

Note

Materials for *Pythium* Flora of Japan VIII Two species of *Pythium*: *P. pyriforme* and *P. oligandrum*

Tomio Kinoshita¹⁾, Takio Ichitani²⁾ and Taeko Okumura²⁾

¹⁾ Hyogo Women's College, Hiraoka-cho, Kakogawa, Hyogo-ken 675-01, Japan

²⁾ Laboratory of Plant Pathology, University of Osaka Prefecture, Gakuen-cho, Sakai, Osaka 593, Japan

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Two species of *Pythium* (*P. pyriforme*, *P. oligandrum*) having spherical sporangia with complex subglobose elements were isolated from the crown of creeping bentgrass [*Agrostis palustris* (cv. Penncross)] and from vegetable field soil, respectively. They are reported for the first time in Japan.

Key Words—bentgrass; Japan; *Pythium pyriforme*; *Pythium oligandrum*; soil.

In the course of a study on a new disease of bentgrass greens during summer 1990 (Miyagawa et al., 1991), *Pythium pyriforme* was isolated from the diseased crown as a non-pathogen. In July 1991, one isolate of *P. oligandrum* was obtained during a survey of *Pythium* spp. in vegetable field soil (Tojo et al., 1992). Both species were maintained as described previously (Ichitani and Kang, 1988) and identified following the methods reported elsewhere (Ichitani et al., 1986). About 200 each of oogonia and oospores chosen at random gave values for diameter.

Part of the work has been reported elsewhere (Kinoshita et al., 1994).

Pythium pyriforme Vaartaja Figs. 1–4, 9–38

At the early stage of culture, colonies on Bacto-CMA submerged with mixed rosette and chrysanthemum patterns; on Bacto-PDA arachnoid with aerial mycelium. Cabbage-rose pattern with less aerial mycelium at the later stage on the latter medium. Main hyphae up to 5.0 μm wide, having constrictions in the upper part of the branching region, septate when old. Sporangia spherical or broadly ovoid, occasionally pyriform or filamentous, 14–18 μm , 16 μm on average, but more usually composed of single or complex combinations of both these elements joined without septa; irregularly swollen sporangia or sporangial elements rarely formed. Germination by germ tubes, especially from smaller sporangia, or by zoospores. Zoospores readily formed after 2 days at 28–31°C. Discharge tubes 2.5–7.5 μm wide and 28–225 μm long, originating from the filamentous elements of the complexes. Oogonia smooth, spherical, 23–31 μm diam, 26 μm on average, intercalary or terminal. Antheridia 1–7, usually 2–4 per oogonium, declinous or monoclinal with a long stalk. Two antheridia, sometimes three, arising from one stalk, the stalk commonly

coiled around the oogonium. Antheridia usually crook-necked with an apical contact to the oogonium, rarely lateral. Oospores plerotic, sometimes aplerotic, 20–28 μm diam, 24 μm on average, wall 1.0–2.0 μm thick. Oospheres became abortive in old cultures.

Cardinal temperatures: minimum 7–10°C, optimum 25–31°C, maximum 37°C. Daily mycelial growth of 28 mm on Bacto-CMA at 25°C.

Description: based on UOP 400 (deposited in the Herbaria, Institute for Fermentation, Osaka, as IFO 32560, and the National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, as MAFF 236743).

Isolation: on selective medium (Fukui et al., 1987) from crown of creeping bentgrass (cv. Penncross) collected at Miki-cho, Kita-gun, Kagawa Prefecture, 19 August 1990, by T. Tani.

Host range: not examined in detail, but non-pathogenic on creeping bentgrass (cv. Penncross).

Aplerotic index was calculated as >65% here, whereas Dick (1990) reported it as <65%. Average wall index obtained here was 65%, coinciding with the index (>55%) reported by Dick (1990).

Although the slender mycelium, plerotic oospores and thinner oospore wall of this isolate differ slightly from those of *P. pyriforme* (Vaartaja, 1965), the fungus may be identified as *P. pyriforme* Vaartaja, considering the similarity of the other main characters.

