

Shun-ichi UDAGAWA* & Yoshikazu HORIE** : *Diplogelasinospora* and its conidial state

宇田川俊一*・堀江義一** : ディプロゲラシノスポラ
とその分生子世代について

Our information on the relations between cleistothecial Ascomycetes (Plectomycetes) and their corresponding Pyrenomycetes has been expanded remarkably with recent discoveries (Cain, 1956; Cain, 1961; Lundqvist, 1967; Aue, et al., 1969; Robison, 1970; and Malloch and Cain, 1971). Even for Discomycetes, it seems almost possible to be connected with several cleistocarpous genera of the Eotterfeziaceae such as *Cleistothelebolus*, *Lasiobolidium*, and *Orbicula* (Malloch and Cain, 1971). Although most of such cleistothecial Ascomycetes were recognized as intermediate groups in the evolutionary line of Ascomycetes and often tentatively placed in the Eurotiaceae, it is generally agreed that several separating taxa at the genus level within the same family were established to accommodate members of these intermediate groups, separating them out from the original genera.

In the process of checking up a lot of ascomycetous fungi isolated from Japanese soil samples, collecting in 1971 has yielded two interesting isolates in the genus *Diplogelasinospora* Cain (1961), which is ascribed to one of such examples. One is known previously as type species from a single collection and the other is considered new because of its morphologically distinctive ascospores. Cultures of these collections on common laboratory media showed that it produced simple conidial structures in some abundance, although Cain did not report any type of conidia when he described the type species *D. princeps*. In addition to reporting isolation of *D. princeps* from Japan, this paper presents the description of the new *Diplogelasinospora* and discusses on related aspect of the new information including associated conidial state to other sordariaceous Plectomycetes.

* Department of Microbiology, National Institute of Hygienic Sciences, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158. 国立衛生試験所.

** Institute of Food Microbiology, Chiba University, Izumi-cho 3-chome, Narashino-shi, Chiba-ken 275. 千葉大学腐敗研究所.

Diplogelasinospora grovesii Udagawa et Horie sp. nov. (Figs. 1, 4, 5)

Cleistotheciis sparsis vel in parvus catervas congregatis, superficialibus vel parum immersis, nigris, sphaericis vel subsphaericis, 320-360 μ diam., dense pilosis; pilis plus minusve flexuosis, olivaceo-brunneis, septatis, levibus, basi 4-5 μ latis. Peridio brunneo, opaco, membranaceo vel parum coriaceo; cellulis externis crassis, obscure angulatis, 6-12 μ diam.; cellulis internis hyalinis. Ascis octosporis, cylindraceutis vel cylindraceuto-clavatis, 155-160 \times 14-16 (-18) μ , contractis et superne rotundatis vel parum truncatis, ad apicem cum annulo indistincto praeditis, in stipitem 25-35 μ longum et sinuatum inferne attenuatis, evanescentibus, in faciculis laxe dispositis; paraphysibus hyalinis, tubularibus vel moniliformibus, septatis, 6-10 μ diam. Ascosporis irregulariter uniseriatis, primo hyalinis, guttulatis, unicellularibus, ellipsoideis, deinde transverse uniseptatis, ad septum non constrictis, omnino (22-) 24-28 (-33) μ longis. Cellula inferiore (sed interdum superiore vel raro duabus) postremo olivaceo-brunnea vel atro-brunnea, opaca, (13-) 16-20 \times 10-14 μ , cum pagina levi vel subtiliter reticulata; foramine germinali indistincto, rotundo, 1.5 μ diam., ad apicem sito. Cellula superiore hyalina, paene levi, extremum contracta et collapsa. Vagina gelatinosa tenui.

Mycelio hyalino vel olivaceo-brunneo, ramoso, septato, 1-5 μ diam., levi vel minute aspero, prope septum plerumque inflato. Neque conidiophoris conspectis. Conidiis (arthrosporis) fere intercalaribus, hyalinis, oblongis vel cylindraceutis, 4-15 \times 1.5-4 μ , utrinque truncatis, levibus, in catenis longis sed saepe disruptentibus. Aleuriosporis solitariis, ex hyphis aereis satis frequenter oriundis, ovatis, 6-7 \times 5-5.5 μ .

Culturis in agaro cum decocto tuberorum et carota effusis, floccosis, comparate tenuibus, cleistotheciis et fructificationibus conidicis abundantibus, griseo-olivaceis; reverso olivaceo-nigro.

Typus: In culturis ex solo, Nakashibetsu-machi, Shibetsu-gun, Hokkaido-Pref., Sept. 19, 1970, NHL 2502.

