

DISCOMYCETE FLORA OF ASIA, PRECURSOR IV:

A NEW SPECIES OF *BIFUSELLA* (RHYTISMATACEAE, HYPODERMATEAE)

ON *CUNNINGHAMIA* IN OKINAWA<sup>1</sup>

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*Cunninghamia lanceolata* Hook., a handsome Taxodiaceous species, has been introduced in experimental plantings in the Yona Demonstration Forest on the island of Okinawa in the Ryukyus. In the course of a survey of forest fungi, the authors noted a rather spectacular disease of this tree in that planting (FIG. 1), and the senior author has twice since recollected the fungus. The causal agent is a species referable to the tribe Hypodermateae of the family Rhytismataceae (usually called the Hypodermataceae). It has the characteristic dumbbell shaped spores which Darker (1932) termed "bifusiform." As is typical of all members of the family, the ascospores are surrounded by a gelatinous sheath both within the ascus and on discharge (FIG. 5), and in this species are often also coiled or twisted (FIG. 7).

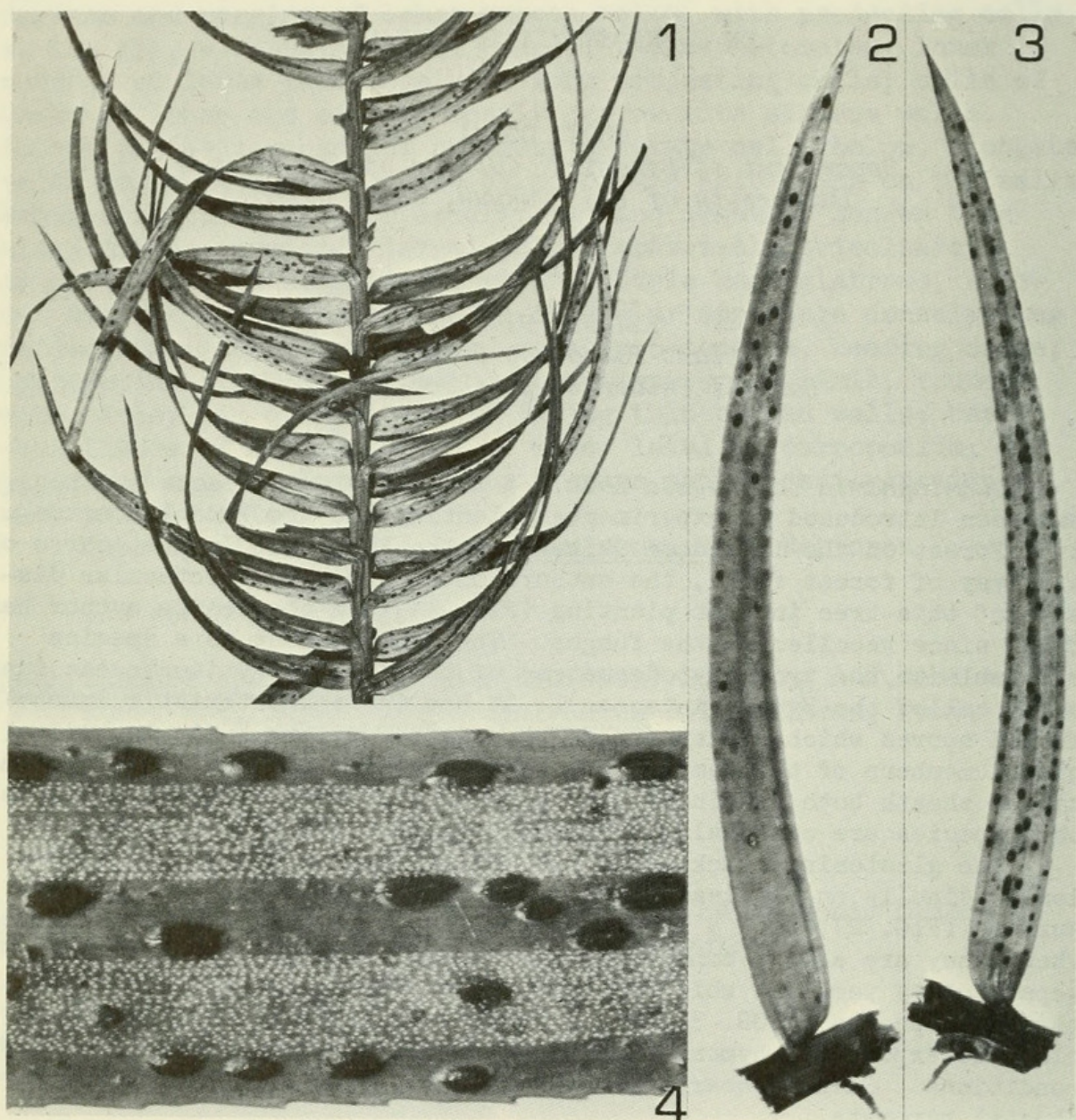
The glistening black ascocarps are elongate ellipsoid, oriented longitudinally on the leaf, a few occurring scattered on the upper surface (FIG. 2), with a larger number occurring on the lower surface where they are almost completely restricted to three rows that are separated by peculiar whitish bands characteristic of the leaves of the host species (FIGS. 3, 4). The ascocarps open by a longitudinal cleft to expose the hymenium in wet weather, closing again under drier conditions. Such ascocarps have been termed hysterothecia or uniapothecial stromata.

