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Phialosimplex, a new anamorphic genus associated with infections in dogs and having phylogenetic affinity to the Trichocomaceae

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Anamorphic members of the ascomycete family Trichocomaceae including *Aspergillus*, *Penicillium*, *Paecilomyces*, *Geosmithia* and *Sagenomella* have been reported from infections in canines. Six clinical isolates (five associated with infections in canines and one from a human source) demonstrated simple phialides producing conidia in long chains and were investigated for their potential relationship to *Sagenomella chlamydospora*, a known agent of canine disseminated mycosis. Phylogenetic analyses of internal transcribed spacer (ITS) and small subunit (SSU) region sequences revealed that all of the canine-associated isolates were distinct from *Sagenomella* species. The new anamorphic genus and species *Phialosimplex caninus* is described to accommodate the clinical isolates. *Sagenomella chlamydospora* and *Sagenomella sclerotialis* are transferred to the new genus as *Phialosimplex chlamydosporus* comb. nov. and *Phialosimplex sclerotialis* comb. nov.

Keywords canine fungal infection, *Monocillium indicum, Phialosimplex caninus, Sagenomella chlamydospora,* Trichocomaceaer

Introduction

Aspergillus, Paecilomyces and *Penicillium* species are among the most frequently reported etiologic agents of opportunistic disseminated mycoses in dogs [1–4]. These fungi are anamorphic members of the ascomycete family Trichocomaceae (Eurotiales). *Geosmithia argillacea* (also known as *Penicillium argillaceum*), another member of the family, was reported recently as the cause of systemic mycosis in a German shepherd dog [5].

In 2003, Gené *et al.* described the new species *Sagenomella chlamydospora* for a fungus that was reported initially as a *Paecilomyces* species and that caused fatal disseminated disease with cervical involvement in a German shepherd dog [6,7]. The anamorphic genus *Sagenomella* was described by Gams in 1978 for *Acremonium*-like fungi producing conidia in connected chains from simple phialides [8]. A relationship between *Sagenomella* and the Trichocomaceae was suggested by the occurrence of *Sagenomella* anamorphs for *Sagenoma viride* [8] and for *Talaromyces ocotl* [9] and later confirmed by phylogenetic analysis of nuclear small subunit (SSU) rRNA gene sequences [10,11]. The SSU data also revealed that *Sagenomella* was not monophyletic, that the species were placed into three groups, and that one species (*S. oligospora*) was excluded from the Trichocomaceae [10,11]. Up to now, no further phylogenetic studies have been done on the genus.

Among *Sagenomella* species, only *S. chlamydospora* has been associated with infection and only the single report from a dog is known [6,7]. Recently, we investigated the identity of six isolates (including five from dogs and one from a human source) that resembled *Sagenomella* species based on morphological analysis. DNA sequences of the rRNA gene internal transcribed spacer (ITS) region

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obtained from these isolates showed a 91–94% sequence similarity to *S. chlamydospora* (AJ519984 based on the ex-type culture) following a GenBank BLAST search. This lack of sequence similarity prompted a further evaluation of the phylogenetic relationship of our isolates with *S. chlamydospora* and other *Sagenomella* species.

Evidence based on phenotypic and molecular analyses of both the ITS and (SSU) rDNA sequences confirm that our six isolates are distinct. We therefore place them within a new anamorphic genus and species in the family Trichocomaceae for which the name *Phialosimplex caninus* gen. et sp. nov. is proposed. The results also show that *S. chlamydospora* and *S. sclerotialis* belong to this genus and thus the new combinations of *Phialosimplex chlamydosporus* (Gené & Guarro) Sigler & Gibas comb. nov. and *P. sclerotialis* (W. Gams & Breton) Sigler & Gibas comb. nov. are proposed. A second report will provide more details about the infections associated with this newly described pathogen.

Materials and methods

Isolates and morphology

Six isolates were sent for characterization to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center (UTHSC) at San Antonio, TX. Due to their unusual phenotypic characteristics, the isolates were subsequently forwarded to the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, AB, for additional studies. Table 1 provides details on the provenance and phenotypic features for the isolates. Colonial features and growth rates were examined on potato dextrose agar plates (PDA; BD Diagnostic Systems, Sparks, MD) incubated at 30ºC and 35ºC for 21 days. For assessing tolerance to cycloheximide, isolates were grown on mycosel agar containing 400 μg^{-1} cycloheximide (BD) and phytone yeast extract agar (PYE; BD) lacking cycloheximide. Media were dispensed into one half of a two-section Petri plate and incubated at 30°C. Color terms and codes are derived from the color standards of Kornerup and Wanscher [12]. Microscopic observations were made from slide culture preparations using cereal agar prepared in-house as the sporulation medium and incubation for 14 or 21 days at 30°C [13].

Sequencing and analysis

Sequences were obtained at the Fungal Molecular Laboratory at the University of Nebraska Medical Center, Omaha, NE, and at the UAMH. Sequences of the ITS region were obtained for the six clinical isolates listed in Table 1 and for available isolates of *Sagenomella* species and *Monocillium indicum* as listed in Table 2, using the methods as described by Henry *et al*. [14] and the sequencing primers ITS1 and ITS4 as described by White *et al*. [15]. Almost complete SSU rDNA sequences were obtained for two clinical isolates (UAMH 10335 and 10337) and for *S. chlamydospora* (UAMH 10961) following procedures for DNA extraction, amplification and sequencing described previously with minor modifications [13]. DNA was extracted using the E.Z.N.A. SP Fungal DNA kit (United Bioinformatica Inc., Saskatoon, SK). The partial SSU region was amplified using primer pair NS1 and NS8 [15]. Sequencing was done with forward primers NS11mun (5'-GCAAATTAC CCAATCCCGAC-3'), NS13mun (5'- TGGTTTCTA GGACCGCCGT-3'), NS151mun (5'-GAAACTCACCA GGTCCAGACA-3') (developed by K.N. Egger, University of Northern British Columbia, Prince George, BC) and reverse primers NS2, NS4 and NS6 [15] using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and run on an ABI 377 Prism Automated Sequencer (Applied Biosystems). The ITS and SSU sequences were edited using Sequencher ver. 4.8 (Gene Codes Corp., Ann Arbor, MI) and aligned manually with sequences retrieved from GenBank using the sequence alignment program Se-Al v2.0a11 [16]. The SSU dataset included sequences available for *Sagenomella* species and for members of the Trichocomaceae obtained by Endo *et al*. and Thanh *et al*. [10,11; Group 1 introns were excluded prior to analysis. Parsimony analyses were performed with PAUP* v.4.0b10 [17] and the robustness of the resultant trees determined using the full heuristic search option (ITS data) and the fast stepwise-addition bootstrap search option for 1000 resamplings (SSU data) [18]. GenBank accession numbers for isolates newly sequenced are listed in Tables 1 and 2.

