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**Materials for Pythium Flora of Japan (II) *Pythium torulosum* and *P. vanterpoolii*
from Golfgreens of Manilagrass or Bentgrass**

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Abstract

Hundreds of isolates of *Pythium torulosum* Coker & Patterson and *P. vanterpoolii* V. Kouyeas & H. Kouyeas were obtained, each as a non-pathogen and pathogen, from spring dead spot of manilagrass and Pythium blight of creeping bentgrass. Both fungi were described and illustrated. Differences between the two species were discussed from biological and pathological points of view.

In the course of the cooperative researches with Kagawa University on spring dead spot of manilagrass (*Zoysia matrella* Merr.) and Pythium blight of bentgrass (*Agrostis palustris* Huds.)¹⁻³, two hundred and thirty-eight isolates of *Pythium torulosum* Coker & Patterson and four hundred and sixty-two of *P. vanterpoolii* V. Kouyeas & H. Kouyeas were obtained. Both fungi were maintained as described previously⁴.

These two species are closely related morphologically and sometimes difficult to separate from each other⁵. This paper deals with a detailed description and drawing of these species, following the method reported previously^{4,6}, and discusses their differences from biological and pathological points of view.

Pythium torulosum (Plates I-1, II-IV)

P. torulosum was more often isolated from turf of manilagrass than from creeping bentgrass. Frequency was low from inner parts of both grasses such as basal segments of the newly developing leaves and high in rhizosphere soils (Table 1). A detailed description of this fungus is as follows.

Sporangia consisting of inflated branched outgrowths, forming toruloid complexes of various sizes. Discharge tubes long and slender, producing zoospores at about 20°C. Oogonia globose, frequently terminal, rarely intercalary, 13–20 μm, average 17 μm diam., smooth. Antheridia 1-2 per oogonium, monoclinal, rarely declinal, originating at a distance of 1–23 μm (usually 5–10 μm, rarely 50 μm) from the oogonial stalk, disappearing soon after fertilization. Antheridial cells allantoid clavate, making apical contact with the oogonium. Oospores spherical, plerotic, 10–18 μm, average 15 μm diam., wall less than 2 μm thick.

Cardinal temperatures: minimum 1–4°C, optimum 25–31°C, maximum 34°C. Daily mycelial growth rate on Bacto-CMA at 25°C 24 mm.

Description: based on UOP 366 (= IFO 32167).

Isolation: UOP 366 (= IFO 32167), on Bacto-CMA, from diseased erect stem tissues of manilagrass collected at Kita-cho, Takamatsu City, Kagawa Prefecture, March 22,

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1988, by T. Ichitani.

Host range: not examined extensively, but proved to be non-pathogenic on Japanese lawngrass (*Zoysia japonica* Steud.) using 3 isolates (16-1, 19-1, 23-1)³⁾ and showed some inhibitory effects on vegetative growth of creeping bentgrass seedlings (37 and 23% reduction in dry weight of aerial part of the seedlings as compared with non-inoculated control at 20 and 25°C, respectively), using 3 isolates (19-1, 23-1, 113-14)⁷⁾.

Pythium vanterpoolii (Plates I-2, V-VII)

P. vanterpoolii, like *P. torulosum*, was more often isolated from turf of manilagrass than from creeping bentgrass, and was also isolated more frequent from inner parts of both grasses such as basal segments of the newly developing leaves, and less common in their rhizosphere soils, unlike *P. torulosum* (Table 1). The morphology of this fungus was essentially the same as reported previously¹⁾, except that the diameters of oogonium and oospore were very variable (13–23 µm and 10–22 µm, respectively) in the present experiment. Detailed morphology of this fungus is as follows.

Sporangia consisting of commonly catenulate complexes of subglobose or irregularly ellipsoidal unbranched outgrowths. Zoospores produced at 20°C. Oogonia globose, thin-walled, terminal or intercalary 13–23 µm, average 20.0 µm diam., smooth. Antheridia 1–2 per oogonium, monoclinal, rarely diclinal, commonly originating at a distance greater than the oogonial diameter from the oogonial stalk, disappearing soon after fertilization. Antheridial cells club shaped or cylindrical, making apical contact with the oogonium. Oospores spherical, plerotic, 10–22 µm, average 18.0 µm diam., wall more than 2 µm thick.

Cardinal temperatures: minimum 1–4°C, optimum 23–25°C, maximum 31°C. Daily mycelial growth rate on Bacto-CMA at 25°C 20.0 mm.

