Ulocladium capsicuma, a new species identified by morphological and molecular phylogenetic data

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A new anamorphic fungus was discovered on leaves of *Capsicum annuum* L. collected in Yunnan of China. Its conidia, conidiophores and colonies resemble the type species of *Ulocladium, U. botrytis*, and the character that conidia are always in chains is similar to *U. chartarum*. Both morphological and phylogenetic analyses based upon ITS and *gpd* (glyceraldehyde-3-phosphate dehydrogenase) gene suggest that the fungus is a species of *Ulocladium* but is distinct from other species in the genus. Thus, the fungus is defined as a new species *Ulocladium capsicuma*.

Keywords: hyphomycetes, taxonomy, ITS, *gpd*, molecular phylogeny

The genus *Ulocladium* Preuss was established in 1851 (Preuss 1851) with *U. botrytis* Preuss as the type species. The genus had been long ignored, until 1967 when several taxa were described by E. G. Simmons and the validity of the genus was ascertained. Since *Ulocladium* was re-expounded by Simmons, several additional species have been described (Mouchacca 1971, Simmons 1982, 1990, Zhang & Zhang 2002).

Members of the genus produce phaeodictyospores on well-differentiated conidiophores which commonly become geniculate through successive lateral renewals of growth. The conidia are fundamentally obovoid, which is the principal morphological characteristic that distinguish this genus from other genera with dematiacious muriform conidia such as *Alternaria* and *Stemphylium* (Simmons 1967). Many of subsequent identification of additional *Ulocladium* species were based primarily upon conidium character-

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istics. Shape, size range, septation, as well as ornamentation of conidia, their formation in chains or not, and the occurrence of secondary conidiophores were used as discriminating characters (Simmons 1967, 1990, Zhang & Zhang 2002). However, it was found that the environmental conditions including media composition, humidity, light cycles, and temperature impacted the conidium growth and development of *Ulocladium* species. For example, conidium characteristics of *U. cucurbitae* and *U. atrum* vary considerably under the different substrate, growth temperature, and incubation days (Leach & Aragaki 1970, Simmons 1982, 1998). Therefore, to identify a new species of *Ulocladium*, growth trends of conidia should be observed and described under different conditions.

The genus *Ulocladium* shares many morphological characters with the genera *Alternaria*, *Stemphylium*, *Nimbya* and *Embellisia*, which also possess phaeodictyospores (Preuss 1851, Wiltshire 1933, Simmons 1967, 1982, 1992). Similarities in conidium shape, septation, pigmentation, and ornamentation among members of these genera have complicated the classification of certain species, as well as the practical identification of some morphologically similar species. Otherwise, the confusion surrounding the taxonomy of *Ulocladium* is revealed by the fact that some typical species have been placed into *Alternaria* and *Stemphylium* since their initial identification, for example, *U. botrytis* Preuss was described as *Stemphylium botryosum* by Saccardo in 1866, *U. atrum* Preuss was transferred to *Stemphylium* by Saccardo (1886), and *U. chartarum* (Pr.) Simmons was originally described as *Alternaria chartarum* by Preuss in 1848.

Previous identification of *Ulocladium* species relied only on conidium shape, size range, septation and ornamentation. However, many of these characters overlap among species. Analysis of molecular characters has become a common tool in modern systematics, and has been used to establish the relationships within many groups of hyphomycetes. For example, 18S, ITS1-5.8S-ITS, and mt SSU rDNA sequences have been used to analyze the phylogenetic relationships among *Alternaria* spp. and the two similar genera *Ulocladium* and *Stemphylium* (Pryor & Glibertson 2000). Chou & Wu (2002) investigated the evolutionary relationships among *Alternaria* spp. and the four other related genera, i.e. *Stemphylium*, *Ulocladium*, *Exerohilum*, and *Nimbya* by analyzing short fragments flanking the ITS regions. In addition, a number of protein coding genes, such as *gpd* (glyceraldehydes-3-phosphate dehydrogenase), have been used to evaluate relationships among different fungal genera or different species in the same genus. For example, ITS and *gpd* sequence data has been used to estimate phylogenetic relationships among *Cochliobolus* / *Bipolaris*, *Pyrenophora* / *Drechslera*, *Seto-*

sphaeria / *Exserohilum* spp. *Stemphylium* spp. (Zhang & Berbee 2001, Berbee *et al*. 1999, Câmara *et al*. 2002), and genera established as sister taxa of *Pleospora* / *Stemphylium* and *Lewia* / *Alternaria* spp. (Pryor & Glibertson 2000). Pryor & Bigelow (2003) used ITS, *gpd* and mt SSU rDNA sequence data to appraise phylogenetic relationships among six *Embellisia* spp. and two *Nimbya* spp. and *Alternaria*, *Ulocladium* and *Stemphylium* spp. To analyze fungal taxa at or below species level, the more variable ITS region is commonly used, and it has been used to examine phylogenetic relationships among *Alternaria* species (Kubaba & Tsuge 1995, Pryor & Glibertson 2002). The purpose of this study is to determine the taxonomic status of a new species of *Ulocladium* isolated from leaf spots of *Capsicum annuum* L. in China, herein after referred to as *U. capsicuma*, based on morphological and phylogenetic data, and then to further the systematic study of *Ulocladium* in general.

Materials and Methods

Sampling

Isolates of *U. capsicuma* were obtained from the spots on living leaves of *Capsicum annuum* L. Necrotic leaves of *Capsicum annuum* L. were collected from Dali, Yunnan province, China in 2003 and were kept wet in plastic petri dishes at 28 °C in order to induce sporulation. For isolates of other species see Table 1.

