

## Saprobic hyphomycetes from China: new records of *Ceratosporium* and *Tetraploa*

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**Abstract** – Five saprobic hyphomycetes with branched conidial arms, *Ceratosporium fuscescens*, *C. gracile*, *C. productum*, *C. verrucosum* and *Tetraploa aristata* var. *sacchari* are first recorded from mainland China. Descriptions and illustrations are provided for each taxon and comments on closely related genera and species are included.

***Iodosphaeria* / *Massarina* / staurosporous fungi / taxonomy**

### INTRODUCTION

During a continuing investigation of saprobic hyphomycetes on fallen leaves, decaying branches, stems and dead wood in forest environments from 1998 to 2009 (Zhao & Zhang, 2003, 2004a b, 2005a b; Zhao *et al.*, 2006, 2007, 2009), five taxa with branched conidia belonging to the genera *Ceratosporium* Schwein. and *Tetraploa* Berk. & Broome were collected. They are described here for the first time from mainland China, as well as compared and discussed with morphologically similar species. The specimens examined are deposited in HMAS (Herbarium of Mycology, Chinese Academy of Sciences) and HSAUP (Herbarium of Plant Pathology, Shandong Agricultural University).

*Ceratosporium* is mainly characterized by micronematous, flexuous, hyaline to pale brown conidiophores, monoblastic, integrated, intercalary, cylindrical, denticulate conidiogenous cells and conidia composed of a central cell and 2-3 divergent pluriseptate branches (Ellis, 1971). Sixteen species names are presently recorded under the genus (<http://www.indexfungorum.org/Names/Names.asp>, 1/1/2009). Hughes (1951) systematically studied the genus and described three species, one of them as new, while recombined or considered

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questionable another five taxa which type specimens were unavailable for examination. Subsequent and recent studies added ten further species to the genus (Rao & Rao, 1970; Holubová.-Jechová. 1988; Matsushima, 1971, 1975, 1981, 1993; Kirschner & Chen 2004). Samuels *et al.* (1987) first built the connection between teleomorphic Ascomycota *Iodosphaeria phyllophila* and *I. ripogoni* (Amphisphaeriaceae) with synanamorphs of *Selenosporella* and *Ceratospodium*.

*Tetraploa* is mainly characterized by micronematous, flexuous, hyaline to pale brown conidiophores, monoblastic, integrated, intercalary, cylindrical conidiogenous cells, and muriform conidia composed of 3-4 columns of cells with appendages (Ellis, 1971). Presently, more than 10 species have been referred to the genus (Ellis, 1949; Sharma, 1978; Arambarri *et al.*, 1987; Rifai *et al.*, 1988; Révay, 1993; Matsushima & Matsushima, 1996; Hatakeyama *et al.*, 2005; Pratibha & Bhat, 2008; Zhao *et al.*, 2009). Among them, only one species, *T. aristata* Berk. & Broome, is known to have a *Massarina* Sacc. (Massarinaceae, Ascomycota) teleomorph (Scheuer, 1991).

Other morphologically similar anamorphic genera producing columned or branched conidia, *Ceratosporella* Höhnelt, *Triposporium* Corda and *Actinocladium* Ehrenb. ex Pers., are comparable with *Ceratospodium* and *Tetraploa*. *Ceratosporella* usually has longer conidiophores and percurrent conidiogenous cells, while *Triposporium* produce percurrent, doliform or lageniform conidiogenous cells and star-like conidia, and *Actinocladium* has percurrent conidiogenous cells and radiate-armed conidia.

## MATERIALS AND METHODS

Field samples were placed separately into plastic or paper bags, taken to the laboratory, and stored in a refrigerator at 4°C before microscopic study or incubated in moist containers (plastic bags or boxes) at room temperature to induce sporulation. Humidity was maintained by adding moistened paper towels. The incubated samples were examined under stereo-microscopes to detect the presence of microfungi after 4-5 days and periodically for up to one month. A piece of mycelia with sporulating structures from natural substrates or agar cultures were picked up and transferred to a slide. Water mounts were used for all observations, measurements, line-drawings and photographs under a Nikon 80i microscope with DIC. Semi-permanent slides were prepared by using lactophenol oil as mounting media and sealed with neutral balsam.

