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Dictyostelids in Japan. X*. Two New Species of *Dictyostelium*, *D. pseudo-brefeldianum* and *D. robustum*

By

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Abstract Two new species of dictyostelids, *Dictyostelium pseudo-brefeldianum* and *D. robustum*, are described based on isolates from forest soils in the temperate regions of Japan. *Dictyostelium pseudo-brefeldianum* is characterized by large but slender sorocarps, white sori, clavate sorophore tips, and elliptical spores without polar granules. *Dictyostelium robustum* is characterized by large and very robust sorocarps, white sori, somewhat capitate or nearly simple sorophore tips, and elliptical spores without polar granules.

In the course of my studies on Japanese dictyostelids, two new species of *Dictyostelium* were obtained. The descriptions and some observations on these species are presented here.

Procedures of isolation, cultivation and observation are the same as those reported previously (Hagiwara, 1989). Type specimens are preserved in the herbarium of the National Science Museum, Tokyo (TNS). Subcultures from the type isolates are kept in the American Type Culture Collection, Maryland, U.S.A.

Dictyostelium pseudo-brefeldianum Hagiwara, sp. nov. (Figs. 1 & 2)

Cultum ad 20°C in agaro non-nutricio cum *Eschericia coli*, sorocarpiis plerumque solitaris, interdum gregariis, non vel pauco ramosis, phototropicis, saepe prostratis; sorophoreis tenuibus, hyalinis, aliquantum sinuosis, 1.0–7.9 (–20.8) mm longis, e basibus dilatatis oriundis, gradatim attenuatis, cum apicibus clavatis, prope basem 7–27(–33) μm crassis, prope apiceum 2.5–10(–17.5) μm crassis; soris albis, globosis, 20–300(–350) μm in diam; sporis hyalinis, ellipsoideis, plerumque 1.6–2.2-plo longioribus quam latioribus, levibus, plerumque 6.0–8.4 × 3.1–4.3 μm, sine polaribus granulis; pseudoplasmodiis radiatis, 0.6–4.4 mm in diam, plerumque sola sorocarpia producentibus.

When cultured at 20°C on non-nutrient agar with *Eschericia coli*, sorocarps

* The title was changed from "The Acrasiales in Japan" because dictyostelids or dictyostelid cellular slime molds, which were treated under this title, are not included in the order Acrasiales at present (Hawksworth et al., 1995). IX: Bull. Natn. Sci. Mus., Tokyo, Ser. B, 12: 99–105, 1986.

usually solitary, sometimes gregarious, usually unbranched, sometimes sparsely and irregularly branched, phototropic, often prostrate; sorophores slender, colorless, sinuous, 1.0–7.9 mm in length, sometimes exceeding 20 mm if prostrate, gradually tapering from conical bases to clavate tips (Figs. 1A & 2C–E), usually surrounded by basal disks (Figs. 1B & 2F), usually consisting of a single tier of cells in a large portion except for both terminal parts, 7–27(–33) μm in diam at a level 100 μm above the bottom, 2.5–10(–17.5) μm in diam at a level 50 μm below the top; basal disks small, 15–40(–80) μm in diam; sori white, globose, 20–300 (–350) μm in diam; spores hyaline, ellipsoid, usually 1.6–2.2 times longer than broad, smooth, mostly 6.0–8.4 × 3.1–4.3 (MD* 6.5–7.7 × 3.4–4.0) μm, without polar granules; pseudoplasmodia with definite radiate streams (Fig. 2A), 0.6–4.4 mm in diam, often migrating with sorophore formation, usually producing single sorogens.

Habitat: In humus and fermentation layers of forest soil in the temperate regions of Japan.

Isolates examined: Ha-3, 4, 9, 11, 12, 19, 24 and 25, 800–1080 m alt., coniferous forests, Mt. Hayachine-san, Iwate Pref., Aug. 1979; MA-6, 8, 21, 25, 42 and 44, 1450–2000 m alt., deciduous and coniferous forests, Mt. Shiomi-dake, Nagano Pref., Aug. 1979; IY-11, 13, 31, 46, 53, 78, 83, 115 and 116, 50–840 m alt., deciduous forests, Mito to Mt. Yamizo-san, Ibaraki Pref., Aug. 1994.

