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Arthroderma olidum, sp. nov. A new addition to the *Trichophyton terrestre* complex

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In 1981, four fungal isolates from hair of the European badger (*Meles meles*) were examined by Dr Phyllis Stockdale at the Commonwealth Mycological Institute, Kew, and deposited in the UK National Collection of Pathogenic Fungi as an undescribed member of the *Trichophyton terrestre* complex. The present paper formalizes the complete description of a new ascomycete taxon, *Arthroderma olidum* following successful recent attempts to re-isolate the same fungus from the soil of Badger holes in South West England. Furthermore, using ribosomal RNA gene sequencing, we show that the asexual form of *A. olidum* is conspecific with the recently described *Trichophyton eboreum* [1] isolated from a human skin specimen in Germany.

Keywords *Arthroderma olidum*, *Trichophyton eboreum*, teleomorph, badger hair, rDNA gene sequences

Introduction

Trichophyton terrestre is a mitosporic species referable to several closely related *Arthroderma* species in the Ascomycete family Gymnoascaceae.

The original description of *T. terrestre* was based upon two fungi collected from soil in Australia [2]. These authors considered that the diagnostic feature of their new species was the production of conidial forms intermediate in morphology between the unicellular microconidia and the multicellular macroconidia of the pathogenic *Trichophyton* species.

Several reports quickly followed of *T. terrestre* isolation from soils in England, California and Germany. In 1961 Dawson & Gentles [3] described *Arthroderma quadrifidum* as an ascomatal form arising spontaneously in a culture of *T. terrestre* initially recovered from the soil of a rabbit burrow in Scotland. These authors went on to show that *A. quadrifidum* was heterothallic [3]. However despite successfully crossing single ascospore isolates derived from *A. quadrifidum* with several European *T. terrestre* strains, they were unable to induce sexual reproduction with the ex-type strain of *T. terrestre* of Durie & Frey, or with Californian isolates.

Following studies of mating compatibility in *T. terrestre*, Pore, Tsao and Plunkett [4] described a second *Arthroderma* species, *A. lenticularum*. They demonstrated three other incompatibility groups, two among Californian isolates (Groups IV, VI) and one from Hungary (Group IX). However, they declined to erect new species for these as each group contained only two isolates. Group IX was later shown to belong to a third *Arthoderma* species, *A. insingulare*, through mating with *T. terrestre* isolates from Canada, Czechoslovakia and the USA [5]. Neither Pore & Plunkett [4], nor Padhye & Carmichael [5] tested their new species by mating with the ex-type strain of *T. terrestre* of Durie & Frey.

In 1981, four fungal isolates from hair of the European badger (*Meles meles*) were examined by Dr Phyllis Stockdale at the Commonwealth Mycological Institute, Kew, UK and deposited in the UK National Collection of Pathogenic Fungi as an undescribed member of the *T. terrestre* complex. The present paper describes this as a new ascomycete taxon, *Arthroderma*

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olidum. Furthermore this study demonstrates that the asexual form of *A. olidum* is indistinguishable from the recently described *Trichophyton eboreum* [1] which was originally isolated from a human skin specimen in Germany.

Materials and methods

Isolation of Arthroderma olidum *from soil around animal holes*

Soil from the entrances of animal holes, predominantly those of the Badger and Rabbit, was collected to a depth of about 3 cm into unused polythene bags, returned to the laboratory and transferred to sterile 9 cm diam Petri dishes, moistened with sterile water and baited with human or horse hair previously sterilized by autoclaving at 15 p.s.i. (121°C) for 15 min. The dishes were incubated at ambient temperature (15–20°C) and examined at intervals over a period of three weeks for the presence of ascomata and conidia of the *Arthroderma* group. All the sites sampled were in wooded localities on the geological formation known as carboniferous limestone in the county of North Somerset, England.

Single-ascospore isolates were obtained from a single ascoma of primary hair bait plate CKC21A, (which later gave the fixed type material NCPF 5111 and the ex type strain 5088; Table 1). The procedure was as follows. A single ascoma was removed from the hair-soil culture CKC21A with a needle and serially transferred by needle three times into drops of sterile water (approx. 0.1 ml each) on the lid of a Petri dish. Under microscopic examination, the last washing water was shown to contain very few microconidia. The ascoma was then transferred to a fourth drop and crushed with a needle point to release the ascospores. The resulting ascospore suspension was again examined and was found to contain many separated ascospores. This was diluted tenfold three times in succession using sterile water. 0.5 ml. aliquots of these dilutions were plated onto Sabourauds agar plates (Oxoid Ltd. Basingstoke, UK). After 24 hours incubation at 30°C several well-separated germlings were removed from the highest dilution plate using a dissecting microscope and inoculated onto fresh plates of the same medium. These isolates were considered as single-ascospore isolates and designated NCPF 5102 through 5109.