Isolation and identification

For primary morphological identification, cultures were obtained by picking a single conidium with a sterile needle and inoculating potato dextrose agar (PDA). Isolates were transferred to PDA slants as well as to 20 mL bottle containing sterile water for long-term storage.

Spore measurements

All isolates were cultured on potato-carrot agar (PCA) and developed under standardized conditions (Simmons & Roberts 1993), ambient temperature approximately 23 °C, cool-white fluorescent light 35–40 cm above the culture surface, timed for 8 h on and 16 h off, and culture dishes not sealed so that relatively low ambient humidity prevents accumulation of free water around colonies. Comparison of sporulation elements were made at different stages of colony development. Conidia and conidiophore samples were taken three, seven, and 15 days after inoculation. For microscopy, samples were mounted in lactophenol and glycerin. Micrographs were taken

using a Olympus BX51-32000 microscope. To confirm species identity, the morphological characters of each isolate were examined for characteristics of the sporulation apparatus and conidium morphology (Simmons 1967, 1982, 1998). Especially, isolates of *U. capsicuma* ($HSAUPIII_0$0035) and *U. chartarum* (ATCC18044) were cultured five times, and significance of conidia differences of these two species was determined by ANOVA (Excel 2003).

DNA extraction, PCR amplification and sequencing

Isolates of *U. capsicuma* ($HSAUPIII_0$0035), *U. cucurbitae* ($HSAUPIII_0$0282), *U. obovoideum* ($HSAUPIII_0$1144 & $HSAUPIII_0$1238), *Alternaria gaisen* (EGS 90-0512), *A. mali* (EGS 38-029), *A. metachromatica* (EGS 38-132), *A. oregonensis* (EGS 29-194), *A. triticina* (EGS 17-061) and *A. triticimaculans* (EGS 40-150) were grown at room temperature for 14 d in 100 mL potato dextrose broth. The mycelia were collected by filtration and ground to fine powder in liquid nitrogen with sterile mortars and pestles. DNA extraction and purifications were conducted according to Pryor & Gilbertson (2000). The DNA concentrations were diluted to a final concentration of 15 ng μL^{-1} for PCR reactions. Primers used for PCR amplification and for sequencing of the internal transcribed spacer region were ITS5 and ITS4 (White et al. 1990), for the *gpd* gene *gpd1* and *gpd2* (Berbee *et al.* 1999).. Amplifications were performed in 0.25 mM of each dNTP, 0.5 mM of each primer, 2.5 mM $MgCl_2$, 2 µL of 5 × buffer PCR buffer II (PE Applied Biosystems, Foster City, CA). TaKaRa Ex Taq™ Hot Start Version DNA polymerase was added at 1.25 u 50 μL^{-1} of reaction mix. 10 ng of genomic DNA template was used in each 50 µL reaction. Amplifications were carried out in a PE Applied Biosytems thermal cycler (model 480) with following parameters: 94 °C for 2 min, 35 cycles 94 °C for 1 min, 55 °C for 30 s, and 72 °C for 1 min, and final extension 72 °C for 10 min. PCR products were checked by electrophoresising in 1 % in 0.5 × TBE buffer (Sambrook *et al.* 1989).

PCR products were purified using DNA fragment Purification Kit Ver. 2.0 (Takara). Both strands of the amplicons were sequenced with an ABI PRISM 377 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

ITS and *gpd* sequenced in this study were deposited in NCBI - GenBank (Table 1). Other ITS and *gpd* DNA sequences of *Alternaria*,

Tab. 1. – Isolates and sequences used in the phylogenetic analysis.

Species	Source	GenBank accession number of ITS	GenBank accession number of *gpd*
Ulocladium alternariae	BMP 31-41-05	AF229485	AY278815
U. atrum	ATCC 18040	AF229486	AY278818
U. botrytis	ATCC 18043	AF229487	AY278817
U. capsicuma	$HSAUPIII_0$0035	AY762940 **	AY762950 **
U. chartarum	ATCC 18044	AF229488	AY278819
U. consortiale	BMP 31-51-001	AY278837	AY278816
U. cucurbitae	$HSAUPIII_0$0282	AY762941 **	AY762951 **
U. obovoideum	$HSAUPIII_0$1144	AY762942 **	AY762952 **
	$HSAUPIII_0$1238	AY762943 **	AY762953 **
Alternaria alternata	EGS 34-016	AF347031	AY278808
A. arborescens	EGS 39-128	AF347033	AY278810
A. brassicicola	EEB 2232	AF229462	AY278813
A. carotiincultae	EGS 26-010	AF229465	AY278798
A. cheiranthi	EGS 41-188	AF229457	AY278802
A. crassa	DGG Acr1	AF229464	AY278804
A. dauci	ATCC 36613	AF229466	AY278803
A. destruens	EGS 46-069	AY278836	AY278812
A. gaisen	EGS 90-0512	AY762944 **	AY762954 **
A. japonica	ATCC 13618	AF229474	AY278814
A. longipes	EGS 30-033	AY278835	AY278811
A. macrospora	DGG Ams1	AF229469	AY278805
A. mali	EGS 38-029	AY762945 **	AY762955 **
A. metachromatica	EGS 38-132	AY762946 **	AY762956 **
A. oregonensis	EGS 29-194	AY762947 **	AY762957 **
A. petroselini	EGS 09-159	AF229454	AY278799
A. porri	ATCC 58175	AF229470	AY278806
A. radicina	ATCC 96831	AF229471	AY278797
A. selini	EGS 25-198	AF229455	AY278800
A. smyrnii	EGS 37-093	AF229456	AY278801
A. solani	ATCC 58177	AF229475	AY278807
A. tenuissima	EGS 34-015	AF347032	AY278809
A. triticina	EGS 17-061	AY762948 **	AY762958 **
A. triticimaculans	EGS 40-150	AY762949 **	AY762959 **
Embellisia leptinellae	EGS 40-187	AY278845	AY278832
E. proteae	EGS 39-031	AY278842	AY278829
Exserohilum pedicillatum	EEB1336	AF229478	AY278824
Lewia ethzedia	EGS 37-143	AY278833	AY278795
L. infectoria	EGS 27-193	AF347034	AY278793
Nimbya caricis	EGS 13-094	AY278839	AY278826
N. scirpicola	EGS 19-016	AY278838	AY278825
Pleospora herbarum	ATCC 11681	AF229479	AY278823
P. tarda	ATCC 42170	AF229481	AY278820
Stemphylium callistephi	EEB 1055	AF229482	AY278822
S. vesicarium	ATCC 18521	AF229484	AY278821

