Shun-ichi UDAGAWA* & Masaki TAKADA**: **The rediscovery of *Aphanoascus cinnabarinus***

宇田川俊一*・高田正樹**: *Aphanoascus cinnabarinus* の再発見

In 1889 an unknown cleistothecial Ascomycete was isolated from alligator excrements in Austria, from where it was subsequently described by Zukal (1890)[9] as *Aphanoascus cinnabarinus*, n. gen., n. sp. The genus has remained monotypic and, so far as we are aware, unreported since it was originally described. Recently our attention has been called to this subject, because Apinis (1968)[1] pointed out the similarities of *Anixiopsis stercoraria* (Hansen) Hansen to *Aphanoascus cinnabarinus*. He stated: "there is no doubt that the present name of isolates of *Anixiopsis stercoraria* (Hansen) Hansen in fact represent *A. cinnabarinus*—Zukal's (1890) generic name has priority against Hansen's (1897) but Cooke's (1875) specific epithet*** has the priority against the other two species names proposed by Hansen and Zukal—therefore, the correct name for this fungus is *Aphanoascus fulvescens* (Cooke) comb. nov." His treatment was followed by Malloch and Cain (1971)[5], although they assigned the genus to a member of the Onygenaceae. On the basis of his observation on new material agreeing in every respect with Zukal's description of *A. cinnabarinus*, however, de Vries (1969)[3] redefined *Anixiopsis* Hansen and regarded the genus *Aphanoascus* as unrelated. Unfortunately Apinis himself stated that "so far it has not been possible to locate Zukal's type specimen", so that his conclusion of this species was apparently not based on the type material.

In the course of a mycological survey on the Japanese soils, the authors frequently encountered a species of beautiful, reddish orange fungus belonging to the cleistothecial Ascomycetes. This species was collected from different localities of Japan in 1967–1968. It has also been collected from tropical soil, Lae, Morobe Dist., Papua and New Guinea. When grown on

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*** *Badhamia fulvescens* Cooke in Grevillea 4: 9, 1875.

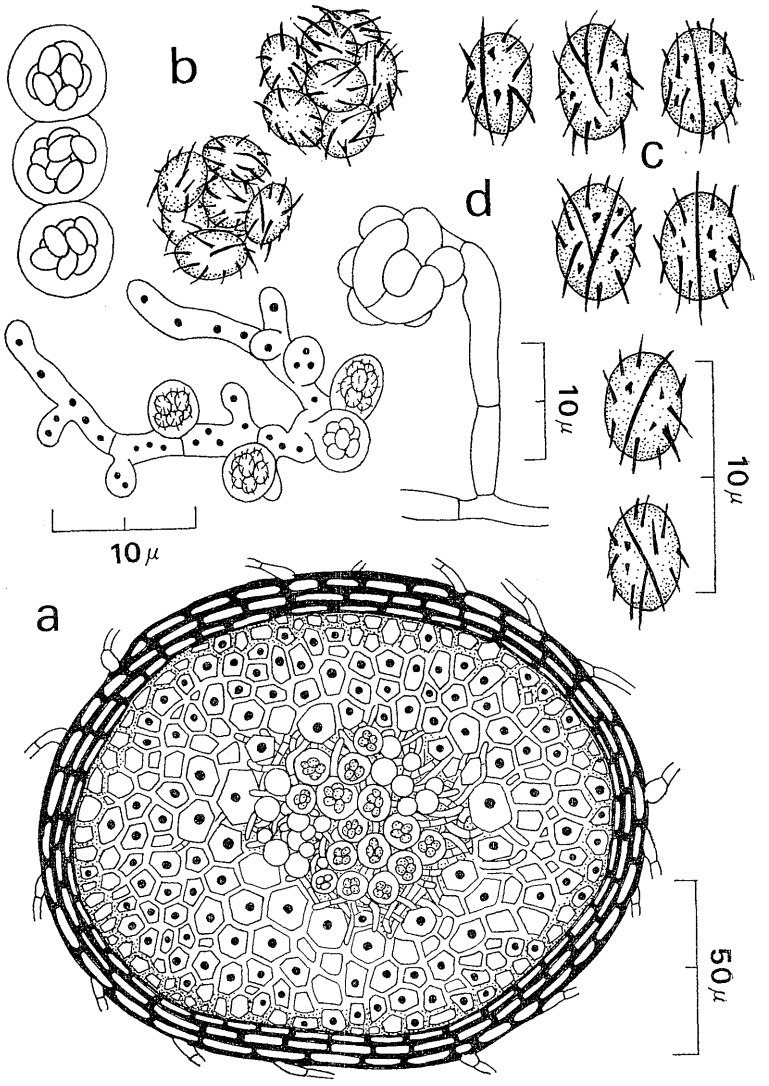

Fig. 1. *Aphanoascus cinnabarinus*. a. Cleistothecium. b. Various stages of asci. c. Ascospores. d. Ascocarp initial.