Cleistothecia scattered or in small groups, superficial or slightly immersed, black, spherical to subspherical, 320-360 μ in diam., densely covered with more or less flexuous, olivaceous brown, septate, smooth hyphae measuring 4-5 μ in diam. at the base. Peridium of cleistothecium brown, opaque, 20-50 μ thick, membranaceous to slightly coriaceous, outer layer consisting of thick-walled, obscurely angular cells measuring 6-12 μ in diam.,

inner layer hyaline, of textura angularis. Asci 8-spored, cylindrical to cylindrical-clavate, 155-160x14-16 (-18) μ , contracted and rounded or slightly

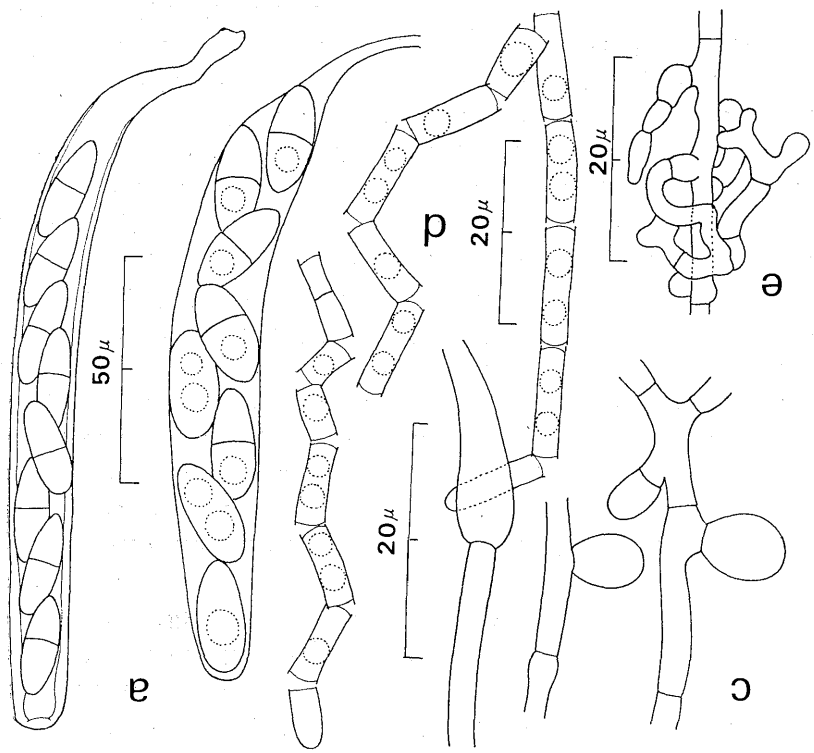
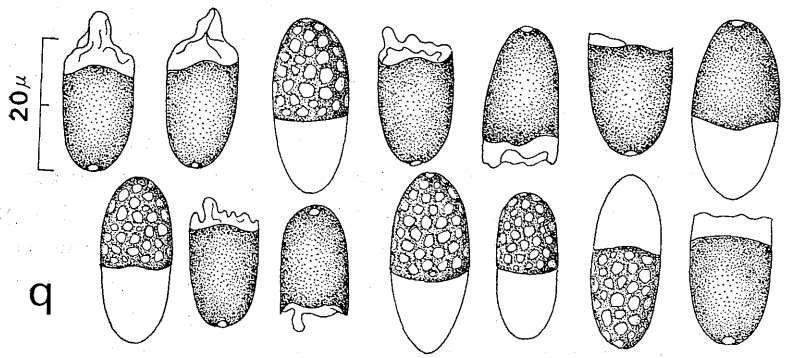


Fig. 1. *Diplogelastinospora grovesii*. a. Asci. b. Ascospores. c. Aleurtospores and mycelium. d. Arthrospores. e. Ascogarp initial.

truncate above, with an indistinct ring-like thickening near the apex, tapering below into a short, sinuous stipe measuring 25-35 μ in length, evanescent, forming a loose fascicle from the base of cleistothecium, mixed with hyaline, tubular to moniliform, septate paraphyses measuring 6-10 μ in diam. Ascospores irregularly uniseriate, rather oblique in arrangement, at first hyaline, guttulate, one-celled, ellipsoid, with both ends rounded or slightly apiculate, then becoming transversely uniseptate, without constriction at the septum, totally (22-) 24-28 (-33) μ in length; lower cell (occasionally upper cell or rarely both cells) changing through olivaceous brown to dark brown and opaque, (13-) 16-20 \times 10-14 μ , with surface smooth to very finely reticulate and walls covered by inconspicuous low ridges, 1-2 μ or more in width of intervening hollow, upper cell of ascospore remaining hyaline, almost smooth-walled, finally empty and contracted into a collapsed pedicel; germinal pore indistinct, circular, 1.5 μ diam., located at the distal end of the dark cell; gelatinous envelope narrow.