## TAXONOMY

*Ceratospodium fuscescens* Schwein., Trans. Am. Phil. Soc. 4: 300, 1832 Fig. 1

Colonies on natural substrate effuse, hairy, scattered, blackish brown. Mycelium mostly superficial, composed of branched, septate, subhyaline, smooth, 3-4 µm wide hyphae. Conidiophores micronematous, flexuous, smooth. Conidiogenous cells monoblastic, integrated, intercalary, determinate, cylindrical or broad conical, pale brown. Conidia pleurogenous, solitary, dry, composed of a central cell and 2-3 divergent arms; arms smooth, 85-150 µm long, 12-16 µm wide

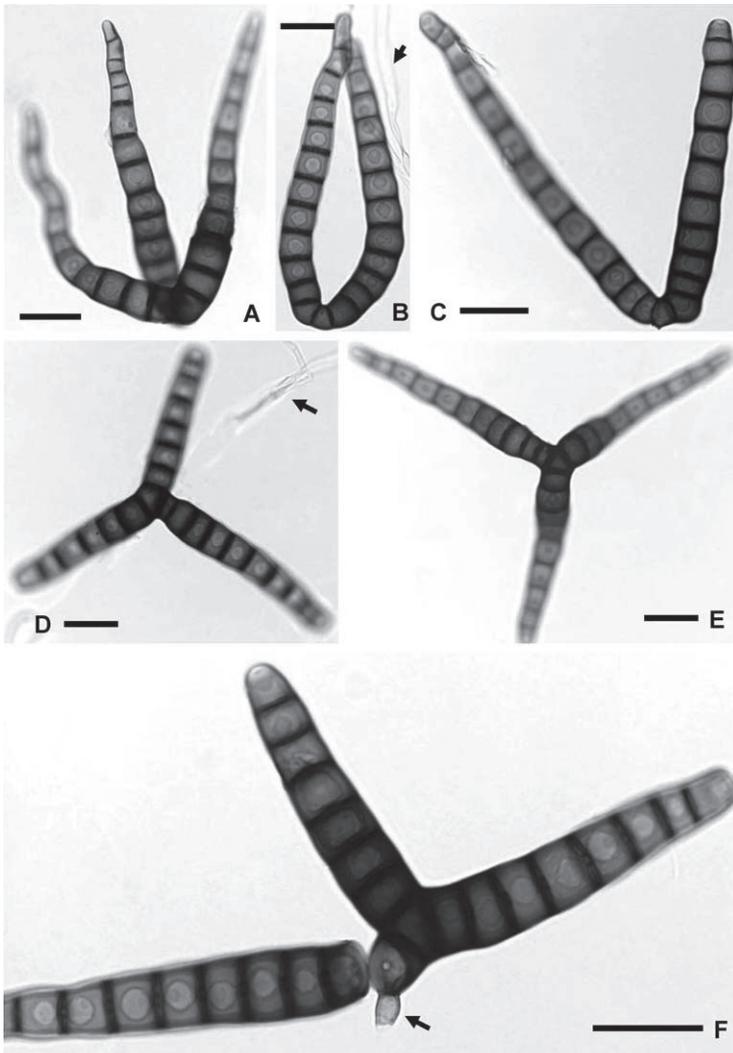


Fig. 1. *Ceratosporium fuscescens* (HMAS 98752): A-F. Hyphae (arrows B, D) and conidia from natural substrate. Bars = 20  $\mu$ m. F. Arrow showing detached conidium with partial conidiogenous cell.

at the dark brown base, tapering to a rounded, pale brown apex, 4-9  $\mu$ m wide, 6-14-septate, usually constricted at the septa, internal cell-like lumina usually visible.

Material examined: On dead twigs, Qianshan mountain, Liaoning Province, 22 Sep. 2004, coll. G.Z. Zhao, HMAS 98752 (= ZGZII<sub>04</sub>152, the collection number).