Description: based on UOP 369 (= IFO 32170).

Isolation: UOP 369 (= IFO 32170), on Bacto-CMA, from diseased basal segment of newly developing leaf of manilagrass collected at Shiroyama-cho, Sakaide City, Kagawa Prefecture, March 22, 1988, by T. Ichitani.

Host range: not examined extensively, but proved to be pathogenic on creeping

Table 1 Isolation of *P. torulosum* and *P. vanterpoolii* from golfgreens of manilagrass and creeping bentgrass (Dec., 1985 – Apr., 1988)

Greens	<i>Pythium</i> spp. Isolated	Newly developing leaf ^{a)}	Erect stem	Root	Rhizosphere soil
Creeping bentgrass	<i>P. torulosum</i>	1/ 612 ^{b)} (0.16)	4/ 612 (0.65)	0/ 612 (0.00)	8/ 816 (0.98)
	<i>P. vanterpoolii</i>	3/ 612 (0.49)	3/ 612 (0.49)	4/ 612 (0.65)	6/ 816 (0.74)
Manila-grass	<i>P. torulosum</i>	9/2052 (0.44)	27/2052 (1.32)	9/2052 (0.44)	83/2736 (3.03)
	<i>P. vanterpoolii</i>	20/2052 (0.97)	17/2052 (0.83)	10/2052 (0.49)	40/2736 (1.46)

a) Basal segment

b) Ratios of samples from which *P. torulosum* or *P. vanterpoolii* was isolated to samples assayed. The numerals in parenthesis are percentage isolation.

Table 2 Growth habits of *P. torulosum* and *P. vanterpoolii*

Culture medium	<i>P. torulosum</i> (15 isolates)	<i>P. vanterpoolii</i> (5 isolates)
Bacto-CMA	No aerial mycelium; colony thin, rosette, sometimes coarsely radiate, rarely radiate	A little aerial mycelium; colony fairly thick, radiate
Bacto-PDA	Moderate aerial mycelium; more autolytic; colony thick and slightly yellow, rosette	Abundant aerial mycelium; less autolytic; colony thick, radiate

bentgrass, Japanese lawngrass and manilagrass, using isolates UOP 314, 315, P-G₃, P-G₇^{1,2}).

Both species were isolated more often from turf of manilagrass than from creeping bentgrass. More isolates of *P. vanterpoolii* than of *P. torulosum* were obtained from inner parts of both grasses, such as the basal segment of a newly developing leaf, but the former was less frequent in rhizosphere soils (Table 1).

Mycelial growth was compared on Bacto-CMA and -PDA for 5-15 isolates between the two species, but no remarkable differences were found. However, *P. torulosum* has a slightly higher optimum temperature and grew slightly faster than *P. vanterpoolii* at 25 and 28°C.

As V. & H. Kouyeas have already pointed out⁵⁾, *P. vanterpoolii* differs from *P. torulosum* in having catenulate sporangia, antheridia originating further from the oogonial stalk and a thick oospore wall. In addition, a slightly lower temperature for mycelial growth was found for *P. vanterpoolii* in the present experiment together with different growth habits on Bacto-PDA (Plate I, Table 2). According to our observations using several hundred isolates, the above differences should be a major key to separate these two species.

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Explanation of Plates

Plate I Growth habits of *P. torulosum* and *P. vanterpoolii*

1: *P. torulosum* UOP 366 (left) and UOP 365 (right), each on Bacto-CMA (upper) and Bacto-PDA (lower).

2: *P. vanterpoolii* UOP 369 (left), UOP 370 (center) and UOP 368 (right), each on Bacto-CMA (upper) and Bacto-PDA (lower).

Plates II–IV Morphology of *P. torulosum* UOP 366

3, 4, 30, 31: Sporangia. 5, 6, 32, 33: Discharge tubes. 7, 34: Empty sporangium with discharge tube. 8, 35: Encysted zoospores. 9–12, 36–38: Germinating zoospores.

13, 14, 39, 40: Empty sporangia. 15, 16, 41, 42: Young oogonia and antheridia.

17–24, 43–50: Oogonia and antheridia. 25–29, 51–53: Mature oospores.

Bar (10 μ m) below fig. 11 and bar (20 μ m) below fig. 23 are applicable to figs. 3–14 and 15–29, respectively. Bar (10 μ m) on fig. 40 is to figs. 30–40. Bars (20 μ m) on figs. 41 and 53 are each to fig. 41 and all figures on plate IV.