Abbreviations:
* ATCC: American Type Culture Collection, Manassas, VA 20108; BMP: B. M. Pryor, Department of Plant Pathology. University of Arizona, Tucson, AZ 85721; DGG: D. G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616; EEB: E. E. Butler, Department of Plant Pathology, University of California, Davis, CA 95616; EGS: E. G. Simmons, Mycological Services, Crawfordsville, IN 47933; HSAUP: Department of Plant Pathology, Shandong Agricultural University, Tai'an, Shandong, P. R. China.
** Sequences obtained in this study.

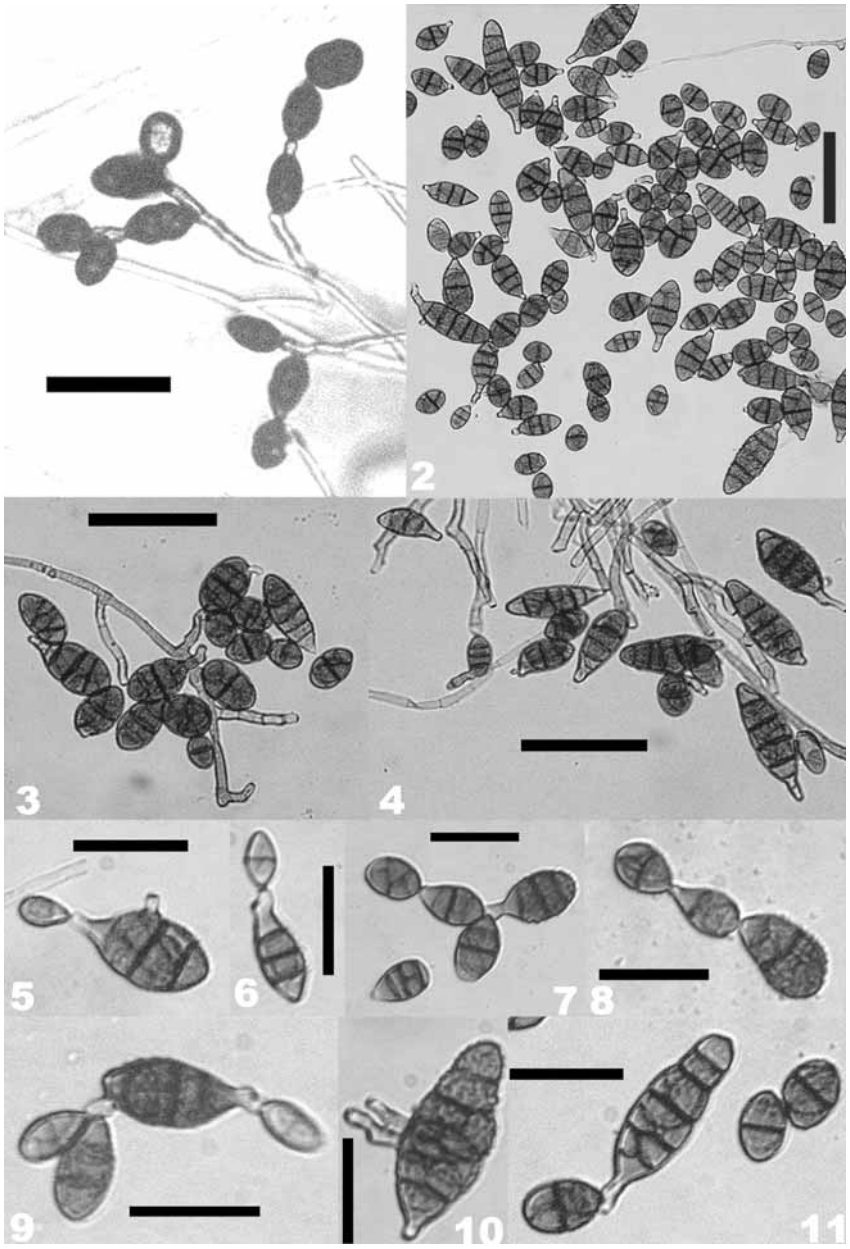
Ulocladium, *Stemphylium*, *Nimbya* and *Embellisia* species used in the analyses were obtained from GenBank. Based on results reported by Berbee *et al.* (1999), Pryor & Bigelow. (2003) and Câmara *et al.* (2002), representative species of three genera (two *Pleospora* species, two *Stemphylium* species and *Exserohilum pedicillatum*) were chosen as outgroups. DNA sequences were edited and aligned using the computer program's SEQUENCHER 3.1 (Gene Codes, Ann Arbor, Michigan) and Clustal X 1.83 (Thompson *et al.* 1997), respectively. Alignments were adjusted manually using the program Genedoc 2.5.000 (http://www.psc.edu/biomed/genedoc/). Trees were generated by both, neighbor-joining (NJ) and maximum parsimony (MP) analysis for ITS and *gpd* sequence datasets using PAUP 4.0b10 (Swofford 2002). The HKY85, JC, K2P, F84 distances calculation was employed for NJ-analysis. For MP-analysis, the heuristic search option with 1000 random addition sequences and TBR branch-swapping options were employed. Gaps were treated as missing data. Bootstrap support for branches in MP and NJ searches were estimated with 1000 pseudoreplicates. Molecular characters were unordered and given equal weight during analysis and all were included in the analysis after removing indels. Partition homogeneity analysis (Farris *et al.* 1995, Huelsenbeck *et al.* 1996) was used to determine if the two data sets (ITS and *gpd*) could be combined, and a combined analysis was run using the parameters described above.