*C. fuscescens*, the type species of the genus, is widely distributed on dead wood and bark of various kinds of tree. Slight differences usually occur between collections from different substrates or localities. Hughes (1951) redescribed *C. fuscescens* based on six collections preserved in IMI and cited conidial arms of

120-210  $\mu\text{m}$  in length. Ichinoe (1970) described a Japanese collection with conidial arms measuring 90-138  $\mu\text{m}$  long. The Chinese collection has shorter conidial arms compared with the specimens revised by Hughes.

***Ceratosporium gracile*** Matsush. Matsush. Mycol. Mem. 2: 3, 1981 Fig. 2

Colonies on natural substrate effuse, punctiform, scattered, olivaceous brown to blackish brown. Mycelium partly superficial and partly immersed, composed of branched, septate, brown, smooth, 5-7  $\mu\text{m}$  wide hyphae. Conidiophores short, macronematous, simple, singly, erect, straight or slightly flexuous, smooth, thick-walled, dark brown, up to 15  $\mu\text{m}$  long, 6-7  $\mu\text{m}$  wide. Conidiogenous cells monoblastic, cylindrical, brown, integrated, terminal or intercalary, determinate. Conidia holoblastic, acrogenous or pleurogenous, solitary, dry, composed of 3 (rarely 2) columns of cells and divergent branches, mostly with 3-5 cells in each column, 30-50  $\times$  22-26  $\mu\text{m}$ , brown to dark brown; basal cell persistent, protuberant, brown to dark brown, cuneiform, truncate at base, 4.5-5.5  $\mu\text{m}$  wide, seceding schizolytically; branches smooth, up to 170  $\mu\text{m}$  long, 8-12  $\mu\text{m}$  wide at the yellowish brown base, tapering to a pale brown apex, 2-3.5  $\mu\text{m}$  wide, 8-15-septate.

Material examined: On decaying branches, Tongbiguan, Gaoligongshan mountain, Yunnan Province, 18 Oct. 2003, coll. G.Z. Zhao, HMAS 90311 (= ZGZI<sub>03</sub>129).

*C. gracile* is close to *Ceratosporium rilstonii* S. Hughes (Hughes, 1951) in having 3-armed conidia, basal cell with a scar and very similar dimensions, but conidiophores of *C. rilstonii* are shorter (up to 5  $\mu\text{m}$ ) and conidial arms are wider at the apex (6-8  $\mu\text{m}$ ). Another species *C. indicum* V.G. Rao & D. Rao (Rao & Rao, 1970, as "*indica*"), is also morphologically similar to *C. gracile* but differs in having very long conidial arms (up to 378  $\mu\text{m}$ ).

*C. gracile* also resembles *Dwibahubeeja indica* N. Srivastava, A.K.Srivastava & Kamal (Srivastava *et al.*, 1995) in conidiophores and conidial features as well as conidial ontogeny. It was originally recorded as a foliicolous fungus on *Calamus tenuis* (Arecaceae) from India. However, *D. indica* is distinct in having hyphopodia and characteristically two-armed, bifurcate conidia in which the basal cells of both arms are consistently fused. A synopsis of *C. gracile* and its similar species is given in Table 1.

The Chinese collection was compatible with the original specimen from Micronesia (Matsushima, 1981) in conidial morphology, texture and dimensions. However, we do not find any mention of conidiophores or conidiogenous cells in Matsushima's original description for further comparison.

***Ceratosporium productum*** Petch, Ann. R. Bot. Gdns Peradeniya, 3: 9, 1906 Fig. 3

Colonies on natural substrate effuse, punctiform, scattered, olivaceous brown to blackish brown. Mycelium mostly superficial, composed of branched, septate, subhyaline, smooth hyphae. Conidiophores micronematous, flexuous, smooth. Conidiogenous cells monoblastic, integrated, intercalary, determinate, cylindrical or broad conical, pale brown. Conidia holoblastic, pleurogenous, solitary, dry, composed of a central cell and 3-4 divergent branches; branches smooth, 50-180  $\mu\text{m}$  long, 8-14  $\mu\text{m}$  wide at the dark brown base, tapering to a thin, pale brown apex, 2.5-5  $\mu\text{m}$  wide, 4-14-septate, internal cell-like lumina usually invisible.

