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Sinomyces: a new genus of anamorphic Pleosporaceae

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ABSTRACT

Sinomyces gen. nov. is described for *Ulocladium alternariae* and two new species from China. These three species differ from *Ulocladium* in producing simple conidiophores with a single, cicatrized apical pore or 1 or 2 short, uniperforate, geniculate sympodial proliferations. Phylogenetic analysis of partial DNA sequences of the glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) gene and a fragment of the *Alternaria alternata* major allergen (Alt a 1) gene, using Maximum-Parsimony (MP), Maximum-Likelihood (ML) and Bayesian approaches, indicates that *Sinomyces* is distinct within the *Alternaria*–*Stemphylium* complex, although its closest relatives could not be determined. *Sinomyces alternariae* comb. nov. and the new species *Sinomyces obovoideus* and *Sinomyces fusioideus* are proposed.

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Introduction

The *Alternaria*–*Stemphylium* complex, which comprises the genera *Alternaria* Nees, *Embellisia* E.G. Simmons, *Nimbya* E.G. Simmons, *Stemphylium* Wallr., and *Ulocladium* Preuss, and, encompasses many species, among which are several economically important plant pathogens. Simmons (1967) surveyed *Alternaria*, *Stemphylium* and *Ulocladium* and emphasized the shapes of juvenile and mature conidia and the modes of conidiophore proliferation. He resurrected *Ulocladium* for species having obovoid and nonbeaked conidia. *Embellisia* was distinguished primarily on the basis of the thick, dark and rigid septa in the conidia, while *Nimbya* produced distoseptate conidia (Simmons 1971, 1989). Inderbitzin et al. (2006) combined molecular and morphological characters in proposing a new genus in the *Alternaria* group, *Crivellia* Inderb. et al., which was monotypic for *Crivellia papaveracea* (De Not.) Inderb. & Shoemaker and was characterized by having cylindrical phragmoconidia.

Recent molecular analyses have revealed that *Alternaria*, *Crivellia*, *Embellisia*, *Nimbya* and *Ulocladium* form a large clade that is in a sister-group relationship with *Stemphylium*, but many of the morphologically defined genera are polyphyletic (Pryor & Gilbertson 2000; Pryor & Bigelow 2003; Hong et al. 2005; Inderbitzin et al. 2006). Thus morphological characters can be misleading. Runa et al. (2009) analyzed the ITS, *gpd* and Alt a 1 sequences of 13 *Ulocladium* species and found only ten species grouped into a well-supported clade. *Ulocladium alternariae* (Cooke) E.G. Simmons and *Ulocladium oudemansii* E.G. Simmons, which possessed the key conidium characteristics of *Ulocladium*, clustered as a separate clade sister to the core *Ulocladium* clade. Conidiophores of *U. alternariae* terminate in a single pore, or occasionally proliferate once sympodially as a geniculation to form a second, uniperforate conidiogenous locus. This is distinct from the conidiophores of typical *Ulocladium* species that produce a sympodial succession of geniculate conidiogenous loci (Simmons 1967).

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During investigations of anamorphic *Pleosporaceae* in China from 2004–2008, we obtained many strains of *Alternaria* spp., *Stemphylium* spp. and *Ulocladium* spp. from leaves of diverse plants and rotten wood. Among them, two species, similar to *U. alternariae* in morphology, were isolated from the leaves of *Tamarix ramosissima* and *Daucus carota*, respectively. Because neither could be identified to any of the known species of *Ulocladium* they appeared to be new. In the current study we characterize the morphology of these fungi and determine their phylogenetic relationships using sequences of *Alt a 1* and *gpd* genes. In light of its unusual morphology in *Ulocladium*, phylogeny and morphology of *U. alternariae* are studied.

Materials and methods

Morphological analysis

Diseased leaves of *Tamarix ramosissima* and *Daucus carota* were collected from Xinjiang and Sichuan provinces in China. Single-conidium cultures were taken from necrotic leaf spots and incubated on potato-dextrose agar (PDA; 20 g white potato boiled and filtered, 20 g dextrose, 20 g agar, 1 L distilled water) at 4 °C. Morphological observations are based on cultures grown on potato-carrot agar (PCA; 20 g white potato boiled and filtered, 20 g carrot boiled and filtered, 20 g agar, 1 L distilled water). ‘Standard conditions’ for the study of morphology are defined as: growth on PCA at ambient temperature (20–23 °C) and under cool-white fluorescent light 35–40 cm above the culture surface, with an 8 h on and 16 h off light cycle (Simmons & Roberts 1993). The holotypes (dried culture) of the new species were deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP). Reference strains were maintained in HSAUP and the collection of the Centraal-bureau voor Schimmelcultures (CBS) Utrecht, The Netherlands.