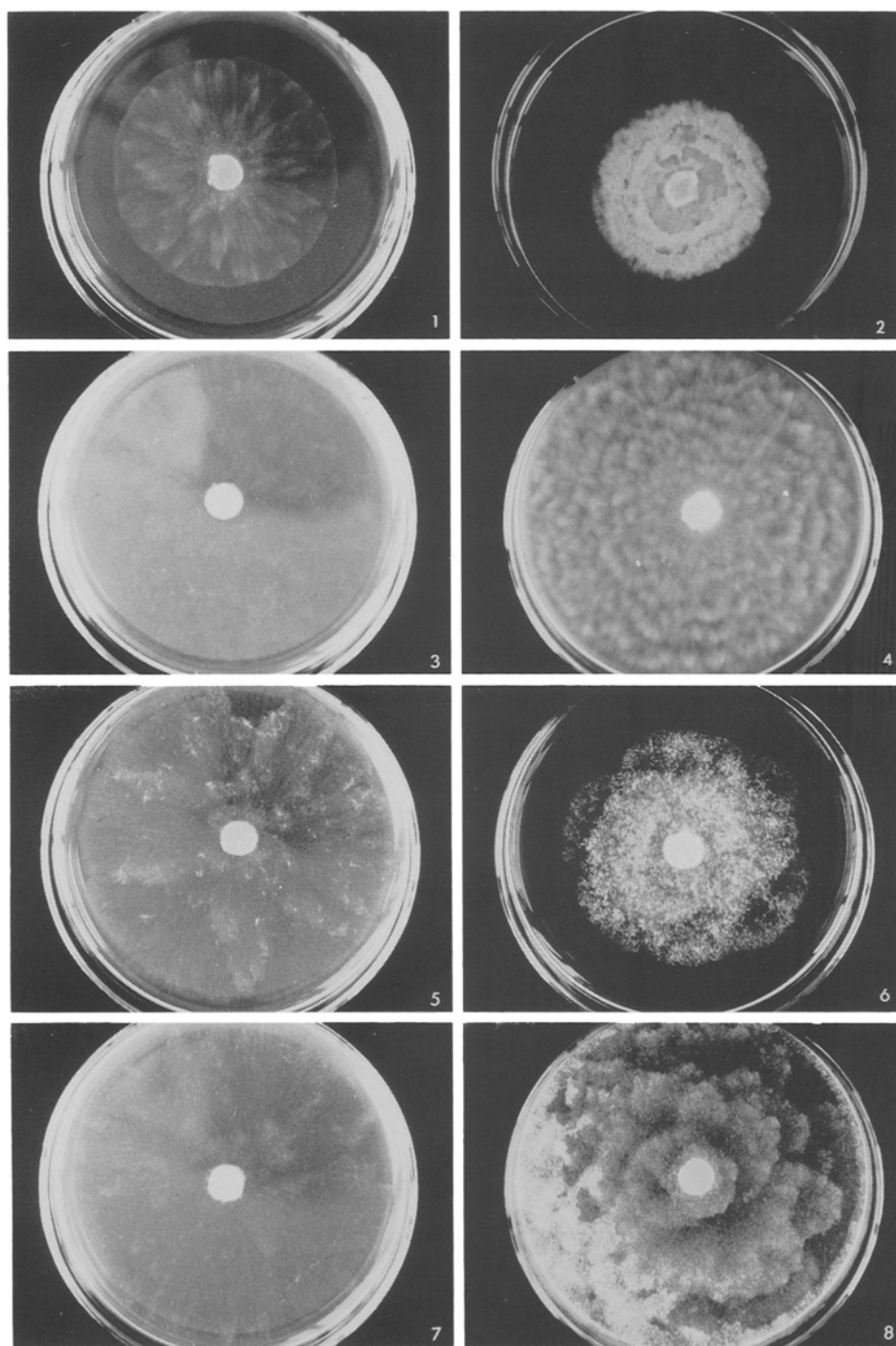
The only other records of *P. pyriforme* appear in two papers from Australia (Vaartaja, 1965; Ward and Ship-ton, 1984) and one from U.K. (Dick and Ali-Shtayah, 1986).

