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A NEW INDETERMINATE STROMATAL TYPE IN *PETROMYCES*

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ABSTRACT

A new species of *Petromyces*, characterized by irregularly-shaped, indeterminately-growing stromata, is described. The anamorph of *P. albertensis* is very similar to *Aspergillus alliaceus* and is recognized as the new species *A. albertensis*.

Key Words: *Petromyces albertensis, Aspergillus albertensis, Aspergillus ochraceus* group, taxonomy.

This article describes a new species of *Petromyces* Malloch & Cain that is characterized by the presence of irregularly-shaped stromata exhibiting indeterminate growth through one to several growing points. The associated *Aspergillus* Micheli anamorph is almost indistinguishable from *A. alliaceus* Thom & Church.

The cultures were grown on cereal agar (Pablum cereal, 50 g; Difco agar, 15 g; distilled water, to 1 liter) and Czapek's solution agar. All colors given in italics are after Ridgway (**1912**). The material was examined by light microscopy, scanning electron microscopy (SEM) and by freeze-fracture (FF) transmission electron microscopy. For SEM, the material was vapor-fixed with osmium tetroxide and air-dried or liquid-fixed in 3% glutaraldehyde followed by 2% osmium tetroxide (both in 0.1 M sodium phosphate buffer, pH 7), washed several times in distilled water, dehydrated in a water-acetone series and critical-point dried. Both preparations were coated with gold and examined in a Cambridge Stereoscan 150 SEM. For freeze-fracturing, the material was suspended in 20% aqueous glycerol, positioned on gold-nickel specimen holders and frozen in molten Freon 22. It was then fractured at −100 C and replicated with an oblique shadow of platinum-carbon and a vertical shadow of carbon using a Balzars BA 360 M high vacuum freeze-etch unit (Moor, **1971**; Tewari *et al.*, **1971**). The replicas were examined in a Philips EM300 transmission electron microscope operated at 80 kV.

Petromyces albertensis Tewari, sp. nov. status imperfectus sit judicatus **Aspergillus albertensis** Tewari, sp. nov. FIGS. 1–14

Coloniae in agaro cereali post dies 14 sub calore 22 C usque ad 7.5 cm diam attingentes, facie mycelii pallide alba, in maturioribus *pallide cinereo-olivea*. Capitula conidica magna *cinnamomea* ad pallide *umbram Saccardonis* secundum aetatem coloniae, 150–300 μm diam. priusquam in densas et divergentes columnas ramosa. Capitula conidica deminutiva, columnaria, eodem colore. Conidiophorae plerumque ad 3500 × 17.6 μm, raro ad 8000 μm longae. Vesiculae ad 95 μm crassae, globosae ad subglobosae. Metulae in cunctis capitulis praeter deminutivissimis inventae, cuneatae, non septatae, in maturioribus elongatae, 4.6–23 × 2–7 μm. Phialidae 5.7–9.5 × 1.7–2.5 μm. Conidica ovata ad subglobosa, levia, 2.3–3.5 × 1.7–3.5 μm. Stromata erecta, sessilia, primo fere globosa et alba, denim vel in uno vel in nonnullis locis colentia prominentia nonnumque ramosa, ad apices alba, digitalia, basi atro-brunnea, ad 3.5 μm alta. Ascocarpae in stromatis ad septem continentibus ante dies 28 inceptae, post ca. dies 56 maturescentes, inostiolatae, usque ad 325 μm diam. Asci 11.7–14.7 × 10.2–14.7 μm. Ascosporae ellipsoideae ad fere globosae, linea elevata, in maturioribus obscura, hyalinae, 4.4–8.8 × 2.9–7.3 μm.

Culture typica Iunio 1968 a J. W. Carmichael a specimine culturae auris in Edmonton (Alberta) isolate et in herbario Universitatis Albertaensis sub numero UAMH No. 2976 deposita.