Type specimen: M-50024 (TNS), ex IY-78.

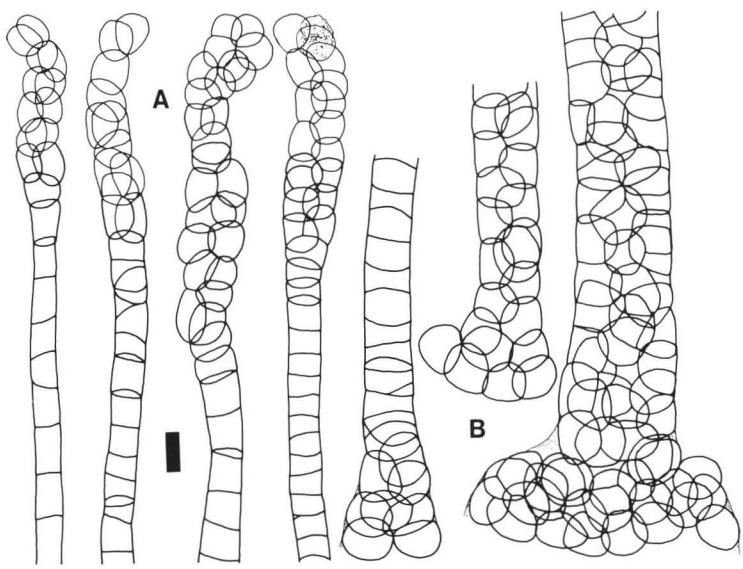

Fig. 1. *Dictyostelium pseudo-brefeldianum.* A. Sorophore tips. B. Sorophore bases. Bar = 10 μm.

* Range of mean spore diameters of the isolates examined.

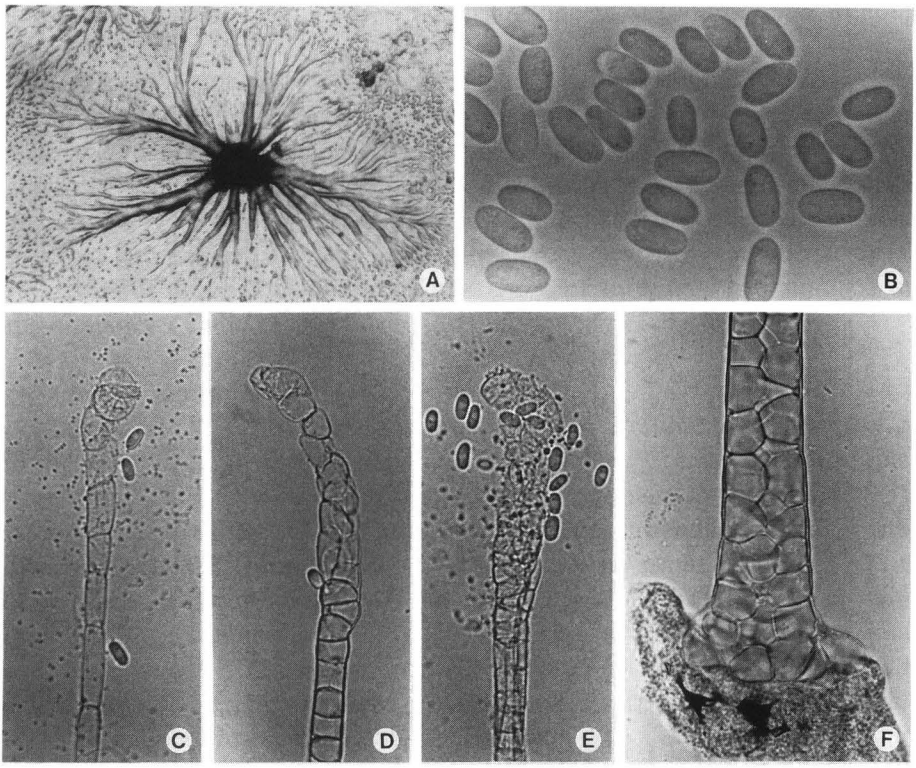

Fig. 2. *Dictyostelium pseudo-brefeldianum.* A. Pseudoplasmodium. ×12. B: Spores. ×1200. C–E. Sorophore tips. ×480. F. Sorophore base. ×480.

