

**PETROMYCES MURICATUS, A NEW SPECIES
WITH AN ASPERGILLUS ANAMORPH**

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ABSTRACT

A new species of *Petromyces* (Ascomycetes: Trichocomaceae) is described and illustrated as *P. muricatus*. It is characterized by pale yellow to flesh colored ascostromata, oblate ascospores with a low equatorial rim and spinulose surfaces, and an *Aspergillus* anamorph belonging to the section *Circumdati* of the subgenus *Circumdati* (= *A. ochraceus* group). The holotype was isolated from grassland soil in the Philippines.

Key Words: ascomycete, Trichocomaceae, *Petromyces muricatus*, *Aspergillus muricatus*, taxonomy, soil fungus, the Philippines.

An interesting *Aspergillus* which produced abundant pale yellow to flesh colored sclerotoid bodies was recently isolated from grassland soil from the Philippines. Light yellow conidial heads with biseriate aspergilla produced in a dense stand over the surface of the colonies on potato-dextrose agar (PDA) indicated that this fungus belongs in the section *Circumdati* of the subgenus *Circumdati* (=the *Aspergillus ochraceus* group after Raper and Fennell (1965)). During the 3 months' observation period, the sclerotoid bodies could be seen to contain one to two ascomata in

which ascospores mature very slowly in cultures on half strength PDA. The ascosporic species of the *A. ochraceus* group was later transferred to the genus *Petromyces* as a new teleomorphic species, *P. alliaceus* Malloch et Cain (Malloch and Cain, 1972). The ascospores of our fungus were oblate with a low equatorial rim and spinulose on the convex surfaces. Since no species of *Petromyces* have been described with ascospores of this ornamentation type, it is here described as a new species.

The cultures were grown in the dark, on several media including: Czapek agar, Czapek-yeast extract agar (CYA) (Klich and Pitt, 1988), malt extract agar (MEA), oatmeal agar and half strength PDA. Colors designated in capitals are from the Kornerup and Wanscher (1978) color standard and Rayner (1970) color chart, and are referred to with the letters M and R, respectively. The spores were examined by scanning electron microscopy (SEM), a Hitachi Model S-4100. For SEM, spores were fixed in 0.5% osmium tetroxide, dehydrated in a graded alcohol series, critical-point dried, and sputter coated with platinum-palladium. Extraction, purification, and determination of ubiquinones from the liquid cultures (a medium composed of 5 g malt extract, 3 g yeast extract, and 30 g glucose per liter of distilled water) of the fungus by high-performance liquid chromatography were carried out as described by Kuraishi et al. (1985).

Petromyces muricatus Udagawa, Uchiyama et Kamiya, sp.nov.
(Figs. 1, 2)

Coloniae in agaro "Czapek-yeast extract" (CYA) effusae, floccosae, radiatim sulcatae, compactae, luteolae vel roseo-bubalinae vel carneae; ascostromata tarde formantia; conidiogenesis ad centrum abundans; reversum dilute aurantiacum vel luteolum. Coloniae in agaro maltoso (MEA) paulo effusae, floccosae, ex mycelio basali coacto tenuiter constantes; ascostromata dispersa, alba vel luteola vel bubalina; conidiogenesis limitata; reversum incoloratum vel dilute aurantiacum vel luteolum. Coloniae in agaro decocto tuberorum (half strength PDA) paulo effusae, floccosae, pro parte maxima planae, ex mycelio basali coacto tenuiter constantes; ascostromata ad centrum abundantia, ex hyphis aeriis et capitulis conidicis laxe obiecta, luteola vel straminea; conidiogenesis sparsa vel profusa; reversum dilute aurantiacum vel salmoneum et agar similis.

Ascostromata dispersa vel saepe confluentia, luteola vel carnea, sclerotioidea, globosa vel subglobosa vel interdum elongata, 400-600 µm diam, 1-2 ascomatibus continentia, multistrata; stratum externum ex cellulis luteolis globosis vel plus minusve angularibus incrassatis 6-16 µm diam compositum. Ascomata non-ostiolata, globosa vel subglobosa, 155-300 µm diam, tarde maturescentia;

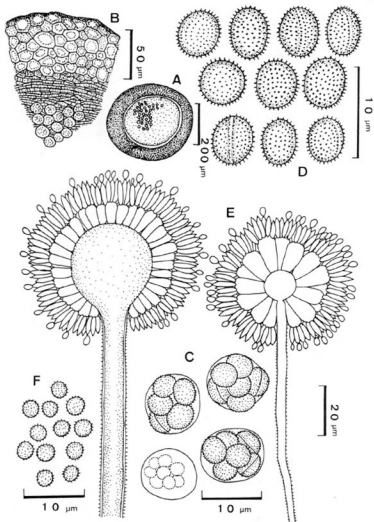


Fig. 1. *Petromyces muricatus*, BF 42515.

