum 15 °C. The fungus was unable to grow at 45 °C.

Specimens examined. Spain, Amposta, Ebro river, from river bank, *K. Ulfig* (CBS 135934, FMR 4032). – USA, Colorado, from human nails, *D.A. Sutton* (holotype CBS H-21344, cultures ex-type CBS 135933, FMR 12125, UTHSC 11-1163).

Notes — The GenBank sequences AB128973.1 (ITS region) and AB128975 (28S rDNA), corresponding to the isolate *E. langeronii* UAMH 7600 from a fingernail, were 99.6 and 100 % (ITS region and 28S rDNA, respectively) similar to those of the type species of *A. curvata*.

Arthrographis globosa Giraldo, Deanna A. Sutton, Cano & Guarro, *sp. nov*. — MycoBank MB804633; Fig. 6

Etymology. Referring to the presence of globose conidia.

Colonies on PDA at 25 °C attaining 22–24 mm diam after 14 d, buttercup yellow (4A7), flat, membranous. On OA and PCA at 25 °C attaining 14–15 mm diam after 14 d, whitish, flat, at first glabrous becoming slightly powdery. On MEA 2 % at 25 °C attaining 4–5 mm diam in 14 d, orange-yellow (4A8), cerebriform, membranous. *Vegetative hyphae* septate, hyaline, with golden pigment accumulation inside, smooth- and thin-walled, 1.5 µm wide. *Conidiophores* absent or poorly differentiated, hyaline, smooth-walled. *Conidiogenous hyphae* simple or branched, 1–1.5 µm wide, thin-walled, forming septa basipetally to form arthroconidia released via schizolythic secession. *Arthroconidia* unicellular, doliiform, ellipsoidal, slightly fusiform or globose, 3–5(–6.5) × 2–4 µm, hyaline, thick- and smooth-walled. Sexual morph, trichosporiella-like synasexual morph and chlamydospores not observed.