Results

Parsimony analysis of the ITS and SSU datasets comprising 45 and 43 taxa of the Trichocomaceae, respectively, yielded trees having similar topologies and in which three distinct clades were well supported. Clade A included our clinical isolates, in addition to *S. chlamydospora* and *S. sclerotialis* (SSU only), whereas Clade B included all other *Sagenomella* species, including the type species, S. *diversispora* (Figs. 1 and 2); however the species of *Sagenomella* included within Clade B differed slightly according to the available cultures or sequences. The ITS dataset comprised 627 aligned characters, of which 256 were constant, 305 were parsimony-informative and 66 were parsimony-uninformative; the tree shown was one of 6 equally parsimonious trees (Fig. 1). Within Clade A, the clinical isolates grouped with 100% support, as pertaining to the

Table 1 Provenance and morphological features of *Phialosimplex* caninus isolates examined

Accession no.[a]	Provenance		Colony characteristics[b]		Conidial sizes[c]		GenBank no. ITS (SSU)
	Host (gender, age)	Specimen(s)	Colony diameter (in cm) on PDAb at 30°C (35°C)	Diffusible yellow pigment on PDA	Conidia in chains (µm)	Conidia in heads (µm)	
UAMH 9569 (UTHSC 99–402)	Canine (M, 14y)	Lymph node, spleen and liver	2.8 (3.2)	+	2.2–3.5 × 1.8–2.7	2.2–3.5 × 1.5(2.5	GQ169311
UAMH 10335 (UTHSC R-3305)	Human	Pleural fluid and tissue	6.5 (7)	+	2.5–4 × 2.7–3.7	3.2–4 × 2–3	GQ169313 (GQ169312)
UAMH 10336 (UTHSC 03-926)	Canine	Lymph node	2.5 (2.2)	+	2.5–3.5 × 2.3–3	2.5–4.2 × 2–3.2	GQ169314
UAMH 10337[T] (UTHSC 03-1073)	Canine	Bone marrow aspirate	7 (6.2)	+	2.5–3.7 × 2.2–3	2.3–4.5 × 1.5–2.5	GQ169315 (GQ169310)
UAMH 10738 (UTHSC 06-1722)	Canine (F, 4y)	Lymph node	5.5 (6.8)	+	2.2–3.5 × 2.2–2.8	2–3 × 1.8–2.5	GQ169316
UAMH 10936 (UTHSC 08-801)	Canine	Vertebral disc	6.2 (5.6)	–	2.2–2.9 × 1.8–2.3	2.2–3.5 × 1.5–2	GQ169317

[a]Abbreviations: T, Type culture, UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, AB; UTHSC, University of Texas Health Science Center, San Antonio, TX.
[b]Determined by recording growth on potato dextrose agar (PDA) after incubation for 21 days.
[c]Recorded as length by width (range).

ITS sequences. Sequence similarity was 99.2% among five isolates but UAMH 10336 was more divergent, having a 97% sequence similarity to the others. This isolate, together with UAMH 9569, demonstrated a slower growth rate, but was not otherwise morphologically distinct (Table 1). The SSU dataset comprised 1739 characters of which 1417 were constant, 179 were parsimony-informative and 143 parsimony-uninformative; the tree shown is one of 1988 equally parsimonious trees (Fig. 2). The SSU sequences for isolates UAMH 10335 and 10337 were identical and only one is shown in Clade A. Also included in this clade were the ex-type culture and several other representatives of *S. sclerotialis*; no ITS sequences were available for this species. In both ITS and SSU analyses, Clade B, comprising *Sagenomella sensu stricto*, is well supported and includes subgroups representing the species *S. diversispora, S. humicola, S. griseoviridis, S. striatispora* and *S. verticillata*. The two teleomorphic species having *Sagenomella* anamorphs grouped in different clades. *Talaromyces ocotl* (anamorph *Sagenomella* sp.) was placed in Clade B. However, *Sagenoma viride* (anamorph *Sagenomella sagenomatis*), was grouped with *Talaromyces flavus* in both analyses and also with *Chromocleista cinnabarina* (shown as Clade C, Figs. 1 and 2). Among unclassified *Sagenomella* isolates (Table 2), UAMH 1655 and 4873 showed 99% ITS similarity with *S. griseoviridis*. UAMH 9571 was close to *T. ocotl* (98% ITS similarity) but failed to produce a teleomorph.

The presence of introns was detected in five SSU sequences and these were removed prior to phylogenetic analysis. The sequences for the two clinical isolates, UAMH 10335 and 10337 (2084 nucleotides [nt]) (Table 1), and *S. griseoviridis* (GenBank AB024591) had a group 1 intron of 357 nt corresponding to subunit position 1165 in a SSU sequence of *Saccharomyces cerevisiae* (GenBank J01353) [19] and to position 943 in a sequence of *Escherichia coli* (GenBank J01695) [20]. Two *S. diversispora* sequences (AB024588 and AB024589) had a group 1 intron of 345 nt at position 564 in *Saccharomyces cerevisiae* and 516 in *E. coli*. Introns were lacking in the sequences for *S. chlamydospora* (UAMH 10961 ex-type culture) and for *S. sclerotialis* AB024592 (CBS 366.77 ex-type culture). More data are required to determine whether the presence of introns has any taxonomic significance within these groups.

Based on molecular analysis, we describe the new genus and species, *Phialosimplex caninus* gen. et sp. nov., for the clinical isolates and propose the transfer of the species *S. chlamydospora* and *S. sclerotialis* to *Phialosimplex* (see Taxonomy). The morphological characteristics differentiating the three accepted species are summarized in Table 3.