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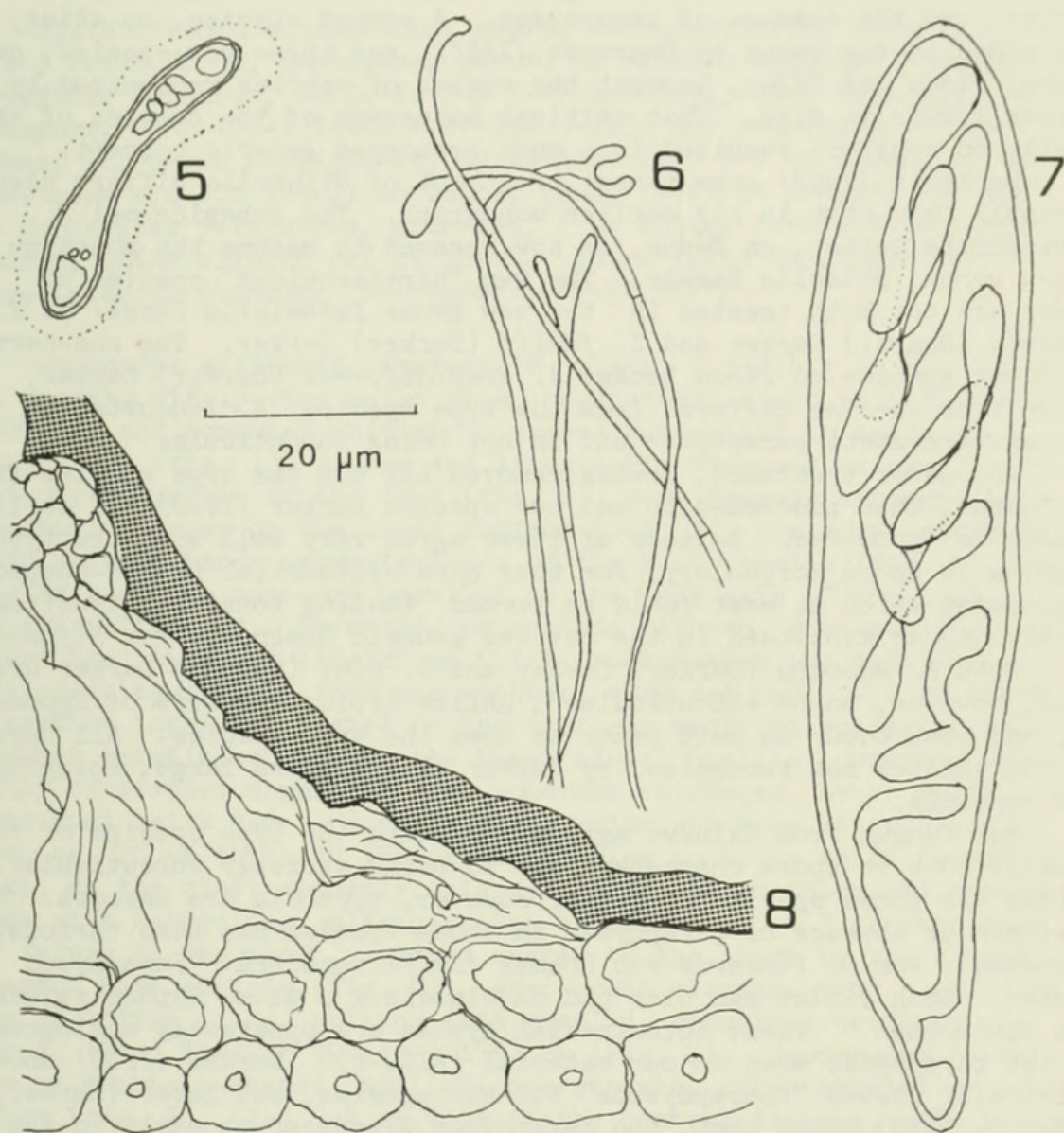
In a recent revision of the family (Darker, 1967) several new genera and new generic concepts have been advanced. Bifusiform ascospores occur in five genera. In *Bifusepta* Darker, on *Vaccinium*, the spores are septate; in the others, as in the Okinawan fungus, the spores are non-septate. In *Duplicaria* Fuckel the ascocarps are sub-spherical, open irregularly, and are not on conifers. The species of