Colonies on cereal agar after 14 da at 22 C about 7.5 cm in diam with white

margins and whitish mycelial mat (FIG. 1) which turns *light grayish olive* upon ageing. Exudate droplets abundant. Colony reverse light cream turning brownish with age. Large conidial heads (FIG. 4) borne on long conidiophores, arising from the mycelial mat initially centrally, *cinnamon-buff* to light *Saccardo's umber* depending on the colony age, splitting into compact divergent columns, 150–300 μm in diam before splitting. Dimunitive conidial heads (FIG. 5) borne on shorter conidiophores arising from the aerial hyphae, columnar, similar in color. Conidiophores (FIGS. 6, 7) commonly up to 3500 × 17.6 μm, rarely up to 8000 μm long, somewhat sinuous, the longer ones faintly yellow, the shorter ones hyaline, with two-layered walls up to 3 μm thick, the outer and inner cell wall surfaces generally smooth but sometimes roughened on the outer or inner sides in parts of the long conidiophores. Vesicles (FIGS. 6, 7) up to 95 μm in diam ranging from globose to subglobose in the larger conidial heads to almost hemispherical in the dimunitive conidial heads, the former with walls up to 3 μm thick and fertile over their entire surfaces, the latter thinner walled and usually with a little barren part. Metulae present in all heads except some of the very dimunitive ones, wedge-shaped, non-septate, elongating with age, 4.6–23 × 2–7 μm. Phialides 5.7–9.5 × 1.7–2.5 μm. Conidia (FIGS. 8–10) oval to subglobose, with up to 1.5 μm long connectives as seen by SEM, appearing smooth by light microscopy but minutely warty by SEM and FF, fractured faces with groups of rodlets as seen by FF, thin-walled, 2.3–3.5 × 1.7–3.5 μm. Stromata (FIGS. 2–4) up to 3.5 mm high, erect, sessile, almost globose and white at first, with age, developing one to several growing points giving rise to white-tipped, grayish and sometimes branched finger-like extensions, the basal part dark brown. Ascocarps (FIG. 11) initiated before 4 wk and maturing by about 8 wk, inostiolate, up to about 7 per stroma, with persistent walls consisting of several layers of brown, thick-walled cells, up to about 325 μm in diam. Asci (FIG. 12) oval to subglobose, eight-spored, evanescent, 11.7–14.7 × 10.2–14.7 μm. Ascospores (FIGS. 13, 14) ellipsoidal to almost globose, with a fine ridge that becomes indistinct with age as seen by light microscopy, clearly seen by SEM in all stages, with an eccentric globule, hyaline, variable in size, 4.4–8.8 × 2.9–7.3 μm.

The type culture was isolated in June, 1968, by J. W. Carmichael from an ear swab from Edmonton, Alberta. It was received by the author in 1971 as *A.*

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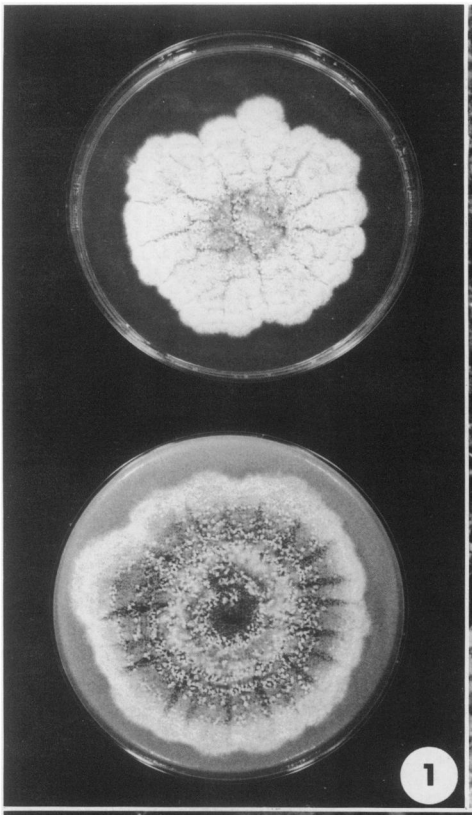
FIGS. 1–4. *Petromyces albertensis.* 1. Colonies on cereal agar (lower) and Czapek's agar (upper) after 14 da growth at 22 C, ×0.5. 2. Colony margin on cereal agar. Note the regularly-shaped stromata at the colony margin (upper) and their irregularly-shaped form in the interior regions (lower), ×8. 3. Colony on cereal agar. Note the proliferating stromata with finger-like extensions, ×2. 4. Colony on cereal agar showing the large, radiate conidial heads and proliferating stromata, ×8.