Plates V–VII Morphology of *P. vanterpoolii* UOP 369

54–57, 79–81: Sporangia. 62, 82: Vesicles. 58–61, 83–85: Empty sporangia with discharge tube. 63, 86: Encysted zoospores. 64, 65, 87: Germinating zoospores.

66, 67, 88, 89: Young oogonia and antheridia. 68, 69, 90, 91: Oogonia and antheridia.

70–77, 92–99: Mature oospores. 78, 100–101: Oospores with degenerated oospheres.

101, 102: Empty spherical bodies (oospores?) after germination by germ tubes.

Bar (5 μ m) below fig. 54 is applicable to figs. 54 and 58. Bar (10 μ m) below figs. 59–60 and bar (20 μ m) below fig. 71 are each to figs. 56, 59–61, 63–65, and 55, 57, 62, 66–78. Bar (10 μ m) on fig. 79 is only to fig. 79. Bar (10 μ m) on fig. 80 and bar (20 μ m) on fig. 88 are to figs. 80–87, 91, 96, 100, 102, and 88–90, 92–95, 97–99, 101, respectively.

Plate I

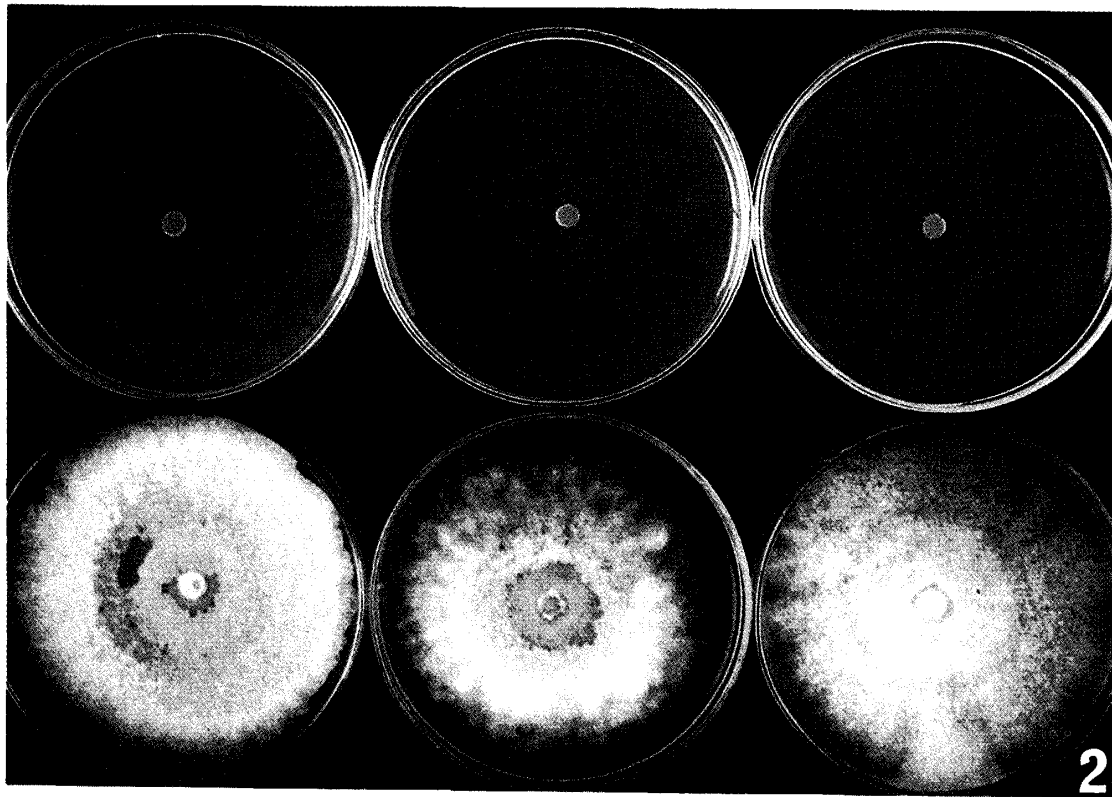
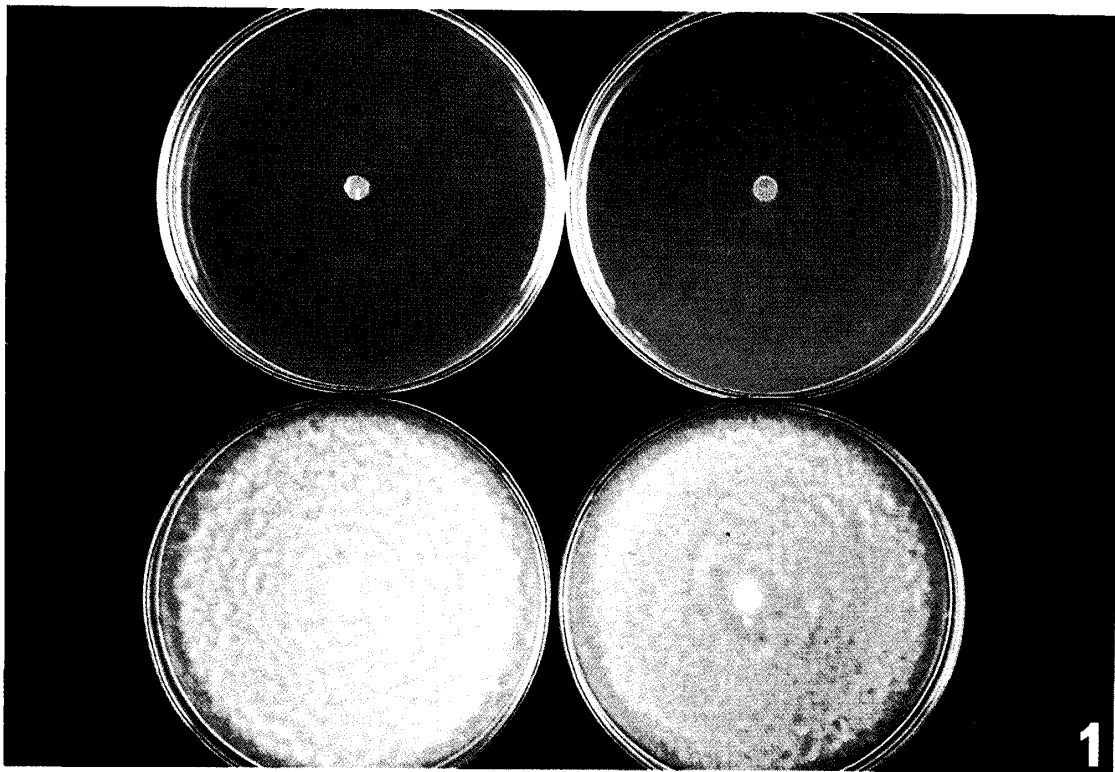


