



## Dictyostelids from Jilin Province, China II

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### Abstract

Four species of dictyostelid cellular slime molds (dictyostelids) in two genera were isolated from forest soil samples collected from Jilin Province in China. *Dictyostelium recurvibasicum* is new to science. Two of four species (*D. longosporum* and *Polysphondylium tenuissimum*) are new to China, and *D. brefeldianum* is recorded for the first time in the northern part of the country, being known previously only from Taiwan. Descriptions and illustrations are provided herein for these species based on our collections.

**Key words:** *Dictyostelium*, *Polysphondylium*, social amoeba, taxonomy

### Introduction

Dictyostelid cellular slime molds (dictyostelids) are a normal component of the soil where they feed upon bacteria and other microbes. These organisms are important agents in bringing quantitative changes to the bacterial flora of the soil and apparently play a role in maintaining the balance between bacteria and other microbes in soil (Raper 1973, 1984). The dictyostelids are a distinct branch of the eukaryotes, separate from plants, fungi and animals. Their cells lack cell walls and resemble animal cells in organization, except for the presence of a contractile vacuole (Fets *et al* 2010). There are relatively few reports of dictyostelids from Jilin Province, China. Bai (1983), He & Li (2008a, b), Ren *et al* (2014) and Liu & Li (2014) investigated the distribution of dictyostelids in several localities in Jilin Province. These studies collectively reported a total of 12 species (Liu & Li 2014).

Jilin Province is one of provinces in northeast China and is located between 122–131° E and 41–46° N. It is characterized by a monsoon climate of medium latitudes in the middle-temperate zone (Liu & Li 2014). Wangqing National Nature Reserve is situated in the northeast of Yanbian in Jilin Province, which is mainly a protected area for Japanese Yew or Spreading Yew (*Taxus cuspidata* S. et Z.), North-east China Tiger (*Panthera tigris ssp. altaica*), *et al*. During June 2009, soil samples for the isolation of dictyostelids were collected from five forestry farms in Wangqing National Nature Reserve. Four species of dictyostelids, including one new species, two new records for China and one new record for Northern China were added to what was known from Jilin Province in this study. This is the second occasion in which a new species of dictyostelids was recovered from Wangqing National Nature Reserve, with the first new species being *D. culliculosum* Yu Li & X.L. He (He & Li 2008b).

### Materials and methods

#### SAMPLING

Field surveys were carried out in five forestry farms located within in Wangqing National Nature Reserve, Jilin Province, in June 2009. All soil samples, each approximately 30–50 g, were collected and placed in sterile whirl-pack plastic bags. Afterwards, soil samples were returned to the laboratory as soon as possible, according to the recommendations of Cavender and Raper (1965). Each sample bag was numbered and preserved in the herbarium of the Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China.

## ISOLATION AND CULTIVATION

The isolation methods used followed those described by Cavender and Raper (1965). Five duplicate plates were prepared for each sample and incubated in an incubator at 23 C with a 12 h light and dark cycle. The aggregations, pseudoplasmodia, and sorocarps appeared from 2 d to 3 wk. The isolates were purified and cultivated for taxonomic studies on non-nutrient water agar plates with *Escherichia coli* pregrown for 12–24 h. Spores from these isolates were frozen in HL 5 media (Cocucci & Sussman 1970) and stored at -80 C in HMJAU, Changchun, China.

## MORPHOLOGICAL OBSERVATION

The locations of each early aggregating clone and sorocarp that developed were marked. The life cycle stages of cell aggregation, pseudoplasmodium, and sorocarp were observed under a Nikon dissecting microscope (SMZ1500) with 0.75–11.25× range (10 × ocular). Slides with fruiting bodies were prepared with water as a mounting medium. Characteristics of spores, stalks, and sorocarps were observed and measured on the slides by using a Nikon light microscope (SMZ1000), with 10× ocular and 10, 40, and 100× (oil) objectives. Photographs were taken with a CANON S70 camera.

## Results

### New species

*Dictyostelium recurvibasicum* Y. Li et P. Liu, sp. nov.

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When cultured at 23 C on non-nutrient agar with *E. coli*, sorocarps (Fig 1A–B) white, typically clustered, erect or semi-erect, usually branched, and sometimes with secondary branches arising from existed branches to give a clustered appearance. Sorophores sinuous, white, generally consisting of one or two tiers of cells, mostly 1.2–4.4 mm high, 7.5–20 µm diam. Bases (Fig 1F–H) recurved, tips (Fig 1E) clavate or obtuse. Sorophores tapering from the hooks to both tips and bases. Sori white, globose, commonly 70–175 µm diam. Spores (Fig 1D) hyaline, oblong to oval, commonly 3.8–7.5 × 3.3–5.2 µm, with prominent polar granules. Cell aggregations (Fig 1C) with indefinitely radiate streams, producing one to several sorogens. Sorogens colorless. Pseudoplasmodia do not migrate.

*Etymology.* Referring to the recurved bases of sorophore.

*Holotype.* HMJAU MR062. Isolated in 2009 from forest soil (Strain 0080–4 from mixed forest soil, 861 m elevation; Strain 0084–4 from coniferous forest soil, 766 m elevation; Strain 0085–5 from coniferous forest soil, 761 m elevation) collected in the Wangqing National Nature Reserve, Jilin Province, China in the same year.