Pythium oligandrum Drechsler Figs. 5–8, 39–85

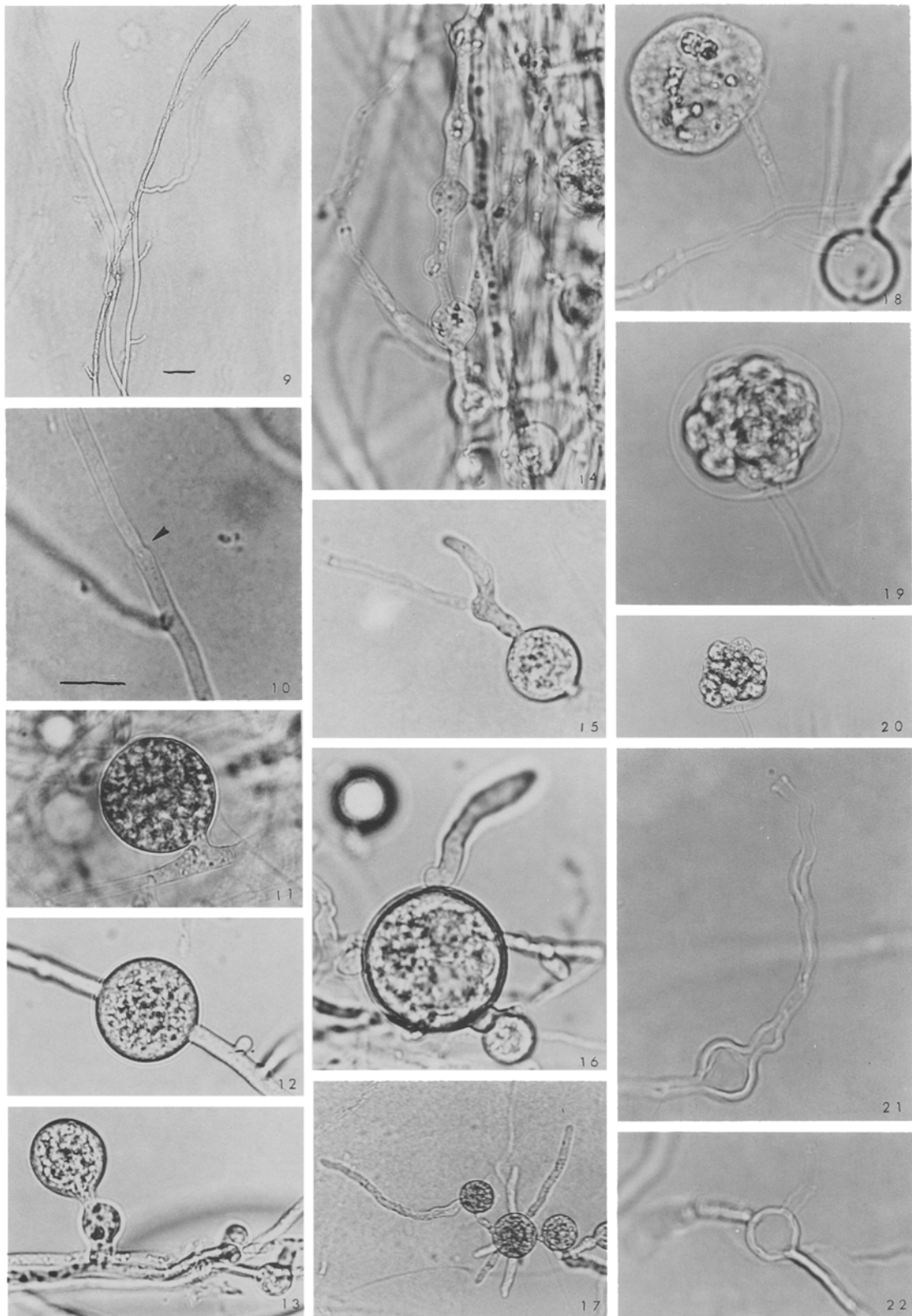
Colonies on Bacto-CMA submerged with a chrysanthemum pattern at the early stage of culture, but no spe-

cial pattern at the later stage; on Bacto-PDA a chrysanthemum pattern with aerial mycelium at all stages. Main hyphae up to $7.5\ \mu\text{m}$ wide, septate when old. Sporangia spherical or broadly ovoid, $12\text{--}38\ \mu\text{m}$, $14\ \mu\text{m}$ on average, contiguous, forming irregular aggregates consisting of one or more subglobose elements with connecting filamentous parts, mostly intercalary, occasionally terminal. Germination by germ tubes or by zoospores. Zoo-

spores readily formed after 2–3 days at $20\text{--}30^\circ\text{C}$. Discharge tubes $4.5\text{--}6.5\ \mu\text{m}$ wide and $18\text{--}55\ \mu\text{m}$ long, mostly formed at the distal end of sporangia. Oogonia spherical, terminal or intercalary, $25\text{--}30\ \mu\text{m}$, $29\ \mu\text{m}$ on average, wall with tapering, sharply pointed projections, $6\text{--}8\ \mu\text{m}$ long, $3\text{--}4\ \mu\text{m}$ diam at the base. Antheridia mostly lacking, but sometimes 1–2 (–3) per oogonium, diclinous, occasionally monoclinal, often adhering