Mating experiments

In an attempt to assess the mating types of the single-ascospore isolates NCPF 5102 through 5109 and the mating type 'tester' strains NCPF 394 and NCPF 494 of *Arthroderma simii* [6] were grown for two weeks on Sabourauds glucose agar (Oxoid Ltd., Basingstoke, UK). Point-inocula, (consisting of aerial mycelium removed from these cultures with a needle), was placed onto the surface of Sabourauds glucose agar plates approx 2 cm apart in combinations of each of the *A. olidum* isolates with each of the tester strains. After 2, 3 and 4 weeks the plates were examined for evidence of a growth stimulation effect between the colonies.

For trial matings of the single ascospore isolates with each other in all possible combinations (Table 2), conidial suspensions were prepared by flooding Sabourauds agar slopes with sterile water. Approximately 0.5 ml of each of two isolates was added to a 9 cm Petri dish containing double-autoclaved garden soil, and sterile human hair. Incubation was at 15–20°C for three weeks, with occasional addition of sterile water if the soil appeared dry. After this time the fungal growth on the hairs was examined for the presence of ascomata using a dissecting microscope. Selected ascomata were examined under higher magnification for the presence of asci and ascospores.

Preparation of fungal genomic DNA

Genomic fungal DNA was prepared using Whatman FTA filter cards excactly as described previously [7]. Briefly, isolates were inoculated onto Sabouraud's (SAC) medium (glucose-peptone agar containing 0.05 mg/ml chloramphenicol) and incubated at 30°C. Two week-old pure cultures were harvested by scraping the surfaces of colonies and suspending in 1 ml volumes of sterile water. Spores and/or hyphal fragments were added to about 10% vol/vol for hyphal fragments, and 2% vol/vol for spores. Mould aqueous suspensions (200 µl) were then applied directly to Whatman FTA microcards (with indicator). FTA cards were then placed, open, in a microwave (Panasonic, 800W) while still damp, and were subjected to 2 cycles of 30 s on full power, with a pause of at least 30 s between each cycle. Filters were subjected to microwaves 4 at a time, in the presence of a pyrex beaker containing 50 ml of sterile water to dissipate excess heat. Dried, processed filters were then stored at room temperature in sealed plastic bags containing dessicant, until needed.

Preparation of filters and PCR amplification of fragments of the nuclear ribosomal repeat region

Punches (2 mm diameter) were removed from dried FTA filters using a Harris micro-punch, washed and used to programme PCR reactions. Washing conditions