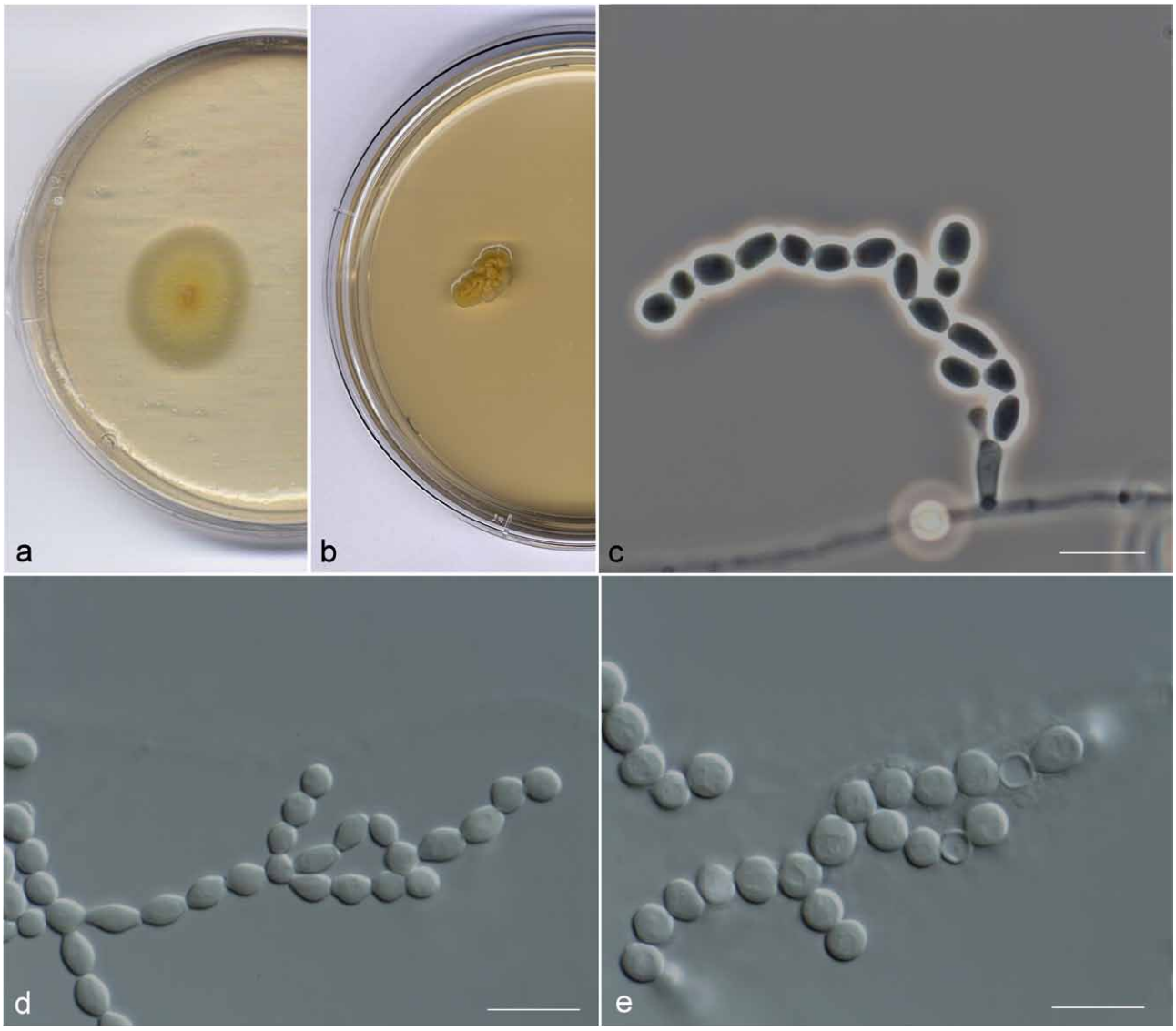

Fig. 6 *Arthrographis globosa* UTHSC 11-757. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c. poorly differentiated conidiophores; d. conidiogenous hyphae fragmenting schizolytically producing ellipsoidal, doliiform, slightly fusiform and globose arthroconidia; e. globose arthroconidia. — Scale bars = 10 µm.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C. The fungus was unable to grow at 37 °C.

Specimen examined. USA, Texas, from human bronchial wash, *D.A. Sutton* (holotype CBS H-21347, cultures ex-type CBS 135397, FMR 12124, UTHSC 11-757).

Arthrographis longispora Giraldo, Deanna A. Sutton, Cano & Guarro, *sp. nov*. — MycoBank MB804631; Fig. 7

Etymology. Referring to the length of the arthroconidia.

Colonies on PDA at 25 °C attaining 18–21 mm diam after 14 d, yellowish orange (4A7), radially folded or rugose at centre and flat toward the periphery, membranous. On OA and PCA at 25 °C attaining 18–21 mm and 8–9 mm diam, respectively, after 14 d, whitish, flat, at first glabrous becoming slightly powdery. On MEA 2 % at 25 °C attaining 11–12 mm diam in 14 d, orange-yellow (4B8), cerebriform at centre and flat toward the periphery, membranous. *Vegetative hyphae* septate, hyaline, with golden pigment accumulation inside, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* poorly differentiated, erect, up to 60 µm long, hyaline, smooth-walled. *Conidiogenous hyphae*, simple, occasionally slightly branched, 1–1.5 µm wide, thin-walled, septating basipetally to form arthroconidia released by schizolythic secession. *Arthroconidia* unicellular, cylindrical with truncated or rounded ends, straight or slightly curved, 5–10(–13) × 1–1.5 µm, hyaline, thin- and smooth-walled. Sexual morph, trichosporiella-like synasexual morph and chlamydospores not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C. The fungus was unable to grow at 37 °C.

Specimen examined. USA, Utah, from human foot, *D.A. Sutton* (holotype CBS H-21345, cultures ex-type CBS 135935, FMR 12101, UTHSC 05-3220).

DISCUSSION

The genus *Arthrographis* was traditionally considered a member of the *Eremomycetaceae*, *Dothideomycetes* (Malloch & Sigler 1988). However, our D1/D2 analysis demonstrated that only the type species, *A. kalrae*, and the new taxa proposed here (i.e., *A. arxii*, *A. chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora*) are members of the family, and that the name *Arthrographis* should be restricted to these species. The other species previously attributed to the genus are phylogenetically distant from the type. *Arthrographis lignicola* belongs to the *Lecanoromycetes*, forming a weakly supported clade with *Sarea resinae*, *Pycnora xanthococca* and *Sarcoletia globosa*. Although a BLAST search using D1/D2 and ITS sequences of *A. lignicola* showed close relationships with other members of