A. Ascostroma containing an ascoma (section). B. A portion of a stroma (section). C. Asci. D. Ascospores. E. Aspergilla. F. Conidia.

peridium tenue, ex cellulis hypharum compositum. Asci singulariter portati, 8-spori, subglobosi vel ovoidei, $10-14 \times 8-10 \mu\text{m}$, evanescentes. Ascosporae hyalinae, oblatae, in ambitu subglobosae vel late ellipsoideae, cum labro aequatorio praeditae, $(4.5-4.8-5.5 \times (3.5-4-4.8 \mu\text{m})$, spinulosae. Status anamorphus: *Aspergillus muricatus*.

Ubiquinonum majus: Q-10(H₂).

Holotypus BF 42515, colonia exsiccata in cultura ex solo gramine, Laguna, Los Banos, in Philippinis, 22.ii.1993, a S. Uchiyama et S. Kamiya isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: Latin, *muricatus*= covered with prickles, referring to the ascospore ornamentation.

Anamorphosis: *Aspergillus muricatus* Udagawa, Uchiyama et Kamiya, anam. nov.

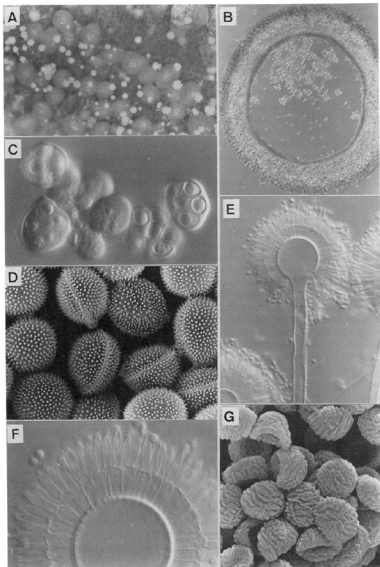
Capitula conidica radiantia, dein secendentia, luteola, usque 300-500 μm diam. Conidiophora plerumque ex mycelio basali oriunda; stipites plus minusve sinuolati, luteoli, 300-2000(-5000) $\times$ 4-10 μm , incrassati, manifeste asperati; vesiculae globosae vel subglobosae, (15-)20-40 μm diam, in toto fertiles. Aspergilla biseriata; metulae (7.5-)10-18(-22) $\times$ (3.5-)5-8 μm ; phialides cylindricae, 7.5-10 $\times$ 2-2.5 μm . Conidia hyalina, globosa vel subglobosa, 2.5-3 μm diam, lobato-reticulata. Status teleomorphus: *Petromyces muricatus*.

Holotypus BF 42515, loc. cit.

Colonies on Czapek agar growing rather restrictedly, attaining a diam of 14-16 mm in 7 days at 25°C, more or less floccose, consisting of a thin mycelial felt, Light Yellow (M. 4A4) or Straw (R); ascostromata slowly developing, not affecting the colony appearance; conidiogenesis profuse; reverse Light Yellow (M. 4A4) or Pale Luteous (R). Colonies on CYA growing rapidly, attaining a diam 45-50 mm in 7 days at 25°C, floccose, rather radially sulcate, consisting of a compact white mycelial felt, at first White, then becoming Light Yellow (M. 4A4) or Rosy Buff to Flesh (R); ascostromata slowly developing; conidiogenesis

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Fig. 2. *Petromyces muricatus*, BF 42515.

A. Ascostromata and conidial heads on half strength PDA in surface view, $\times 10$. B. Ascostroma containing an ascoma (section), $\times 100$. C. Asci, $\times 1400$. D. Ascospores (SEM), $\times 3500$. E. Aspergillum, $\times 400$. F. Metulae and phialides, $\times 1000$. G. Conidia (SEM), $\times 5000$.



abundant in the central area and scattered in the submarginal zones; margins broad, thin; exudate clear; odor musty; reverse Light Orange (M. 5A4) or Pale Luteous (R), with surrounding agar colored in the same shades. Colonies on MEA growing rather rapidly, attaining a diam of 40-48 mm in 7 days at 25°C, floccose, consisting of a thin mycelial felt, producing scattered ascostromata, White to Pale Yellow (M. 4A3) or Buff (R); conidiogenesis limited; exudate scattered, clear; reverse uncolored to Light Orange (M. 5A5) or Pale Luteous (R). Colonies on half strength PDA growing rather rapidly, attaining a diam of 46-48 mm in 7 days at 25°C and rather slowly at 30°C, floccose, mostly plane, raised at center up to 4-5 mm high, consisting of a thin mycelial felt enmeshing abundant ascostromata at center, loosely covered by aerial hyphae and conidial heads, Pale Yellow (M. 4A3) or Straw (R); conidiogenesis sparse to profuse; margins entire, narrow; exudate clear; odor indistinct; reverse and agar Pale Orange (M. 5A3) or Salmon (R).