Material examined: On decaying branches, Huangshan mountain, Anhui Province, China, 10 Aug. 2002, coll. G. Z. Zhao, HSAUP<sub>02</sub>0952-3 (= ZGZII<sub>02</sub>152-3).

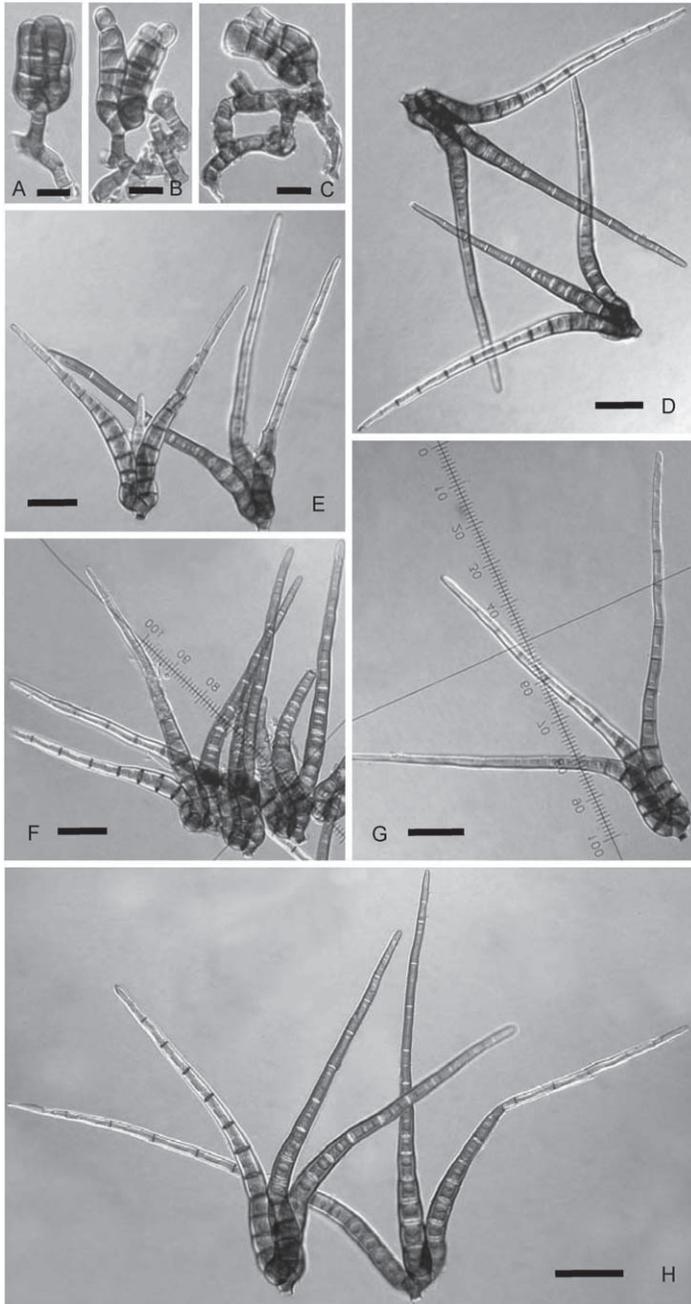


Fig. 2. *Ceratosporium gracile* (HMAS 90311): A-C Hyphae, conidiophores and developing conidia from natural substrate. Bars = 10  $\mu$ m. D-H. Mature conidia from natural substrate. Bars = 20  $\mu$ m.