FIGS. 5–10. *Petromyces albertensis* (on cereal agar). 5. Dimunitive columnar conidial heads, ×25. 6. Larger conidial heads, ×130. 7. The smaller dimunitive head. Note that the metulae are absent, ×650. 8. Conidial chains. Air-dried SEM preparation. The shrinkage is artefactual. Note the long connectives (arrowheads) and minute warts on the conidial surface, ×7300. 9. Conidia. Freeze-fracture preparation. Note the minute warts and groups of rodlets on the conidial fractured face. Upper left—concave fracture, lower—convex fracture. The arrow in the lower right corner indicates the direction of shadowing, ×29,300. 10. A higher magnification view of the conidial convex-fractured face. The arrow in the lower right corner indicates the direction of shadowing, ×104,000.

FIGS. 11–14. *Petromyces albertensis* (on cereal agar). 11. SEM preparation of a stroma cut with a razor blade after critical point drying. Note the six ascocarps in sectional views, ×115. 12. Asci, ×940. 13. Ascospores. A few ascospores are seen in sectional views (arrowheads) while many others show the equatorial ridges, ×1460. 14. Ascospores. SEM preparation same as in FIG. 11. Note the equatorial crests and variation in the size of ascospores, ×3600.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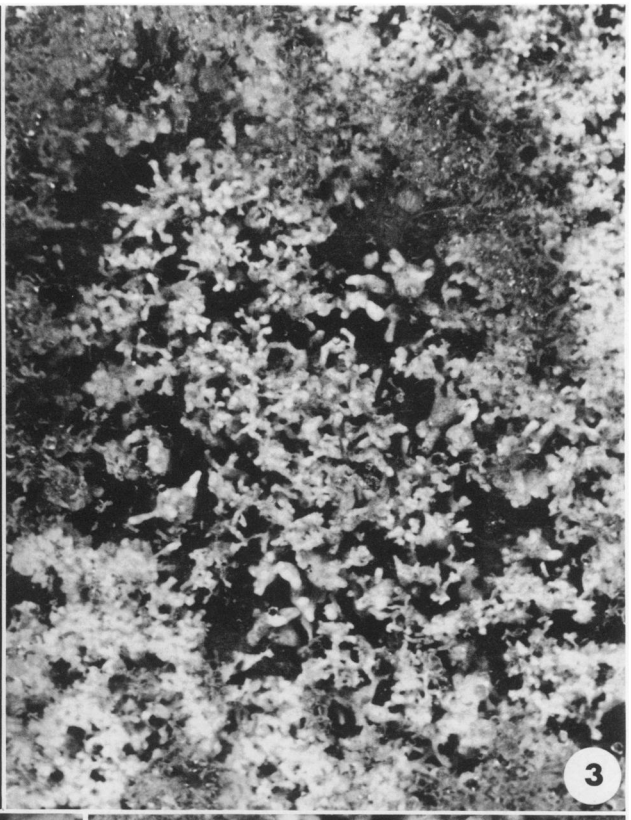