Dictyostelium pseudo-brefeldianum is characterized by large but slender sorocarps, white sori, clavate sorophore tips, and elliptical spores without polar granules. According to the terminology in Hagiwara (1989), its sorophores are "gigantic" in length, and its sorophore tips may be "simple" and "capitate" in the proper sense because most of them become suddenly thicker than in any other part of the upper sorophores near the top (Fig. 1A). Its spores are not oblong but elliptical in shape, viz., their sides are not parallel but curved (Fig. 2B).

Dictyostelium pseudo-brefeldianum fits well the species concept of *D. mucoroides* Brefeld emended by Raper (1984). However, I believe that Raper's emendation of *D. mucoroides* deviates from Brefeld's original description and illustration, which was indicated by Hagiwara (1984a), and moreover Raper's species concept of *D. mucoroides* is too broad including seven or more morphologically distinct groups I recognize as separate species such as *D. arabicum* Hagiwara, *D. brefeldianum* Hagiwara, *D. clavatum* Hagiwara, *D. firmibasis* Hagiwara, *D. implicatum* Hagiwara, *D. longosporum* Hagiwara and *D. medium*

Table 1. Comparison of some morphological features among *Dictyostelium pseudo-brefeldianum* and its related species.*

	Sorophores			Spores		References
	Length**	Width of bases***	Shape of tips	Shape	Size (MD, μm)	
D. pseudo-brefeldianum	large to gigantic	thin	simple to compound, clavate	elliptical	6.5–7.7×3.4–4.0	
D. arabicum	large	thick	compound, clavate	oblong	6.3–6.9×3.6–3.9	Hagiwara (1991)
D. brefeldianum	large	thin	simple, typically capitate	oblong	5.9–6.7×3.3–3.7	Hagiwara (1984a, 1989)
D. clavatum	large	moderate	compound, clavate	oblong	5.4–5.5×2.9–3.1	Hagiwara (1992)
D. firmibasis	gigantic	thick	simple, somewhat capitate	elliptical	6.9–8.4×2.9–3.6	Hagiwara (1989)
D. implicatum	large	moderate	simple, acuminate	elliptical	7.4–8.4×4.0–4.8	Hagiwara (1989)
D. longosporum	large	moderate	simple, typically capitate	elliptical	7.4–8.2×3.3–3.6	Hagiwara (1983)
D. medium	large	moderate	simple, somewhat capitate	elliptical	6.4–7.0×3.4–3.7	Hagiwara (1992)

Table 2. Comparison of some morphological features among *Dictyostelium robustum* and its related species.*

	Sorophores		Spores		References
	Width of bases***	Shape of tips	Shape	Size (MD, μm)	
D. robustum	very thick	simple, somewhat capitate	elliptical	7.7–8.8×3.4–4.1	
D. firmibasis	thick	simple, somewhat capitate	elliptical	6.9–8.4×2.9–3.6	Hagiwara (1989)
D. giganteum (=*D. magnum*)	moderate	simple, somewhat capitate	oblong	6.5–7.4×3.6–4.2	Hagiwara (1983)
D. septentrionalis	thick	compound, typically capitate	elliptical	8.4–9.5×4.5–5.6	Hagiwara (1989)

* Small sorocarps and prostrate ones in each species were excluded in the comparisons.

** Categories of the sorophore length follow Hagiwara (1989), viz., the sorophores usually exceeding 3.5 mm but not or rarely more than 6 mm are "large", and ones often or usually exceeding 6 mm are "gigantic".

*** Sorophore bases are tentatively grouped into five categories, "very thin", "thin", "moderate", "thick" and "very thick", which indicate the sorophore bases at 100 μm above the bottom not or rarely exceeding 10 μm, 30 μm, 50 μm, 70 μm and more in width, respectively.