Plate II

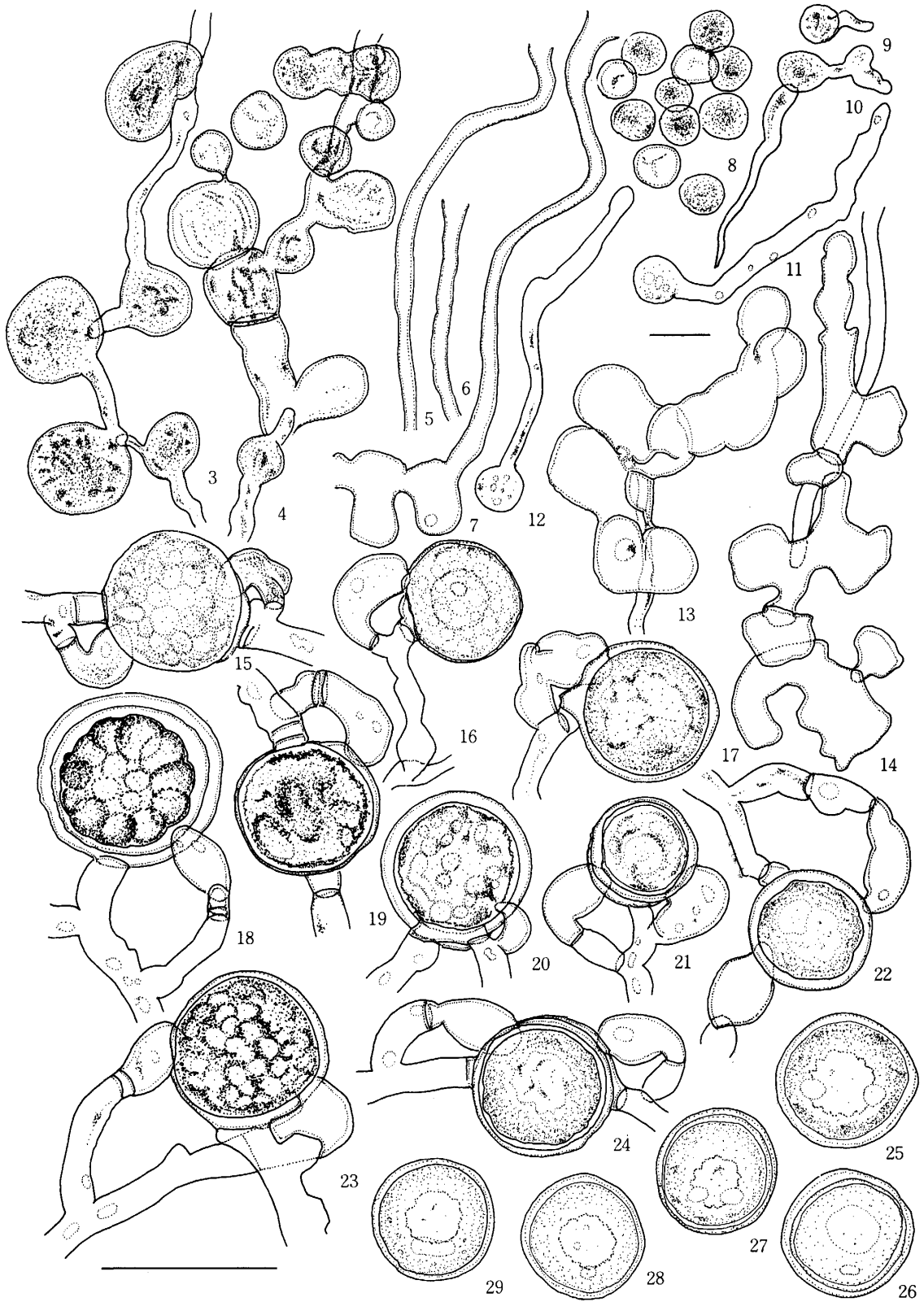


Plate III