Conidium germination was studied on PCA, at ambient temperature (20–23 °C) and under cool-white fluorescent light 35–40 cm above the culture surface, with an 8 h on and 16 h off light cycle (Simmons & Roberts 1993). The statistics presented here are based on measurement of 50 mature conidia and 30 conidiophores at 100× magnification in lactic acid.

DNA extraction, PCR amplification, DNA sequencing

Thirty-six strains belonging to 35 species were used for phylogenetic analyses. Among them, the two unknown strains from *Tamarix ramosissima* and a strain of *Crivellia* were sequenced *de novo*; all other sequences were retrieved from GenBank (Table 1). Genomic DNA was isolated from mycelium grown on PCA plates following the protocol of Pryor & Gilbertson (2000). Portions of *Alt a 1* and *gpd* gene regions were amplified using the primers targeting the conserved regions of these genes in several species of *Ulocladium* and *Alternaria* (Hong et al. 2005). Reaction mixtures contained 5 µL of 10× ThermoPol reaction buffer [200 mM Tris–HCl, pH 8.3, 100 mM KCl, 100 mM (NH₄)₂SO₄ and 1 % Triton X-100], 5 µL of 10 mM MgSO₄, 20 ng template genomic DNA, 4 pM of each primer, 4 µL of 2.5 mM dNTPs, 2.5 U of AmpliTaq polymerase (TaKaRa, Dalian, China); total volume was adjusted to 50 µL with deionized water. The PCR thermal cycling program of *Alt a 1* and *gpd* regions was conducted using

previously described reaction conditions (Pryor & Gilbertson 2000; Hong et al. 2005). The PCR products were purified using DNA fragment Purification Kit Ver. 2.0 (TaKaRa). PCR products were cloned into the pGM-Teasy vector following the manufacture’s protocol of the pEASY-T3 Cloning Kit (China) and transformed into DH5α. Sequences were performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator chemistry (Applied Biosystems Inc., Foster City, California). The sequences of both strands of each fragment were determined for sequence confirmation.

Phylogenetic analysis

Alt a 1 and *gpd* sequences were aligned using Clustal X 1.81 (Thompson et al. 1997). The alignments were improved manually where necessary, and deposited in TreeBASE (TB2: S10509). Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford 2002). A partition homogeneity test (Swofford 2002) was run to determine whether sequence data for *gpd* and *Alt a 1* genes regions could be combined.

MP analysis, in which searches for most parsimonious trees was conducted with the heuristic search algorithm with tree-bisection-reconnection (TBR) branch swapping. For each search, 1000 replicates of random stepwise sequence addition were performed and 100 trees were saved per replicate. Optimal trees were identified using heuristic searches based on 1000 random addition replicates retaining clades compatible with the 50 % majority rule in the bootstrap consensus tree.

For ML analysis, MODELTEST v. 3.06 (Posada & Crandall 1998) was used to identify the model of DNA substitution that best fits. MODELTEST analyses using Akaike Information Criterion (AIC) selected the GTR + G + I model of nucleotide frequencies (A = 0.2336, C = 0.3332, G = 0.2204, and T = 0.2128) with the shape parameter of the gamma distributed (α = 1.1627) to accommodate rate variations among sites. Confidence values were estimated using bootstrap analysis (100 replicates), which were summarized as a 50 % majority rules consensus tree in PAUP.

The model of evolution in Bayesian analysis was also GTR + G + I estimated by MODELTEST v. 3.06. Posterior probabilities (PP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run for 600 000 generations and trees were sampled every 100th generations (resulting 6001 total trees). Burn-in was set at 25 100 generations (i.e. 251 trees), well after the likelihood values converged to stationary, leaving 5750 trees from which the consensus trees and PP were calculated. Bayesian analyses were repeated five times for improved sampling of tree space and to guard against local optima in searches.

Results

Morphological and cultural studies

In culture on PCA under standard conditions the three unknown isolates were fast growing with yellow brown to dark brown,

Table 1 – Species and sequences database accession numbers used in this study.