Hagiwara. Therefore, I cannot accept Raper's concept of *D. mucoroides* although it is widely accepted.

Dictyostelium pseudo-brefeldianum is macroscopically similar to *D. brefeldianum* in the outline of sorocarps except for somewhat longer sorophores, therefore it is difficult to distinguish between the two species under a dissecting microscope. But the new species is easily distinguished microscopically from *D. brefeldianum* by clavate sorophore tips and elliptical and somewhat larger spores. Also from other related species, the present species is distinguished by the combination of dimensions of sorophores, shape of sorophore tips, and size and shape of spores (Table 1).

Dictyostelium robustum Hagiwara, sp. nov. (Figs. 3 & 4)

Cultum ad 20°C in agaro non-nutricio cum *Eschericia coli*, sorocarpiis plerumque solitaris, interdum gregariis, non vel pauco ramosis, phototropicis, interdum prostratis; sorophoreis robustis, hyalinis, aliquantum sinuosis, 0.4–11.6 mm longis, e basibus dilatatis oriundis, gradatim attenuatis, cum apicibus aliquantum capitatis, prope basem 5–114 μm crassis, prope apiceum 2–12 μm crassis; soris albis, globosis, 50–400(–640) μm in diam; sporis hyalinis, ellipsoideis, plerumque 1.9–2.6-plo longioribus quam latioribus, levibus, plerumque 7.1–9.6×3.2–4.4 μm, sine polaribus granulis; pseudoplasmodiis radiatis, 0.3–6.8(–8.0) mm in diam, plerumque sola sorocarpia producentibus.

When cultured at 20°C on non-nutrient agar with *Eschericia coli*, sorocarps usually solitary, sometimes gregarious, usually unbranched, sometimes sparsely and irregularly branched, phototropic, sometimes prostrate; sorophores robust, colorless, sinuous, 0.4–11.6 mm in length, gradually tapering from conical, round or clavate bases (Figs. 3B–D & 4D, E) to somewhat capitate or nearly simple tips (Figs. 3A & 4C), sometimes with supporting cells and supporters, often surrounded by basal disks, 5–114 μm in diam at a level 100 μm above the bottom, 2–12 μm in diam at a level 50 μm below the top; basal disks 45–180 μm in diam, sometimes well-developed and 205–650 μm in diam; sori white, globose, 50–400 (–640) μm in diam; spores hyaline, ellipsoid, usually 1.9–2.6 times longer than broad, smooth, mostly 7.1–9.6×3.2–4.4 (MD 7.7–8.8×3.4–4.1) μm, without polar granules; pseudoplasmodia with definite radiate streams (Fig. 4A), 0.3–6.8 (–8.0) mm in diam, rarely migrating without sorophore formation, usually producing single sorogens.

Habitat: In humus and fermentation layers of forest soil in the temperate regions of Japan.

Isolates examined: Ha-1, 6, 9, 14, 22 and 23, 780–1020 m alt., coniferous forests, Mt. Hayachine-san, Iwate Pref., Aug. 1979; Ch-37, 45, 48 and 53, 1220–1380 m alt., deciduous forests, Mt. Chokai-san, Akita Pref., Sept. 1983, reported as "*Dictyostelium firmibasis* variant" in Hagiwara (1984b); MB-2, 1050 m alt., deciduous forest, Mt. Haku-san, Ishikawa Pref., Sept. 1985; IY-52, 78,