Table 2 Isolates of *Monocillium* and other *Sagenomella* species examined and sequenced

UAMH No.	Species name (Type status)	Source	Location	Other collection numbers	GenBank no. ITS (SSU)
1499	*Monocillium indicum* (T)	Soil	India	IMI 62202	GQ169328
929	*Sagenomella diversispora* (T, *Scopulariopsis diversispora*)	Soil	Netherlands	CBS 354.36 MUCL 9029	GQ169318
1419	*Sagenomella diversispora* (T, *Paecilomyces variabilis*)	Soil	Canada	DAOM 87662 CBS 430.67	GQ169319
1655	*Sagenomella sp.*	Air	USA.		GQ169320
2873	*Sagenomella diversispora*	Soil	Canada		GQ169321
2888	*Sagenomella striatispora*	Soil	Canada	CBS 394.69 IMI 148005	GQ169327
2890	*Sagenomella humicola* (T, *Paecilomyces humicola*)	Soil	Canada	IMI 113166 ATCC 18506 CBS 427.67	GQ169323 (GQ169322)
4873	*Sagenomella sp.*	Wood	Canada		GQ169324
9571	*Sagenomella sp.*	Plant root	USA	CBS 113280	GQ169325
10961	*Sagenomella chlamydospora* (T)	Canine	Spain	CBS 109945 IMI 387422	(GQ169327)

Abbreviations: T = Ex-type culture; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; DAOM, National Mycological Herbarium, Ottawa, ON; IMI, CABI Genetic Resource Collection, Egham, UK; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, AB.

Taxonomy

Phialosimplex Sigler, D.A. Sutton, Gibas, Summerbell & Iwen gen. nov.

Mycobank MB513392

Coloniae albae, flavo-albae ad pallide aurantiaco-griseae. Incrementum nullum ex agaro cum cycloheximido. Hyphae ramosae, septatae, angustae, hyalinae. Cellulae conidiogenae phialides simplices, laterales, plerumque monophialidicae, interdum proliferentes et foramen secundum formantes (polyphialides). Phialides hyalinae, cylindricae, interdum deorsum vel prope medium modice inflatae, cum collari indistincto. Conidia vel in catenis longis vel in capitulis aggregata, hyalina, levia, subglobosa, pyriformia, obovoidea vel ovoidea cum basi truncata. Chlamydosporae et sclerotia absunt vel adsunt. Teleomorphosis ignota. Genus *Sagenomella* simile. Affinitas generis anamorphosis: Ascomycota; ordo: Eurotiales; familia: Trichocomaceae.

Species typica: *Phialosimplex caninus* Sigler, D.A. Sutton, Gibas, Summerbell & Iwen sp. nov

Colonies are pale, white, cream to yellowish white. Hyphae are narrow, branched, septate. Conidiogenous cells are simple phialides borne laterally on the vegetative hyphae or occasionally on short, unbranched conidiophores. Conidiogenous cells are simple phialides (mostly monophialidic) that sometimes proliferate to form a second opening (polyphialidic). Phialides are narrow, cylindrical to slightly swollen at the base or below the midpoint and taper to a narrow neck with indistinct collarette. Conidia are borne in long chains or aggregate in heads and are hyaline, smooth, subglobose, pyriform, obovoid or ovoid with a truncate base. Chlamydospores and sclerotia are absent or present. A teleomorph is unknown. *Phialosimplex* is an anamorphic genus morphologically resembling *Sagenomella* and placed within the order Eurotiales, family Trichocomaceae.

Phialosimplex caninus Sigler, D.A. Sutton, Gibas, Summerbell & Iwen sp. nov.

Table 1. Fig. 3 A–C and Fig. 4 A–E.

Etymology: associated with dogs

Mycobank MB513393

Coloniae fere celeriter crescentes ad 30ºC et 35ºC, velutinae vel pulveraceae vel fasciculatae, planae vel sulcatae, flavo-albae ad pallide aurantiaco-griseae. Incrementum nullum in agaro cum cycloheximido. Cellulae conidiogenae phialides simplices, laterales, plerumque monophialidicae, raro proliferentes et foramen secundum formantes (polyphialidicae). Phialides hyalinae, cylindricae, interdum deorsum vel prope medium modice inflatae, cum collari indistincto ad apicem, 4.5–16 µm longae, 2–4 µm latae ad basim, et 0.7–1.5 µm latae ad apicem. Conidia in catenis longis, hyalina, levia, subglobosa, 2.2–4 µm longa et 1.8–3.7 µm lata. Conidia in capitulis aggregata obovoidea, 2–4.5 µm longa et 1.5–3.2 µm lata. Ascomata, sclerotia, et chlamydosporae absunt.

Holotype: UAMH 10337 is preserved as a dried colony and living culture. It was isolated from bone marrow aspirate from a canine, USA.

Colonies on PDA after 21 days at 30°C (Fig. 3, left column) are velvety to powdery, sometimes fasciculate in the centre, flat to radially furrowed, occasionally sectoring, yellowish white (4A2) [12] to orange grey (6B2). Colonies are moderately fast growing, attaining diameters of 5.5 to 7 cm in 21 days, except two isolates (UAMH 9569 and UAMH 10336) that are slower growing, attaining diameters