Species name	Collection no. ^a	GenBank no.	
		<i>gpd</i>	Alt a 1
<i>Alternaria alternata</i>	EGS 34-016	AY278808	AY563301
<i>Alternaria arborescens</i>	EGS 39-128	AY278810	AY563303
<i>Alternaria brassicae</i>	BMP 0322	AY562414	AY563309
<i>Alternaria brassicicola</i>	EEB 2232	AY278813	AY563311
<i>Alternaria cetera</i>	EGS 41-072	AY562398	AY563278
<i>Alternaria cinerariae</i>	EGS 33-169	AY562413	AY563308
<i>Alternaria ethzedia</i>	EGS 37-143	AY278795	AY563284
<i>Alternaria euphorbiicola</i>	EGS 42-049	AY562417	AY563314
<i>Alternaria japonica</i>	ATCC 13618	AY278814	AY563312
<i>Alternaria longipes</i>	EGS 30-033	AY278811	AY563304
<i>Alternaria macrospora</i>	DDG Am1	AY278805	AY563294
<i>Alternaria metachromatica</i>	EGS 38-132	AY562404	AY563285
<i>Alternaria mouchaccaae</i>	EGS 31-061	AY562399	AY563279
<i>Alternaria petroselini</i>	EGS 09-159	AY278799	AY563288
<i>Alternaria photistica</i>	EGS 35-172	AY562402	AY563282
<i>Alternaria porri</i>	ATCC 58175	AY278806	AY563296
<i>Alternaria radicina</i>	ATCC 96831	AY278797	AY563286
<i>Alternaria solani</i>	ATCC58177	AY278807	AY563299
<i>Alternaria sonchi</i>	EGS 46-051	AY562412	AY563307
<i>Alternaria smyrnii</i>	EGS 37-093	AY278801	AY563289
<i>Sinomyces fusoides 1</i>	CBS 124114	HM209081	HM209085
<i>Sinomyces fusoides 2</i>	HSAUP1986	HM209082	HM209086
<i>Sinomyces obovoideus</i>	CBS 123375	EU862547	EU862546
<i>Sinomyces alternariae</i>	BMP 0352	AY278815	AY563316
<i>Crivellia papaveracea</i>	CBS 427.50	HM209083	HM209084
<i>Embellisia allii</i>	EGS 38-073	AY278827	AY563322
<i>Embellisia telluster</i>	EGS 33-026	AY562419	AY563325
<i>Nimbya caricis</i>	EGS 13-094	AY278826	AY563321
<i>Nimbya scirpicola</i>	EGS 19-016	AY278825	AY563320
<i>Stemphylium botryosum</i>	ATCC 42170	AY278820	AY563274
<i>Ulocladium atrum</i>	ATCC 18040	AY278818	AY563318
<i>Ulocladium botrytis</i>	ATCC 18043	AY278817	AY563317
<i>Ulocladium chartarum</i>	ATCC 18044	AY278819	AY563319
<i>Ulocladium consortiale</i>	CBS 201-67	FJ266494	FJ266509
<i>Ulocladium solani</i>	CBS 123376	EU855805	EU855801
<i>Ulocladium subcucurbitae</i>	CBS 121491	EU855803	EU855807

Newly generated sequences in bold.

^a ATCC, American Type Culture Collection, Manassa, VA, USA; BMP, B.M. Bryor, Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DGG, D.G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616, USA; EEB, E.E. Butler, Department of Plant Pathology, University of California, Davis, CA 95616, USA; EGS, E.G. Simmons, Mycological Services, Crawfordsville, IN 47933, USA.

cottony colonies that attained 35–45 mm diam in 7 d. Conidiophores typically arose from the tip cell of a hypha that was morphologically and functionally distinct from vegetative hyphae. A single conidium was produced from a pore at the tip of the conidiophore; occasionally the conidiogenous cell proliferated sympodially to form 1 or 2 uniperforate geniculations, each of which produced a single conidium. Conidia were dictyosporous, yellow brown to dark brown and obovoid to fusiform, tapering from a broad apex to a narrow base. Additionally, conidia did not produce 'false beaks' (i.e. secondary conidiophores arising from conidia still attached to the conidiogenous cell).