FIGS. 1-4. Photomicrographs of *Bifusella cunninghamiicola* on the dead leaves of *Cunninghamia lanceolata*. FIG. 1. Portion of a branch with ascocarps,  $\times 1$ . FIG. 2. Upper surface of a leaf with scattered ascocarps,  $\times 2$ . FIG. 3. Lower surface of the same leaf with ascocarps in three longitudinal rows,  $\times 2$ . FIG. 4. Ascocarps at a greater magnification, longitudinally oriented clefts visible, developing almost wholly between the two longitudinal whitish bands on the lower leaf surface,  $\times 16$ . All photographs from the holotype specimen, taken by Howard H. Lyon, Department of Plant Pathology, Cornell University.



the remaining three genera were all at one time or another referred to the genus *Bifusella* von Höhnelt, have ellipsoid to linear ascocarps, and occur on coniferous hosts. It is to this "*Bifusella* complex"



FIGS. 5-8. Camera lucida drawings of *Bifusella cunninghamicola*, from the holotype specimen,  $\times 1000$ . FIG. 5. Mature ascospore showing the thick gelatinous sheath and various inclusions within the spore. FIG. 6. Apices of paraphyses. FIG. 7. An ascus containing eight, mostly vermiform, bifusiform ascospores. FIG. 8. Vertical freezing microtome section through the extreme edge of an ascocarp, the thick-walled epidermal cells at the base, with even thicker-walled hypodermal cells below them, the host cuticle (shown shaded) raised by the ascocarp and adhering closely to the upper surface of the ascocarp, the cells of the stroma mostly gelatinized at the margin and difficult to differentiate, at the upper portion of the section at the left with cells more typical of the stroma, forming a brown-walled textura angularis.



(Darker, 1967) that the species on *Cunninghamia* belongs.

*Bifusella* was erected by von Höhnelt (1917) for a single species, *B. linearis* (Peck) v. Höhn., on the needles of *Pinus strobus* L. He noted as generic distinctions that the ascocarps are subcuticular (rather than intraepidermal as in *Hypoderma*), the bifusiform shape of the spores, and the absence of paraphyses. A second species, on *Abies*, was added to the genus by Dearness (1926), and three new species, on *Abies*, *Picea* and *Pinus*, brought the number of species recognized by Darker (1932) to five. That critical monograph of the species of this family on conifers resulted in a much broadened generic concept.