Results

Morphological Study

Colonies (on PCA at 23 °C): Within 1–3 d after inoculation, radiating vegetative hyphae on and in the agar gave rise to a few upright, hyaline superficial or immersed hyphae. A few young subhyaline conidiophores and conidia were present. By the end of day three, conidia of two types, smooth-obovoid and punctulate-elliptical were abundant, but their pigmentation was still not darker than that of conidia incubated for 7 d and 15 d. The two disparate spore morphologies remained distinct from the time of initiation until maturity, none being a developmental conversion product of the other. At this stage the spores were approximately 7–9 × 8 µm, with 1 transverse or longitudinal septum, L/W = 0.9–1 (not shown in Figs. 1–11).

At day seven, the dark colony was densely conidial (at center) to openly but still abundantly conidial near its circumference. The obovoid and elliptical spores characteristic of the early growth stage were still found, but some of them became broad obovoid and oblong-elliptical (i.e. nearly mature spores), darker than in day 1–3,



Figs. 1–11. Conidiophores and conidia of *U. capsicuma* from ex-type culture on PCA. **1.** Characteristic pattern of sporulation (15 days). **2.** Proportion of several types of conidia (15 days). **3 & 4.** Primary conidiophores (15 days). **5–6 & 7.** Secondary conidia with secondary conidiophores (7 days). **8 & 9.** Terminal chains of conidia (7 days). **10 & 11.** Primary conidia with secondary conidiophores (7 days). Scale bars: 1–4. = 50 µm, 5–11. = 20 µm.

and often developed some cellwall ornamentation. Moreover, some of those nearly mature spores had generated one or two short secondary conidiophores that were produced laterally at various intervals along each spore (Figs. 5–10). Each secondary conidiophore had 1–2 conidiogenous sites. The primary conidiophores were often erect or ascending, dilute brown, smooth or rarely inconspicuously roughened, up to approximately 20–30 × 4.5–6 μm, septate, bearing solitary conidia from 1–2 perforate geniculations. Conidia were broad obovoid or oblong-elliptical, smooth to inconspicuously roughened, golden brown to olivaceous, up to approximately 26.5–42.5 × 7.5–12 μm, L/W = 1.7–4, with (1–) 2–6 transverse and 1–3 longitudinal or oblique septa. In addition, some juvenile conidia were produced in chains (Fig. 1).

At day 15 (Figs. 1–4), colonies became darker. Conidiophores were abundant, erect or ascending from both submerged and superficial hyphae, simple or branched, dilute golden brown, up to approximately 160–240 × 5–7 μm, septate, smooth or rarely inconspicuously roughened, bearing solitary conidia at 3–6 perforate geniculations or at 2–3 widely separated sporogenous pores. The mature conidia became finally broad obovoid or oblong- elliptical, dark reddish brown, smooth to inconspicuously roughened, approximately 32–52.5 × 8.5–14 μm (mean: 40.5 × 12.5 μm), L/W = 1.9–4.2 (mean: 2.8), with (1–) 3–8 transverse and 1–6 longitudinal or oblique septa in any or all of the transverse divisions, base broadly conical to rounded, apex broadly rounded, sometimes with a false beak due to germinatation, usually remaining solitary but somemtimes also in chains (Fig. 1). Unlike many other *Ulocladium spp*., in *U. capsciuma* only little variations of morphological characters were observable when grown on different media like PCA, PDA and V8 (data not shown).

U. capsiuma is morphologically distinct from similar species of *Ulocladium* by a combination of characters (Table 2): (i) Conidia reached a large size range, highly L/W ratio and more numerous transverse septa; (ii) more and longer conidiophores; (iii) most of the nearly mature conidia finally developed a broadly obovoid and oblong-elliptical shape; (iv) the mature conidia were often in chains on the natural or artificial substrates. In general, *U. capsicuma* has bigger conidia with higher L/W quotient and longer conidiophores than all other species of the genus (Table 2). In contrast to most other *Ulocladium*-species, the conidia of *U. capsicuma* are smooth and only rarely inconspicuously roughened.