Table 1 Strains used in the phylogenetic analysis

Taxon	Strain/sequence	Other strain designation & origin	EMBL No.	Sequence Ref.	Strain Ref
Arthroderma benhamiae	CBS623.66	NCPF 410	Z98015		[21]
" *cajetani*	NCPF 499	Sai Ex Type	AM182489, AM182490	This paper	
" *ciferrii*	CBS 272.66	UAMH 2534, Ex Type	AB 40681, CGE7844	[22]	[23]
" *cuniculi*	NCPF 5073	CKC R1d, rabbit hole soil	AM182491, AM182492	This paper	
" "	NCPF 5085	CKC 18b rabbit hole soil	AM182493, AM182494	This paper	
" "	CBS 492.71	Rabbit hair and soil, UK	ACU000609		
" "	IMI 96244	ATCC 28442	AJ390382	[24]	[17]
" *curreyi*	CBS 138.26	ATCC 13550	AY 176726	[25]	
" "	CBS 130.70	Soil, Netherlands	ACU000610	[26]	
" *gertleri*	CBS 598.68	Ex Type *T. vanbreuseghemii*	AG ITS 5986	[27]	[28]
" *grubyii* as *Nannizia*	NCPF 487	Ex Type, ATCC 14419	AM182495, AM182496	This paper	
" *incurvatum*	CBS174.64	Ex Type, NCPF 236	AY176738		
" "	CBS 161.69	Human isolate ? A. gypseum	AF168129		
" *insingulare*	NCPF 469	UAMH 3441 sai ex Type	AM182750, AM182751	This paper	[5]
" "	NCPF 470	UAMH 3442 sai ex Type	AM182752, AM182753	This paper	[5]
" *lenticularum*	NCPF 467	UAMH 2931 Authentic for type	AM182754, AM182755	This paper	[4]
" "	NCPF 468	UAMH 2932 Authentic for type	AM182910, AM182911	This paper	[4]
" *melis*	NCPF 5079	Ex Type, Badger hole, Czech.	AM182912, AM182913	This paper	[18]
" *multifidum*	IMI 125889	Mouse hole, Czechoslovakia	AM182914, AM182915	This paper	
" "	NCPF 5078	IMI 125890 Bat droppings, Czechoslovakia	AM182916, AM182917	This paper	
" "	NCPF 5083	CKC17a Rabbit hole soil, UK	AM182918, AM182919	This paper	
" "	NCPF 5086	CKC20a Rabbit hole soil, UK	AM182920, AM182921	This paper	
" "	NCPF 5074	CKC10c Mole hole soil, UK	AM182922, AM182923	This paper	
" "	NCPF 5075	CKC10d Mole hole soil, UK	AM182924, AM182925	This paper	
" "	NCPF 5076	IMI 94205 sai ex Type	AM182926	This paper	[17]
" "	NCPF 5077	IMI 94206 sai ex Type	AM182927	This paper	[17]
" *olidum*	NCPF 5069	IMI 261046 Badger hair, UK	AM182928, AM182929	This paper	
" "	NCPF 5070	IMI 261047 Badger hair, UK	AM182930, AM182931	This paper	
" "	NCPF 5071	IMI 261048 Badger hair, UK	AM183276, AM183277	This paper	
" "	NCPF 5072	IMI 261049 Badger hair, UK	AM183278, AM183279	This paper	
" "	NCPF 5081	CKC16a Badger hole soil, UK	AM183280, AM183281	This paper	
" "	NCPF 5082	CKC16b Badger hole soil, UK	AM183282, AM183283	This paper	
" "	NCPF 5084	CKC17b Rabbit hole soil, UK	AM183284, AM183285	This paper	
" "	NCPF 5087	CKC 20b Rabbit hole soil, UK	AM183286, AM183287	This paper	

Table 1 (*Continued*)

Taxon	Strain/sequence	Other strain designation & origin	EMBL No.	Sequence Ref.	Strain Ref
" "	NCPF 5088	CKC 21a Badger hole soil, UK	AM183290, AM183291	This paper	
" "	NCPF 5089	CKC 7.b Badger hole soil, UK	AM183288, AM183289	This paper	
" *otae*	CBS 496.86	Ex Type of *A. otae*	AJ000611	[25]	[29]
" *quadrifidum*	NCPF 362	IMI 84307 sai ex Type	AM183292, AM183293	This paper	[3]
" "	NCPF 363	IMI 84308 sai ex Type	AM183294, AM183295	This paper	[3]
" "	ATCC 22954	UAMH 3183, authentic for Type	AY 176728	[24]	
" "	CBS 464.62	Human infection, Netherlands	AQ ITS 4646	[26]	
" *silverae*	UAMH 6517 (quoted as 6715 by Untereiner *et al*.)		AY176729	[24]	[30]
" *simii*	NCPF 494	sai ex Type	AM183265	This paper	[31]
" *uncinatum*	NCPF 364	sai Ex Type	AM183296, AM183297	This paper	[3]
" "	NCPF 216	Ex Type of *T. ajelloi,* garden soil	AM183263, AM183264	This paper	[32]
" "	CBS 615.65		AB075329	[33]	
" "	CBS 315.65	Soil, USA	AUN 000607	[26]	
" "	CBS 316.65	Soil, USA	AUN 000608	[26]	
" *vanbreuseghemii*	NCPF 452	sai Ex Type, RV 27960	AM183266, AM183267	This paper	[34]
Chrysosporium vespertilium	RV 27093	IMI 357403, Ex Type	AJ007846.1	[24]	[35]
Epidermophyton floccosum	NCPF 634	Human infection UK	AM183268, AM183269	This paper	
Microsporum canis	NCPF 5066	Human infection UK	AM183275	This paper	
Tichophyton eboreum	DSMZ 16978	Ex Type	AJ876907	[1]	[1]
Trichophyton erinacei	NCPF 652	Human infection UK	AM183270	This paper	
" *terrestre*	NCPF 602	Ex Type, Australia	AM183271, AM183272	This paper	[2]
" *interdigitale*	NCPF 80	Human infection UK	AM183272, AM183274	This paper	

were 2 washes for 1 min each with 100 µl of Whatman FTA wash reagent, followed by 2 washes for 1 min each with 100 µl of TE buffer (10 mM Tris-HCL pH 7.5, 0.1 mM EDTA). Washed filters were then dried for 5 min at 55°C on a dry heat block, and PCR reaction mixes were added directly to the washed and dried FTA filter punches.