Darker's (1967) more recent treatment of *Bifusella* differs significantly from that in his earlier monograph. The subepidermal *B. striiformis* Darker, on *Pinus*, is now removed to become the monotype of a new genus, *Soleella* Darker. The two "nervisequious" species on *Abies* are now both treated in the new genus *Isthmiella* Darker as *I. abietis* (Dearn.) Darker and *I. faulii* (Darker) Darker. The non-nervisequious species on *Picea* became *I. crepidiformis* (Darker) Darker. These four species differed from the type species, *B. linearis*, in having persistent paraphyses and in not being subcuticular.

The newer treatment, having removed all but the type species from the genus, then proceeded to add two species Darker (1932) had earlier placed in *Hypoderma*. Neither of these agree very well with the type species in spore morphology, for they have cylindrical or rod-shaped ascospores which at most could be termed "tending toward a bifusiform condition" as mentioned in the revised generic diagnosis for *Bifusella*. Both *B. saccata* (Darker) Darker and *B. pini* (Dearn.) Darker are said, however, to be subcuticular<sup>3</sup>, unlike typical members of *Hypoderma*, and both occur on soft pines as does the type species. All three of the species now recognized by Darker also possess large, conspicuous pycnidia.

Our fungus from Okinawa agrees well with the type species of *Bifusella* both in spore characters and in being strictly subcuticular. Unlike the three species on *Pinus*, however, pycnidia are unknown. The presence or absence of paraphyses in those species has been variously reported. For *B. linearis* von Höhnelt (1917) reported: "Paraphysen fehlen. Doch findet man hier und da einzelne 1  $\mu$  dicke Hyphen zwischen den Schläuchen." These interthecial hyphae are apparently analogous to the paraphyses seen in our material (FIG. 6). Darker (1932) unequivocally stated "Aparaphysate" for the species, but later (Darker, 1967, p. 1413) notes that "the paraphyses disappear so early in the development that the species has come to be regarded as aparaphysate." Of the two species he transfers from *Hypoderma*, he states that in *B. pini* the paraphyses disappear early, while in *B. saccata* "the paraphyses though more persistent finally undergo lysis and at maturity may be lacking." In our fungus the paraphyses certainly do not disappear early, since they may be found profusely in apparently completely mature ascocarps which open widely to expose the hymenium in moist weather. While it is possible that the paraphyses undergo lysis at maturity or post-maturity in the Okinawan species, this has not yet

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<sup>3</sup>. *B. pini* was stated by Darker (1932) to be intraepidermal.



been demonstrated.

*Bifusella cunninghamiicola* Korf & Ogimi, sp. nov.

*Hysterothecia ellipsoidea*, nigra, subcuticularia,  $0.3-0.4 \times (0.5-) 0.6-0.75$  mm, amphigena; stroma ex textura angulari formata, parietibus brunneis, cellulis  $3-6 (-8) \mu\text{m}$  diam.; asci late clavati, octospori, J—,  $(17-) 19-22 \times 90-110 (-120) \mu\text{m}$ ; ascospores bifusi-formes, plerumque vermiformes, ad isthmum  $(2-) 3-4 \mu\text{m}$  latae, ad extremitates  $4.5-5.5 (-6.5) \mu\text{m}$  latae,  $28-36 (-40) \mu\text{m}$  longae, hyalinae; paraphyses filiformes, graciles,  $1 \mu\text{m}$  latae, persistentes, circinatae, apice clavato vel difformi. Intra folia *Cunninghamiae lanceolatae* in Okinawa. Holotypus: CUP-JA 3437.

