On the Specific Connection of *Cordyceps entomorrhiza* and *Tilachlidiopsis nigra*.

By

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With 1 Plate & 2 Text-figures.

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It has long been known about the close relation between Deuteromycetes (Fungi Imperfecti) and Ascomycetes.

Some fungi formerly treated as imperfect are found to be some stage in the life-histories of Ascomycetous fungi and others to be the conidial forms, although there are many species of imperfect fungi which remain to be determined or probably never exist their perfect stages.

When *Tilachlidiopsis nigra* of Stilbaceae was found in 1929, the writer presumed that it may be a conidial form of some *Cordyceps*. Ever since, he tried to search the perfect stage of that species, and collections were made in the field of Japan. In type locality, approximately fifty specimens have been obtained, although all of them were conidial form. In early summer of last year, it was the good fortune of the writer to collect two specimens which had both the synnemata and stromata on the same fruit-bodies among thirteen specimens collected at Mt. Takao, near of Tokyo. Thereafter, other collections were made frequently in the same locality from June to September, but never found again the stromatiferous fruit-bodies. One stromatic specimen is illustrated in the plate. This is surely *Tilachlidiopsis nigra*. Many conidiiferous branches are attached laterally on a stalk and one stromatic head at the top of it. The writer compared this with *Cordyceps entomorrhiza* [DICKSON] LINK with some conclusions. Before entering into comparison, the writer will make some notice about the latter.

As C. G. LLOYD (1913) and T. PETCH (1933) pointed out, *Cordyceps gracilis* DUR. et MONT. and *C. entomorrhiza* LINK had long been confused since BERKELEY's treatment. The former is widely distributed species which the writer also collected two times in Japan. On the contrary, the latter is a very rare one which was originally named from England and since never found in there. In France, there are some reports on this species with different specific names. TULASNE, in his epoch making work "Select. Fung. Carp." reported this species as *Torrubia cinerea* TUL. and

for *C. gracilis* he named erroneously as *C. entomorrhiza*. Also some reports are found from Germany.

Thus, this is confined to a part of Europe. This has two kinds of conidial stages, one of which is named *Hirsutella Eleutheratorum* (*Isaria Eleutheratorum*) as recognized by J. SCHROETER, G. LINDAU, W. MIGULA and others. These two forms occur on same insect body, which is like to the relation between *Cordyceps militaris* LINK and *Isaria farinosa* FR. Lately, PETCH reported that he saw the conidial branch attached on the same fruitbody with *C. entomorrhiza*. He named this as *Stilbella setiformis*.

The results of the writer's comparison between *C. entomorrhiza* and *T. nigra* are as follows:

1. Host relations. The hosts of these two species are necessarily confined to the insects belonging to Carabidae of Coleoptera. *Carabus nemorensis* and *Carabus hortensis* are known to be the hosts of *C. entomorrhiza*. Its conidial form "*Hirsutella Eleutheratorum*" is reported to grow on *Carabus* sp., *Calathus* sp., *Leptura* sp., and *Apion flavipes*. *T. nigra* springs from the larvae and imagoes of *Carabus insulicola*, *C. mayasanus* or *Damaster Lewisi*.

2. Stipe (Clava). Stipes of *C. entomorrhiza* are "cylindrical, bent back, about 4-6 cm. in length, and 1-2 mm. thick, black or blackish, fuscous, smooth and glabrous, but toward apices glauco-cinereous and delicately pulverulent". Usually only one stipe springs up from the head of the host larva or the upper surface of the frontier segment of the host imago. Stipes of *T. nigra* are cylindrical, very hard, about 3-7 cm. in length and 0.5-1.5 mm. in diam., wavelly bent, terete or somewhat compressed, black and smooth, laterally with many conidiiferous branches (synnemata). One to ten stipes spring up from the head or the segments of host.

3. Stromatic head. In *C. entomorrhiza*, the head is apically seated, obovate-globose, 3-4 mm. in diam., at first whitish-cinereous, becoming later pale fulvo-violaceous, with its surface mamillated with semi-protruding perithecia. In *T. nigra* (cf. Fig. 1; Fig. 2, D; Fig. A, C of plate) has one apically seated head, which is about 3 mm. in diam., obovate-globose, mamillated and black in its surface, with several synnemata among the protruding perithecia. Peridium is about 22 μ thick, composed of several layers of pseudoparenchymatous cells, dark brown. Substances of the head are composed of minute and compactly woven pseudoparenchymatous cells which have thinner walls than that of peridium, dark violet, when immersed in formalin discolouring to reddish-violet.

4. Perithecia. Perithecia of *C. entomorrhiza* are globose, thickly crowded, very obtuse. Spores are linear, straight, 1.5-2 μ broad, hyaline, segmented into numerous secondary spores which are about 7-10 μ in length.