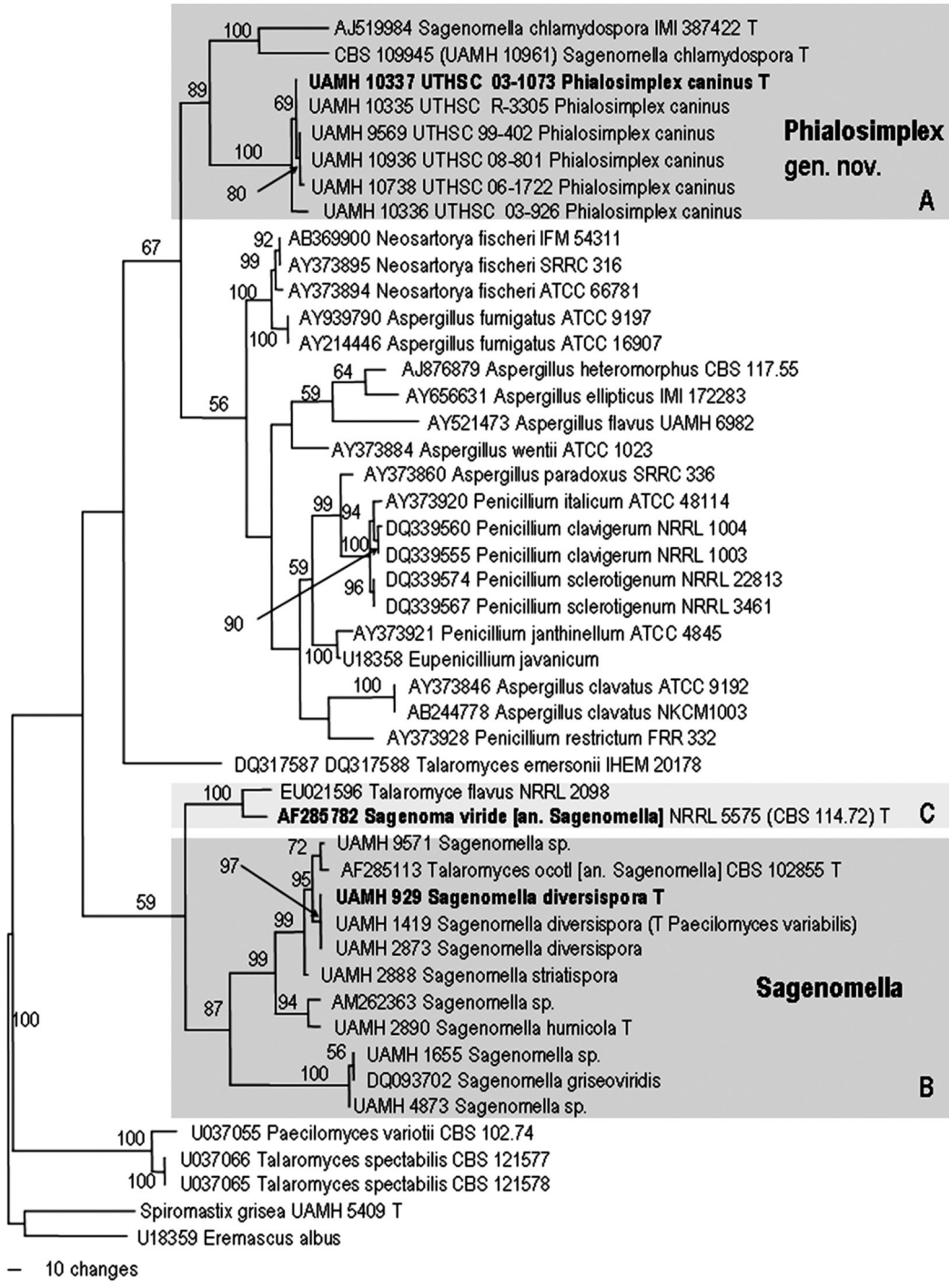

Fig. 1 One of six equally parsimonious trees (CI 0.458, RI 0.542, HI 0.771) inferred from maximum parsimony analysis of internal transcribed spacer (ITS) rDNA sequences showing the placement of *Phialosimplex* and *Sagenomella* species within the Trichocomaceae. *Spiromastix grisea* and *Eremascus albus* are outgroup taxa. Bootstrap values above 50% are shown. The GenBank accession number together with the culture collection number for each isolate is listed if available. GenBank accession numbers for newly sequenced isolates are listed in Tables 1 and 2. T v ex-type culture.

of 2.5 to 2.8 cm (Table 1). Growth rates on PDA at 35°C are nearly equivalent (Fig. 3 middle column). Most isolates (except UAMH 10936) produce a diffusible yellow pigment on PDA or PYE. Colonies on PYE after 21 days at 30°C are similar macroscopically to those on PDA, but the diffusible pigment is darker golden yellow (5B7) to brown