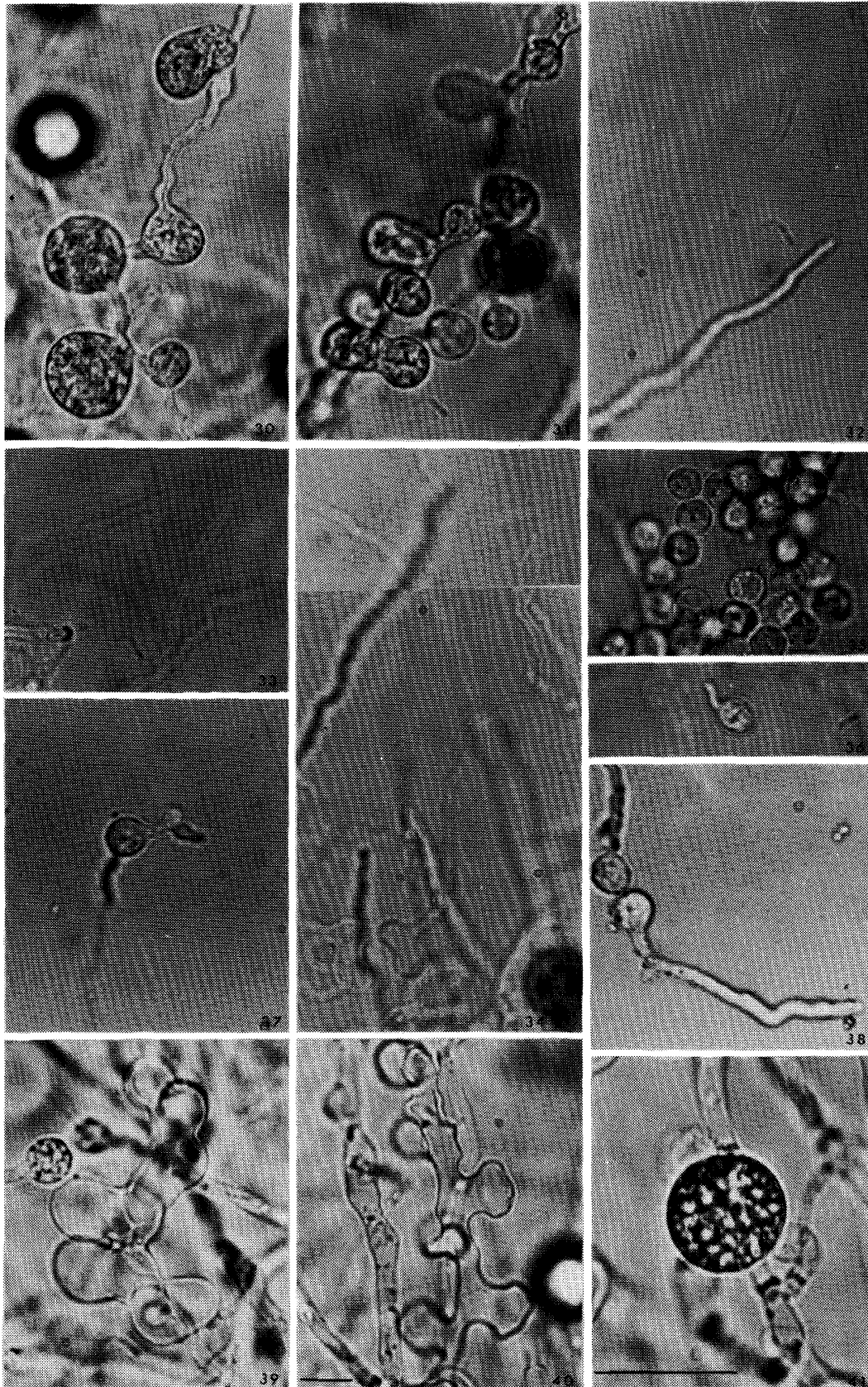


Plate IV

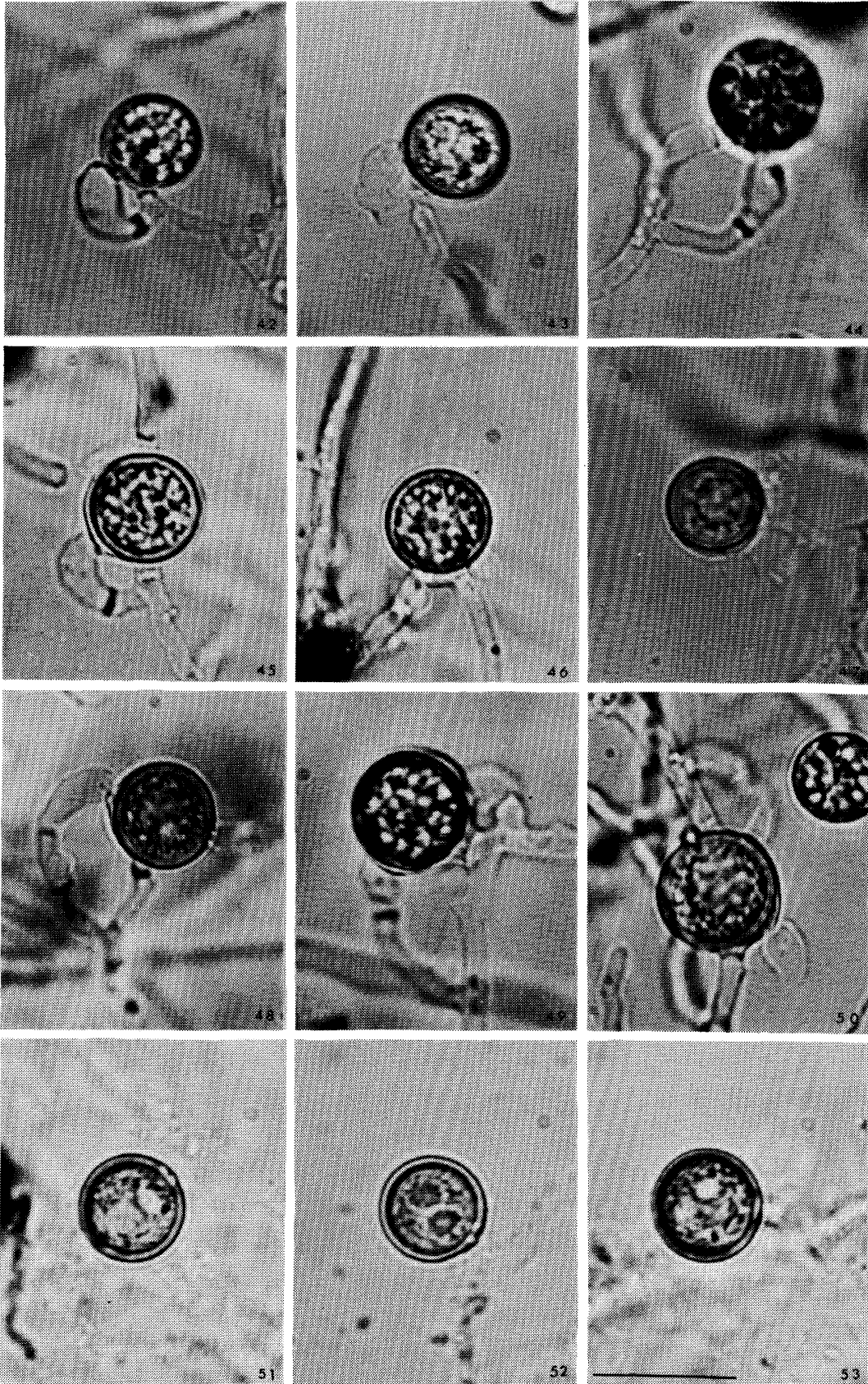