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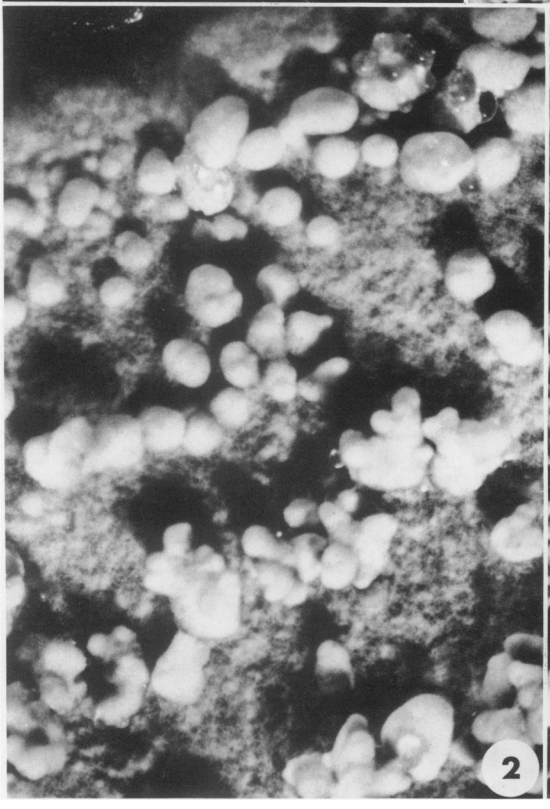
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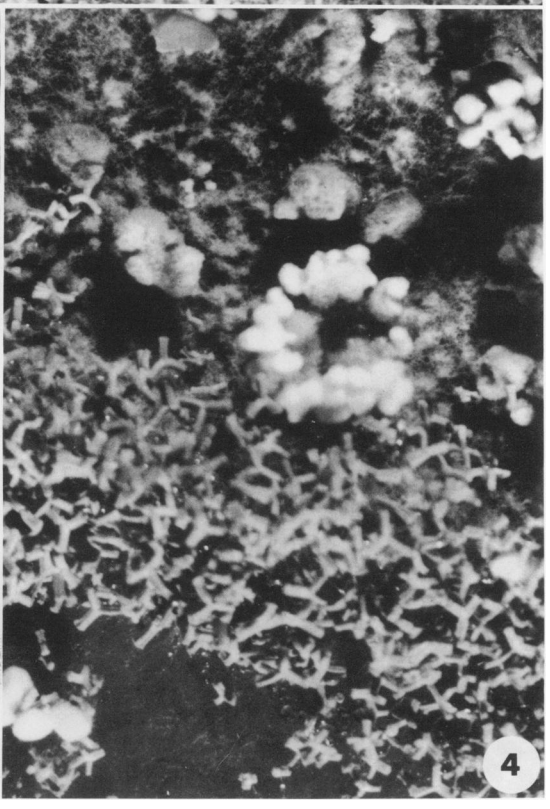
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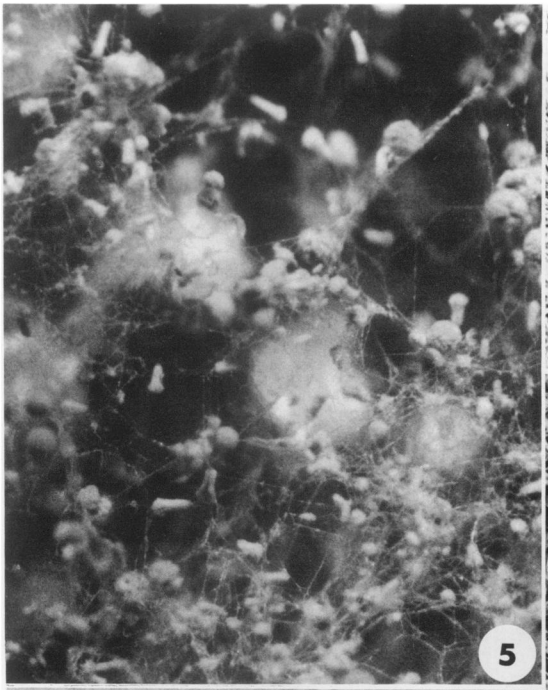
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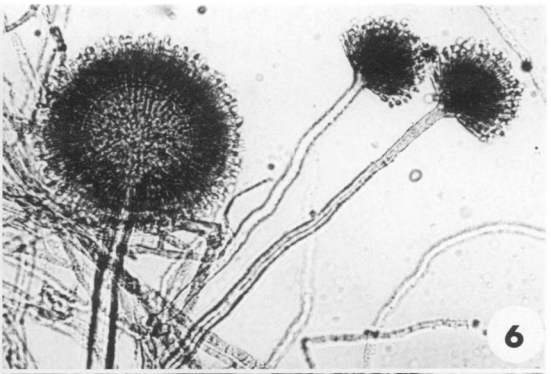
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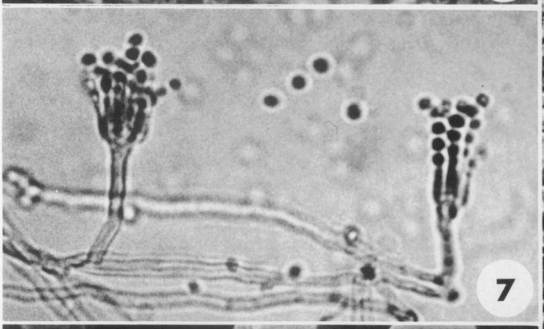
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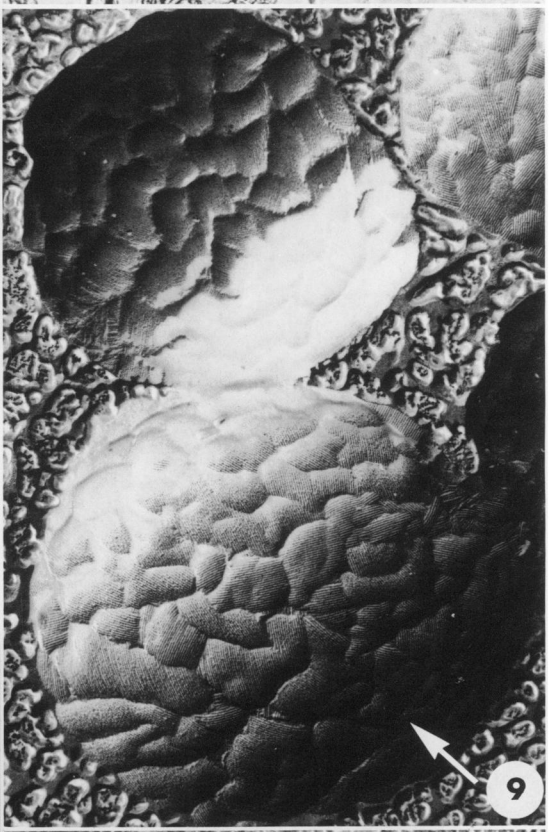
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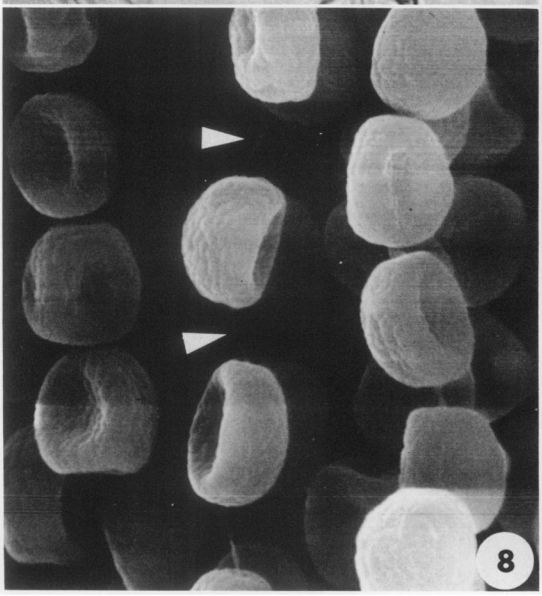
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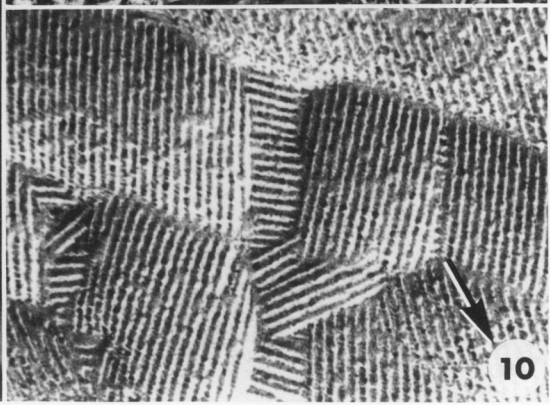