Phylogenetic analyses

The size of DNA fragments was of 449–451 bp for Alt a 1, and 472–508 bp for *gpd*. Alignment of the Alt a 1-*gpd* fragments

resulted in a 966-character data set. Of these, 349 characters were parsimony informative, and 516 were constant. Results of the PHT between the two loci ($P = 0.113$) indicated the Alt a 1 and *gpd* data sets could be combined and analyzed. *Stemphylium botryosum* was used as outgroup based on results from previous studies (Pryor & Gilbertson 2000; Pryor & Bigelow 2003; Hong et al. 2005).

Parsimony analysis yielded six equally parsimonious trees of 1339 steps with a consistency index (CI) = 0.5063, retention index (RI) = 0.6824, rescaled consistency index (RC) = 0.3455, homoplasy index (HI) = 0.4937. The MP tree (Fig 1), which was obtained from the analysis of combined DNA sequences of *gpd* and Alt a 1, grouped *Ulocladium alternariae* and the two unknown strains clustered independently of each other in one clade with a high bootstrap support (100 %). Two strains isolated from *Tamarix* formed a branch with 100 % bootstrap



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Fig 1 – Phylogenetic tree of *Sinomyces* and five similar genera of anamorphic *Pleosporaceae* generated from MP analysis based on combined data set of *Alt a 1* and *gpd* sequences. Number in the front of ‘/’ represents parsimony bootstrap values and number after the ‘/’ represents ML bootstrap values. Bootstrap values <70 % are not shown. Thickened branches indicate Bayesian PP $\geq 95\%$. The tree is rooted with *Stemphylium botryosum*.

support. The strain isolated from *Daucus* was joined to this branch with high bootstrap support (80 %). *Ulocladium alternariae* formed a strongly supported subclade in a sister relationship to the clade that included the *Tamarix* and *Daucus* strains. Based on MP phylogenetic analysis (Fig 1), and consistent with previous results (Pryor & Bigelow 2003; Hong et al. 2005), *Embellisia*, the *Alternaria infectoria* group and *Nimbya* formed a single, well-supported clade. The analysis revealed

several well-supported terminal clades, including *Sinomyces*, but there was no internal support to show relationships among the clades. The topologies generated from ML and Bayesian analyses were essentially similar to that of the parsimony analysis. The ML and Bayesian trees were therefore not shown, but the bootstrap values of ML ($\geq 70\%$) and statistically-supported clades ($\geq 95\%$) are marked in the parsimony trees (Fig 1).

Taxonomy

Phylogenetic analyses (MP, ML and Bayesian) for the two gene regions (*Alt a 1* and *gpd*) indicate that *Ulocladium alternariae* and the *Daucus* and *Tamarix* strains are distinct from any of the genera of the *Alternaria* group (Fig 1). This result is consistent with morphological observations.

Conidiophores of *U. alternariae* and the *Daucus* and *Tamarix* strains produce few, more or less widely spaced conidiogenous loci. As a result the conidia always appear to be solitary. This differs from typical *Ulocladium*, where conidia appear to be held in clusters because of the close spacing of the conidiogenous loci, which tend to repeatedly proliferate sympodially. Moreover, conidia of some typical *Ulocladium* species produce short conidiophores directly and when conidia arise from these secondary conidiophores, they are closely spaced and appear to be catenate. We have not observed this in *U. alternariae* or the *Daucus* and *Tamarix* strains. These fungi are similar to *Alternaria* because of their simple conidiophores and terminal conidiogenous loci (Simmons 1967). However, *Alternaria* is distinguished by its ovoid apically tapered, often catenate conidia. Conidia of *U. alternariae* and the *Daucus* and *Tamarix* strains are dictyosporous, which distinguishes them from *Crivellia*, *Embellisia* and *Nimbya* (Simmons 1971, 1989; Inderbitzin et al. 2006), which have phragmosporous conidia. Finally, *U. alternariae* and the *Daucus* and *Tamarix* strains differ from *Stemphylium* by the sympodial/geniculate proliferation of the conidiogenous cells; conidiogenous cells in *Stemphylium* proliferate percurrently.

The combined molecular/phylogenetic and morphological observations strongly indicate that *U. alternariae* does not belong in *Ulocladium* but that it cannot be accommodated in any of the genera that are close to *Ulocladium*. The results also show that the two unknown fungi that we isolated, respectively from *Daucus* and *Tamarix*, are closely related but distinct from each other but are closely related to *U. alternariae*. Thus we propose a new genus for them:

***Sinomyces* Yong Wang bis & X.G. Zhang, gen. nov.**

Mycobank no.: 513490

Etym.: Sino (Latin) referring to the country of collection.