Phylogenetic analysis

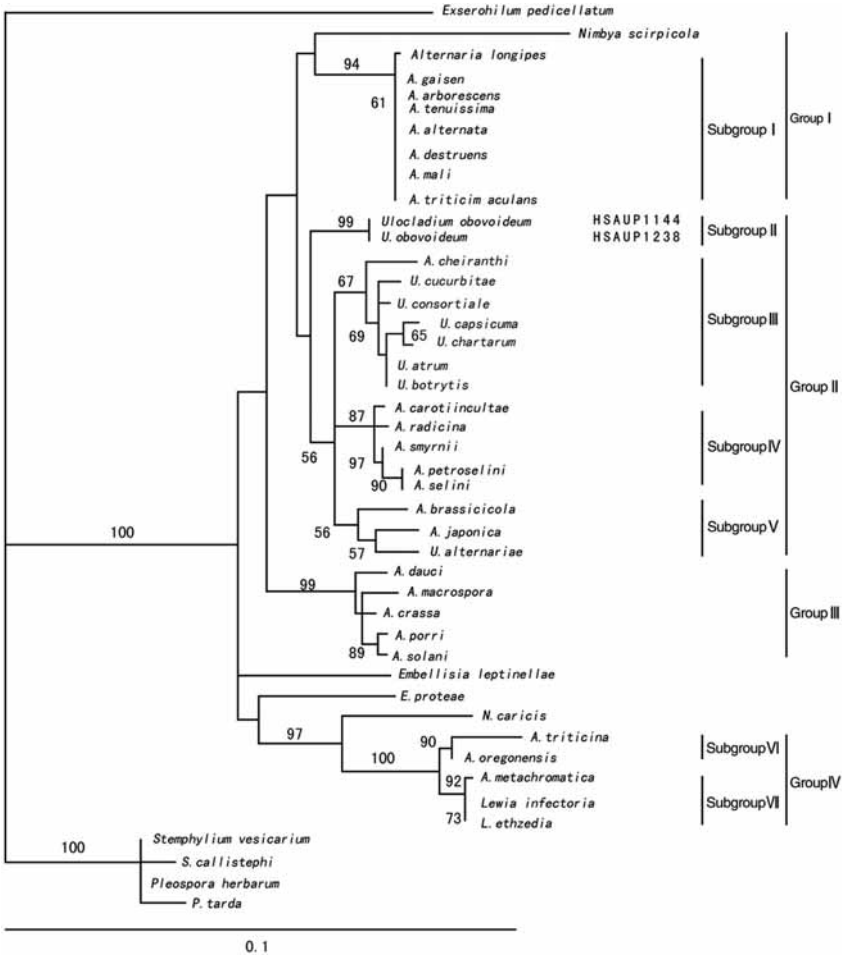
All phylogenetic analysis provided almost the same tree topology. The neighbor-joining tree offered a better resolution; phyloge-

Tab. 2. – Morphological characteristics of *Ulocladium*.

Species	Conidiophores	Conidia shape, chains	Conidial ornamentation	Conidia size	Transverse septa	Longitudinal or oblique septa
U. capsicuma F. Xue & X. G. Zhang sp. nov.	Up to 160–240 µm, 3–6 perforate geniculations	Obovoid or elliptical, broad obovoid and oblong-elliptical, mature conidia in chains	Smooth or rarely inconspicuously roughened	32–52.5 × 8.5–14 µm, av. 40.5 × 12.5 µm, L/W = 1.9–4.2, av. 2.8	(1–) 3–8	1–5
U. chartarum (Simmons 1967)	Up to 40–55 µm, 1–6 perforate geniculations	Obovoid and short elliptical, solitary or commonly in chains	Smooth to verrucose	24.6–32.4 × 12.3–21.6 µm, av. 26.4 × 14.7 µm, L/W = 1.5–2, av. 1.8	1–5	1-5
U. cucurbitae (Simmons 1982, 1998)	Up to 80–120 µm, 2–5 perforate geniculations	Obovoid, narrowly long-elliptical or cylindrical and spherical, not in chains	Distinctly verrucose	27–35 × 9–12 µm, av. 31 × 11 µm, L/W = 1.0–3.6, av. 2.3	(1–) 3–6	1-3
U. atrum (Preuss 1852)	Up to 110 µm, 8 perforate geniculations	Obovoid or broad elliptical, and subspherical or sarciniform, rarely in chains	Distinctly verrucose	16.5–19.8 × 13.2–18.7 µm, av. 18.6 × 16.0 µm, L/W = 1.0–1.3, av. 1.2	1–3	1–4

Tab. 2. – continued.