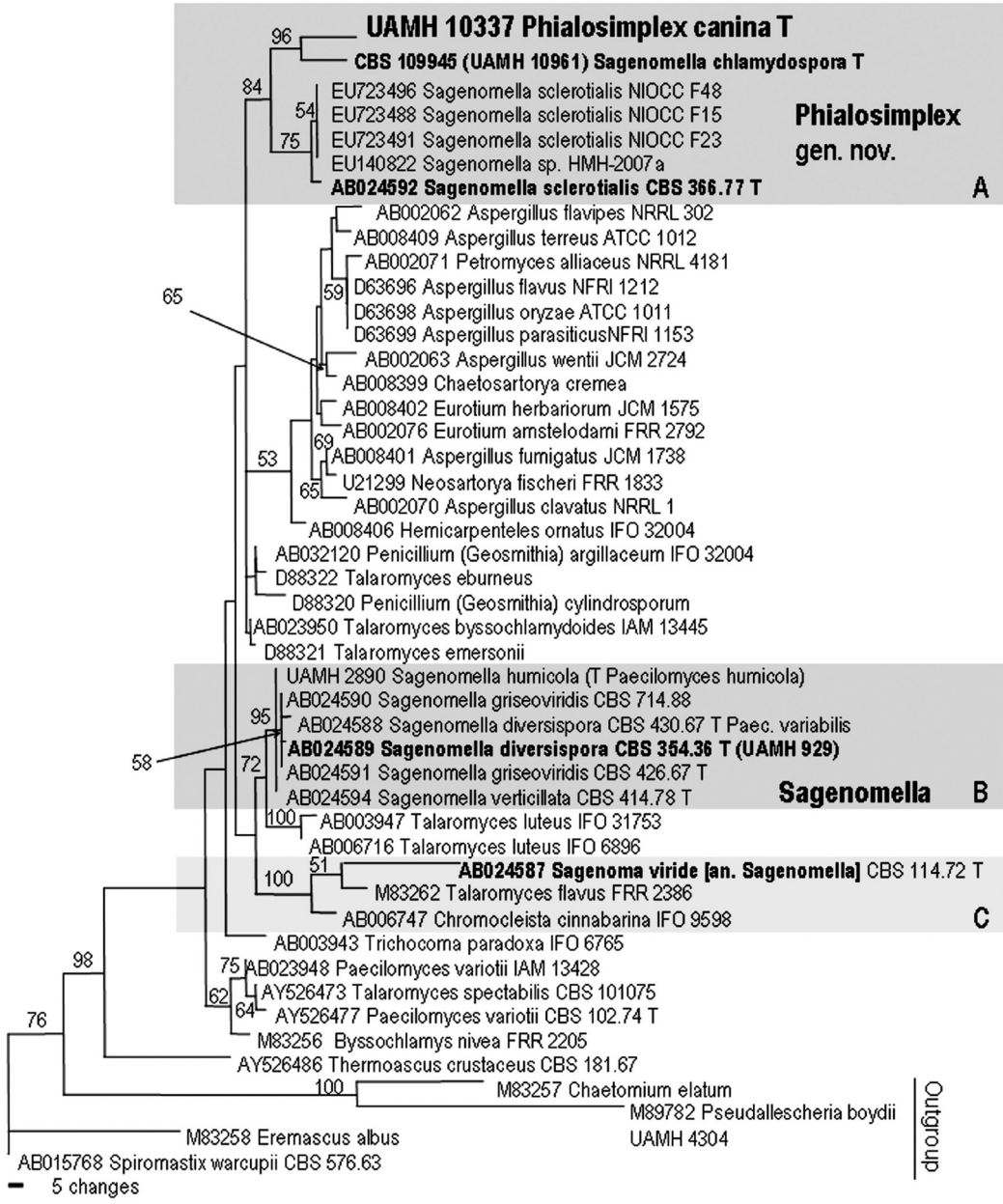

Fig. 2 One of 1988 equally parsimonious trees (CI 0.639, RI 0.731, HI 0.361) inferred from maximum parsimony analysis of small subunit (SSU) rDNA sequences showing the placement of *Phialosimplex* and *Sagenomella* species within the Trichocomaceae. Bootstrap values above 50% are shown. Outgroup taxa are *Chaetomium elatum*, *Pseudallescheria boydii*, *Eremascus albus* and *Spiromastix warcupii*. The GenBank accession number together with the culture collection number for each isolate is listed if available. GenBank accession numbers for newly sequenced isolates are listed in Tables 1 and 2. T = ex-type culture.

(6D/E8) (Fig. 3 right column). All isolates were inhibited in the presence of cycloheximide. Conidiogenous cells are simple phialides borne laterally on the vegetative hyphae or occasionally on short, unbranched conidiophores. Phialides are typically monophialidic, sometimes proliferating to form a second opening (polyphialidic), and are narrow, cylindrical to slightly broader at the base or swollen below the midpoint, tapering at the neck and bearing an indistinct collarette (Fig. 4 A–E). They measure 4.5–16 µm (average 11.9 µm) long, 2–4 µm (average

Table 3 Phenotypic characteristics of *Phialosimplex* species

Species	Conidial size[a] (µm)	Conidial shape	Conidia in heads present	Chlamydospores present	Sclerotia present	Cycloheximide tolerance[b]	Diffusible pigment present[c]	Growth at 35°C[c]
Phialosimplex caninus	2.2–4 × 1.8–3.7	Subglobose	+	–	–	–	+(yellow)	+
Phialosimplex chlamydosporus[d]	2.3–5.8 × 1.7–3	Oval to pyriform	+	+	–	–	–	+
Phialosimplex sclerotialis[e]	3–4.5 × 1.5–2	Ovoid with truncate base	ND	–	+	ND	ND	+

[a]Conidia formed in chains; recorded as length by width (range).
[b]Determined by recording growth on mycosel agar after 21 days incubation.
[c]Recorded on potato dextrose agar after 21 days incubation.
[d]Based on our observations of the ex-type culture and the original description [7].
[e]From original description of Sagenomella sclerotialis [8; ex-type culture not examined.
ND – not determined.

2.1 µm) wide at the base and 0.7–1.5 µm (average 1.1 µm) at the tip. Conidia are smooth, hyaline, single-celled and produced in long chains or in heads (Fig. 4 B,C). Conidia in chains are subglobose, 2.2–4 µm long by 1.8–3.7 µm wide. Conidia in heads are obovoid, 2–4.5 µm long by 1.5–3.2 µm wide. Ascomata, sclerotia and chlamydospores are absent.

Phialosimplex chlamydosporus (Gené & Guarro) Sigler comb. nov. Table 3, Figs. 3D and 4F–G

Mycobank MB513394

Basionym: *Sagenomella chlamydospora* Gené & Guarro, J. Clin. Microbiol. 41:1723, 2003.

P. chlamydosporus differs from *P. caninus* by developing abundant solitary chlamydospores borne laterally on short unbranched or branched stalks (Table 3). Chlamydospores are subglobose to globose or obovate, somewhat thick-walled, hyaline and measure 2.8–5.5 µm long by 2.5–4.8 µm wide (Fig. 4F). Phialides are simple, narrow, cylindrical to slightly swollen centrally, and measure 3–14 µm long, 1.5–2.3 µm wide at the base, 1–1.5 µm wide at the tip (Fig. 4G). Conidia are smooth, hyaline and single-celled and produced in chains or in heads. Conidia in chains are oval to pyriform, 2.3–5.8 µm long and 1.7–3 µm wide, while those in heads are 1.8–3.5 µm long by 1.3–2 µm wide. Colonies on PDA after 21 days at 30°C and 35°C are white, thin, glabrous with patches of felty aerial mycelium (Fig. 3D). Growth is similar on PYE. No diffusible pigment is produced.

Phialosimplex sclerotialis (W. Gams & Breton) Sigler comb. nov.

Mycobank MB513395

Basionym: *Sagenomella sclerotialis* W. Gams & Breton, Persoonia 10: 109, 1978.

Based on the original description [8], *P. sclerotialis* is distinguished from other *Phialosimplex* species by the presence of white globose sclerotia in older cultures and by the absence of chlamydospores (Table 3). *P. sclerotialis* is similar to the other species in having pale colonies, in being thermotolerant, growing faster at 33–36°C than at 24°C, and in producing conidia in chains from simple phialides measuring 5–15 µm long, 1.2–1.8 µm wide at the base and 1.0 µm wide at the tip.

Discussion

Based on combined morphological and molecular data, this report describes the new anamorphic genus *Phialosimplex* encompassing one new species and two species formerly accommodated in *Sagenomella* (Figs. 1 and 2). *Phialosimplex* and *Sagenomella* produce conidia in long chains from phialides similarly to other anamorphic genera within the Trichocomaceae, including *Aspergillus*, *Penicillium, Geosmithia*, *Paecilomyces* and *Torulomyces.* In *Phialosimplex*