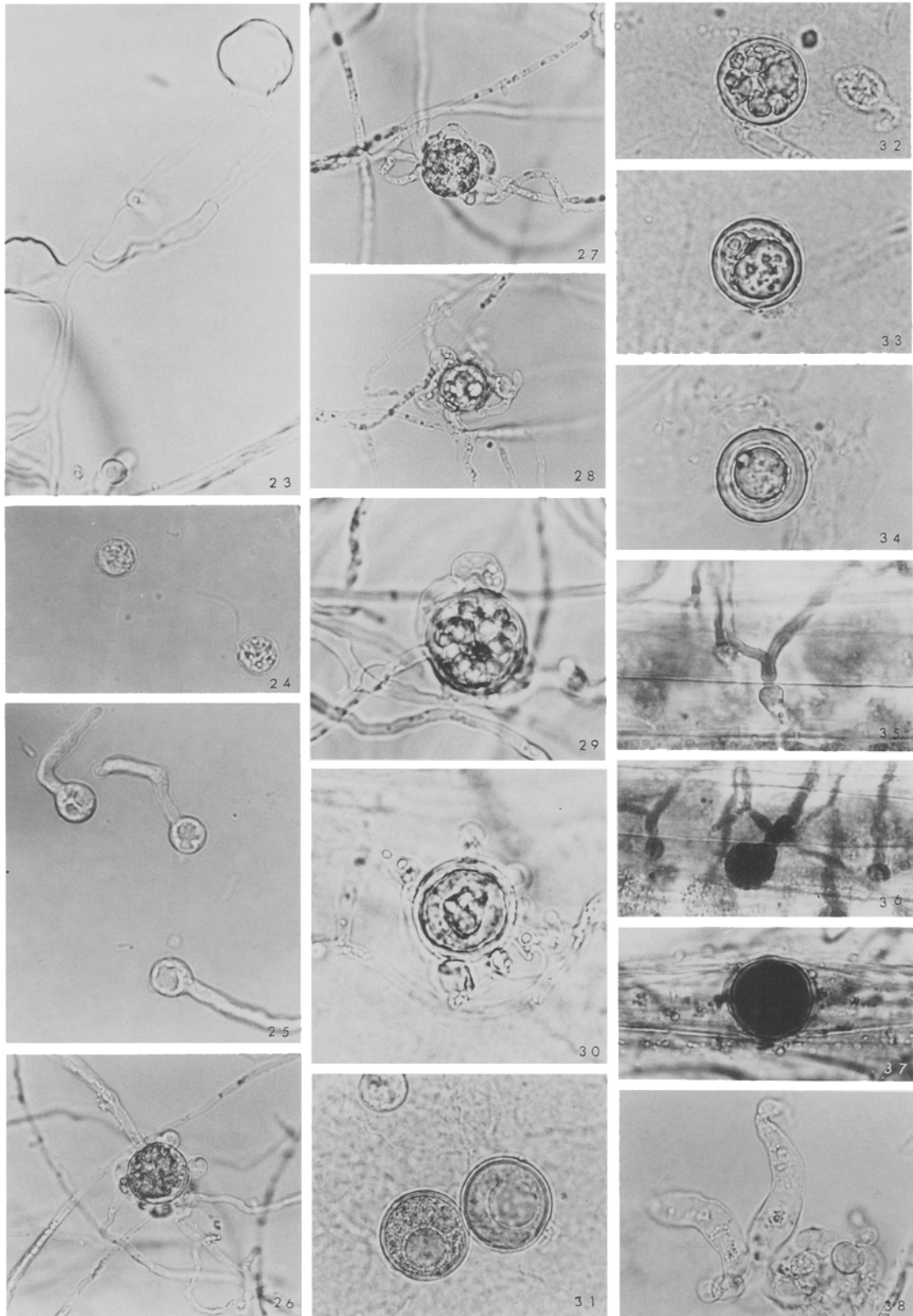


Figs. 1–8. Growth habits of *P. pyrlobum* UOP400 (1–4) and *P. oligandrum* UOP399 (5–8) on Bacto-CMA (left) and Bacto-PDA (right) incubated at 25°C for 2 days (1, 2, 5, 6) and 16 days (3, 4, 7, 8), respectively.