oat-meal agar at 25°C, these cultures appear to represent *A. cinnabarinus* Zukal in most respects. For a comparative study, living culture of *A. cinnabarinus* kept at the Centraalbureau voor Schimmelcultures was obtained through the courtesy of Dr. J. A. von Arx This culture (CBS No. 424.69), according to the list (1970)[2], was isolated by E. Härri. However, an attempt to compare it with our isolates was unsuccessful since no cleistothecium has been observed on the CBS culture. In view of Apinis' misinterpreted knowledge on the genus *Aphanoascus*, it seems appropriate to review the fungus on the basis of the additional informations provided by these collections. Description from our observations of the collections is as follows:

Aphanoascus cinnabarinus Zukal in Ber. Deutsch. Bot. Ges. 8: 296 (1890). (Figs. 1 & 2).

Colonies on oat-meal agar growing rapidly, plane, consisting of a thin mycelial felt in which scattered cleistothecia are embedded, surface appearing slightly flocculent, light orange to reddish orange, conidial structures not produced; reverse pale reddish orange to dark brown.

Cleistothecia superficial, yellowish orange to orange red, subspherical to ovate, with base often slightly flattened to hemispherical, 350–600 μ in diameter, later confluent in small groups, attaining up to 1 mm or more in diameters, smooth, loosely invested with reddish orange pigmented hyphae. Peridium membranaceous, pseudoparenchymatous to sclerotioid, 12–20 μ thick, outer part yellowish brown to reddish brown, consisting of several layers of hyphal strands, inner part hyaline, consisting of sclerotioid masses of thick-walled, isodiametric cells measuring 8–16×6–14 μ. Ascus formation slow outwards from the centre in 4–5 weeks or more; at maturity the inner tissue of cleistothecium completely disorganized. Asci irregularly disposed, 8-spored, hyaline, borne as

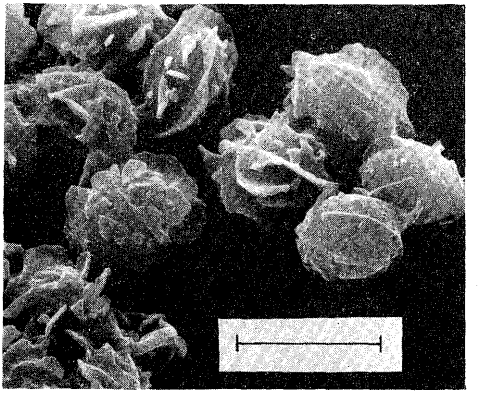

Fig. 2. *Aphanoascus cinnabarinus*. Scanning electron micrograph of ascospores (scale: 5μ).

lateral branches from ascogenous hyphae, in short chains, globose to subglobose, 9.5–11μ in diameter, evanescent. Ascospores hyaline to pale yellow, at maturity reddish-colored, broadly elliptical, 4–5.5(–6)×3–3.5μ, becoming more or less thick-walled in age, ornamented by irregular, often anastomosing ridges; ridges longitudinal to oblique or even transverse, 0.5–0.8μ thick. Mycelium hyaline to pale yellowish orange, branched, 1–3μ in diameter, septate, encrusted with pigmented granules, often aggregated into bundles. Conidial state unknown.

On potato-carrot agar essentially as on oat-meal but growing somewhat more slowly, usually developing cleistothecia fairly abundantly, in somewhat deeper shades near dark red.

On malt agar spreading, orange to reddish orange, more or less floccose, ripening process of cleistothecia extremely delayed; colony reverse dark green to dark blue-green with surrounding agar similarly colored.

At 37°C, grows slower than at 25°C, with production of fructification much reduced and immatured.

The above description of *A. cinnabarinus* is based upon the cultures NHL 2673, isolated from pepper field soil, Minamikushiyama-mura, Minamitakagi-gun, Nagasaki-ken, Sept. 1, 1967, and NHL 2674, isolated from burned forest soil, Ôhito-machi, Tagata-gun, Shizuoka-ken, Apr. 17, 1968. Since none of Zukal's specimens of *A. cinnabarinus* exist, isolate NHL 2673 is designated as the neotype. Dried specimen and slides of this isolate are kept at the National Institute of Hygienic Sciences, Tokyo. Pure cultures of the above isolates are also deposited both at the Institute for Fermentation, Osaka and the Centraalbureau voor Schimmelcultures, Baarn. An additional culture of *A. cinnabarinus*, NHL 2675, was also isolated from paddy-field soil, Shuzenji-machi, Tagata-gun, Shizuoka-ken, Sept. 18, 1968. The latest Japanese isolate agrees in essential morphological characters with the above description, but differs in having somewhat smaller ascospores (3–4×2–3 μ). One culture from New Guinea was of special interest, because it produced much larger ascospores. It may be regarded as new and a full treatment will be published later. Further additional strains representing this species have been repeatedly encountered among the fungi isolated from soils in temperate and tropical areas of the Pacific.

