

A Mycoparasitic Ascomycete *Sypastospora parasitica* on the Entomopathogenic Fungus *Paecilomyces tenuipes* Growing in *Bombyx mori*

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While studying *Bombyx mori* "Zipnuae" inoculated with an entomopathogenic fungus, *Paecilomyces tenuipes*, a mycoparasitic hymenoascomycete with a long neck was isolated from the silkworm rearing room. The fungus was identified as *Sypastospora parasitica* which has been recognized as a hyperparasite on various moniliaceous hyphomycetes. A descriptive illustration is presented in this study.

KEYWORDS: Mycoparasite, *Paecilomyces tenuipes*, Silkworm, *Sypastospora parasitica*

During the cultivation of entomopathogenic fungi, *Paecilomyces tenuipes* (Peck) Samson, which is the authentic name of commercially known *P. japonica*, a mycoparasitic fungus with dark brown ascomata emerged to contaminate about 2,000 inoculated silkworm larvae and was identified as *Sypastospora parasitica* (Tul.) P. F. Cannon & D. Hawksw.

The genus *Sypastospora* P. F. Cannon & D. Hawksw., of Ceratostomataceae in the order Sordariales, was erected to accommodate *Melanospora* species having a long neck and cylindrical spores by Cannon and Hawksworth (1982) in their reappraisal of the genus *Melanospora* Corda. Both genera are characterized by the membranous ascomal wall, the evanescent asci and the release of immature ascospores into the ascomal cavity. The distinguishing features are ascomal neck structure, ostiolar setae and ascospores. The neck of *Sypastospora* consists of parallel hyphae instead of the pseudoparenchymatous cells of *Melanospora*. The ostiolar setae are not observed in *Sypastospora* but are present in *Melanospora*. *Sypastospora* has cylindrical to doliiform ascospores with truncate ends, while *Melanospora* has ellipsoid, but never cylindrical, ascospores.

This fungus was first described as *Sphaeronema parasitica* Tul. on *Isaria crassa* by Tulasne (1857). Eight years later, the brothers Tulasne relocated the species to *Melanospora* (Tulasne and Tulasne, 1865). However, Saccardo (1883) doubted the correctness of this disposition and suggested it to be *Ceratostoma* owing to the light-colored ascomata. Ellis and Everhart (1897) published the same fungus as *Ceratostoma biparasiticum* in North America and Keissler (1931) as *Naemosphaerella chalaroides* in Vienna. Doguet (1951) treated this species as a member of *Melanospora* in broad sense.

The species *Sypastospora parasitica* was the only member of the genus until Horie *et al.* (1986) reported the new species, *S. boninensis* Horie, Udagawa & P. F. Cannon from Japanese cultivated soil.

The isolate was collected from the silkworm rearing room in September, 1998 by the junior author, where fifth instars of *Bombyx mori* L. "Zipnuae" were inoculated with *Paecilomyces tenuipes* to do experimental research. The rearing room has been maintained at 24°C and 65% RH before the inoculation and at 20°C and 80% RH afterwards. A mycoparasite with its host fungus was isolated and kept on corn meal slants at 4°C until this study.

Three duplicates of the isolate were cultured in V8 juice agar with calcium carbonate. General observations, measurements and photography of characteristic structures were made on fresh material mounted in water or 70% ethanol. Sizes represent 25 measurements in ascospores and less than 10 in other structures. Photographs were taken with a Nikon FX-35DX on an Olympus BH-2 stereo microscope with normarski. The author citations follow Kirk and Ansell (1992).

Morphological and cultural characteristics were described and the isolate was stored in the Department of Sericulture and Entomology (JM1110), NIAST, RDA.

Sypastospora parasitica (Tul.) P. F. Cannon & D. Hawksw., 1982. *Bot. J. Linn. Soc.* 84: 152.

Figs. 1-12.

≡ *Sphaeronema parasitica* Tul., 1857. *Ann. Sci. Nat., Bot.* IV 8: 40.

≡ *Melanospora parasitica* (Tul.) Tul. & C. Tul., 1865. *Sel. Carp. Fung.* 3: 10.

= *Ceratostoma biparasiticum* Ellis & Everh., 1897. *Bull. Torrey Club* 24: 127.