Table 1. Synopsis of *Ceratosporium gracile* and its similar species

Species (Reference)	Conidiophores ( $\mu\text{m}$ )	Conidium morphology			
		Number of columns	Width of each column( $\mu\text{m}$ )	Length of columns/ arms ( $\mu\text{m}$ )	Number of septa in each column
<i>Ceratosporium gracile</i> (Matsushima, 1981)	undescribed	2-3	6-10	36-192	5-24
<i>Ceratosporium gracile</i> This paper	15 $\times$ 6-7	3, rarely 2	8-12	Up to 170	8-15
<i>Ceratosporium indicum</i> (Rao & Rao, 1970)	1.4-4.2 $\times$ 2.8-5.6	2-3, rarely 4	3.6-7.2	140-378	14-23
<i>Ceratosporium rilstonii</i> (Hughes, 1951)	2-5 $\times$ 4-6	2-3	14-17	130-190	8-14
<i>Dwibahubeeja indica</i> (Srivastava <i>et al.</i> , 1995)	undescribed	2	3-10	30-280	4-20
<i>Tetraploa aristata</i> var. <i>sacchari</i> (Sharma, 1978)	undescribed	2-3	5-6	45-350	3-12 (b spore; Ellis, 1949)
<i>Tetraploa aristata</i> var. <i>sacchari</i> This paper	indistinguished	3, rarely 2 or 4	6.5-8	60-180	9-14

Hughes (1951), when examining the type specimen of this species, doubted whether this fungus was possibly a *Tripospermum* Sp. for lacking 4-armed conidia. In the Chinese collection however, the distinct 4-armed conidia were observed. They fit well with Petch's original description except in lacking 2-armed conidia. Occurrence of 2 or 4-armed conidia may not be considered a stable feature.

*C. productum* is very similar to *C. fuscescens* in conidial morphology, but they can be distinguished by at least two features: first, the conidial branches in *C. fuscescens* are 2-3 and tapering to a blunt rounded apex, while those of *C. productum* are 3-4 and tapering to a more tender and fragile apex; secondly, cell-like lumina are usually distinct in *C. fuscescens*, but not in *C. productum*.

***Ceratosporium verrucosum*** R. Kirschner & Chee J. Chen,  
Mycologia 96: 918, 2004

Fig. 4

Colonies on natural substrate effuse, hairy, scattered, blackish brown. Mycelium partly superficial and partly immersed, composed of branched, septate, subhyaline, smooth, 2-3  $\mu\text{m}$  wide hyphae. Conidiophores micronematous, flexuous, smooth. Conidiogenous cells monoblastic, integrated, intercalary, determinate, pale brown. Conidia pleurogenous, solitary, dry, composed of a basal cell and 2-4 divergent arms; arms strongly verrucose, up to 49-89  $\mu\text{m}$  long, 12-14  $\mu\text{m}$  wide at the blackish brown base, tapering to a pale brown apex, 2.5-4  $\mu\text{m}$  wide, 6-11-septate, apical cells subhyaline, sometimes elongated, slightly curve.