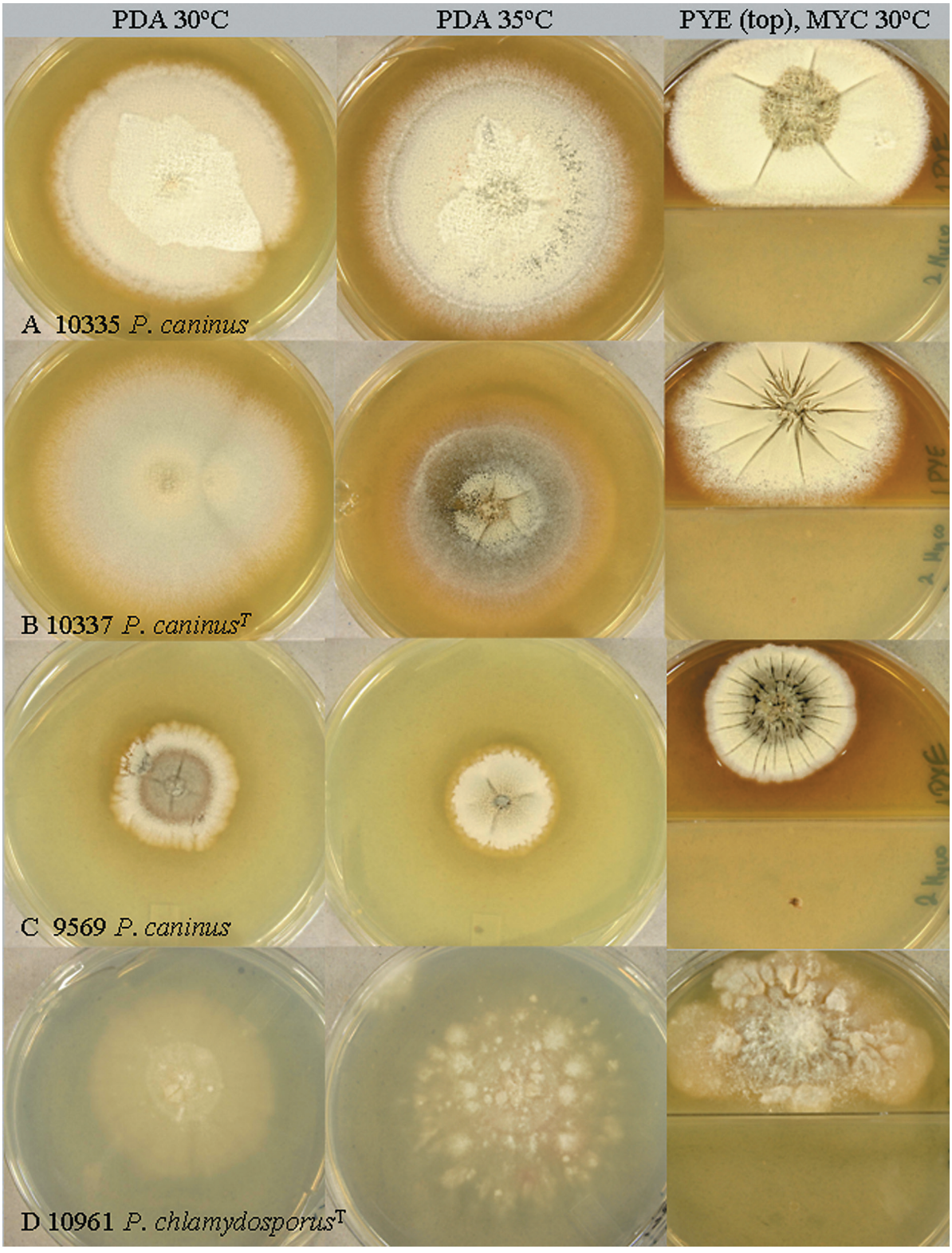

Fig. 3 Colonial morphologies of *Phialosimplex caninus* and *P. chlamydosporus* are shown after 21 days incubation on PDA at 30°C (left column), PDA at 35°C (middle column) and on PYE and Mycosel agar in biplates at 30°C (right column). Figs. A to C show faster and slower growing isolates. Yellow diffusible pigments are present on PDA for *P. caninus*, but are stronger on PYE.