Typus: *Sinomyces fusoides* Yong Wang bis & X.G. Zhang

Anamorphic Pleosporaceae. Mycelium ex hyphis, simplicia vel ramosa, septatae, subhyalinae, laeves. Conidiophora copiosa, recta vel acclivia, simplicia vel ramosa, ex lateribus hypharum submersarum aeriarumque oriunda, sympodialis, septata, subhyalinae vel aureo-brunnea, simplicia, terminals vel raro semel vel bis crebre unus-terebro genicula, laevis. Conidia solitaria, obovoidea vel fusoides, aureo-brunnea vel atro-brunnea, ad quin exhibitio reprobate rostrum. Mycelium subhyaline simple or branched, septate, smooth. Conidiophores abundant, subhyaline to pale brown, erect or ascending from submerged and aerial hyphae, simple or branched, sympodial, septate, smooth, simple with an apical pore or 1 or 2 uniperforate geniculations present at times, originating laterally or apically from attached, primary conidium. Conidia solitary, obovoid

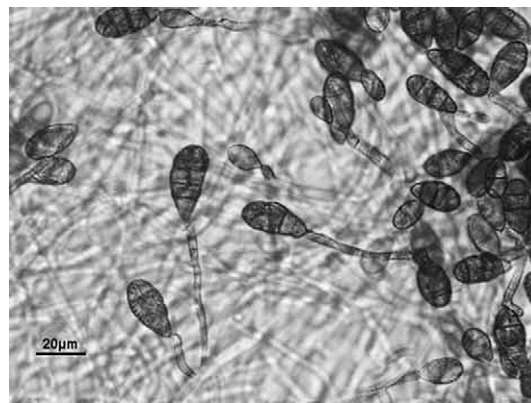


Fig 2 – *Sinomyces fusoides* from the holotype. Conidiophores and conidia (40× magnification).

or fusoid, golden brown to dark brown, without producing a false beak.

***Sinomyces fusoides* Yong Wang bis & X.G. Zhang, sp. nov.** (Figs 2 and 3)

Mycobank no.: 513491

Etym.: fusoides (Latin) referring to the fusoid conidia of this fungus.

Coloniae in PCA, 35–40 diam in seven diebus ad 23 °C, auctus velosi, sporulatione abunda, conspicue concentricae zonatae. Hyphae vegetativae septatae, ramosae, subhyalinae, 4–5 μm crassae, laeves. Conidiophora copiosa, recta vel acclivia, simplicia vel ramosa, ex lateribus hypharum praecipue submersarum aeriarumque oriunda, pallide-brunnea, septata, 25–60 × 3.5–4 μm ($M = 43 \times 3.7 \mu m$, $n = 30$), terminals vel raro semel vel bis crebre unus-terebro genicula, laeves. Conidia 31–39 × 11–15 μm ($M = 36 \times 13 \mu m$, $n = 50$), solitaria, obclavata vel fusoides, aureo-brunnea vel atro-brunneae, 3–6 transverse septata et 1–3 longiseptata, laeves vel dense pustulata, ad quin exhibitio reprobate rostrum.

Holotype: China: Xinjiang Province: Kashi, 40°12'30"N, 76°30'27"E, on diseased leaves of *Tamarix ramosissima*,

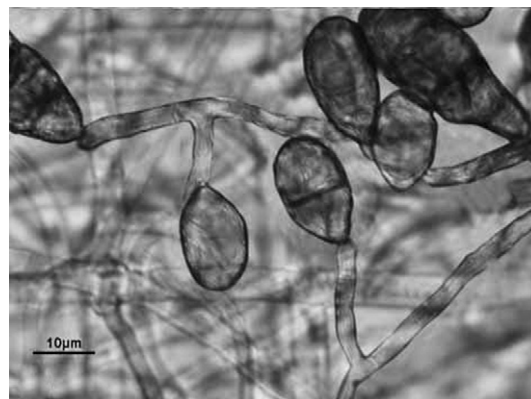


Fig 3 – *Sinomyces fusoides* from the holotype. Sporulation apparatus (100× magnification).