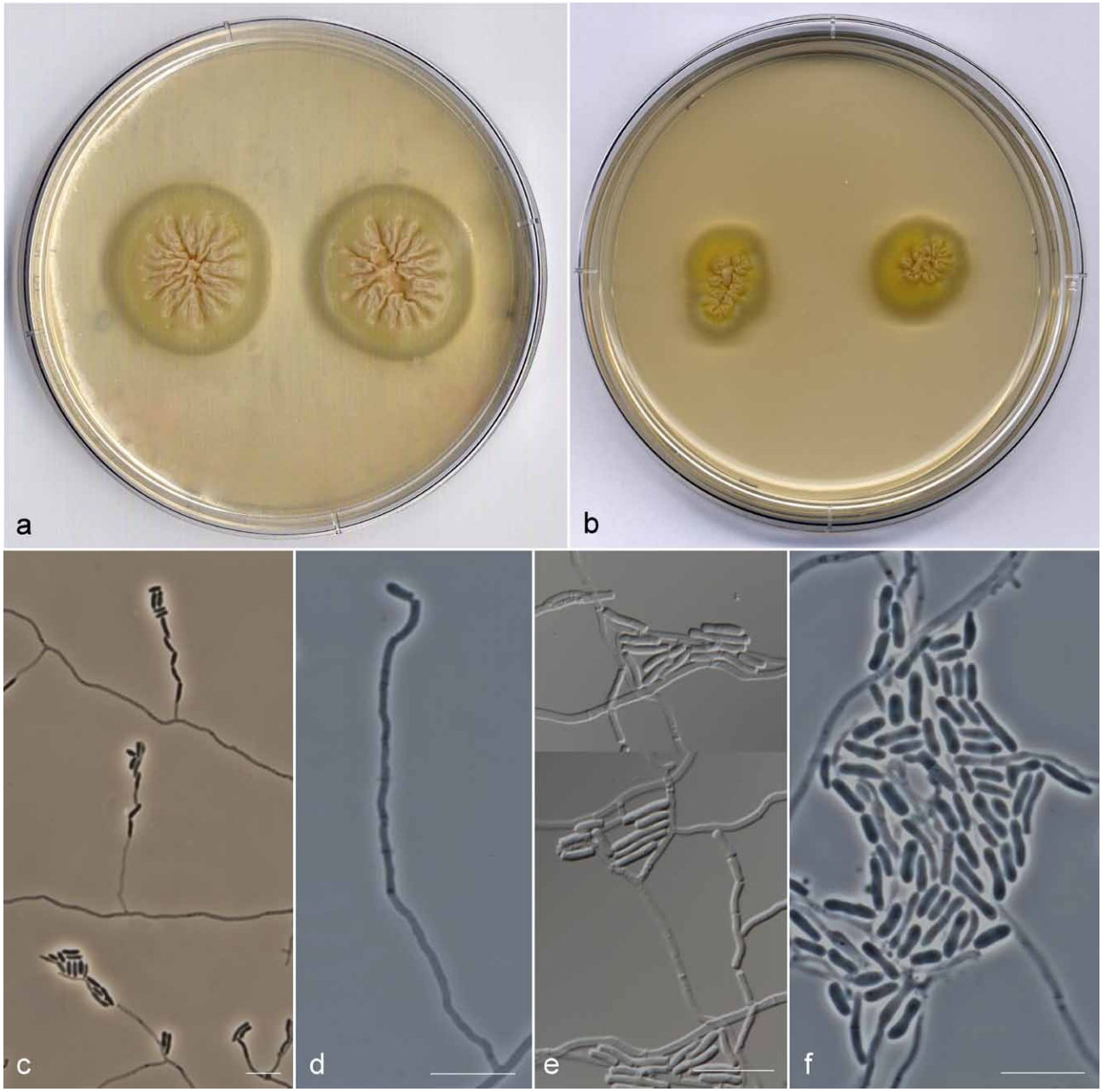

Fig. 7 *Arthrographis longispora* UTHSC 05-3220. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c, d. poorly differentiated conidiophores; e, f. cylindrical arthroconidia with truncate or rounded ends. — Scale bars = 10 µm.

that class, we could not include more sequences of *Lecanoromycetes* in our phylogenetic analysis due to the difficulties in performing a reliable alignment. *Arthrographis pinicola* and *A. alba* are accommodated in the *Eurotiomycetes*, more particularly, the former in *Eremascaceae*, closely related to *Eremascus albus*, and the latter in the *Gymnoascaceae*, closely related to *Leucothecium emdenii. Arthrographis alba* was described based on several isolates from different origins and, although some morphological similarity with the anamorph of *L. emdenii* was already mentioned, none of those isolates developed the sexual morph (Gené et al. 1996). The present study reveals that sequences from D1/D2 and ITS of both species are practically identical (data not shown), which indicates that *A. alba* must be considered the asexual morph of *L. emdenii.* The genus *Leucothecium* was described by von Arx & Samson (1973) to accommodate ascomycetes with yellowish globose ascomata, hyaline peridium, bivalve-lenticular ascospores and asexual morphs with hyaline arthroconidia. Currently, the genus comprises two species, *L. emdenii*, the type species, and *L. coprophilum*, both traditionally included in *Gymnoascaceae* on the basis of the morphology of the asexual morph and ascospores (von Arx & Samson 1973, Valldosera et al. 1991).