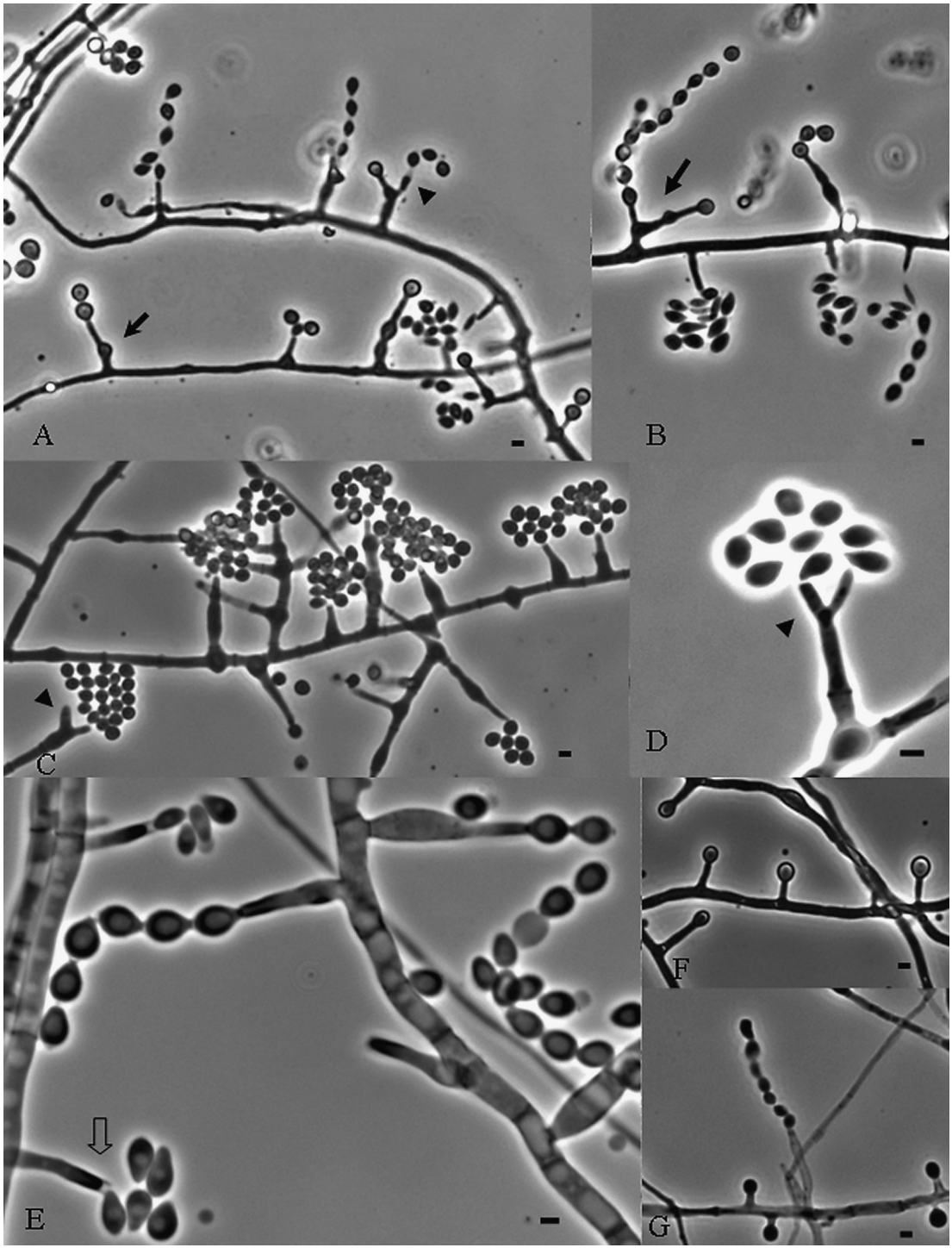

Fig. 4 Microscopic morphologies of *Phialosimplex caninus* (A-E) and *P. chlamydosporus* (F-G) are shown from slide culture preparations using phase contrast microscopy. Figures A-E show conidia in chains and in heads produced from phialides that are narrow, cylindrical to slightly broader at the base or swollen near the midpoint (arrows) and that taper to an indistinct collarette (open arrow, Part E). Phialides are typically monophialidic but sometimes polyphialidic (arrowheads). Isolates shown are UAMH 10337, 10738 and 10936. Figures F-G show chlamydospores, phialides and conidia of *P. chlamydosporus* (UAMH 10961). All scale bars = 2 μm. D and E were taken with an oil immersion objective.

and *Sagenomella*, phialides are simple and produced laterally on hyphae; are cylindrical to centrally swollen, and may proliferate to form a second opening (polyphialides). In the other genera, phialides are typically borne on complex branched conidiophores (except *Torulomyces*); are often flask-shaped, and do not proliferate. Although *Torulomyces* has been distinguished from *Penicillium* by its solitary phialides, the type species (*T. lagena*) has been reclassified in *Penicillium* with a *Eupenicillium limoneum* teleomorph [21]. Similarly, several species of *Geosmithia* are now classified within *Penicillium* because *Geosmithia sensu stricto* comprises anamorphs of Bionectriaceae.

Phialosimplex species differ from *Sagenomella* species by having lightly pigmented conidia, by producing conidia also in heads and by demonstrating good growth at 35ºC. The species accepted here, including *P. caninus, P. chlamydosporus* and *P. sclerotialis*, are differentiated by conidial shape, presence of diffusible yellow pigments on PDA and the presence of chlamydospores or sclerotia (Table 3).

In addition to *P. chlamydosporus*, a species known thus far only from disseminated mycosis in a dog [6,7], *P. caninus* is recognized here as another potential agent of canine infection. In the case of infection involving *P. chlamydosporus,* and in four of the five cases of disseminated canine disease reported here for *P. caninus*, the fungus was isolated from lymph nodes or vertebrae (Table 1). As noted by Zanatta *et al.* [4], swelling of the lymph nodes is considered an early sign of mycotic discospondylitis in dogs, and the disease usually progresses to involve numerous sites. Based on the relatively small number of cases examined thus far, it is not clear whether *P. caninus* is associated with a particular type of clinical disease.

We are aware of one published report of probable *P. caninus* infection. Mackie *et al*. described a case of granulomatous lymphadenitis and splenitis in a dog in which the fungus involved was identified tentatively as *Monocillium indicum* [22]. Although the isolate from that case was not available for study, it was described as producing conidia in fragile chains from solitary, centrally swollen phialides. An unpublished photograph available to the senior author (LS) revealed phialides strongly resembling those described here for *P. caninus.* A potential relationship between *P. caninus* and *M. indicum* was evaluated by morphological examination and ITS sequence comparison of the ex-type culture (UAMH 1499, Table 2). *M. indicum* differs from *P. caninus* by producing sclerotia and by having phialides that are conspicuously thick-walled and refractile in the lower region, swollen near the middle and tapered to a thin-walled, sinuous apex. Some phialides terminate in a swollen vesicle that collapses resembling a flared cup shaped collarette, but these collapsed structures do not produce conidia. The sequence of *M. indicum* could not be aligned with members of the Trichocomaceae; therefore it was excluded from the analysis shown here. A Blast search with the ITS sequence revealed closest relatives among the Hypocreaceae but there were no sequences showing high similarity. According to Gams [23], *Monocillium* species are anamorphs of *Niesslia* species, which are now placed in the family Niessliaceae of the Hypocreales [24].

The third species in *Phialosimplex, P. sclerotialis*, may also have potential to cause infection. In the SSU analysis (Fig. 2), the subgroup comprising *P. sclerotialis* includes five isolates. One is reported in the GenBank record (EU140822) as being from a case of human keratitis in Taiwan. Although the record states that the isolate represents a new species, we were unable to find a published description. The other isolates are from environmental sources, including the ex-type culture (GenBank EU140822, CBS 366.77), obtained from fodder grasses in France, and three isolates from deep sea basin in India. No ITS sequences were available for members of this group, but pair-wise comparison of SSU sequences revealed that type isolate differed by only 2 nt from the others.

Currently, the Mycobank [available at: http://www.mycobank.org] and Index Fungorum [available at: http://www.indexfungorum.org] list 13 and 12 *Sagenomella* species respectively. One species listed on Mycobank appears to be unpublished. With the transfer of *S. chlamydospora* and *S. sclerotialis* to *Phialosimplex* (Clade A, Fig 2), and the placement of *Sagenoma viride* (anamorph *Sagenomella sagenomatis*) outside *Sagenomella* (Clade C), six species are substantiated in our analysis as members of the genus *Sagenomella* (Clade B). These are *S. diversispora*, the type species (including its synonym *Paecilomyces variabilis*), *S. griseoviridis* , *S. humicola*, *S. striatispora*, *S. verticillata* and the *Sagenomella* anamorph of *T. ocotl.* Pigmented conidia are found in *S. diversispora* (roughened and smooth conidia of varying shapes) and *S. striatispora* (limoniform striated conidia) [8,25]. *S. humicola* produces brown chlamydospores and hyaline limoniform conidia [8,25]. Faintly pigmented conidia occur in *S. griseoviridis* and in *S. verticillata* [8] as well as in *T. ocotl* [9] but the latter two species often demonstrate phialides borne on branched conidiophores. *S. griseoviridis* has been considered close to *S. verticillata* and this is confirmed by our SSU data. *S. oligospora*, having strongly warty conidia [8], was excluded from the Trichocomaceae by Endo *et al*. [10]. No sequences are available for the three remaining *Sagenomella* species so their disposition has not been resolved by the present or prior molecular analyses [10,11]; however, none of the species matches *Phialosimplex* morphologically. *Sagenomella bohemica* and *Sagenomella ryukyuensis* produce phialides on branched conidiophores and the latter also expresses a *Talaromyces* teleomorph [26,27]. *Sagenomella alba* fails to grow above 27°C [8]. No species of *Sagenomella*, as

redefined here, has been associated with animal or human infection. This apparent absence of pathogenicity is correlated with an inability to grow at 35–36ºC among the isolates examined by us (Table 2) and others [8,9].