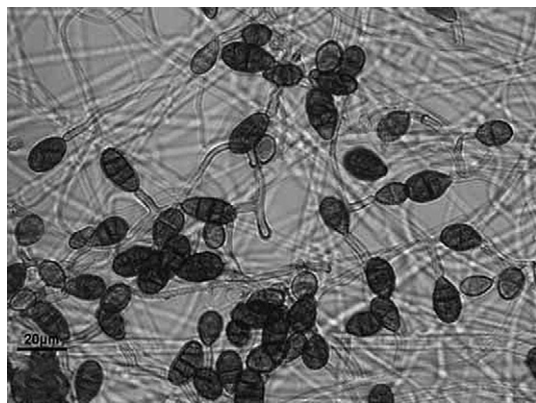


Fig 4 – *Sinomyces obovoideus* from the holotype. Conidiophores and conidia (40× magnification)

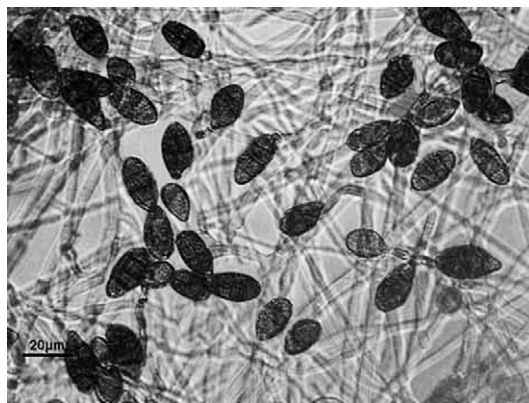


Fig 6 – *Sinomyces alternariae* from the living cultures of BMP 0352. Conidiophores and conidia (40× magnification).

Y. Wang, Jul. 2006, HSAUP2708 (dry agar culture), holotype designated herewith. Living ex type culture. CBS 124114 = HSAUP2708.

Colonies 35–40 mm diam in 7 d at 23 °C on PCA, yellow brown to dark brown, rapidly covering entire 9-cm-diam Petri dish. Concentric zonation of growth pronounced; sporulation abundant. Mycelium subhyaline, hyphae branched, smooth, septate, 4–5 μm wide. Conidiophores abundant, erect or ascending from submerged and aerial hyphae, simple or branched, dilute brown, septate, smooth, 25–60 × 3.5–4 μm ($M = 43 \times 3.7 \mu\text{m}$, $n = 30$), simple with an apical pore or occasionally 1 or 2 close, uniperforate geniculations. Conidia 31–39 × 11–15 μm ($M = 36 \times 13 \mu\text{m}$, $n = 50$), solitary, obclavate to fusoid, golden brown to dark brown, 3–6 transverse septa and 1–3 longitudinal septa, smooth to depressed pustulate, lacking a false beak (secondary conidiophores).

Additional specimens examined: on diseased leaves of *Tamarix ramosissima*. **China:** Xinjiang Province: Yili, 43°14'35"N, 85°21'43"E, Y. Wang, Sep. 2008 (HSAUP1986).

Sinomyces obovoideus Yong Wang bis & X.G. Zhang, **sp. nov.** (Figs 4 and 5)

MycoBank no.: 513492

Etym.: obovoideus (Latin) referring to the conidial shape.

Coloniae in PCA 40–45 diam in seven diebus temperatura 23 °C, auctus velosi, sporulatione abunda, conspicue concentricae zonatae. Hyphae vegetativae septatae, ramosae, subhyalinae, 3.5–4 μm crassae, laeves. Conidiophora copiosa, recta vel acclivia, simplicia vel ramosa, ex lateribus hypharum praecipue submersarum aeriarumque oriunda, subhyalinae, septata, 20–50 × 3–3.5 μm ($M = 40 \times 3.2 \mu\text{m}$, $n = 30$), terminals vel raro 1–2 crebre unus-terebro genicula, laeves. Conidia 18–26 × 13–17 μm ($M = 22.5 \times 15 \mu\text{m}$, $n = 50$), solitaria, obovoidea vel ellipsoidea, atro-brunneae, 2–3 transverse septata et 2–3 longiseptata, laeves vel dense pustulata, ad quin exhibitio reprobate rostrum.