Cleistothecial initials arise from two or more different parent hyphae as morphologically similar side branches, then coiling several times about one another.

Mycelium hyaline to olivaceous brown, branched, septate, 1-5 μ in diam., smooth to minutely roughened, often swollen near the septum up to 6-8.5 μ . Conidial structures of arthrospore- and aleuriospore-type. Conidiophores lacking or not distinctly differentiated from vegetative hyphae. Conidia mostly borne as an intercalary cell, hyaline, oblong to cylindrical, 4-15 \times 1.5-4 μ , with both ends truncated, smooth-walled, often sliding into a slimy mass measuring 12-20 μ in diam.; solitary aleuriospores formed fairly frequently along the hyphae, ovate, 6-7 \times 5-5.5 μ .

Cultures on potato-carrot agar spreading broadly, floccose, comparatively thin, developing abundant black cleistothecia on the agar surface, partially obscured by a limited development of aerial hyphae, grayish olive to dark olive gray, conidial structures abundantly produced throughout the entire colony but not affecting the colony appearance; reverse olive black, often assuming a slight pinkish tint. At 37°C, only very slight growth.

Habitat: on soil of beet-field, Nakashibetsu-machi, Shibetsu-gun, Hokkaido-Pref., Sept. 19, 1970.

Holotype: NHL 2502, has been preserved at the Herbarium, National

Institute of Hygienic Sciences, Tokyo. Subcultures derived from the type have been deposited in the culture collection of the Institute for Fermentation, Osaka, and the Centraalbureau voor Schimmelcultures, Baarn.

In cultural appearance, *D. grovesii* is essentially indistinguishable from *D. princeps*. Microscopically, however, the longer and finely reticulate dark cell of ascospores would suffice for the identification of new taxon. In contrast to the definitely pitted ascospores of *D. princeps*, the pits in the ascospore wall are hardly seen but this appears to be a consequence of the reduced tendency to develop a completely smooth-walled character.

The species is named after the late Dr. J. W. Groves, who was the first collector of *Diplogelasinospora*.

Diplogelasinospora princeps Cain, in Can. J. Bot. 39 : 1670. 1961. (Figs. 2, 3)

Cleistothecia superficial, spherical to subspherical, 325–400 μ in diam., dark olivaceous brown to olive black, covered with flexuous, septate, brown, hyphal-like hairs, measuring 4–5 μ in diam. at base. Peridium membranaceous to slightly coriaceous, late becoming cephalothecoid, dark olivaceous brown to dark brown, consisting of angular, outer cells, 6–12 μ in diam., at maturity breaking. Asci 8-spored, cylindrical, 160–200 \times 16–20 μ , with a short stipe, soon evanescent. Paraphyses numerous, more or less monilioid, swollen up to 10 μ in diam. Ascospores irregularly uniseriate, at first hyaline and elliptical, becoming two-celled by the formation of a single, median transverse septum, more or less constricted, totally 20–27 \times 10–15 μ , with both ends rounded, with surfaces in both cells marked by conspicuous pits about 1–1.5 (–2) μ in diam.; lower cell (or sometimes upper cell) becoming dark brown to nearly black, opaque, 12–16 μ in length; other cell hyaline persistently, rarely darkened, later collapsing; germinal pore provided at the distal end of the dark cell; gelatinous envelope thin.

Conidial structures represented by hyaline, cylindrical, smooth, terminal or intercalary arthrospores measuring 7–14 \times 2–3 μ , and hyaline, ovate to elongate, smooth, aleuriospores measuring 8–12 \times 3.6–5 μ , which are borne singly and laterally on aerial hyphae.

Cultures on potato-carrot agar spreading, thin, with loose aerial growth in light brownish gray shades, cleistothecia scattered throughout as black dots, conidial structures few in number; reverse dark olive gray. At 37°C., luxuriant growth with no production of cleistothecia.