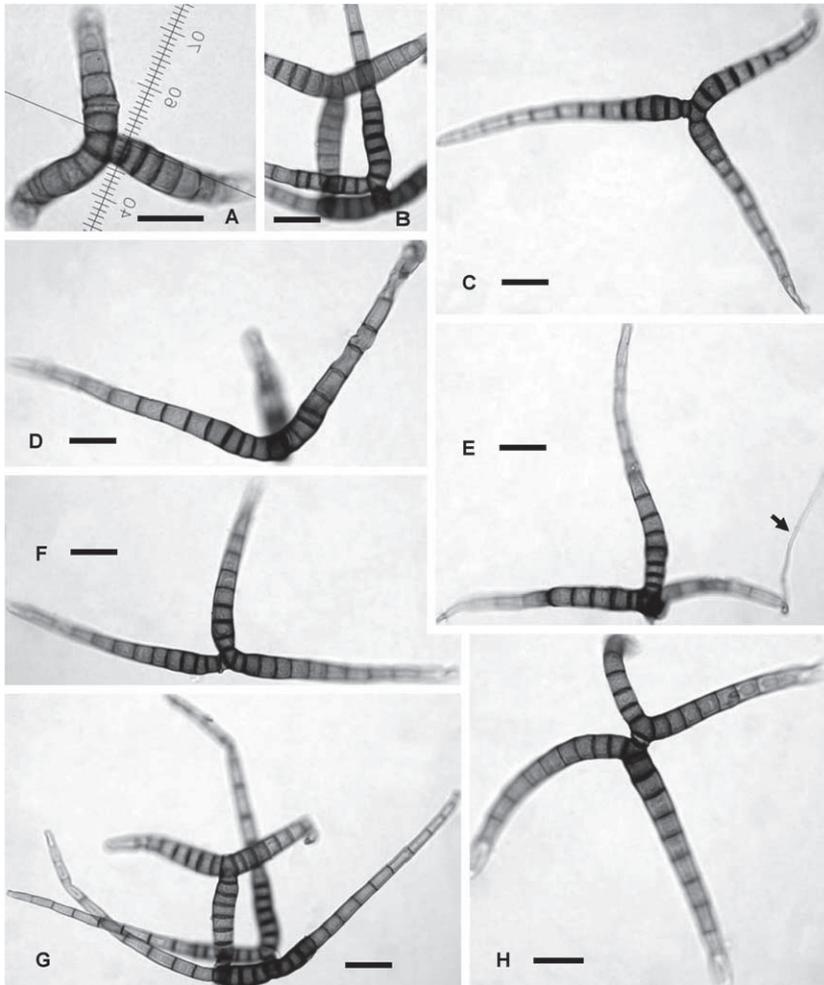


Fig. 3. *Ceratosporium productum* (HSAUP<sub>02</sub>0952-3): A-H. Conidia from natural substrate. Bars = 20  $\mu$ m. E. Arrow showing germinating hypha from conidial arm apical cell.

Materials examined: On dead bamboo culms, Huangshan mountain, Anhui province, China, 10 Aug. 2002, coll. G. Z. Zhao. HSAUP<sub>02</sub>0958-3 (= ZGZII<sub>02</sub>158-3); HSAUP<sub>02</sub>1038-1 (= ZGZII<sub>02</sub>238-1). On decaying bract of bamboo, Bawan, Gaoligongshan mountain, Yunnan Province, 14 Oct. 2003, coll. G. Z. Zhao, HMAS 90417 (= ZGZII<sub>03</sub>081).

*C. verrucosum* is distinct from other members of the genus in having strongly verrucose conidial arms, and it was also originally reported on dead bamboo culm from Taiwan (Kirshner & Chen, 2004). Among the three Chinese collections, the specimen HSAUP<sub>02</sub>1038-1 produces longer, thinner and sometimes slightly curved conidial arm apical cells, which are slightly different from Taiwan's specimen.



Fig. 4. *Ceratosporium verrucosum*: A-I. Conidia from natural substrate. A-F. Conidia from HSAUP0958-3; G-I. Conidia from HSAUP1038-1. A,C-I, Mature conidia. Bars = 20 µm. B. Developing conidium. Bar = 10 µm.

*Tetraploa aristata* var. *sacchari* N.D. Sharma, J. Indian. Bot. Soc. 57: 104, 1978

Fig. 5

Colonies on natural substrate effuse, punctiform, scattered, olivaceous brown to blackish brown. Mycelium superficial. Conidiophores and hyphae micronematous, branched and anastomosing to form a network, flexuous, hyaline