Plate V

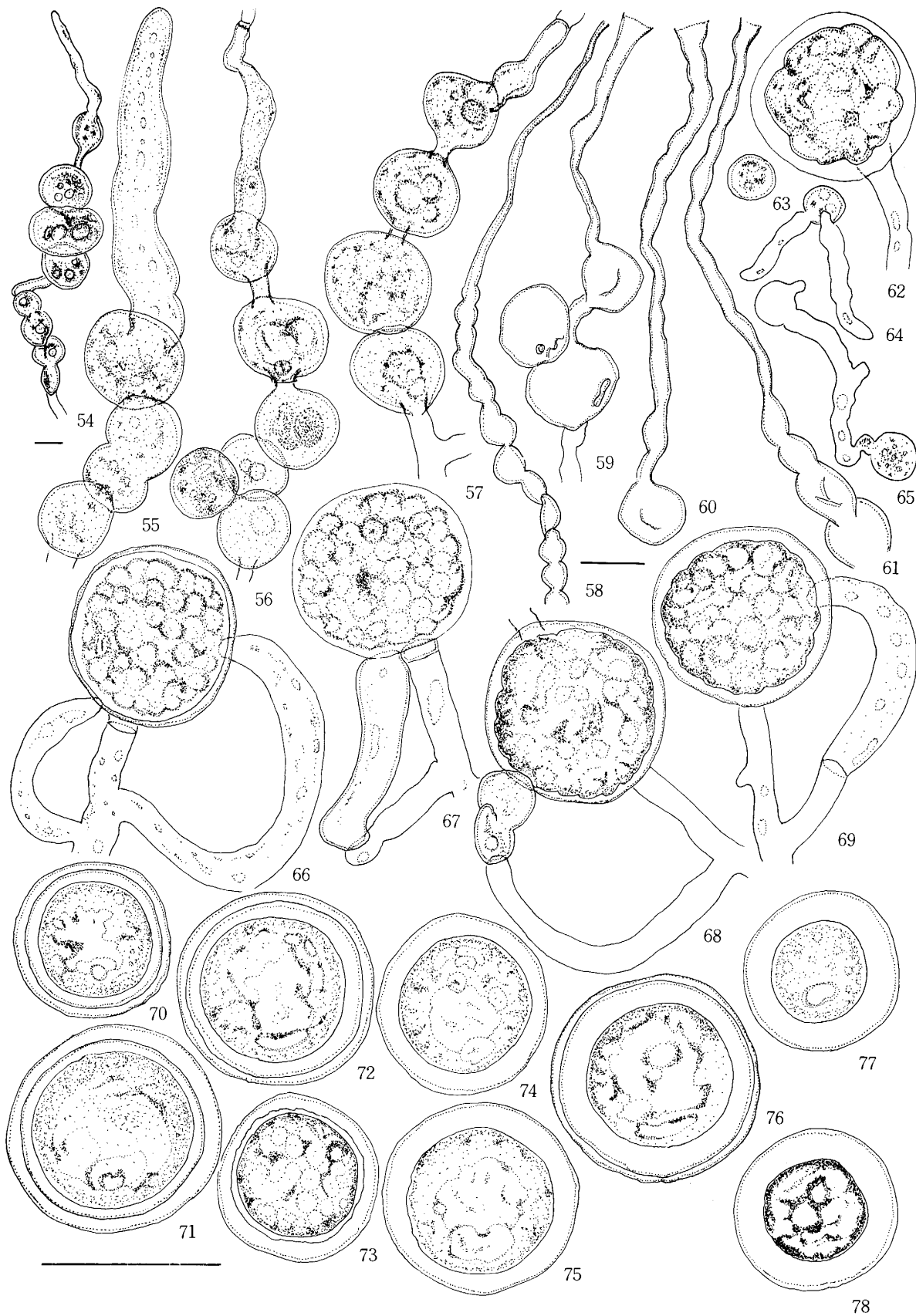