The writer's specimen of *T. nigra* has its perithecia (Fig. 1) ovate with somewhat long necks, protruding their ostioles on the surface of head, 0.7–0.8 mm. long, and 0.3 mm. broad. The peripheral layer of its perithecium is similarly composed with that of *C. entomorrhiza*, but the cavity of perithecium of the former is filled with totally sterile and hyaline hyphae. In spite of the writer's prudent observation, no ascogenous hyphae and primary asci were seen.

5. Conidial stage. As already has been stated, *C. entomorrhiza* is reported to have two kinds of conidial forms, which are named "*Hirsutella Eleutheratorum* and *Stilbella setiformis*." According to PETCH, the fruit-bodies of the former arise "from the larvae either singly or in groups of up to a dozen. In general, they are about a centimetre long, linear, terete or irregularly flattened, simple, or expanded and branched at the apex, brown, becoming black, whitish towards the apex. Each clava or branch terminates in a small subglobose, or ovoid, or laterally flattened head, the shape of the head depending upon that of the stalk. Sometimes a larva bears one long clava, instead of numerous shorter ones. In one example, the insect bore a clava, 6 cm. high, slender below, expanding upwards and bifurcating at the apex into a flabelliform lobe on one side and a repeatedly branched stem on the other, the ultimate branches being slender and bearing small conidial heads". Conidia are ellipsoid, 6–7 μ long and 3.5 μ broad, hyaline (according to LINDAU), but PETCH pointed out that is much greater than that found in the examples in RABENHORST, *Fungi Europaei* No. 1218. *S. setiformis* "occur as lateral branches on the stems of the *Cordyceps* clavae. The head is waxy, and pale yellow or pale brown in the dried specimens. The conidiophores are linear, simple or branched, 24–40 μ high, 0.75 μ diam., arising in clusters

Fig. 1. Longitudinal section of stromatic head of *Tilachlidiopsis nigra*, $\times 30$.

from the apices of the stalk hyphae and forming a compact head. The conidia are terminal, minute, hyaline, oval, oblong-oval, or subpyriform, 1.5–3.5 $\times$ 0.75–2 μ , or globose, 1–2 μ diam." *T. nigra* is a *Stilbella* on the dark-coloured stalk. Its conidiiferous branches (synnemata or coremia) grow laterally or apically on the stalk, rarely in clusters, 3–8 mm. long and 0.3 mm. in diam., white, smooth, with one terminal conidial head. It is globose, sometimes compressed, 0.5–1 mm. in diam., white. The apically

seated one on stalk is more larger, but the same in its contexture. Conidiophores cover the head, and are cylindric, toward base thinner, $16-17 \times 2\mu$, each with one sterigma. Rarely two short conidiophores seat on common cylindrical hypha. Conidia (fig. B of plate) are cylindric, hyaline, with pointed base and obtuse end, $10-12 \times 1.7-2\mu$. The writer examined many spores and found that mingled with long one, very short spores are to be seen, especially on the specimen which has the sterile stroma, ranging their length from 7 to 4.2μ in length and 2.1 to 2.8μ in diam., just as the size of spores of *Stilbella setiformis*.

Fig. 2. Semi-diagrammatic drawings.

A *Hirsutella Elcutheratorum*. B *Cordyceps entomorrhiza*. C The same, with *Stilbella setiformis* as a lateral branch. D *Tilachlidiopsis nigra* with one stromatic head. E The same, two of them having one large conidial head on each stipe.

Conclusions.

The specimen of *Tilachlidiopsis nigra* which has the sterile stroma is the unique connecting link between this species and *Cordyceps entomorrhiza*. These two species has the identical hosts and clavae. Also, in respect of the stromatic head and conidial stage, they have some affinity with each other. The writer supposes either of the two has derived from the other. In this case, the writer would rather call them the "connected species" than the "same species". This fact is not always indicating that the genus *Tilachlidiopsis* is one of the conidial forms of *Cordyceps*.

Cordyceps entomorrhiza [DICKSON]. LINK, Handb. III, p. 347 (1833);

FR. Summa Veg. Scand. p. 381 (1849); SACC. Syll. Fung. II, p. 567 (1883); LLOYD, Myc. Writ. IV, Let. 47, p. 15 (1913) et Let. 51, p. 4 (1914) et VI, p. 915, pl. 143, fig. 1633 (1920); PETCH in Trans. Brit. Myc. Soc. XVIII, p. 54 (1933); MAINS in Proc. Amer. Phil. Soc. LXXIV, p. 264, pl. III, fig. c (1934).

Syn. *Sphaeria entomorrhiza* [DICKSON, Plant. Crypt. Brit., Fasc. I, p. 22, pl. III, fig. 3 (1785)] FR. Syst. Myc. II, p. 324 (1822).