Ascocarps ellipsoid, hysteroioid, black,  $0.3-0.4 \times (0.5-) 0.6-0.75$  mm, opening widely when moist by a longitudinal cleft that is faintly fimbriate and somewhat whitish to expose the pallid to beige disc, amphigenous (fig. 1), sparse and irregularly disposed on the glabrescent upper surface of the leaf (fig. 2), more densely arranged into 3 longitudinal rows on the lower surface (fig. 3), the rows separated by broad whitish bands characteristic of the host species (fig. 4), the long axis of the ascocarp parallel to the long axis of the leaf, subcuticular (fig. 8). Spermatia or conidia unknown, presumably absent. In section: hymenium  $110-120 \mu\text{m}$  thick; subhymenium and medullary tissue indistinguishable from one another, very reduced,  $5-10 \mu\text{m}$  thick, of textura intricata seated on the scarcely modified epidermal cells, somewhat gelatinized toward the basal edges, hyaline; covering stroma mostly of textura angularis with somewhat thickened, brown walls, the individual cells  $3-6 (-8) \mu\text{m}$  in diam, paler and more hyphoid at the base, ca.  $20 \mu\text{m}$  thick at the sides, flaring to  $40 \mu\text{m}$  thick toward the cleft, at the cleft suddenly enlarged to ca.  $80 \mu\text{m}$  thick and provided there with subhyaline, glassy-walled apices ca.  $15 \times 3 \mu\text{m}$  that form the fringe at the cleft, covered nearly to the cleft by the closely adherent host cuticle (fig. 8). Asci broad clavate, with a pore not blue in iodine (J—), arising from gnarled, repeating croziers, 8-spored,  $(17-) 19-22 \times 90-110 (-120) \mu\text{m}$  (Fig. 7). Ascospores bifusi-form or dumbbell-shaped, hyaline, non-septate, often twisted or vermiform (fig. 7),  $(2-) 3-4 \mu\text{m}$  wide at the central isthmus, ends swollen to  $4.5-5.5 (-6.5) \mu\text{m}$ , length when not coiled  $28-36 (-40) \mu\text{m}$ , surrounded in the ascus and after discharge by a thick, hyaline, gelatinous sheath (fig. 5), oil drops and other inclusions mostly polar and not in the isthmus. Paraphyses about as long as the asci, gracile, mostly unbranched below, ca.  $1 \mu\text{m}$  broad, remotely septate, often apically branched one or more times with tips usually circinate, apices usually clavately swollen or sometimes irregularly distorted,  $1.5-2 (-3) \mu\text{m}$  broad, hyaline, persistent (fig. 6).

SPECIMENS EXAMINED: On leaves of *Cunninghamia lanceolata*, 20-50 m elev., Yona Demonstration Forest, Yona, Okinawa, Ryukyus, 19. IV. 1969, Korf & Ogimi CUP-JA 3437, HOLOTYPE (CUP), ISOTYPES: DAOM, K,



TNS, Dept. Forestry, University of the Ryukyus, Naha, Okinawa. PARATYPES: Same substrate, same locality, 10-100 m elev., 10. II. 1971, *Ogimi*, Dept. Forestry, University of the Ryukyus, Naha, Okinawa; Same substrate, same locality, 2. V. 1971, *Ogimi*, Dept. Forestry, University of the Ryukyus, Naha, Okinawa.

There are two other members of the Rhytismataceae reported on the leaves of *Cunninghamia lanceolata*, but both should be easily distinguished on field characters as well as microscopic features. Neither species has the constricted ascospores so characteristic of *Bifusella cunninghamicola*. In the case of *Lophodermium petrakii* Durrieu (1957) the long filiform ascospores ( $56-72 \times 1.5-2.0 \mu\text{m}$ ) are borne in subcuticular ascocarps which are scattered on the leaf with the clefts randomly oriented. Darker's supposition that the reports of *Lophodermium pinastri* (Schröd. per Hook.) Chev. (Teng, 1932), and of *L. uncinatum* Darker (Teng, 1933), both on this host from China represent instead Durrieu's species are at least partially confirmed by studies of the junior author. The undestroyed portion of the Chinese National Fungus Herbarium is on deposit in CUP (part of this collection is also on deposit at BPI). The report of *L. pinastri* is based on Teng 1005, but that specimen was also cited when Teng (1933) apparently reidentified it as *L. uncinatum*. While the Teng 1005 cannot be located at CUP, another specimen cited in 1933 is deposited as *L. uncinatum*, Teng 1940. This specimen is clearly *L. petrakii*. The intraepidermal ascocarps of the second species, *Hypoderma handelii* Petrak<sup>4</sup>, are also not regularly aligned as in *B. cunninghamicola*, and the bacillar ascospores are shorter and 1-septate ( $13-22 \times 4-6 \mu\text{m}$  fide Petrak, 1947). Another fungus which might conceivably be confused with these is *Lophiosphaera orientalis* Hino & Katumoto (1964) which also occurs on the leaves of *Cunninghamia*, but the ascostromata of that species are deeply immersed