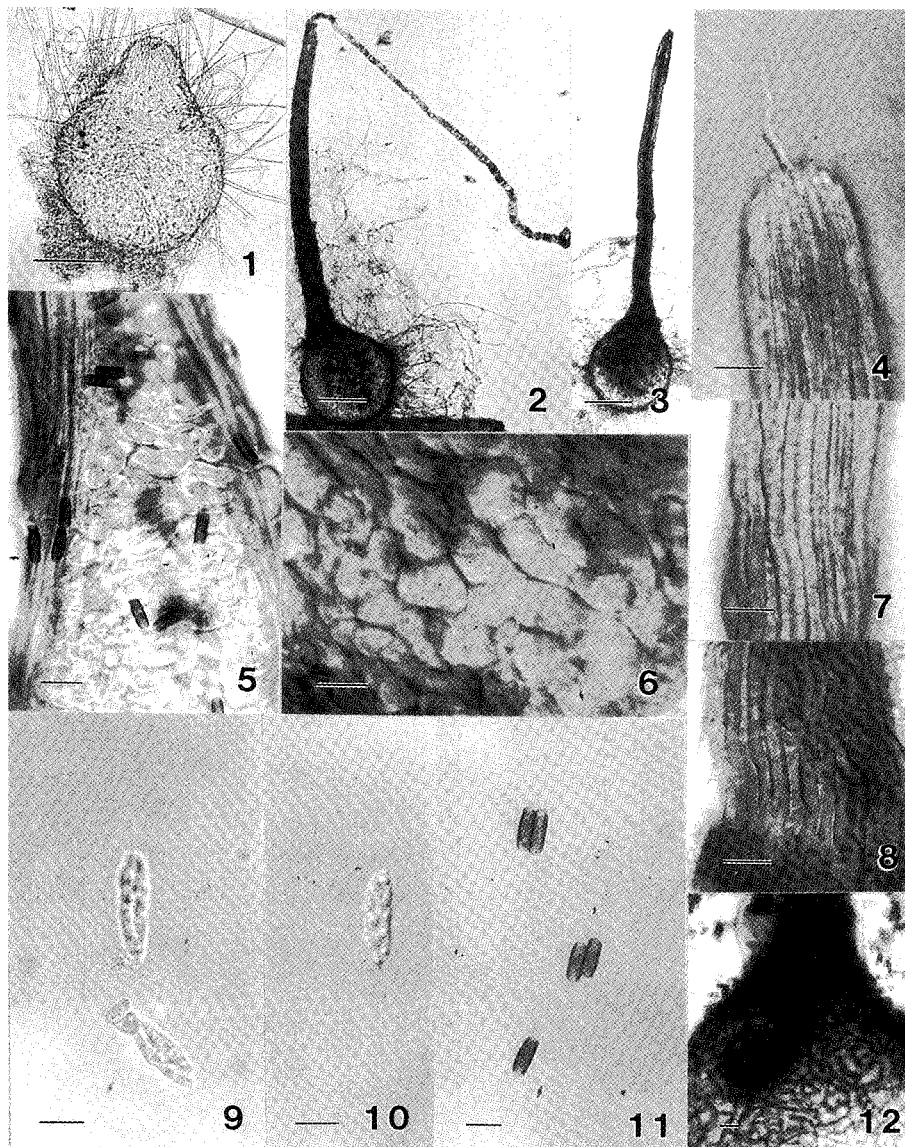
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= *Naemosphaerella chalaroides* Keissl., 1931. *Ann. Naturhist. Mus. Wien* 45: 295.

Colonies cottony, with aerial mycelium. Ascomata perithecial, superficial to partly immersed in the hyphae of the host fungus, subglobose to globose, light brown when young, dark brown at maturity, glabrous or with occasional hyphae emanating from the body wall when young, 150.2~175.9 μm in diameter, with a long cylindrical neck. Vegetative hyphae hyaline, aerial. Ascomal walls membranous, subtransparent, light brown, 6.4~8.6 μm thickness, *textura angularis*. Ascomal neck, straight or slightly curved, glabrous, composed of somewhat parallel hyphae, 511.6~1014.3(~1058.4) μm long,

44.1~61.7 μm wide at the base, 22.1~35.3 μm at the top, tip truncate to obtuse, without ostiolar setae. Individual hyphae in neck smooth, pale brown, hyphoid, 42.2~62.2 \times 2.2~4.4 μm . Asci in a fascicle, clavate, short-stipitate, biseriate, 22.7~34.1 \times 6.8~11.4 μm , evanescent at early stage, 8-spored. Ascospores 1-celled, cylindrical, pale to dark brown, smooth-walled, 4.5~9.1 \times 2.2~3.2 μm , 1- to 2- guttulate, apices truncate, 2 terminal germ pores.

The Korean material shows a wider range of spore length 4~9 μm than other isolates of 5~6 or 6~7 μm (Petch, 1938; Ellis and Everhart, 1897; Doguet, 1955), which phenomenon was also observed by Cannon and Hawksworth (1982). The length of ascomal neck sometimes reaches up to 1.5 mm



Figs. 1-6. Light micrographs of *Syspastospora parasitica* (JM1110). 1. Immature ascoma with hyphae emanating from ascomal walls. 2. Cirrus, a spore horn, from ascoma. 3. Ascomal neck and cavity filled with ascospores. 4. Obtuse tip of ascomal neck. 5. Internal structure of ascomal neck composed of parenchyma cells. 6. Ascomal wall of *textura angularis*. 7-8. Ascomal neck composed of individual hyphae. 9-10. Young asci having two-layered spores. 11. Mature ascospores. 12. Beginning of ascomal wall with dark intricated hyphae. Scale bars: 1-3 = 100 μm ; 4-12 = 10 μm .

with age.

This species was widely reported from several countries and particularly common on *Paecilomyces* Bainier species such as *P. tenuipes*, *P. farinosus* (Holm) Brown & Smith but also known on *Beauveria bassiana* (Bals.) Vuill., *Hirsutella floccosa* Speare and *Verticillium lecanii* (Zimm.) Vigas (Cannon and Hawksworth, 1982).

The isolate was originally identified by R. A. Samson on a visit to Seoul in November, 1999.

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