In conclusion, *Phialosimplex caninus* and *P. chlamydosporus* are here reported as additional members of the Trichocomaceae having potential to cause opportunistic disseminated mycoses in dogs. While comparison of ITS (or other) sequences is now often very helpful in determining the identity of an unknown isolate, the sequence databases still have many gaps, as we found in this study. Although the closest match to our clinical isolates was a species of *Sagenomella*, the paucity of *Sagenomella* species available for comparison led to the detailed comparison reported here and to the discovery of a novel genus.

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References

1 Foley JE, Norris CR, Jang SS. Paecilomycosis in dogs and horses and a review of the literature. *J Vet Intern Med* 2002; **16**: 238–243.
2 Schultz RM, Johnson EG, Wisner ER, *et al*. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. *J Vet Intern Med* 2008; **22**: 851–859.
3 Watt PR, Robins GM, Galloway AM, O'Boyle DA. Disseminated opportunistic fungal disease in dogs:10 cases (1982-1990). J Am Vet Med Assoc. 1995; **207**: 67–70.
4 Zanatta R, Miniscalco B, Guarro J, *et al*. A case of disseminated mycosis in a German shepherd dog due to *Penicillium purpurogenum*. *Med Mycol* 2006; **44**: 93–97.
5 Grant DC, Sutton DA, Sandberg CA, *et al*. Disseminated *Geosmithia argillacea* infection in a German Shepherd dog. *Med Mycol* 2009; **47**: 221–226.
6 García ME, Caballero J, Toni P, *et al*. Disseminated mycoses in a dog by *Paecilomyces* sp. *J Vet Med A Physiol Pathol Clin Med* 2000; **47**: 243–249.
7 Gené J, Blanco JL, Cano J, García ME, Guarro J. New filamentous fungus *Sagenomella chlamydospora* responsible for a disseminated infection in a dog. *J Clin Microbiol* 2003; **41**: 1722–1725.
8 Gams W. Connected and disconnected chains of phialoconidia and *Sagenomella* gen. nov. segregated from *Acremonium*. *Persoonia* 1978; **10**: 97–110.
9 Heredia G, Reyes M, Arias RM, Bills GF. *Talaromyces ocotl* sp. nov. and observations on *T. rotundus* from conifer forest soils of Veracruz State, Mexico. *Mycologia* **93**: 528–540.
10 Endo M, Thanh NT, Yokota A, Gams W, Sugiyama S. Phylogenetic analysis of *Sagenomella* and relatives based on nuclear 18S ribosomal RNA gene and determination of ubiquinone system. *Biseibutsu Bunrui Kenkyukai Puroguramu oyobi Shoroku* 1998; **18**: 35–36.
11 Thanh NT, Endo M, Yokota A, Gams W, Sugiyama J. Phylogenetic analysis of *Sagenomella* and relatives based on nuclear 18S ribosomal RNA gene sequences with the determination of the ubiquinone system. *Ann Rept ICBiotech* 1998; **21**: 307–318.
12 Kornerup A, Wanscher JH. *Methuen Handbook of Color*, 3rd edn. Methuen: London1978.
13 Sigler L, Gibas CFC. Utility of a cultural method for identification of the ericoid mycobiont *Oidiodendron maius* confirmed by ITS sequence analysis. *Stud Mycol* 2005; **53**: 63–74[Available online at http://www.cbs.knaw.nl/publications/].
14 Henry T, Iwen PC, Hinrichs SH. Identification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. *J Clin Microbiol* 2000; **38**: 1510–1515.
15 White T, Burns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand DH, Innis MA, Sninsky JJ, White TJ, (eds) *PCR Protocols. A Guide to Methods and Applications*. San Diego: Academic Press Inc, 1990; 315–322.
16 Rambaut A, Se-Al:Sequence Alignment Editor Version 2.0a11. Department of Zoology, University of Oxford, UK. c2002 Aug 2008. shttp://tree.bio.ed.ac.uk/software/seal/т.
17 Swofford DL. PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4. USA, MA, Sunderland:Sinauer Associates, Inc., 2002. http://paup.csit.fsu.edu/.
18 Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985; **39**: 783–791.
19 Gargas A, DePriest DT, Taylor JW. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Mol Biol Evol* 1995; **12**: 208–218.
20 Gutell RR. Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. *Nucleic Acids Res* 1993; **21**: 3051–3054.
21 Stolk AC, Samson RA. The ascomycete genus *Eupenicillium* and related *Penicillium* anamorphs. *Stud Mycol* 1983; **23**:1–149.
22 Mackie JT, Padhye AA, Sutherland RJ, Lamb WA, Davis S. Granulomatous lymphadenitis and splenitis associated with *Monocillium indicum* infection in a dog. *J Vet Diagn Invest* 2004; **16**: 248–250.
23 Gams W. *Cephalosporium-artige Schimmelpilze (Hyphomycetes).* Gustav FischerStuttgart1971.
24 Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW. Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycol Res* 2004; **108**: 864–872.
25 Onions AHS, Barron GL. Monophialidic species of *Paecilomyces*. *Mycol Pap* 1967; **107**:1–25.
26 Fassatiová O, Pe˘c˘ková M, Prášil K, Vášová M.. Rare micromycete findings from Bohemian peloids. *Novit Bot Univ Carol Praha* 1990; **6**: 21–31.
27 Ueda S, Udagawa S-I. *Sagenoma ryukyuensis*, a new thermotolerant ascomycete. *Mycotaxon* 1984; **20**: 499–504.

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