Holotype: **China:** Sichuan Province: Leshan, 29°21'36"N, 103°26'24"E, on diseased leaves of *Daucus carota*, Y. Wang, Jul. 2005, HSAUP1144 (dried culture), holotype

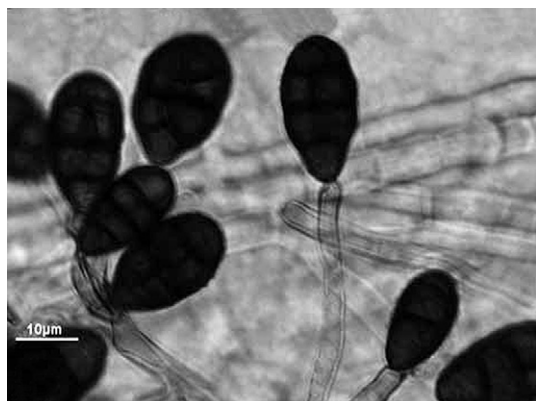


Fig 5 – *Sinomyces obovoideus* from the holotype. Sporulation apparatus (100× magnification).

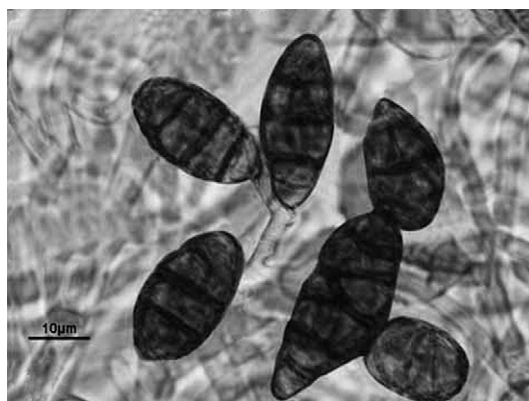


Fig 7 – *Sinomyces alternariae* from the living cultures of BMP 0352. Sporulation apparatus (100× magnification).

Table 2 – Distinguishing characteristics of two new species and a new combination in *Sinomyces* under ideal growth conditions.

Species	Conidium				Conidiophore
	Shape	Size (µm)	Transverse septa	Longitudinal or oblique septa	Dimensions (µm, length × width at base)
<i>Sinomyces fusoides</i>	Obclavate to fusoid	31–39 × 11–15	3–6	1–3	25–60 × 3.5–4
<i>Sinomyces obovoideus</i>	Obovoid to ellipsoid	18–26 × 13–17	2–3	2–3	20–50 × 3–3.5
<i>Sinomyces alternariae</i>	Obovoid to broadly ellipsoid	22–31 × 13–17	3–5	1–2	10–30 × 4–5

designated herewith. Living ex type culture: HSAUP1144 = CBS 123007.

Colonies 45–50 mm diam in 7 d at 23 °C on PCA, yellow brown to dark brown, rapidly covering entire 9-cm-diam Petri dish. Concentric zonation of growth pronounced; sporulation abundant. Mycelium subhyaline, hyphae branched, smooth, septate, 3.5–4 µm wide. Conidiophores abundant, erect or ascending from submerged and aerial hyphae, simple or branched, subhyaline, septate, 20–50 × 3–3.5 µm ($M = 40 \times 3.2 \mu\text{m}$, $n = 30$), smooth, simple with an apical pore or occasionally 1–2 close, uniperforate geniculations. Conidia 18–26 × 13–17 µm ($M = 22.5 \times 15 \mu\text{m}$, $n = 50$), solitary, dark brown, obovoid to ellipsoid, 2–3 transverse septa and 2–3 longitudinal septa, smooth to depressed pustulate, false beak (secondary conidiophores) lacking.

***Sinomyces alternariae* (Cooke) Yong Wang bis & X.G. Zhang, comb. nov. (Figs 6 and 7)**

MycoBank no.: 513493

Basionym: *Sporidesmium alternariae* Cooke, Handbook of British Fungi. 1: no. 1440 (1871).

Stemphylium alternariae (Cooke) Sacc., Syll. fung. 4: 523 (1886).

Ulocladium alternariae (Cooke) E.G. Simmons, Mycologia, 59: 82 (1967).

Colonies 40–45 mm diam in 7 d at 23 °C on PCA, yellow brown to dark brown, rapidly covering entire 9-cm-diam Petri dish. Concentric zonation of growth pronounced; sporulation abundant. Mycelium pale yellow brown, hyphae branched, smooth, septate, and 4–6 µm wide. Conidiophores abundant,

erect or ascending from submerged and aerial hyphae, simple or branched, golden brown, septate, 10–30 × 4–5 µm ($M = 22 \times 4.5 \mu\text{m}$, $n = 30$), smooth, simple with an apical pore or occasionally 1–2 uniperforate geniculations. Conidia 22–31 × 13–17 µm ($M = 27 \times 15.5 \mu\text{m}$, $n = 50$), solitary, obovoid to broadly ellipsoidal, golden brown to olivaceous, 3–5 transverse septa and 1–2 longitudinal septum, smooth to depressed pustulose, false beak (secondary conidiophores) lacking.