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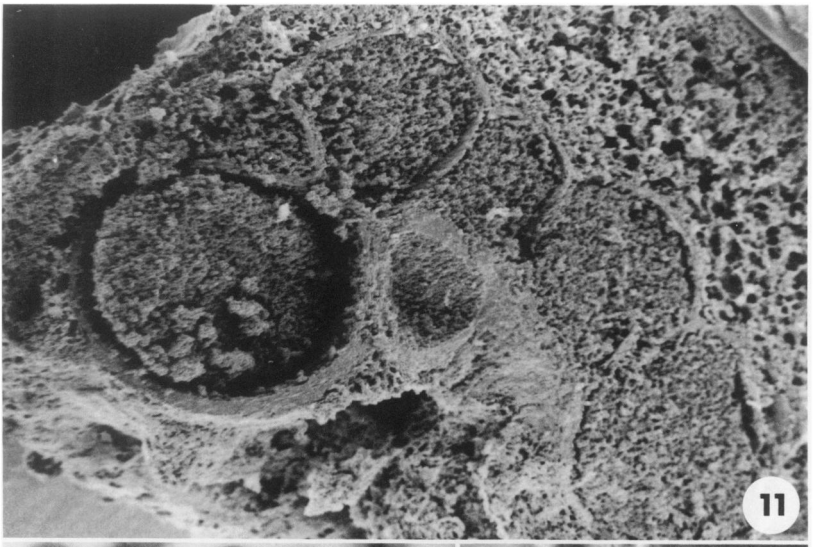
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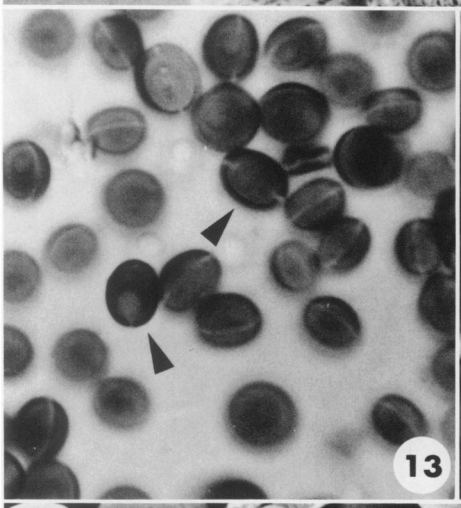
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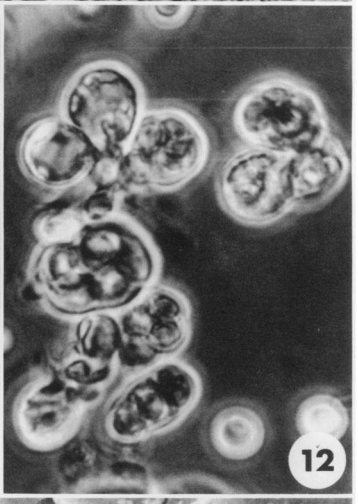
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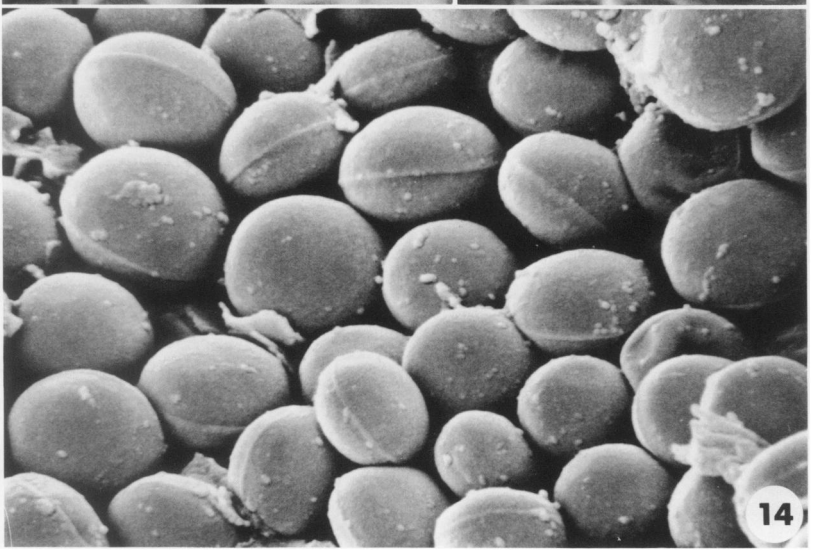
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alliaceus. It has remained unchanged, with the author, during these many years of cultivation and was named as *P. alliaceus* in the author's study on the development of stromata in this fungus (Tewari, **1983**).