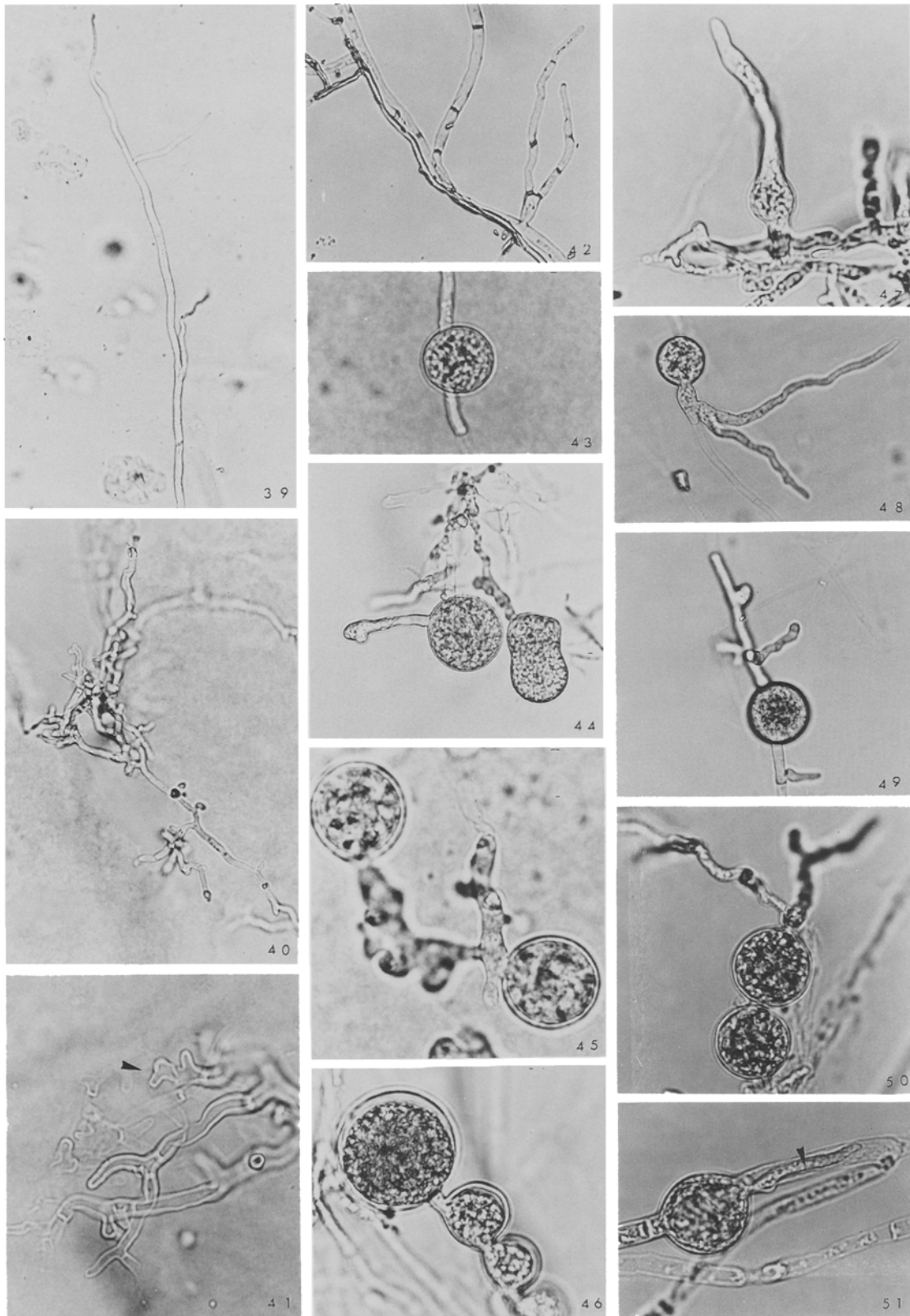
Figs. 9-22. Morphology of *P. pyrilobum* UOP 400.

9: Mycelia. 10: Hypha having constriction (arrow). 11-14: Sporangia. 15-17: Direct germination of sporangia. 18-20: Vesicles. 21-22: Empty sporangia.