The Internal Transcribed Spacer 1 (ITS1) region of the nuclear rDNA gene repeat was PCR amplified using primers ITS5 and ITS2 [8]. Amplification of a region of the large subunit gene (LSU) was performed using the primers described in [9]. For PCR, reactions (100 µl) were performed in the presence of 200 µM of each dNTP, 250 nM of the appropriate primers, 2U

Table 2 Results of crossing single ascospore strains. NCPF numbers of the single ascospore isolates employed in test crosses are given along the x and y axes. +++, abundant development of ascomata; 0, no development of ascomata

	5102	5103	5104	5105	5106	5107	5108	5109
5102		+++	+++	+++	+++	0	+++	+++
5103			0	0	0	+++	0	0
5104				0	0	+++	0	0
5105					0	+++	0	0
5106						+++	0	0
5107							+++	+++
5108								0

of HotStar Taq polymerase (Qiagen, Valencia, CA, USA) and a single filter punch. Following enzyme activation at 94°C for 15 min, reactions were subjected to 40 thermal cycles with the following parameters: 94°C (15 s), 55°C (15 s), 72°C (90 s) on a GeneAmp PCR systems 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). Amplification success was evaluated by electrophoresis of a fraction of total amplification products in 1.2% w/v agarose gels run for 45 min at 120 V in Tris-borate buffer. For sequencing of PCR products, the remainder of the PCR reactions were adjusted to final concentrations of 10% w/v PEG 8000, and 10 mM $MgCl_2$, and centrifuged for 10 min at 12 000 rpm in a bench-top centrifuge. The resulting DNA pellets were washed in 75% ethanol, air-dried, re-suspended in sterile water and subjected to automatic sequencing using the commercial service available at the Advanced Biotechnology Center (Imperial College, London, UK). Sequences from the various organisms were submitted to BLAST searches against fungal sequences in existing synchronized EMBL and Genbank DNA databases [10]. Sequence alignments were performed with Clustal W version 1.82 [11], and the final alignments were hand-edited. Phylogenetic inferences were made from Distance trees constructed using the Kimura 2 parameter measure and Neighbour Joining obtained through the PHYLIP package version 3.5 [12]. The ITS tree was rooted with *A. curreyi*, which was the isolate least related to *A. olidum*. For the LSU tree rooting was performed with *A. silverae,* since the *A.curreyi* sequence was too closely related to *A. olidum* in this region. Unfortunately, no comparable ITS1 sequence exists in the databases for *A. silverae*. The final consensus trees generated from each data set were drawn using TreeView [13], and bootstrap values greater than 50% are indicated. The trees were not scaled (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Results

Mycological examination of Arthroderma olidum

Experiments to assess mating types using tester strains of *Arthroderma simii* were unsuccessful due to complete lack of stimulation in any of the combinations of strains. However, experimental crossing of single-ascospore isolates from ascomata of soil sample 21A produced large numbers of ascomata in some crosses and none in others (Fig. 1A–C; Table 2). Ascomata were shown to contain viable ascospores (Fig. 1D) by serial dilution and plating of pure ascospore suspensions (data not shown). In conclusion the fungus was shown to be heterothallic, with two mating types. In the absence of stimulation by either the plus or minus tester strains of *A. simii*, isolates NCPF 5102 and NCPF 5107 were designated 'x' mating type and NCPF 5103, 5104, 5105, 5106, 5108 and 5109 were designated as 'y' (see Table 2). Colonies of the conidial state on Sabouraud Dextrose Agar (Oxoid) incubated at 30°C grew to 40 mm diameter in two weeks. They were flat, finely powdery, dull whitish to pale cream in colour, with an indistinct feathery, almost colourless edge. The reverse showed no colouration. A strong smell was emitted, reminiscent of smoked fish. Microconidia were abundant, unicellular, peg-shaped and borne in a sessile manner along the sides of undifferentiated hyphae (Fig. 2A). Macroconidia were also numerous, of varying lengths and ranged from two- to nine-celled (Fig. 2A). The dimensions of conidia present were similar to those seen in soil hair mixtures as described below. Sclerotial masses, consisting of thickened hyphae with curved tips tightly inter-woven, became abundant as the culture aged (Fig. 2B and C). All isolates failed to grow at 37°C. These morphological features are very similar to those of the recently described species *Trichophyton eboreum* [1].