Petromyces albertensis shows much more robust growth on cereal agar than on Czapek's solution agar (FIG. 1). On the latter, the fungus differentiates much more slowly.

The fungus described has an anamorph belonging to the *A. ochraceus* group and produces asci in one to several inostiolate ascocarps within upright stroma. It therefore belongs to the genus *Petromyces* which so far has only one species, *P. alliaceus* Malloch & Cain (Raper and Fennell, **1965**; Malloch and Cain, **1972**; Fennell, **1977**; Christensen, **1982**). The stromata in *P. alliaceus* are gray-black, white-tipped, and ovate to ellipsoidal whereas in *P. albertensis* they are irregular in shape since the dark brown basal part bears one to several grayish, white-tipped, finger-like, indeterminately growing extensions. The determinate form of the stromata (and sclerotia) almost appears to be a rule in species belonging to *Aspergillus* and their teleomorphs (Raper and Fennell, **1965**). *Petromyces albertensis* and two other fungi, the latter described as strains of *Aspergillus niger* van Tieghem and *A. flavus* Link (Rai *et al.*, **1969**), would appear to be exceptions to this rule. Rai *et al.* (**1969**) interpreted such a behavior in the latter two strains as reflecting *in situ* germination of the sclerotia. However, since this represented a phase in the maturation of sclerotia, such a behavior should have been recognized as indicating the presence of indeterminately growing sclerotia. It should also be mentioned that the stromata (or sclerotia) of many species of *Aspergillus* and their teleomorphs are white-tipped, indicating the potential of indeterminate growth. However, this indeterminate growth is expressed only in a few fungi of this group, including *P. albertensis*.

The ascospores in *P. alliaceus* are reported to have an equatorial furrow (Raper and Fennell, **1965**; Malloch and Cain, **1972**) whereas in *P. albertensis* they have an equatorial ridge. The dimunitive heads in members of *A. ochraceus* group characteristically have biseriate elements (Christensen, **1982**). *Aspergillus albertensis* differs from these in some of the dimunitive heads having only uniseriate elements.

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