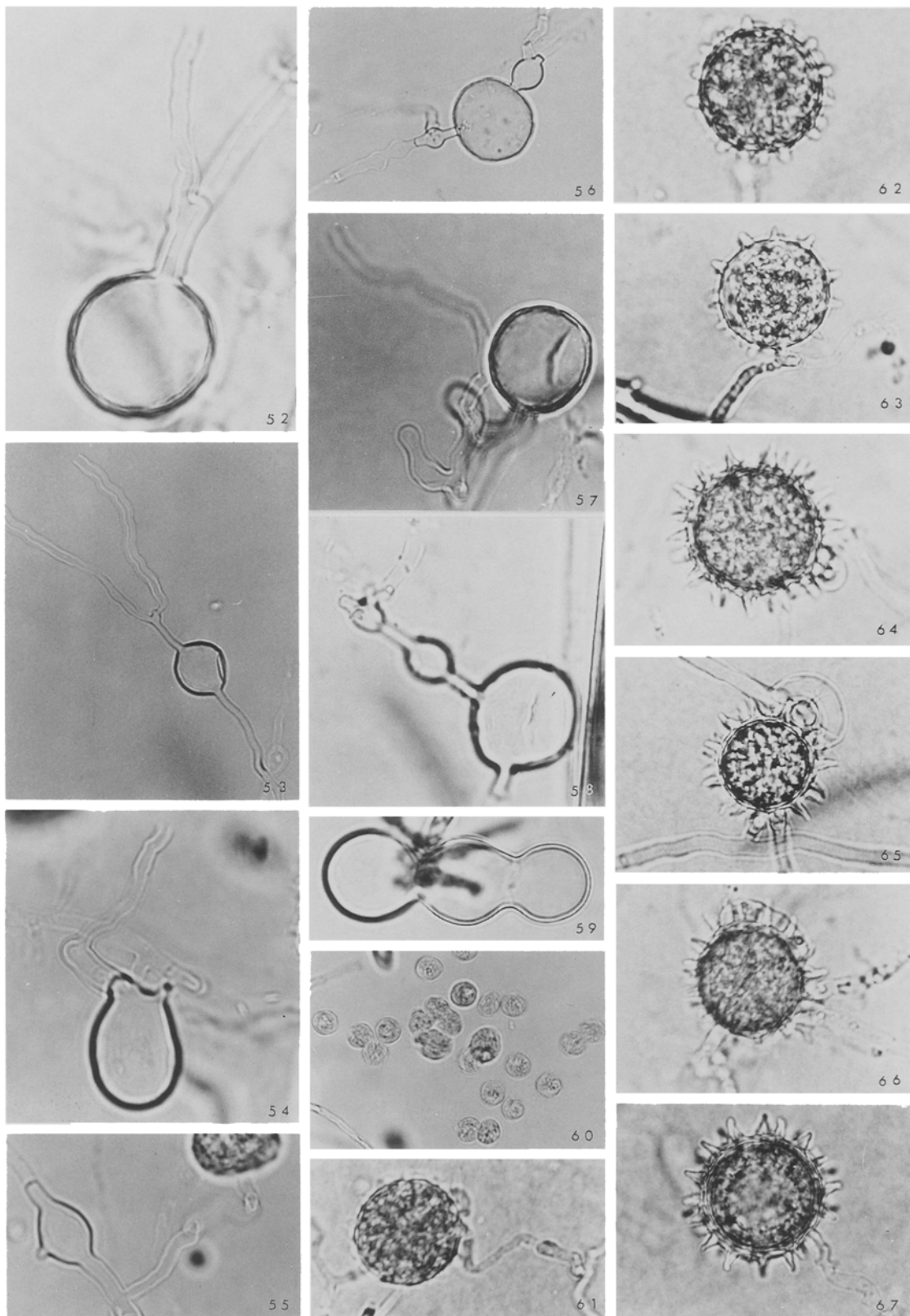


Figs. 23-38. Morphology of *P. pyriformis* UOP 400.

23: Empty sporangia. 24: Encysted zoospores. 25: Germinated zoospores. 26-30: Oogonia and antheridia. 31-34: Oospores. 35-37: Propagules in leaf tissues of bentgrass. 38: Appressoria.