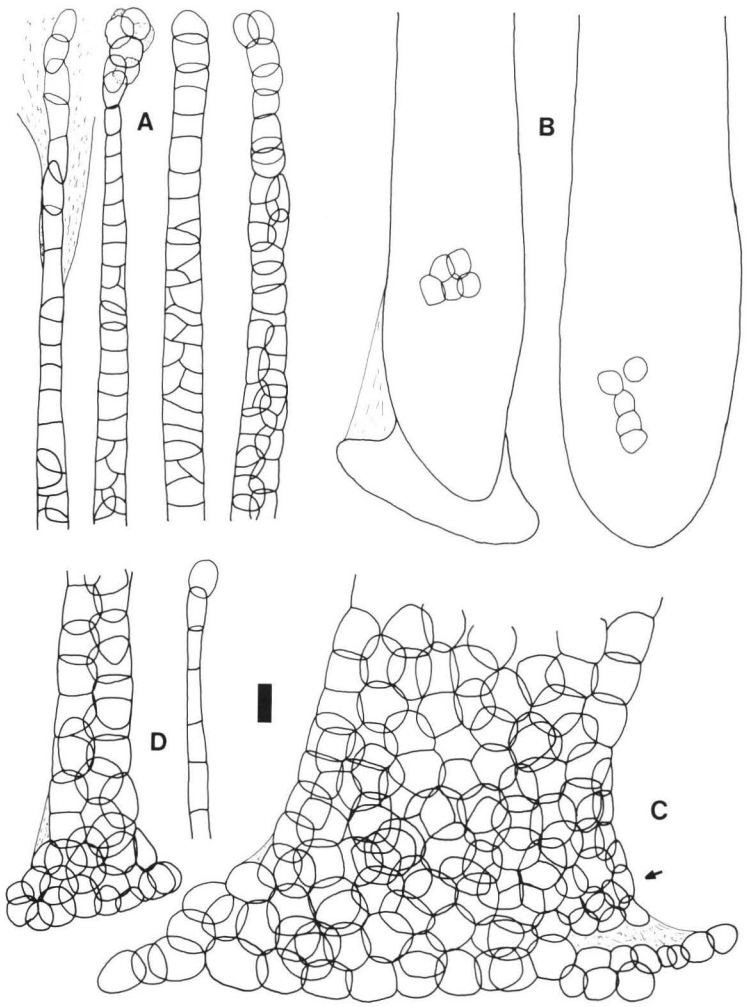

Fig. 3. *Dictyostelium robustum.* A. Sorophore tips. B. Sorophore bases. C. Sorophore base with supporting cells (arrow). D. Sorophore tip and base of a small sorocarp. Bar = 10 μm for A, C, D and 20 μm for B.

Fig. 4. *Dictyostelium robustum.* A. Pseudoplasmodium. ×12. B. Spores. ×1200. C. Sorophore tip. ×480. D, E. Sorophore bases. ×120. F. Well-developed basal disk in the isolate Ch-45. ×120. G. Part of a sorophore with a supporter (arrow). ×480. H. Migration without sorophore formation in Ch-53. ×12. I. Short and robust sorocarps in Ch-53. ×12.