Molecular characterization of A. olidum

It has previously been shown with a variety of fungi that the highly conserved LSU rRNA-coding sequence in combination with the more variable non-coding ITS1 sequence allow the identification of many conidial fungi to species level, and are good indicators of phylogenetic relatedness [see for example 14,15]. Thus PCR products corresponding to both regions of the *A. olidum* isolates were sequenced automatically and the sequences generated were compared to known DNA sequences in the available databases, and to a variety of strains housed in the NCPF (see Table 1).

Blast searches [10] using LSU sequences from the various *A. olidum* strains failed to reveal any very closely-related sequences in the existing databases. However, sequences corresponding to the ITS1 regions of our *A. olidum* isolates were indistinguishable from that of a recently described dermatophyte relative, *Trichophyton eboreum* [1]. To further examine genetic relationships between these organisms, the LSU and ITS1 sequences of the organisms listed in Table 1 were independently aligned. The results of Neighbour Joining phylogenetic analysis of the ITS1 and LSU data sets are represented as rooted, un-scaled consensus trees in Fig. 3. Analysis of the LSU regions placed the 10 *A. olidum* isolates in a single group distinct from members of the *T. terrestre* complex (Fig. 3A), with

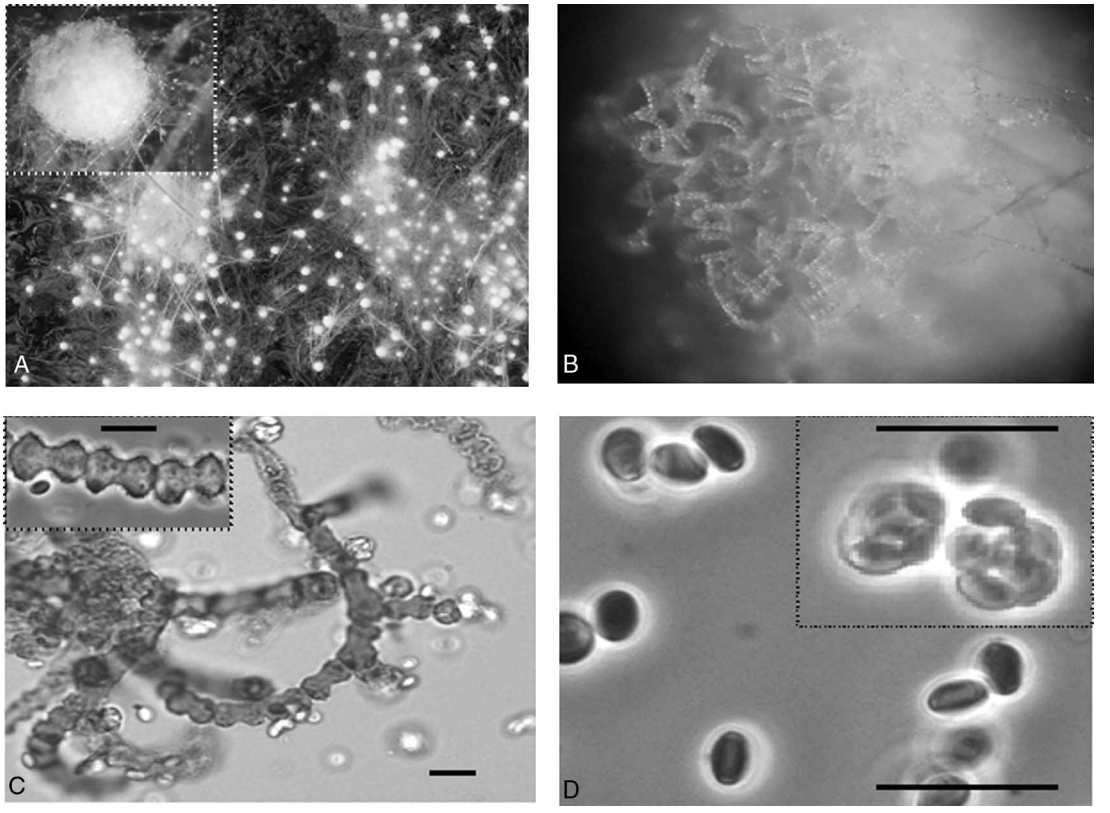

Fig. 1 Ascomatal features of *Arthroderma olidum*. Panel A: Low magnification photograph of the results of mating cross between single-ascospore isolates (NCPF 5102 and 5104) from ascomata of soil sample 21A (NCPF 5088). Panel B: Peridial hyphae of an individual ascoma under incident light. Panel C: The same at higher magnification under transmitted light. Panel D: Single ascospores and asci (inset). Scale bars in Panels C and D represent 10 μm.