Morphologically, the Japanese fungus agrees closely in most respects

with the original description and illustration by Zukal, although no conidial structures were found in our isolates on the common media. Zukal described its conidial state as "*Botrytis*-artigen Conidienträger" suggestive of arthroaleuriospores type. Whether or not the conidial state was actually associated with Zukal's *A. cinnabarinus* may be still open to speculation but it is believed that our isolates represent *A. cinnabarinus*.

Aphanoascus is characterized by the production of its spherical, cinnabar, non-ostiolate ascocarps, which are surrounded by loose wefts of encrusted hyphae and have a sclerotioid inner tissue, irregularly disposed globose asci, and ellipsoid-globose and hyaline to reddish orange ascospores ornamented with several narrow ridges. Usually the cells of sclerotioid inner tissue are very slowly disorganized from the centre of ascocarp and converted into asci. The asci may be borne in short chains. This pattern of ascus formation is strongly suggestive of those seen in most members of the genera *Eupenicillium* (Scott, 1968)[6] and *Hemicarpenteles* (Udagawa and Takada, 1971)[8]. The absence of conidial structures in our isolates as well as the colony pigmentation and the morphology of ascocarp initials would separate the fungus from the latter two genera.

As suggested by von Arx (personal communication), *Aphanoascus* may also be related to a thermophilic genus *Thermoascus* Miehe (Stolk, 1965)[7]. The general appearance of ascocarps, especially in respect of their reddish orange pigmentation, and the morphology of ascospores are quite similar in these two genera. However, it is separated from *Thermoascus* by the production of catenulate asci and irregularly ornamented ascospores, and the growth behavior at high temperature.

Finally, as already mentioned by de Vries (1969)[3], this fungus is entirely distinct from *Anixiopsis* Hansen (1897)[4]. The latter is clearly distinguished by smaller, brownish, thin-walled ascocarps, early developing asci, and more regularly ornamented ascospores.

Because of the close affinity to *Thermoascus* and *Eupenicillium* (or *Hemicarpenteles*), the genus *Aphanoascus* is apparently classified under the family Eurotiaceae.

We are indebted to Dr. J. A. von Arx and the staffs of the Centraalbureau voor Schimmelcultures for helpful suggestions.

Summary

On the basis of our observation on cultures isolated recently from soil collections in Japan, a cleistothecial ascomycete *Aphanoascus cinnabarinus* Zukal is redescribed and illustrated. These collections appear to be the only extant representatives of the species. Taxonomical relationship of the genus is also discussed. *Aphanoascus* is separated definitely from *Anixiopsis* by characters of ascocarps, ascus development and ascospores.

References

1) Apinis, A. E. 1968. Mycopathol. et Mycol. Appl. **35**: 97–104. 2) Centraalbureau voor Schimmelcultures. 1970. List of cultures. Supplement 1 to the 27th edition (1968). 39 p. 3) De Vries, G. A. 1969. Mykosen **12**: 111–122. 4) Hansen, E. C. 1897. Bot. Zeit. **55**: 127–131. 5) Malloch, D. & R. F. Cain. 1971. Can. J. Bot. **49**: 839–846. 6) Scott, B. De 1968. The genus *Eupenicillium* Ludwing. CSIR, Pretoria, S. Africa, 150 p. 7) Stolk, A. C. 1965. Antonie van Leeuwenhoek **31**: 262–276. 8) Udagawa, S. & M. Takada. 1971. Bull. Nat. Sci. Mus. Tokyo **14**: 501–515. 9) Zukal, H. 1890. Ber. Deutsch. Bot. Ges. **8**: 295–303.

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閉子のう殻を形成する子のう菌の一種 *Aphanoascus cinnabarinus* は 1889 年にオーストリアでワニの糞から発見され記載されたが，その後再び記録されず，また当時の標本も現存していない。著者らは日本各地の土壌から *A. cinnabarinus* と同定される菌株を再発見し，記載を補うと同時に，neotype として指定した。本種は朱赤色の大きい子のう果を形成，最初菌核様の内部組織が徐々に成熟して子のうに移行する特徴をそなえている。最近，英国の Apinis は *Anixiopsis* と *Aphanoascus* を同一の菌として新組合せを提案したが，著者らの知見から両者は別個の属であることが確認された。上記の子のう形成様式は *Eupenicillium* など土壌産子のう菌類にみられる分化型の一つで，子のう胞子の分散遅化現象と考えられる。*Aphanoascus* は集落の色調，子のう果形成原基の形態などの違いや，分生子構造を形成しない点で類似属とは区別される。また，好温性菌 *Thermoascus* とは連鎖状に生ずる子のう，不規則な粗面の胞子，高温での生育状態などで相違が認められる。