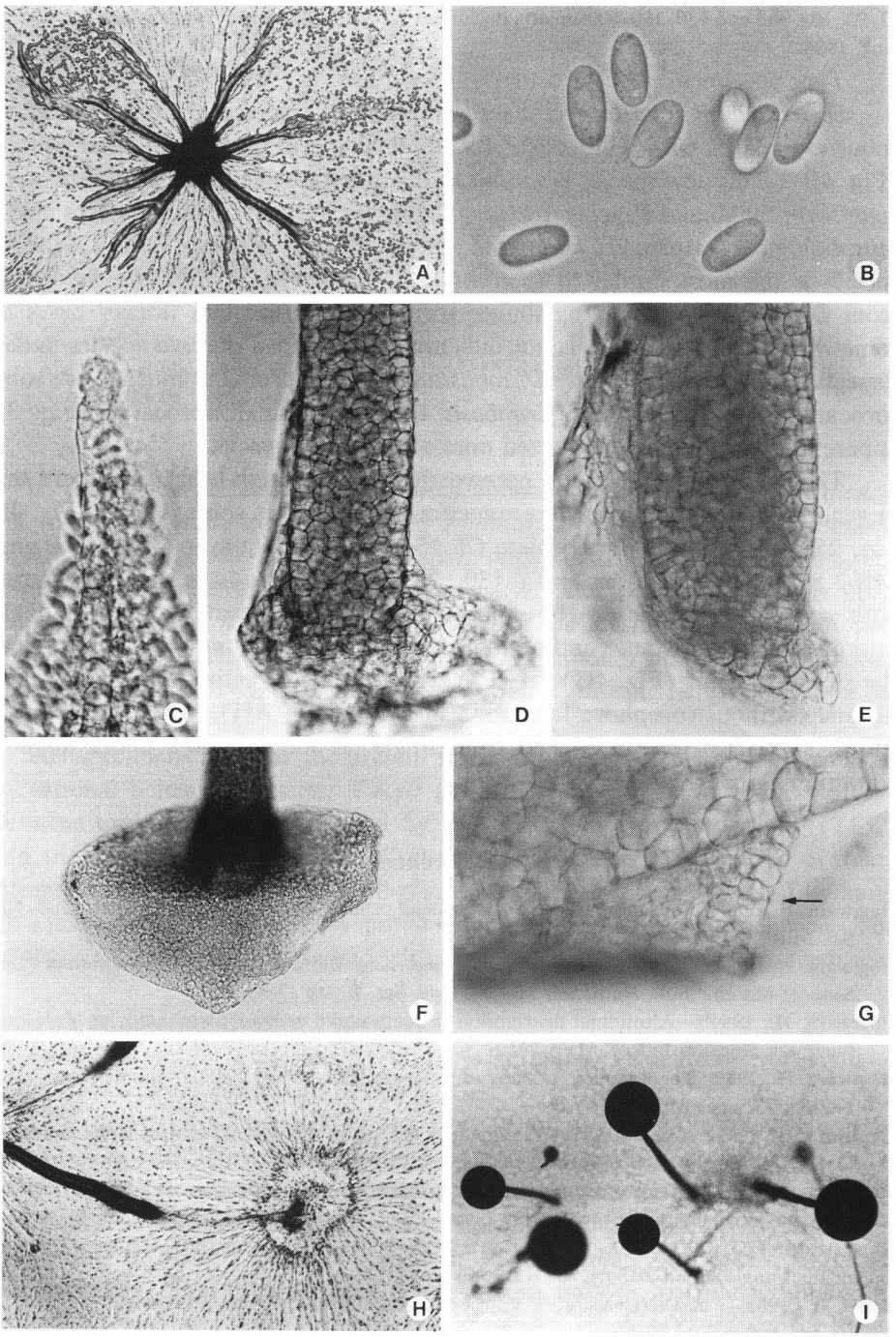

Fig. 4.

87, 95, 103, 115 and 130, 100–1000 m alt., deciduous forests, Mito to Mt. Yamizo-san, Ibaraki Pref., Aug. 1994.

Type specimen: M-50025 (TNS), ex Ch-53.

Dictyostelium robustum is macroscopically characterized by very large and robust sorocarps with white sori. Its elliptical spores don't have polar granules (Fig. 4B). This new species is similar to three described species, *D. firmibasis*, *D. giganteum* Singh and *D. septentrionalis* Cavender, in the size of sorocarps. When morphological features are compared among the four species (Table 2), the new species is the most similar to *D. firmibasis*. However, *D. robustum* is different from *D. firmibasis* in having longer sorophores with much thicker bases and somewhat larger spores. It is not difficult to distinguish the two species under a dissecting microscope because *D. robustum* sparsely produces much more robust sorocarps than those of *D. firmibasis* though its small sorocarps (Fig. 3D) apparently resemble medium-sized ones of the latter species.

The following respects are noteworthy. 1) Although basal disks were small in general, quite large disks were sometimes produced in some isolates (Fig. 4F), viz., 250 and 365 μm in the isolate Ch-45, 205 and 350 um in Ch-48, 650 μm in MB-2, and 260 and 470 μm in IY-130. 2) Pseudoplasmodia sometimes migrated with sorophore formation, but most of the pseudoplasmodia stood up after migrating a short distance and often produced supporters under the sorophores at the standing point (Fig. 4G). 3) Pseudoplasmodia sometimes migrated a short distance without sorophore formation in Ch-53 (Fig. 4H). 4) A few small but robust sorocarps were produced from single pseudoplasmodia under unknown conditions in Ch-53 (Fig. 4I).

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