Ascostromata developing as irregular masses of swollen hyphae, scattered or often confluent in a layer, Pale Yellow or Flesh (R), sclerotoid, globose to subglobose or sometimes elongate, 400-600 μm in diam, containing 1-2 ascomata, multi-layered; outer layer consisting of pale yellow, globose to rather angular, thick-walled cells measuring 6-16 μm in diam; inner layer of hyaline, angular, rather thin-walled cells measuring 10-22 μm in diam. Ascomata non-ostiolate, globose to subglobose, 155-300 μm in diam, maturing slowly from the center outwards after 6 weeks (on half strength PDA at 30°C) or more; peridium about 5-6 μm thick, consisting of a compact hyphal tissue. Asci irregularly disposed on the ascogenous hyphae, borne singly from croziers, 8-spored, subglobose to ovoid, 10-14 $\times$ 8-10 μm , evanescent. Ascospores hyaline, oblate, subglobose to broadly ellipsoidal in face view, with a low equatorial rim, (4.5-)4.8-5.5 $\times$ (3.5-)4-4.8 μm , spinulose on the convex surfaces.

Conidial heads radiate, splitting into 3-4 columns in age, Light Yellow (R), up to 300-500 μm in diam. Conidiophores mostly arising from the basal mycelium; stipes more or less sinuous, pale yellowish, 300-2000(-5000) $\times$ 4-10 μm , with walls thick and distinctly roughened; vesicles globose to subglobose, (15-)20-40 μm in diam, fertile over the entire surface. *Aspergilla* biserial; metulae variable in size, (7.5-)10-18(-22) $\times$ (3.5-)5-8 μm , more or less widening upward; phialides cylindrical, 7.5-10 $\times$ 2-2.5 μm . Conidia hyaline, globose to subglobose, 2.5-3 μm in diam, with walls lobate-reticulate (under SEM). Diminutive conidiophores present.

Major ubiquinone: Q-10(H₂).

At 37°C, growth is somewhat reduced, but ascostromata production is increased.

Specimen examined: BF 42515 (holotype), in dried culture isolated from grassland soil, Laguna, College of Agriculture, University of the Philippines at Los Banos, the Philippines, Feb. 22, 1993. The holotype has been deposited with the Natural History Museum and Institute, Chiba, Japan.

Petromyces muricatus becomes the third species of the *A. ochraceus* group known to produce a sclerenchymatous stroma bearing one to several ascomata (Malloch and Cain, 1972; Tewari, 1985). The type species, *P. alliaceus*, is characterized by non-ostiolate ascomata produced within a black stroma, and hyaline, oblate ascospores with a thin equatorial furrow or very low crests and smooth convex surfaces (Raper and Fennell, 1965; Malloch and Cain, 1972; Subramanian and Rajendran, 1981; Horie et al., 1993). Ascus and ascospore development is extremely slow or the stromata may remain abortive. The *Aspergillus* anamorph is characterized by tan-colored conidial heads, biseriate aspergilla with a globose to pyriform vesicle, and subglobose to ovoid, smooth-walled (micro-tuberculate under SEM) conidia (Raper and Fennell, 1965; Christensen, 1982; Klich and Pitt, 1988; Horie et al., 1993). It was recently transferred to the *A. wentii* group by Kozakiewicz (1989), due to the common characters of micro-tuberculate conidia, two series of conidiogenous cells and, ecologically, inhabitation of soil.

The second species, *P. albertensis*, is morphologically so similar to the type species that reliable separation becomes difficult. Only one notable character in *P. albertensis* is the irregular-shaped, indeterminately growing stromata.

Petromyces muricatus can be easily distinguished from these known species by the pale yellow to flesh colored ascostromata, fewer (usually one, sometimes two in number) ascomata in the ascostroma, smaller ascospores with spinulose convex surfaces, and light yellow conidial heads. The anamorph of *P. muricatus* clearly belongs to the section *Circumdati* of the subgenus *Circumdati* (Gams et al., 1985). The ornamentation of the conidia of the fungus might be interpreted to indicate a relationship to the species of the lobate-reticulate category in this section (Kozakiewicz, 1989). Among the species, the fungus somewhat resembles *A. sclerotiorum* Huber and *A. sulphureus* (Fres.) Wehmer in certain macroscopic and microscopic features. Besides the development of sclerotoid bodies to the ascostromata, the fungus differs from both species of *Aspergillus* in having light color in the colony reverse on MEA, longer conidiophores and larger metulae.

ACKNOWLEDGMENTS

We thank Prof. R.T. Hanlin, University of Georgia, for reviewing this manuscript. The junior authors wish to gratefully acknowledge Dr. H. Iino, Showa Women's University, for supplying the soil samples in the Philippines.

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