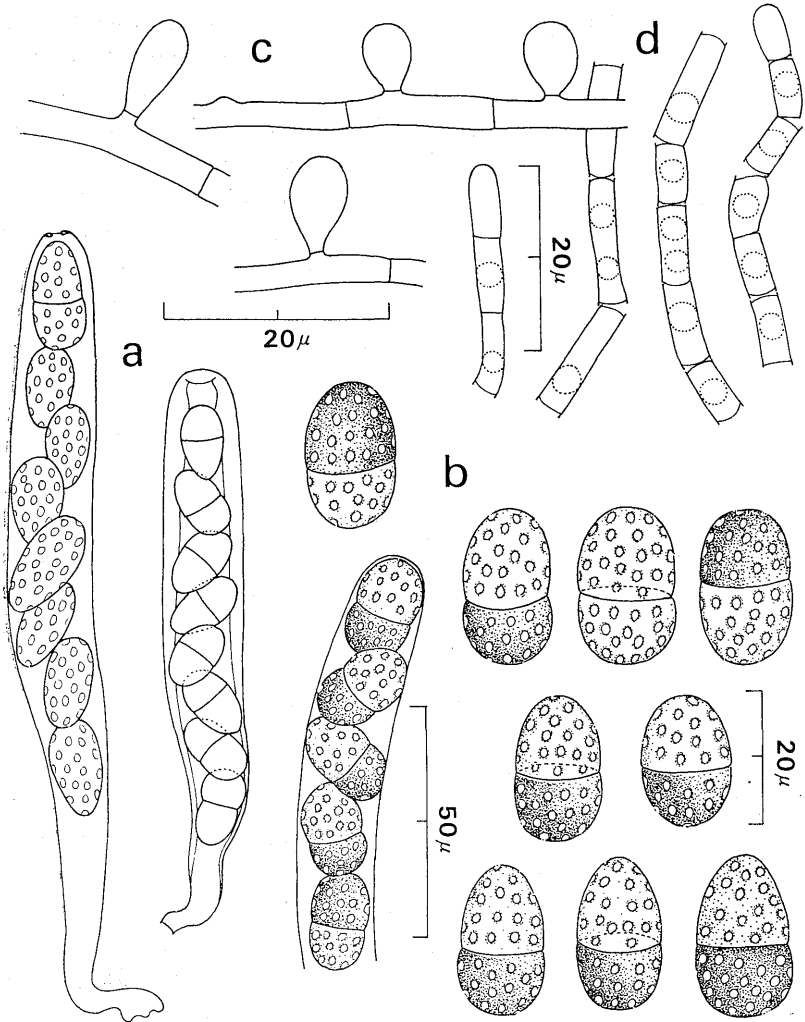


Fig. 2. *Diplogelasinospora princeps*. a. Asci. b. Ascospores.
c. Aleuriopores. d. Arthrospores.

Habitat : on soil, Amamioshima Isl., Oshima-gun, Kagoshima-Pref., April 10, 1971, NHL 2504.

Specimen examined : DAOM 107740, in culture from flax seed, Caledonia Springs, Ontario, Canada, Jan. 5, 1942, leg. J. W. Groves (type).

This is the first record outside of the type locality. Measurements obtained in our observation on the Japanese isolate, as recorded above, are in almost complete agreement with Cain's original description and result from examination of the type material (DAOM 107740). An imperfect state, of arthrospore- and aleuriospore-type, not mentioned in the type description was also found on the Japanese and Canadian materials. Its aleuriospores are somewhat larger than those of *D. grovesii*, while both species have nearly identical arthrospores.

Discussion In the early study, Cain (1961) stated that *Diplogelasinospora*, like *Anixiella*, has derived from *Gelasinospora* as an adaptation to fruiting in unexposed locations and delayed dispersal of the ascospores. Subsequently it was explained by Lundqvist (1967) as follows: "*Anixiella* has one-celled spores and seems to be hardly more than a cleistocarpous *Gelasinospora*, although meriting the rank of a genus. In *Diplogelasinospora* the spores are two-celled, one of the cells being hyaline. It is worth noticing that the hyaline cell is pitted too, but indistinct, indicating that this spore type derives its origin from a taxon with one-celled or two-celled, brown spores pitted all over."

The relationship between *Anixiella* and *Gelasinospora* seems to be essentially natural by Maniotis' observation (1965) on a spontaneous and cleistothecial mutant of *G. calospora*. In relation to the taxonomical position of *Diplogelasinospora*, however, the writers should like to call attention to the fact that ascospore ornamentation is not always a traceable feature as phylogenetic evidence for discussing problems connected with the origin and evolution of the sordariaceous fungi. The occurrence of the smooth perispore in *D. grovesii* probably is to be regarded as the result of a kind of morphogenetic segregation. Despite the basic differences shown by its pitted ascospores and arthrospore-type conidial state, *Diplogelasinospora* might be placed in the vicinity of a cleistoascomycete, *Zopfiella*, in which several species have cephalothecoid peridium, broadly clavate, evanescent asci, two-celled ascospores which consist of dark cell and broad pedicel-like hyaline cell, and ovate aleuriospores. In our observation on *D. grovesii*, the general characteristics of its ascospores are strongly suggestive of *Z. latipes* (Lundqvist, 1969; Malloch and Cain, 1971) and *Z. pilifera* (Udagawa and Furuya, 1972). Association with a *Humicola* state was noted in the original