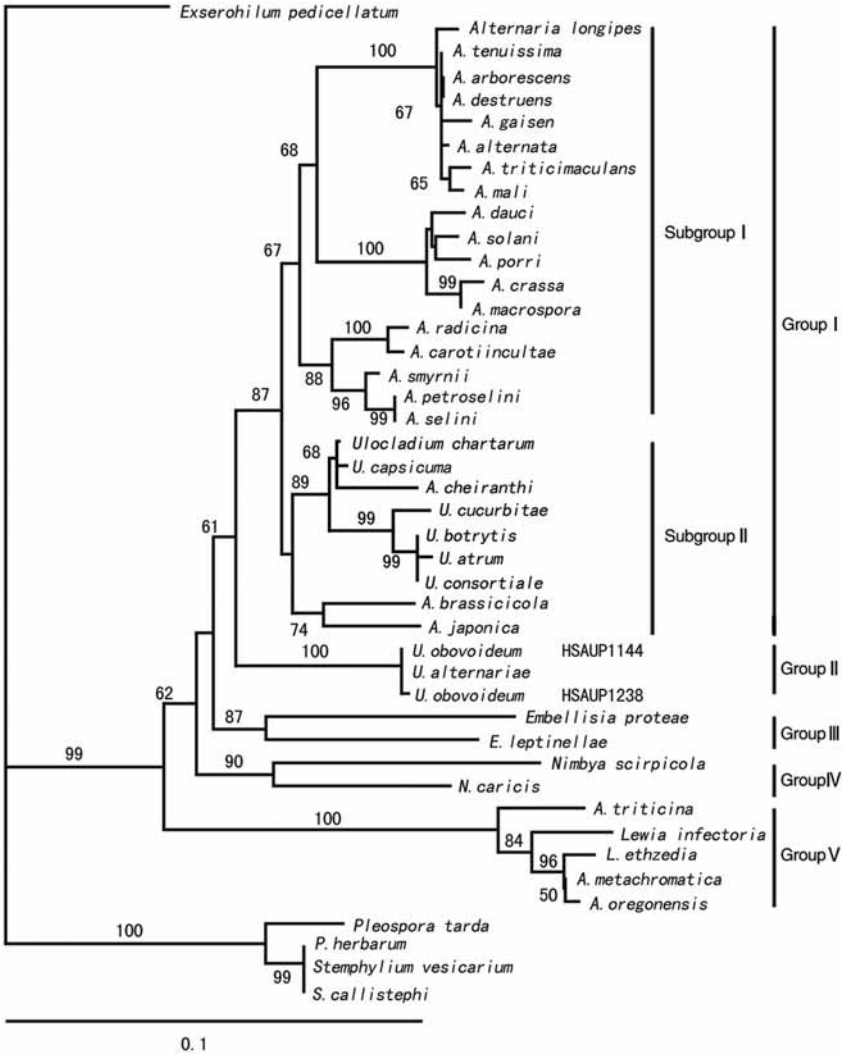
Species	Conidiophores	Conidia shape, chains	Conidial ornamentation	Conidia size	Transverse septa	Longitudinal or oblique septa
U. consortiale (Simmons 1967)	Up to 60 µm, 1–8 perforate geniculations	Obovoid or long elliptical, solitary or occasionally in chains	Smooth to pustulose or rarely verrucose	20–29 × 10.8–15.4 µm, av. 23.5 × 12.4 µm, L/W = 1.6–2.2, av. 1.9	1–5	1–6
U. botrytis (Preuss 1851)	Up to 80–100 µm, 2–10 perforate geniculations	Obovoid, broad ovoid or broad elliptical, not in chains	Rarely smooth, distinctly roughened to verrucose	13.9–24.6 × 7.7–15.4 µm, av. 18.5 × 11.5 µm, L/W = 1.2–2.3, av. 1.6	2–3	1–2
U. alternariae (Simmons 1967)	Up to 20 (seldom to 50) µm, 1 – perforate geniculations	Obovoid or broad elliptical, not in chains	Smooth or depressed pustulose	21.6–30.8 × 14.0–20 µm, av. 25.5 × 16 µm, L/W = 1.1–2, av. 1.6	3–5	1–2
U. obovoideum (Simmons 1967)	Up to 100 µm, 1–9 perforate geniculations	Obovoid, broad elliptical to broad obovoid, not in chains	Distinctly punctulate to roughened	23–28 × 14–18 µm, av. 25.5 × 16 µm, L/W = 1.5–1.6, av . 1.55	1 prominent in median	1–3



Figs. 12. Neighbor-joining tree generated from ITS1-5.8s rDNA-ITS2 sequences under the HKY85 substitution model. Values above branching nodes indicate bootstrap support (>50 %) obtained from bootstrap analysis with 1000 pseudoreplicates. The scale bar indicates the total nucleotide differences between taxa.

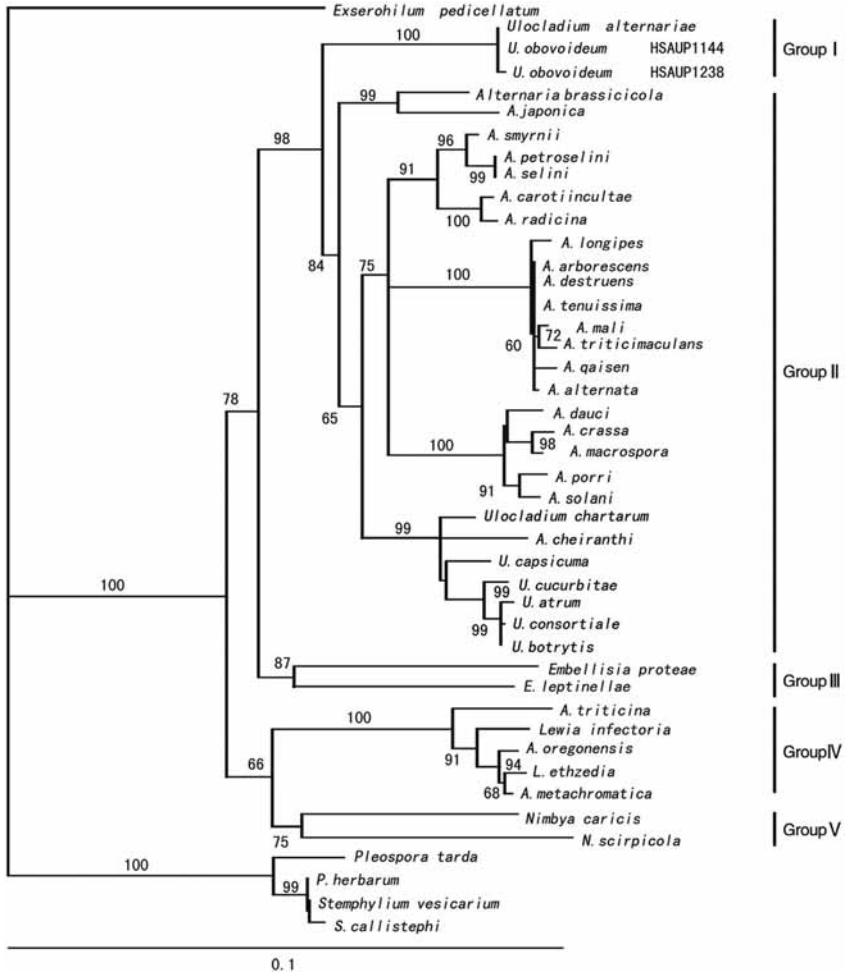
netic trees using the HKY85 distance algorithms are shown in Figs. 12–14.