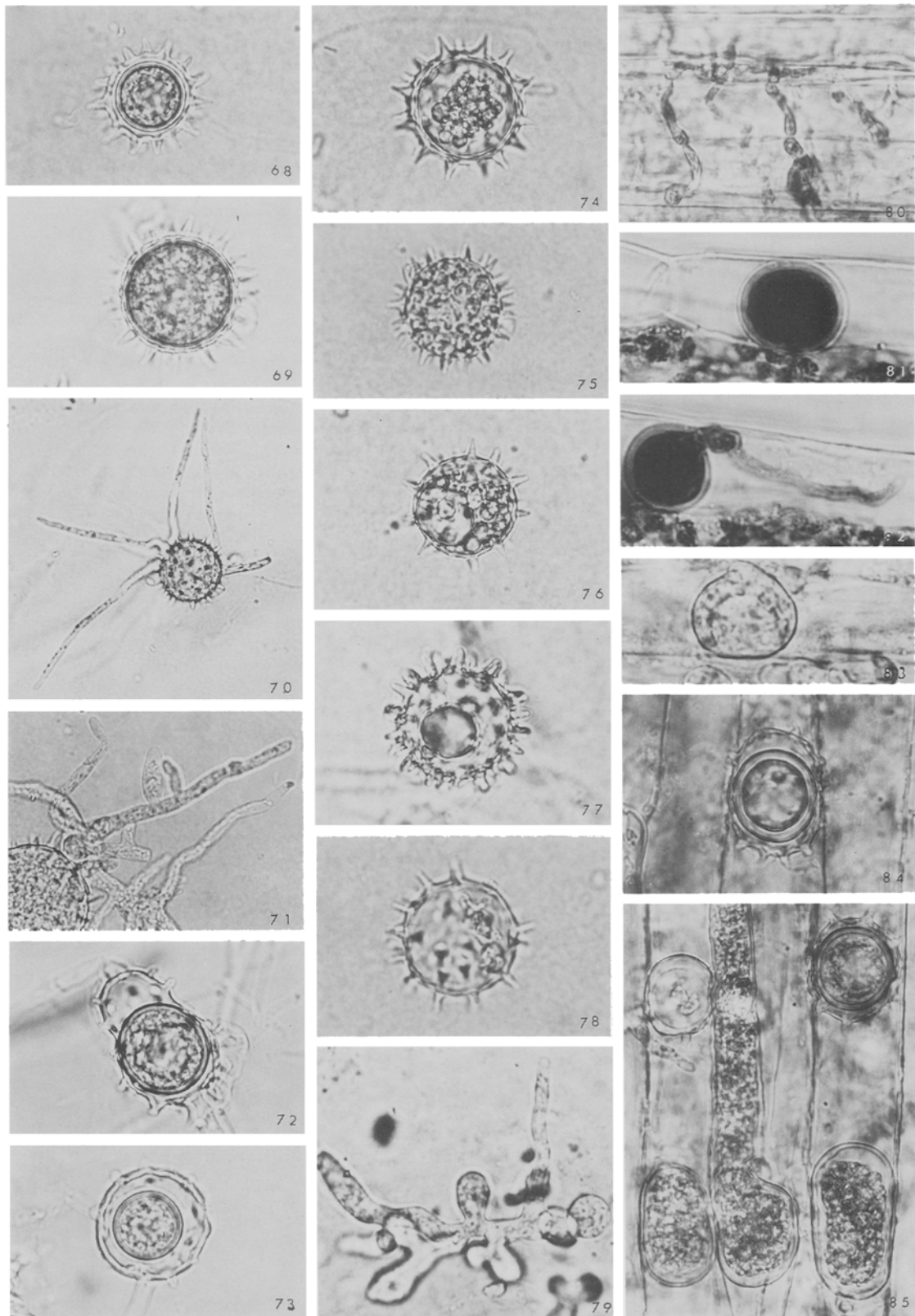


Figs. 39-51. Morphology of *P. oligandrum* UOP 399.
39, 40: Mycelia. 41: Hypha with short pocket (arrow). 42: Old septate hyphae. 43-46: Sporangia. 47-51: Direct germination of sporangia [germination (arrow in fig. 51) may occur inside a hypha].



Figs. 52-67. Morphology of *P. oligandrum* UOP 399.

52-59: Empty sporangia. 60: Encysted zoospores. 61, 64-66: Oogonia and antheridia. 62, 63: Young oogonia. 67: Oospores.



Figs. 68–85. Morphology of *P. oligandrum* UOP 399.

68–69, 72–78: Oospores (72: Inside an abnormal-shaped oogonium). 70, 71: Germinated oospores. 79: Appressoria-like body. 80–85: Propagules in leaf tissues of bentgrass. Bars (20 μ m) on figs. 9 and 10 are applicable to figs. 17, 20, 26–28, 39, 40, 42–44, 48, 49, 53, 68, 70 and to the rest to the figures, respectively.

lengthwise to the oogonium, often with one or two transverse constrictions and appearing lobate at the early stage of culture. Oospores plerotic, sometimes aplerotic, 22–31 μm diam, 26 μm on average, wall 1.0–2.0 μm thick. Oospheres became abortive in old cultures.