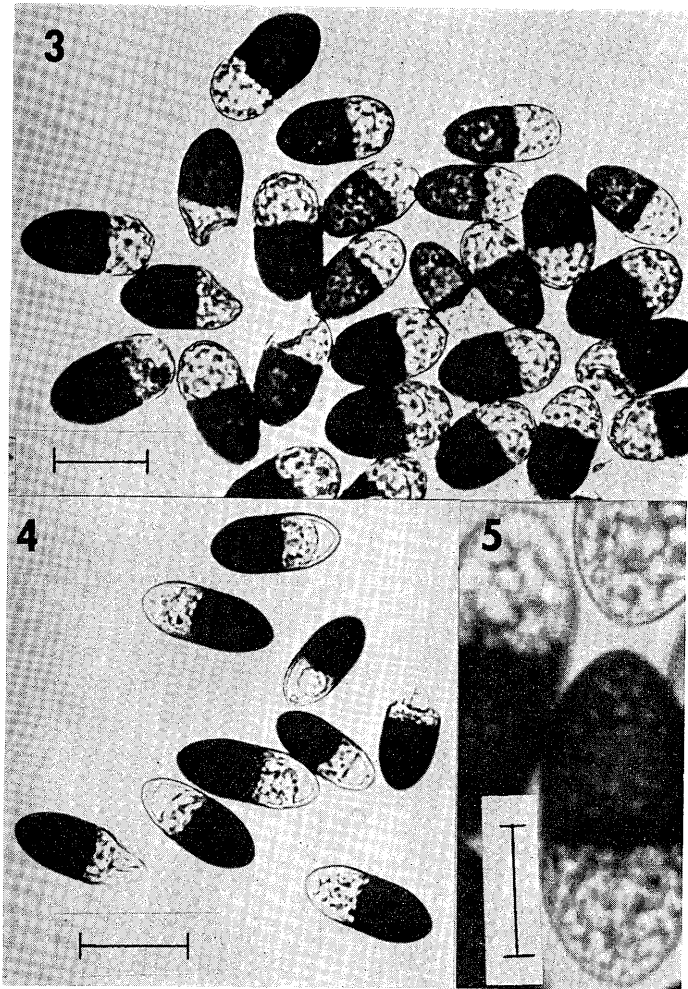


Fig. 3. Ascospores of *Diplogelasinospora princeps*. (scale: 20μ)

Figs. 4, 5. Ascospores of *Diplogelasinospora grovesii*. (scales: 4= 20μ , 5= 10μ)

description of *Z. latipes*, but our observation on the Japanese collection of this species revealed only production of small aleuriospores bearing a resemblance to those of *Diplogelasinospora*.

Arthrospore-type conidia are reported as the imperfect states of certain cleistocarpous Ascomycetes such as *Anixiopsis* (de Vries, 1969), *Ascocalvatia*

and *Xynophila* (Malloch and Cain, 1971), and *Xylogone* (Arx and Nilsson, 1969), but there is no expected relationship between these fungi and *Diplogelasinospora*. More exact placement of this genus must await the further isolation of additional fungi along with the evolutionary line toward the well defined sordariaceous Pyrenomycetes.

Summary

Two species of *Diplogelasinospora*, *D. grovesii* Udagawa et Horie sp. nov. and *D. princeps* Cain, are described on the basis of isolates from Japanese soil. Both isolates in culture produce aleuriospores and arthrospores, as well as seen in the type material of *D. princeps* (DAOM 107740). This is the first report of a conidial state for *Diplogelasinospora*.

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Diplogelasinospora 属は閉子のう殻を形成するソルダリア科の子のう菌類として知られ、現在まで 1961 年にカナダ産亜麻種子から分離培養された一例があるにすぎない。日本産土壌試料から子のう菌類を検索中、この属と思われる 2 株が分離されたので、それらの形態を精査したところ、1 株はタイプ種 *D. princeps* の記載とよく一致し、他の株は網目型の表面をもった子のう胞子を形成し新種であることが分った。これを *D. grovesii* と命名し記載した。日本産の 2 株にはアルーリオ胞子および分節胞子型の分生子世代が発見されたので、オタワの中央農業研究所 (DAOM) から標本を借用し検討したところ、カナダ産株にも同様の分生子世代が確認された。しかし、これら分生子世代の存在については未報告であったため本報告において追加記載し、またこれらの新知見をもとに関連諸属との相互関係を論議した。