A. multifidum as nearest relative. This same grouping was also recovered from analysis of the ITS1 sequences, but this time included the recently described *T. eboreum* sequence. As was described for *T. eboreum* [1], *Chrysosporium vespertilii* (93% similarity) was identified as nearest neighbour (Fig. 3B). This species was also grouped together with *Trichophyton* anamorphs of *Arthroderma* by Vidal *et al*. [16]. Moreover, the various *A. olidum* isolates shared over 99% sequence similarity in the ITS1 and LSU regions, with good concordance of groupings between the two trees. In summary, the combination of our morphological and genetic data strongly suggests that *Arthroderma olidum* is the perfect state of the recently described *Trichophyton eboreum*.

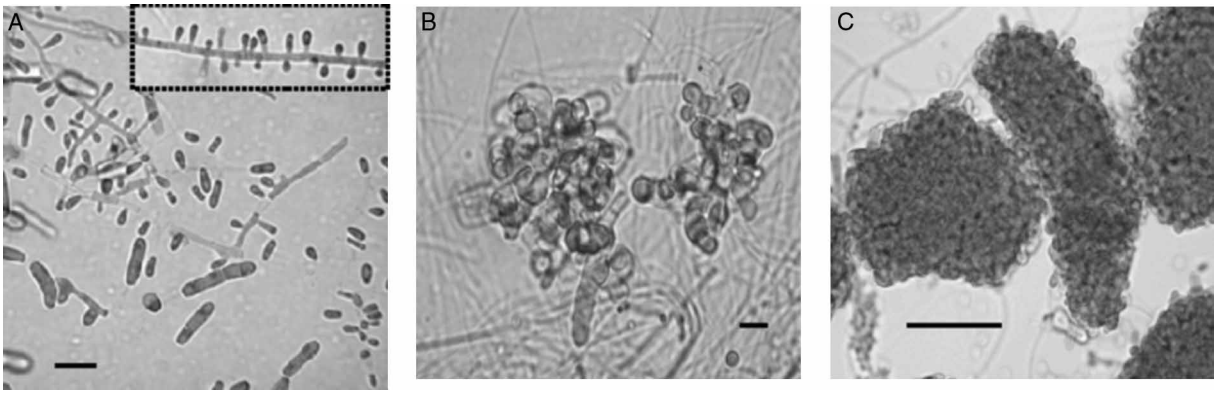

Fig. 2 Microscopic features of the conidial state of *Arthroderma olidum*. Panel A: Range of micro- and macroconidia produced. Panels B and C: Young and older sclerotial masses produced upon prolonged incubation. Scale bars represent 10 μm (Panels A and B) and 100 μm (Panel C).