Cardinal temperatures: minimum 4–7°C, optimum 28°C, maximum 37°C. Daily mycelial growth of 15 mm on Bacto-CMA at 25°C.

Description: based on UOP 399 (deposited in the Herbaria, Institute for Fermentation, Osaka, as IFO 32559, and the National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, as MAFF 236742).

Isolation: isolation on VP₃ medium (Ali-Shtayeh et al., 1986) at 25°C, using oven-disinfected cucumber seeds buried within 24 h at 30°C as bait in vegetable field soil (35.3% water content) collected from the University Farm, University of Osaka Prefecture, Sakai, Osaka, 9 July 1991, by E. Nakazono.

Host range: not examined.

Aplerotic index was calculated as 67–73% here, whereas Dick (1990) reported it as 60–68%.

Although the plerotic oospores of this isolate also differ slightly from those of *P. oligandrum* (Drechsler; 1930, 1946), the fungus may be identified as *P. oligandrum* Drechsler, considering the similarity of the other main characteristics.

P. oligandrum has been reported as an aggressive mycoparasite (Deacon, J. W., 1976) and mycoparasitism of this fungus is under investigation (Kinoshita et al., 1994).

There have been no previous records of *P. oligandrum* from Japan.

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Literature cited

- Ali-Shtayeh, M. S., Ho, C.-L. and Dick, M. W. 1986. An improved method and medium for quantitative estimates of populations of *Pythium* species from soil. *Trans. Br. Mycol. Soc.* **86**: 39–47.
- Deacon, J. W. 1976. Studies on *Pythium oligandrum*, an aggressive parasite of other fungi. *Trans. Br. Mycol. Soc.* **66**: 383–391.
- Dick, M. W. 1990. "Keys to *Pythium*," pp. 9–32. College of Estate Management, White knights, Reading.
- Dick, M. W. and Ali-Shtayeh, M. S. 1986. Distribution and frequency of *Pythium* species in parkland and farmland soils. *Trans. Br. Mycol. Soc.* **86**: 49–62.
- Drechsler, C. 1930. Some new species of *Pythium*. *J. Wash. Acad. Sci.* **20**: 398–418.
- Drechsler, C. 1946. Several species of *Pythium* peculiar in their sexual development. *Phytopathology* **36**: 781–864.
- Fukui, R., Kitano, S., Takamatsu, S. and Ichitani, T. 1987. Taxonomic consideration on two closely related species, *Pythium paddicum* and *P. polypapillatum*. *Bull. Univ. Osaka Pref., Ser. B* **39**: 21–31.
- Ichitani, T. and Kang, H. T. 1988. Materials for *Pythium* Flora of Japan (I): Plant pathogenic *Pythium irregulare*. *Bull. Univ. Osaka Pref., Ser. B* **40**: 19–26.
- Ichitani, T., Takamatsu, S. and Stamps, D. J. 1986. Identification and pathogenicity of three species of *Pythium* newly isolated from diseased wheat and barley just after thawing in Japan. *Ann. Phytopath. Soc. Japan* **52**: 209–216.
- Kinoshita, T. and Ichitani, T. 1994. Hyphal interactions between a mycoparasite, *Pythium oligandrum*, and *P. ultimum*. Abstract presented at annual meeting of the Phytopathological Society of Japan, Tsukuba (in Japanese).
- Kinoshita, T., Ichitani, T. and Tani, T. 1994. Identification of two species of *Pythium* from bentgrass and upland field soil. *Ann. Phytopath. Soc. Japan* **59**: 750 (in Japanese).
- Miyagawa, T., Tani, T. and Ichitani, T. 1991. A new *Pythium* disease causing heavy damage on bentgrass greens during summer season. Abstract presented at annual meeting of the Nishi-Nippon Division of the Mycological Society of Japan. p. 8 (in Japanese).
- Tojo, M., Nakazono, E., Hotta, K. and Ichitani, T. 1992. Seasonal variations of *Pythium* spp. in vegetable field soil. *Proc. Kansai Pl. Prot.* **34**: 11–16 (in Japanese).
- Vaartaja, O. 1965. New *Pythium* species from South Australia. *Mycologia* **57**: 417–430.
- Ward, D. E. and Shipton, W. A. 1984. Root rot of papaw caused by *Pythium pyrlobum*. *Australasian Plant Pathology* **13**: 25–27.