Torrubia cinerea TUL. Select. Fung. Carp. I, p. 61 (1861) et III, p. 15, pl. I, fig. 11 (1865).

Cordyceps cinerea (TUL.) SACC. Mich. I, p. 320 (1879) et Syll. Fung. II, p. 570 (1883); COOKE, Veg. Wasp. p. 73, pl. I, fig. 6 (1892); MASSEE in Ann. Bot. IX, p. 152 (1895); LINDAU in Engl. Pflz. Fam. I, 1, p. 369, fig. 246 E (1897).

Cordyceps carabi QUÉL. in Assoc. franç. Avanc. Sc. XXI Suppl. p. 7, pl. IV, fig. 19 (1897); SACC. Syll. Fung. XIV, p. 661 (1899); MIGULA, Krypt. Fl. III, pars 3, p. 765 (1913).

Torrubia Eleutheratorum [NEES] SCHROET. in Krypt. Fl. Schles. III, pars 2, p. 277 (1908) (pro parte).

Cordyceps Eleutheratorum [NEES] MIGULA, Krypt. Fl. III, pars 3, p. 765 (1913) (pro parte).

Hab. Among fallen leaves of *Fagus*, on a shady slope of the woods (by TULASNE).

England (Type locality):

(Host): *Carabus nemorensis* and *Carabus hortensis*.

Distr. Europe: England, France, Germany.

Conidial Form I (On the fruitbodies of perfect stage).

*Stlbella setiformis** [VAHL] PETCH in Trans. Brit. Myc. Soc. XVIII, p. 55 (1933).

Syn. *Clavaria setiformis* VAHL in Naturhist. Selsk. Skrivt. II, p. 50 (1793).

Conidial Form II (Frequently accompanied with perfect stage).

*Hirsutella Eleutheratorum** [NEES] PETCH in Naturhist. (1932) p. 45 et in Trans. Brit. Myc. Soc. XVIII, p. 35 (1933).

Syn. *Isaria Eleutheratorum* [NEES, Syst. p. 86, fig. 84 (1817)] FR. Syst. Myc. III, p. 274 (1829); SACC. Syll. Fung. IV, p. 587 (1886); COOKE, Veg. Wasp. p. 74 (1892); LINDAU in Rabenhorst, Krypt. Fl. IX, p. 325 (1910).

Torrubia Eleutheratorum [NEES] SCHROET, l.c. (pro parte).

Cordyceps Eleutheratorum [NEES] MIGULA, l.c. (pro parte).

Hab. Germany (Type locality):

(Host): *Carabus* sp., *Calathus* sp., *Leptura* sp., and *Apion flavipes*.

Distr. Europe: Germany, Austria, Belgium, Italy, France.

Connected Species.

Tilachlidiopsis nigra YAKUSHIJI et KUMAZAWA in Bot. Mag. Tokyo XLIV, p. 461 (1930).

Syn. *Isaria nigra* YAKUSHIJI et KUMAZAWA l.c. p. 42 et 73.

Nom. Jap. *Osamusi-take*.

Hab. On the ground under deciduous trees.

Japan:

Prov. Musasi; Tokyo, Bot. Gard. Imp. Univ. (YAKUSHIJI et KUMAZAWA, Jul.-Aug. 1929; KOBAYASI, Jul.-Aug.-Oct. 1929-1936), Mt. Takao (KOBAYASI Jun.-Jul. 1936).

Prov. Kaga: Kanazawa (specim. in Herb. 4th High School).

(Host): *Carabus insulicola* CHAUD. "*Awo-osamusi*". (Coll. in Bot. Gard. Imp. Univ. and at Kanazawa).

Carabus mayasanus BATES. "*Mayasan-osamusi*". (Coll. at Mt. Takao, det. by MASAYO KATÔ).

Damaster Lewisii RYE. "*Ko-maimaikaburi*". (Coll. at Mt. Takao, det. by HARUO TAKASIMA).

Distr. Japan (endemic).

- * Now I retain the two names "*Stilbella setiformis* PETCH and *Hirsutella Eleutheratorum* PETCH" for facultative use, which, strictly speaking, should be rejected, following to the rules of nomenclature.

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Explanation of Plate II.

Tilachlidiopsis nigra.

- A Fruitbodies on various hosts. $\times 18/23$.
Three of left growing on *Carabus mayasanus*, lower left one having stromatic head. Upper right one growing on *Damaster Lewisii*, others on imago and larvae of *Carabus insulicola*.
- B A conidiophore (left) and several conidia of various sizes. $\times 4000$.
- C A stromatic head and several conidial branches. $\times 5$.
-

A

B

C

Y. KOBAYASI: On the Specific Connection of *Cordyceps entomorrhiza*
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KOBAYASI phot. & del.