The ITS1-5.8S -ITS2 rDNA dataset covered a total length of 549 base pairs. Neighbor-joining analysis with HKY85 distance setting of the ITS dataset yielded 311 least squares trees (Fig. 12) which differed primarily in minor changes within the *A. alternata* species-group. The *Ulocladium* branch was supported with a bootstrap value of 67 %, which was similar with the result of MP-analysis. *Ulocladium capsiuma* formed a subclade together with *U. chartarum* sup-



Figs. 13. Neighbour-joining tree generated from *gpd* sequences under the HKY85 substitution model. Values above branching nodes indicate bootstrap support (>50 %) obtained from bootstrap analysis with 1000 pseudoreplicates. The scale bar indicates the total nucleotide differences between taxa.

ported by a bootstrap value of 65 %. *Alternaria gaisen*, *A. mali* and *A. triticimaculans* clustered within the *A. alternata* species-group with a bootstrap support of 61%. *Alternaria. metachromatica*, *A. oregonensis*, *A. triticina* formed a clade together with the *Lewia infectoria* species-group supported by 100 % bootstrap value. The *A. brassicicola* species-group (*A. brassicicola*, *A. japonica* and *U. alter-*



Figs. 14. Neighbour-joining tree generated from combined ITS and *gpd* sequences under the HKY85 substitution model. Values above branching nodes indicate bootstrap support (>50 %) obtained from bootstrap analysis with 1000 pseudoreplicates. The scale bar indicates the total nucleotide differences between taxa.

nariae) was weakly supported (56 %), which was similar to the result of Pryor & Bigelow (2003). The *A. brassicicola* species-group as well as the *A. radicina* species-group were closer related to *Ulocladium* than to the *A. alternata* and *A. porri* species-group. The filament-beaked *A. porri* species-group formed a distinct clade with a bootstrap support of 99 %. *Nimbya scirpicola* (anamorph: *Macrospora scirpicola*) clustered together with the *A. alternata* species-group with bootstrap support less than 50 %, while *N. caricis* was included in the *Lewia infectoria* clade with a strong bootstrap value of 97 %.

Embellisia leptinellae and *E. proteae* did not constitute a distinct clade in the NJ analysis and formed only an unstable clade in MP analysis (tree not shown) with a weak bootstrap support (< 50 %).

The alignment of *gpd* sequences resulted in a 592-character dataset. Six least squares NJ trees (Fig. 13) had similar topology but were somewhat different from the ITS tree. *Ulocladium capsiuma* formed a clade together with *U. chartarum* and *Alternaria cheiranthi* with a bootstrap support of 68 %. *Ulocladium alternariae* was not included in the *Alternaria brassicicola* clade but clustered together with *U. obovoideum* with a 100 % bootstrap support. The relationship among the the *A. brassicicola, A. porri, A. alternata* and *A. radicina* species-groups and the *Ulocladium* group became explicit. The subclades brassicicola, porri, alternata and radicina species-groups composed a large clade together with the *Ulocladium* group with 87 % bootstrap support. *Ulocladium alternariae* and *U. obovoideum* formed a distinct clade. *Embellisia* spp. and *Nimbya* spp. clustered in distinct clades with strong bootstrap supports of 87 % and 90 % respectively. In the alternata species-group, *A. triticimaculans* and *A. mali* formed a subclade with 65 % support. A subclade in the *Lewia infectoria* species-group was formed by *Lewia ethzedia*, *A. metachromatica* and *A. oregonensis* with 94 % bootstrap support.

Results of the partition-homogeneity test (P = 0.145) indicated that the ITS and gdp gene trees reflect the same underlying phylogeny. Therefore these datasets were combined and analysed.

Neighbor-joining analysis of the combined dataset yielded only one least squares NJ tree (Fig. 14). The relationships revealed in the analysis of the combined dataset were similar to those observed in analysis of the *gpd* dataset with the exception that the *Nimbya* group formed one clade together with the *L. infectoria* species group (bootstrap support = 66 %). In the analysis of the combined dataset, *U. capsiuma* was grouped within the subclade composed of *U. cucurbitae*, *U. atrum*, *U. consortiale* and *U. botrytis* with a high bootstrap value of 99 %.

Taxonomy

Ulocladium capsicuma F. Xue & X. G. Zhang **sp. nov.** – Figs. 1.–11.