The ascomycete *Faurelina indica* also produces an arthrographis-like asexual morph similar to the *Arthrographis* anamorph of *E. langeronii* (von Arx 1978, von Arx et al. 1981). The genus *Faurelina*, with a coprophilous habitat, was originally included in the family *Chadefaudiellaceae*, *Microascales* (Locquin-Linard 1975, Cannon & Kirk 2007). This genus was characterised by pustulate or hemispherical ascomata, a peridium composed of vertical rows of dark cells, asci arranged in vertical chains and striate, and pale brown ascospores (Guarro et al. 2012). Our D1/D2 analysis revealed a well-supported relationship of *F. indica* with the *Dothideomycetes*, although distantly related to the genus *Arthrographis* and other members of *Eremomycetaceae*. Réblová et al. (2011), based on LSU sequences analysis, demonstrated the relationship of *F. indica* with the *Didymellaceae*, including it in the *Pleosporales*. The exclusion of *F. indica* from *Microascales* correlates with the morphological features of the asexual morph, since in that order the asexual morphs are characterised by percurrently proliferating conidiogenous cells (annellides) usually belonging to the genera *Scopulari-*

opsis, *Graphium*, *Scedosporium*, *Cephalotrichum* and *Wardomycopsis* (Valmaseda et al. 1986, Zhang et al. 2006, Réblová et al. 2011).

Eremomyces langeronii was traditionally considered to be the sexual morph of *A. kalrae* (von Arx 1978, Malloch & Sigler 1988). However, this connection was questioned by Sigler & Carmichael (1983), and later by Gené et al. (1996), arguing that both species produced different RFLP patterns. The present study confirms that *E. langeronii* and *A. kalrae* are not conspecific. *Eremomyces langeronii* was initially described as *Pithoascus langeronii* (von Arx 1978), later being transferred to the genus *Pithoascina* by Valmaseda et al. (1986). Malloch & Sigler (1988) accommodated this species in the genus *Eremomyces* (*Eremomycetaceae*) (Malloch & Cain 1971, Malloch & Sigler 1988) together with *E. bilateralis* and *Rhexothecium globosum*. Members of the *Eremomycetaceae* are characterised by cleistothecial ascomata, clavate to ovoid, evanescent asci, unicellular, hyaline to pale yellow-brown ascospores, arthrographis-like or trichosporiella-like asexual morphs and a coprophilous habitat (Malloch & Sigler 1988). Similar morphological features such as non-ostiolate dark ascomata and hyaline, unicellular ascospores can be also found in species of *Pseudeurotiaceae* (*incertae sedis*, Lumbsch & Huhndorf 2010), but the members of this family display pale-brown or olive-brown ascospores at maturity and asexual morphs with poorly differentiated conidiophores sympodially producing subspherical to ovoidal conidia. Our study demonstrated that the family *Eremomycetaceae* encompasses the genera *Arthrographis* s.str., *Rhexothecium* and *Eremomyces* (91.4–95.3 % intergeneric similarity in D1/D2 sequences). The latter now is restricted only to *E. bilateralis*, which is the type species of the genus. *Eremomyces bilateralis* is distinguished from *Arthrographis* s.str. and *Rhexothecium* by DNA sequence data (92.8 %, 89 % and 76.4 % similarity in D1/D2, *ACT1* and ITS sequences, respectively) and by its cephalothecoid peridium, dark coloured colonies and the absence of an asexual morph (Malloch & Cain 1971).

The multilocus sequence analysis revealed the existence of four new species in *Arthrographis*, *A. curvata* being the only one that showed both sexual and asexual morphs in culture. Its ascomata and ascospores are similar to those of *A. arxii*; however, in *A. arxii* the ascomata are immersed, and the ascomata and ascospores are larger (75–160 µm diam and 2.7–5 × 1.8–2.6 µm, respectively). The asexual morph of *A. curvata* differs from *A. arxii* and *A. kalrae* mainly by less differentiated and poorly branched conidiophores, the presence of curved, sessile conidia and a restricted growth at 40 °C. Another fungus that also produces curved, cashew-nut shaped conidia is the dermatophyte *Trichophyton phaseoliforme*, but this species is a member of *Eurotiomycetes*, produces pycnidium-like conidiomata and cigar-shaped macroconidia in clusters (de Hoog et al. 2011). *Arthrographis arxii* differs from *A. kalrae* in producing shorter and wider (3.5–5 × 2–2.5 µm) 1-septate arthroconidia.