Plate VI

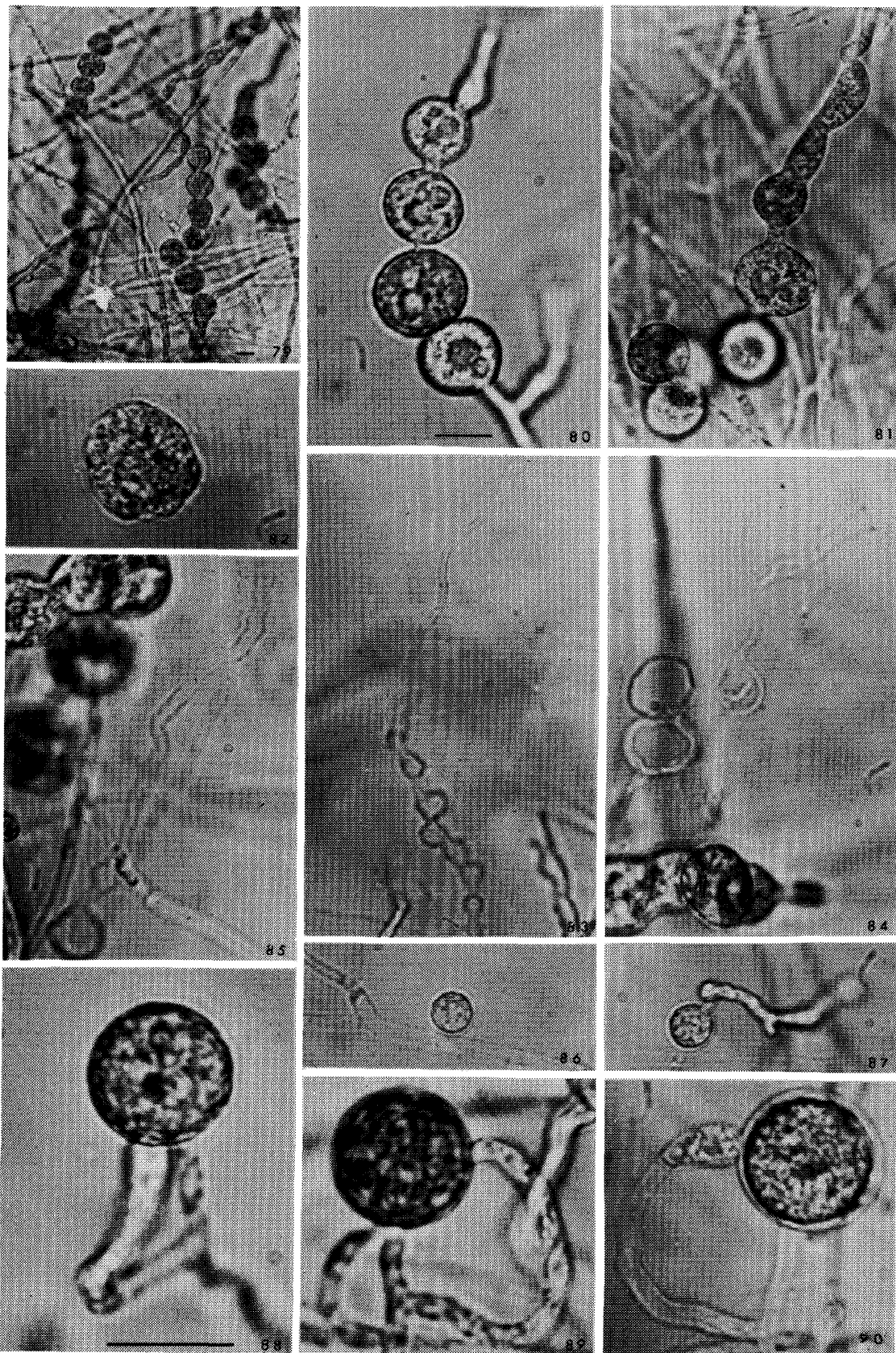


Plate VII