Ex culturis in agaro "potato-carrot" descripta. Coloniae cineraceous-brunneae vel atro-brunneae, rapide crescentes. Mycelium ex hyphis septatis, ramosis, subhyalinis vel dilute brunneis compositum. Conidiophora copiosa, recta vel acclivia, simplicia vel ramosa, ex lateribus hypharum submersarum aeriarumque oriunda, dilute aureo-brunnea, septata, usque ad 5–7 μm crassa, plerumque 160–240 μm longa, usque 3–6 genicula sporifera sed vulgo 2–3 remote disjuncta. Conidia initio anguste obovoidea vel oblongo-ellipsoidea, levia, denique crassa obovoidea vel ellipsoidea, dilute aureo-brunnea vel olivacea, tenue laevia vel verrucosa; 32–52.5 × 8.5–14 μm; septis transversalibus 3–8 et longitudinalibus obliquisve 1–6 per

partitionem transversam praedita; basim subacuta vel rotundata, ad apicem rotundata, plerumque solitaria, interdum rostrum spurium et sporiferum difficientia.

Holotypus. – Hab. in foliis vivis *Capsici annui* L. Burm.f. Yunnan Provincia, Sina, 8 VII 2003, leg. X. Feng, Holotypus: $HSAUPIII_0$0035.

Material examined. – Isolated from disease leaves of *Capsicum annuum* L., Dali, Yunnan province, China, by F. Xue. The type specimen (Holotypus: $HSAUPIII_0$0035 dried specimen and ex-type culture) deposited at the Herbarium of Plant Pathology of Shandong Agricultural University (HSAUP) culture collection.

Discussion

Phylogenetic analysis revealed the evolutionary relationship between the new species and other species of *Ulocladium*. *U. capsicuma* was included in the *Ulocladium* clade and was strongly supported in all parsimony and neighbor-joining analyses. The tree based on ITS datasets (Fig. 12) showed that *U. capsiuma* clustered together with *U. chartarum* and there were only minor nucleotide differences between their ITS sequences. The topology of the *gpd* tree (Fig. 13) was slightly different from the ITS tree in the placement of *U. capsiuma* and *U. chartarum*. *Alternaria cheiranthi* was included in the subclade of *U. capsiuma* and *U. chartarum*, though it does not possess the typical characters of *Ulocladium*. The conidiophores of *A. cheiranthi* arise singly or in groups without marked geniculation; the conidia are mostly solitary, seldom in chains of 2, 3 or more, variously shaped, often pyriform conidia, with numerous transverse, longitudinal and oblique septa. All these characters indicate that the conidia are not produced in dense clusters on closely geniculate conidiophores and the base of conidia is not initially conical. But the conidia of *A. cheiranthi* on the upper surface of culture medium are clearly more abundant than in other species of *Alternaria*, which is somewhat similar with *Ulocladium*.

The combined tree (Fig. 14) provided strong bootstrap support (99 %) for the *Ulocladium* group and the placement of the new taxon was shifted from the moderately supported subclade of *U. chartarum* (65 % in ITS tree and 68 % in *gpd* tree) to a subclade containing *U. cucurbitae*, *U. atrum*, *U. consortiale* and *U. botrytis*, which was only weakly-supported (< 50 %). However, the high bootstrap support values may not be treated as definite values because of high degree of sequence identity (> 99 %) in *Ulocladium* sequences (Pryor & Bigelow 2003). In parsimony analysis, *U. capsiuma* was always clustered with *U. chartarum* and the subclade composed of *U. cucurbitae*, *U. atrum*, *U. consortiale* and *U. botrytis* was well supported. This result suggests that the two *Ulocladium* subclades mentioned above can be recognized as monophyletic in phylogenetic analysis. The topological change in the *Ulocladium* group is due to the high degree of sequence identity in *Ulocladium* sequences.

Although molecular phylogenies are unable to fully resolve relationships within closely related fungus, they provide insights into the current classification of genera or species based on existing morphological features. It appears that diagnostic characters displayed by *Ulocladium* and some distinct monophyletic groups of *Alternaria*, such as the alternata group and infectoria group, might be of some taxonomic distinction. We suggest that *Ulocladium* should be maintained as a distinct genus and the characters that circumscribe this genus should be revised. The taxonomic placement of *U. alternariae* and *U. obovoideum* should be changed so that new species could be accommodated in *Ulocladium* genus appropriately. The viewpoint of Pryor & Bigelow (2003) that the infectoria species-group which is phylogenetically basal to both the *Nimbya* and *Embellisia* clade would require genus designation also, should be recognized as an important change to the taxonomic structure of *Alternaria* species-group. However, at least three important questions need to be solved before any firm definition of a new genus can be drawn: 1) representative species that are usually considered as atypical species have to be identified; 2) more species should be included; and 3) the characters used as taxonomic criteria should be integrated as a systematic conception.

Simmons (1998) studied the *U. atrum* group. He recognized *U. multiforme* and *U. dauci*, which had been misidentified as *U. atrum*, as new species. With the increasing study of multiplex conidial morphology, it can be predicted that more new taxa which are similar to *U. atrum* will be recognized as new species. In addition, the type species *U. botrytis* was also included in the *U. atrum* group in phylogenetic analysis with a strong bootstrap support, which indicates that this group dominates the *Ulocladium* genus. As a new species, *U. capsiuma* appears to be an initial supplement to *U. chartarum* clade in phylogenetic trees, which provides a valuable information to understand the conception of *Ulcladium*.

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