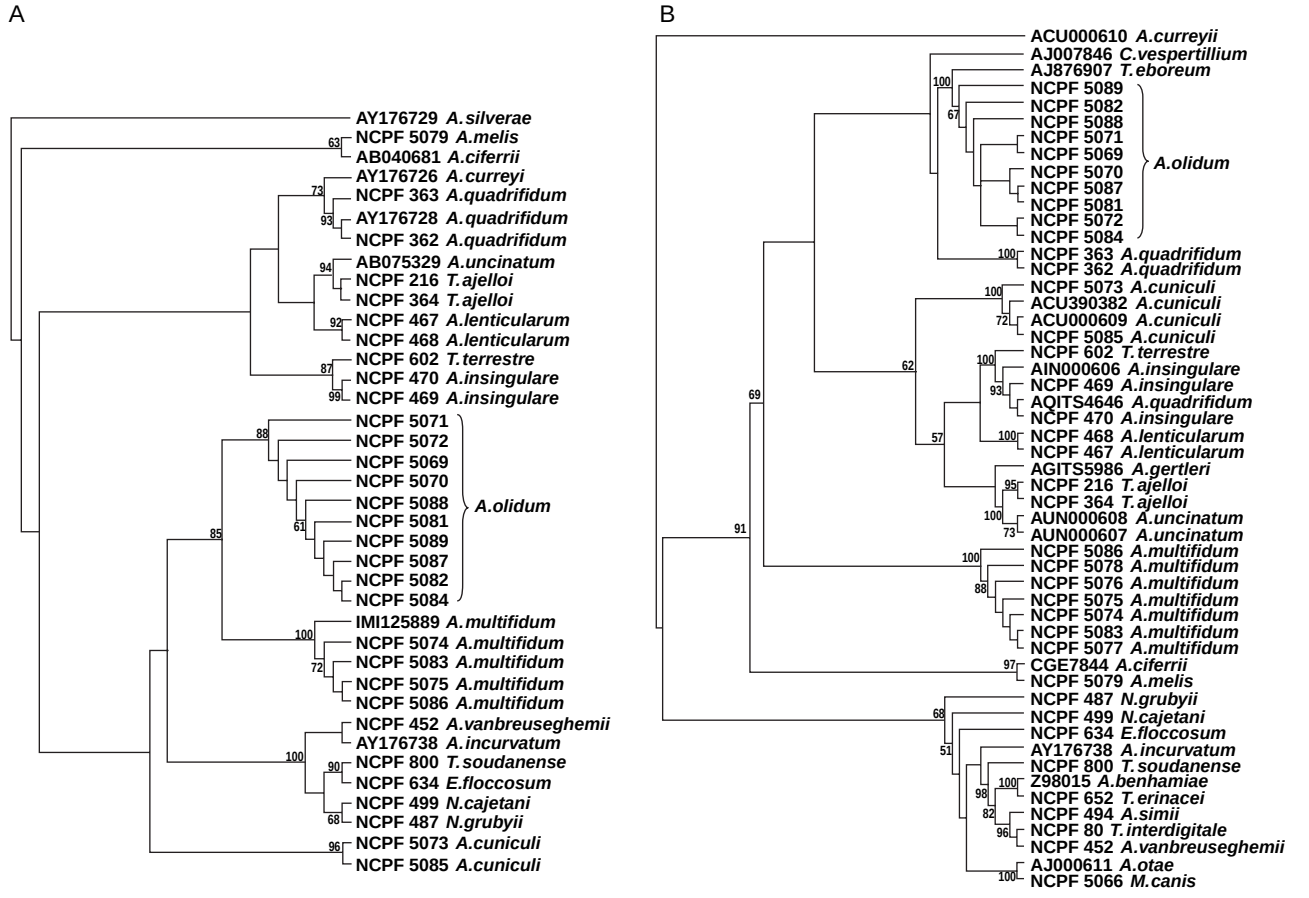

Fig. 3 Phylogenetic analysis of the LSU (Panel A) and ITS1 (Panel B) datasets. Rooted Neighbour Joining consensus tress are drawn. Bootstrap values above 50% are indicated. The EMBL accession and NCPF numbers are listed in Table 1. Trees were rooted using the sequences of *Arthroderma silverae* and *Arthroderma curreyii* for the LSU and ITS1 datasets, respectively. Note that phylogenetic analyses indicate that EMBL AQITS4646 is almost certainly *A. insingulare* and not *A. quadrifidum* as originally suggested (see ITS tree; Panel B).

Arthroderma olidum *sp. nov.*

Fungus heterothallicus. Ascomata globosa, pallido-flava, 300–800 μm diam. Peridium 50–100 μm crassum. Hyphae peridiae septatae, arcuatae, interdum ramosae, cellulis constrictis concinnis vel aliquantis aconcinnis, 5–7 μm diam 7–10 μm longis latitude, in apicibus helicoideis. Asci hyalini, 6.3–7.3 μm diam, hyaline, subglobosi, tenuitunicati evaniescenti, octospori. Ascosporae 2.8–3.8 × 1.8–2.3 μm hyalinae laeves, lenticulares, in concursu lutiae.

Conidia unicellularia 2.8–5.0 × 1.3–2.3 μm, hyalina laeves, clavata, hiliis basalis planis. Conidia multicellularia 6–30 × 2–6 μm. cylindrica. 1–8 septata, laeves. Sclerotia dura, alba constans ex hyphis intertextis, apicibus incurvatibus.

Heterothallic. Ascomata globose, white to pale yellow, 300–800 μm. diameter. Peridium 50–100 μm. thick. Peridial hyphae septate, curved, occasionally branched, 5–7 μm wide, composed of symmetrically to slightly asymmetrically constricted, roughened, dumb-bell shaped cells 7–10 μm long. Some peridial hyphae terminate in smooth-walled coiled appendages. Asci subglobose, thin walled evanescent, 6.3–7.3 μm, 8 spored. Ascospores hyaline, smooth, oblate, 2.8–3.8 μm diam, 1.8–2.3 μm thick. Yellow in mass.