<sup>4</sup>. This species is referred to as *H. cunninghamiae* (Keissl.) Teng by Darker (1967), but Petrak's name is the correct one for the fungus at species rank. Although *H. strobicola* Tub. f. *cunninghamiae* Keissler (1924) was validly published, Teng (1936) did not know of this paper and adopted Keissler's epithet only from a herbarium label. Teng's name is not validly published under Art. 36 of the International Code of Botanical Nomenclature since he provided neither a new nor a reference to a previously published Latin description or diagnosis. When Petrak (1947) reworked Keissler's Chinese material he cited primarily a later paper (Keissler, 1937), decided the fungus deserved species rank, and gave it a new name rather than adopting Keissler's epithet for the forma (as he is permitted to do under the Code). The somewhat confusing synonymy is:

*Hypoderma handelii* Petrak (as "*Handelii*"), Sydowia 1: 371. 1947.  
 = *Hypoderma strobicola* Tub. f. *Cunninghamiae* Keissl., Akad. Wiss. Wien Anzeiger, Math.-Naturwiss. Kl. 61: 13. 1924.  
 = [*Hypoderma Cunninghamiae* Teng (as "(Keissl.) Teng"), Sinensia 7: 261. 1936, not validly published.]



and the ascospores 1-septate, fusoid, and appendaged; its asci are bitunicate, as is characteristic of the Lophiostomataceae, to which the species seems correctly referred.

As delimited and redefined here, the genus *Bifusella* is to be accepted for two, or possibly four, species, all of which occur on the leaves of conifers. All of the other species assigned to this genus which occur on conifers have been excluded by Darker (1967), and have been discussed above. Two other species which do not occur on conifers have, however, been referred to the genus, "*B. vaccinii* Tehon" and *B. acuminata* (Ellis & Everh.) Bonar & Cooke. The first of these is now the type species of *Bifusepta*, *B. tehonii* Darker, and occurs on the stems of *Vaccinium*. The second, a species not mentioned by Darker (1967), occurs on the culms of Juncaceae and Cyperaceae, and was originally placed in the genus *Duplicaria*. Neither of these species now appears to be congeneric with *Bifusella linearis* or *B. cunninghamiicola*, and the junior author will discuss both at greater length in his monographic study of *Duplicaria* now in preparation.

It is not yet known how serious this disease is in Okinawa, nor whether any successful control measures can be found. Though *Cunninghamia lanceolata* is widely grown in Asia, this disease has apparently never been noted before its discovery in the experimental forest at Yona. Whether the fungus was brought to Okinawa with the seeds of *Cunninghamia*, or possibly exists also on some other Taxodiaceous or Pinaceous host plant endemic to Okinawa, is also unknown. The senior author is continuing his studies of the disease.

## 要 旨

*Cunninghamia lanceolata* のこれまでに知られていない病気が沖縄の実験森林に発生した。病因はここに記述されている *Bifusella cunninghamiicola* Korf & Ogimi sp. nov. であります。この種は *Bifusella* 属の type である *B. linearis* と比較されています。 *B. cunninghamiicola* は持続性のある側線を有している反面, *B. linearis* は無側線であります。また, *Bifusella* 属は Hypodermateae に属する他の四属 (*Dupli-*



*caria*, *Bifusepta*, *Isthmiella*, *Soleella*) ともここに比較されています。*Bifusella cunninghamicola* と *C. lanceolata* に発生するこれと混同されやすい他の三種の菌類 *Lophodermium petrakii*, *Hypoderma handelii* (= *H. cunninghamiae*) と *Lophiosphaera orientalis* との相異点も検討されています。

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