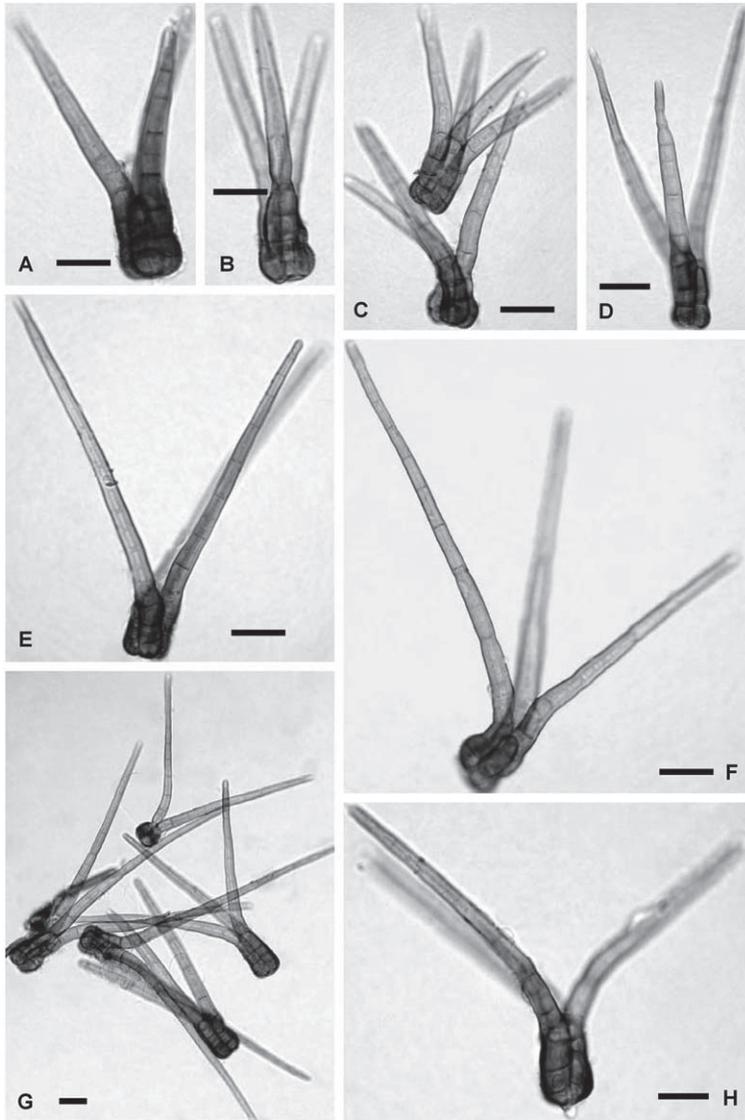


Fig. 5. *Tetraploa aristata* var. *sacchari* (HSAUP<sub>02</sub>0856-2): A-H. Conidia from natural substrate. Bars = 15 µm.

to pale yellowish brown. Conidiogenous cells monoblastic, integrated, intercalary, determinate, cylindrical. Conidia holoblastic, pleurogenous, solitary, dry, composed of 3 (rarely 2 or 4) columns of cells, mostly with 2-3 cells in each column, septa sometimes distinctly constricted; conidial body  $19-31 \times 14-20$  µm, brown to dark brown; the columns develop closely at the base, diverging apically and topped with hard setiform appendages in each column. Setose appendages pale brown except their brown base, smooth, up to 60-180 µm long, 6.5-8 µm wide at the base, 2-4 µm wide at the apex, 9-14-septate.

Material examined: On decaying stalk of bulrush, Huangshan mountain, Anhui Province, China, 10 Aug. 2002, coll. G. Z. Zhao, HSAUP<sub>02</sub>0856-2(= ZGZII<sub>02</sub>156-2).

Because this fungus produces secondary conidia in abundance, Sharma (1978) erected the variety *T. aristata* var. *sacchari*. Judging from Sharma's illustrations, the conidia mainly consist of 2-3 columns of cells, unlike those of 4 columns of cells in secondary conidia of *T. aristata* described by Ellis (1949). Sharma mentioned also that conidial body measures 12-15 × 12-14.5 μm, while the Chinese collection has larger conidial body (19-31 × 14-20 μm). We consider the difference due to dissimilar measurement criteria.

*T. aristata* var. *sacchari* shares a similar conidial morphology with *C. gracile*. They can be differentiated on the basis of conidiophores morphology and ontogeny. Conidiophores in *Ceratosporium* are irregularly branched, with branches often disposed in right-angles and denticulate conidiogenous cells, although they were not mentioned in *C. gracile* original description (Matsushima, 1981). As a result, detached conidia usually show a basal protruding cell. In *Tetraploa* however, the micronematous conidiophores are usually indistinct from the intercalary conidiogenous cells (Ellis, 1971), and detached conidia usually lack a basal protruding cells (Ellis, 1949; Sharma, 1978).

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