Unicellular conidia hyaline, smooth, clavate, 2.8–5 × 1.3–2.3 μm with a flat basal scar, 0.8–1.0 μm. Multicellular conidia 6–30 × 2–6 μm cylindrical with 1–8 septa, thin walled, smooth. Sclerotia, hard, white, 100–200 μm, formed of closely inter-twined hyphae of widths up to 10–20 μm with incurled tips.

Type material consisting of formalin-fixed ascomata on hair/soil (derived by crossing single-ascospore isolates NCPF 5102 and NCPF 5104) is deposited as NCPF 5111 at the HPA Mycology Reference Laboratory, Myrtle Road. Bristol, UK. In addition, ascomata

from crosses of other single-ascospore isolates were killed and kept.

Living isolate NCPF 5088 is designated as the ex-type strain as it is derived from soil/hair mixture CKC21A, which was used to generate the single-ascospore parents of the type material. Also kept were three other isolates from soil of badger holes, two from soil of rabbit holes (Table 1) and eight single ascospore isolates NCPF 5102–5109, (two of 'x' mating type and six of 'y' mating type) derived from an ascocarp of the type material before fixation.

Discussion

The present concept of *T. terrestre* is as a group of closely related species with indistinguishable conidial forms. Although it is able to grow in the presence of the cycloheximide, used in primary selective media for diagnosis of dermatophytosis, members of the *T. terrestre* complex can readily be distinguished microscopically from the pathogenic *Trichophyton* species. As such, *T. terrestre* is frequently encountered as a contaminating mould in dermatological and veterinary skin specimens showing no microscopic evidence of fungal infection. Little is known of its distribution in soil, and nothing of the relative origins of the various sexual species in the group. The strong keratinolytic abilities of the geophilic *Trichophyton* species suggest they may be specialized in the decomposition of animal hair and dead animals.

Arthroderma cuniculi and *A. multifidum* were both closely associated with rabbits in Scotland [17], and some strains of *A. lenticularum* were from soil of 'gopher' holes in California. The possibility thus arises that *A. olidum* might be associated with the European badger, since early isolates were from badger hair and 4 of 6 badger holes sampled in the present work yielded this species. However, two isolates were also obtained from rabbit holes with evidence of occupation (rabbit droppings at the entrance), and it may be that *A. olidum* is more associated with the limestone-derived soil than with an animal species. There is scope for further fieldwork to clarify this. *Arthroderma melis*, consisting of a single collection from badger hole soil in the Czech Republic [18] is clearly different from *A. olidum*. *A. melis* is partly characterized by production of a red pigment and our work shows it to be genetically distinct.

A. olidum has been shown to be distinguishable from other members of the *T. terrestre* group by the production of hard sclerotia in the mycelium. In addition the mycelium smells strongly of smoked fish, a feature remarked upon by Stockdale, who was so struck by it that she 'had to rush home and buy kippers for tea' (Stockdale, personal communication). Genetic analyses of two separate regions of the nuclear ribosomal repeat also support the distinction between *A. olidum* and the rest of the *T. terrestre* group. However, the range of conidia produced by *A. olidum* does not formally distinguish it, nor does the detailed morphology of the sexual phase, which is very similar in most *Trichophyton* teleomorphs. Several authors have previously commented that *Arthroderma* species are more readily distinguished by their conidia rather than by their ascomatal characters [19,20].

The advent of modern molecular techniques has revolutionized the study of such closely related groups of species. The present work shows clearly that the geophilic *Arthroderma* species form a phylogenetically close grouping, and that *A. olidum* is distinct from the previously described species of this group. One possible explanation for species divergence in the group could be their relative isolation due to their association with different burrowing animal species.

Finally, the recently described *Trichophyton eboreum* Brasch & Gräser is genetically indistinguishable from *A. olidum*, at least over ITS1 sequence reported for *T. eboreum* [1]. The description of colonial morphology of *T. eboreum* is also remarkably similar to *A. olidum*. Brasch & Gräser describe and illustrate the sclerotial bodies we found in our isolates, but interpret them as 'cleistothecium-like structures'. They also noted the strong smell of the fungus, which they described as perceptible even from closed Petri dishes. In conclusion, we believe that *A. olidum* and *T. eboreum* have been shown to be conspecific. *Trichophyton eboreum* therefore appears to be the correct name for the anamorphic state of *Arthroderma olidum*.

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