Arthrographis chlamydospora is characterised by the production of repeatedly branched conidiophores, cuboid or doliiform arthroconidia and numerous chlamydospores. The species morphologically closest to *A. chlamydospora* is *A. kalrae*, but the latter differs by exhibiting yellowish to tan colonies, thin-walled arthroconidia, good growth at 40 °C and the presence of the trichosporiella-like synasexual morph. The other two new species, *A. globosa* and *A. longispora*, share several phenotypic features, i.e. absence of trichosporella-like synasexual morph, membranous colonies, inability to grow at 37 °C and resistance to high doses of cycloheximide. *Arthrographis globosa* can easily be distinguished by its globose to doliiform arthroconidia, and *A. longispora* by its poorly differentiated conidiophores producing large cylindrical arthroconidia. Other species of *Arthrographis* s.lat. unable to grow at 37 °C are *A. alba*, *A. lignicola* and *A. pinicola*. *Arthrographis alba* produces white colonies, pseudodichotomously branched conidiophores and, in our study, this species was susceptible to high doses of cycloheximide (2 g/L); *A. lignicola* can be distinguished by its lemon-yellow to olive-green colonies with a diffusible brown pigment, narrow branched conidiophores and yellow arthroconidia; and *A. pinicola* produces floccose conidiomata composed by repeatedly branched conidiophores and is susceptible to low doses of cycloheximide (Sigler & Carmichael 1983, Sigler et al. 1990, Gené et al. 1996).

In this study we observed some morphological variability in *A. kalrae*, with the presence of some characteristics not previously reported for this species. Such variations, however, did not correlate with genetic differences in any of the three loci sequenced. Several isolates showed chlamydospores that were terminal or intercalary, solitary or catenulate, hyaline or pigmented. In the protologue of *Oidiodendron kalrai*, based in the strain CBS 693.77, Tewari & Macpherson (1971) reported the occasional presence of oval to round, thick-walled chlamydospores; however, Sigler & Carmichael (1976) did not mention these structures and only reported the sessile conidia of the trichosporiella-like synasexual morph. The UTHSC 05-17 isolate produced infertile ascomata morphologically similar to the ascomata produced by *A. arxii* and *A. curvata*, but in that isolate these structures were smaller (37–70 µm diam). That isolate also produced abundant conidiophores with whorls of short chains of clavate or cylindrical arthroconidia. The clavate conidia was reported by von Arx (1978) in the description of the asexual morph of *E. langeronii*, but probably this description was based on a single strain of this species and not on the ex-type strain of *A. kalrae*.

The genus *Arthropsis* was established by Sigler et al. (1982) with *A. truncata* as the type species, to accommodate species with dark arthroconidia, joined by adjacent connectives and developed from undifferentiated conidiogenous hyphae. Until now the species of this genus have not been associated to any sexual morph. Our D1/D2 sequence analysis demonstrates that *Arthropsis* is polyphyletic and unrelated to *Arthrographis* s.str. *Arthropsis hispanica* and *A. cirrhata* fall into the *Onygenales*, as do other species previously included in *Arthrographis*. Other arthroconidial anamorphs of the *Onygenales* are included in the genus *Malbranchea*. However, *Malbranchea* is morphologically distinguished by its branched and arcuate fertile hyphae, straight in some species, that produce alternate arthroconidia (Sigler & Carmichael 1976). Our analysis placed the only available living strain of *A. microsperma* (UAMH 4290) in the *Helotiales* (*Leotiomycetes*). *Arthropsis microsperma* was originally described by Berkeley & Broome (1873) as *Oidium microspermum* and later transferred to *Arthropsis* by Sigler & Carmichael (1983) based on its arthroconidial ontogeny. Therefore, the name of this species should be reconsidered because *Oidium* anamorphs are currently associated with members of the *Leotiomycetes* (Braun & Cook 2012). Finally, *Arthropsis truncata* is related to members of the *Sordariomycetes*. Although such type of asexual morphs have not been described in that class, humicola-like asexual morphs similar to the *Humicola* synasexual morph of *A. truncata* are present in some species of *Chaetomium* (Gené & Guarro 1996, Seifert et al. 2011). Further studies with a greater number of taxa of *Sordariomycetes* are needed to ascertain a defined position for *A. truncata* within this class.

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