Specimen examined: U.S.A., on commercial carrot seed, 1991, B. M. Pryor, BMP 0352 (dried culture) – epitypus. BMP 0352 – Ex-epitypus.

In *Sinomyces*, *S. fusoides* is unique for its obclavate to fusoid conidia. Moreover, conidia of *S. fusoides* are bigger than those of the other two species. *Sinomyces fusoides* possesses more transverse septa than *S. obovoideus*, and the length of conidiophores differentiates *S. fusoides* from *Sinomyces alternariae*. Additionally, *S. fusoides* has fewer geniculations in its conidiophores than *S. alternariae*. *Sinomyces obovoideus* shows greater morphological similarity to *S. alternariae*. These two species produce mostly obovoid to ellipsoid conidia, but conidia of *S. obovoideus* are shorter than those of *S. alternariae*. *Sinomyces obovoideus* forms fewer transverse and longitudinal septa than *S. alternariae*. The conidiophores of *S. obovoideus* are longer than those of *S. alternariae*. The morphological characters of conidia and conidiophores in these three *Sinomyces* species are shown in Table 2.

Sinomyces shares many morphological characters with *Ulocladium*, *Alternaria*, *Crivellia*, *Embellisia*, *Nimbya* and *Stemphylium*. These seven allied genera can be distinguished in the following key

1. Apical cell or region of conidiophore slightly to distinctly swollen.....*Stemphylium*
Apical cell or region of conidiophore not swollen.....2
2. Basal region of conidium tapered.....3
Basal region of conidium blunt 4
3. Conidiophore geniculations terminal or intercalary, often multiperforate, closely or widely spaced*Ulocladium*
Conidiophore geniculations terminal, at most or with 1 or 2 closely spaced uniperforate geniculations*Sinomyces*
4. Conidia mainly dictyosporous*Alternaria*
Conidia mainly phragmosporous 5
5. Conidia cylindrical.....*Crivellia*
Conidia distoseptate*Nimbya*
Conidia with thick, dark and rigid septa.....*Embellisia*

Discussion

We recognize *Sinomyces* on the basis of the shared morphological and molecular characteristics of its species. The genus is distinguished on the basis of conidial shape, size range, septation and the dimension of conidiophores. Although the phylogenetic analysis presented here is unresolved internally and does not show interrelationships among the included genera, it is clear that *Sinomyces* is distinct. Conidial morphology and this indicates that conidial shape is not a reliable indicator of phylogeny and may be a result of convergent evolution (Campbell et al. 2006; Inderbitzin et al. 2006). which is generally consistent with the result of Inderbitzin et al. (2006). Sequences of *gpd* and the Alt a 1 region of the three *Sinomyces* species are 99 % similar. This situation is seen also in some species in *Alternaria* (McKay et al. 1999).

In 1967 there was no living culture typical of *Ulocladium alternariae* (Simmons 1967). Since then Strain BMP 0352 was isolated by Pryor in 1991 (Hong et al. 2005); and according to E. G. Simmons (pers. comm.), this culture is representative of this species. The morphology of this species was described and illustrated here based on study of this representative culture. The original description of this species as a *Sporidesmium* species was based on the moniliform arrangement of conidia as seen under low magnification (Cooke 1871). Saccardo (1886) placed it in the *Stemphylium* because of its conidial characters. Hughes (1953) suggested that some hyphomycete species should be classified into different genera primarily upon the types of conidiophore and conidial development. Because this species produces abundant obovoid conidia but conidiophore is not apically swollen and does not proliferate, key morphological characters of *Stemphylium*, Simmons (1967) removed this species from *Stemphylium* and classified it in *Ulocladium*. The geniculations on conidiophores of *Ulocladium* are often multiperforate and closely or widely spaced, while conidiophores of *Sinomyces* typically do not proliferate or at most produce 1 or 2 geniculations, which are uniperforate. Because *U. alternariae* possesses these essential characteristics of *Sinomyces*, it is transferred to that genus.

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