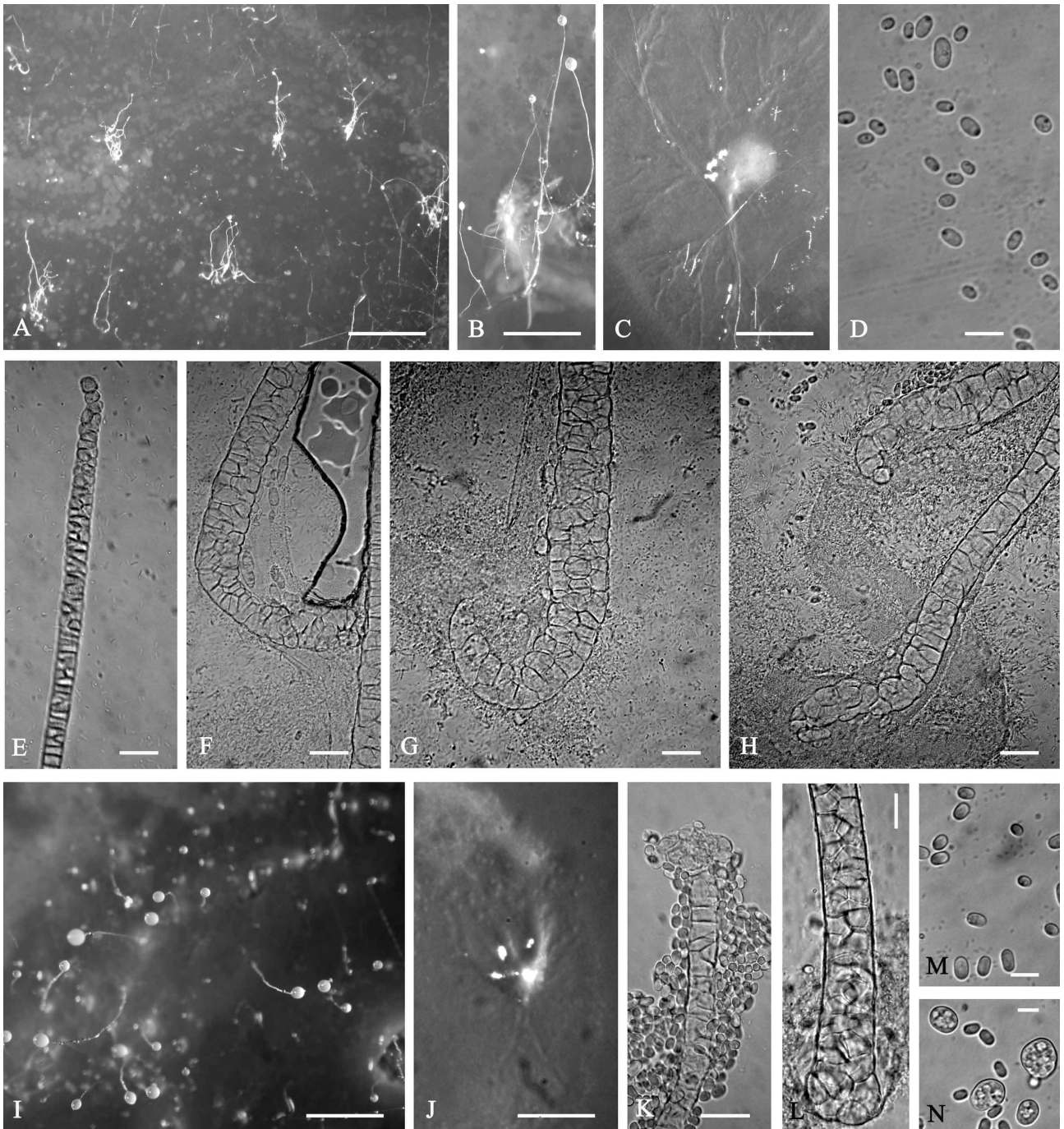
*Known distribution.* China.

*Commentary.* Sorocarps of *Dictyostelium recurvibasicum* closely resemble those of another clustered dictyostelid—*D. monochasioides* H. Hagiw. (Raper 1984). This species is clearly distinguishable from *D. monochasioides* in the following four respects. First, sorocarps of *D. recurvibasicum* are rarely solitary and mostly erect or semi-erect, whereas those of *D. monochasioides* are solitary or gregarious and erect or inclined. Second, the sorophores of *D. recurvibasicum* consist of one or two tiers of cells. However, the sorophores of *D. monochasioides* are delicate, consisting of single tier of cells except for the bases. Third, *D. recurvibasicum* has hook-like sorophore bases, this is the primary difference from *D. monochasioides* and other species of *Dictyostelium*. Last, the spores of *D. recurvibasicum* are oblong to oval. They are slightly rounder and smaller than those of *D. monochasioides* (spores are elliptical 2.8–3.8 × 4.4–6.4 µm to elongate ellipsoid or recurved 3.4–4.6 × 7.6–9.6).

### New records for China

*Dictyostelium longosporum* H. Hagiw., Bull. Natn. Sci. Mus., Tokyo, Ser. B, 9(4): 155 (1983).

Sorocarps (Fig 1I) solitary, unbranched, phototropic, prostrate. Sorophores colorless, sinuous, 0.5–4.6(–30) mm high, bases (Fig 1L) expanded, tips (Fig 1K) capitate, tapering from bases to tips. Sori white, globose, 75–380 µm diam. Spores (Fig 1M) hyaline, ellipsoid, usually 5.0–8.8 × 3.0–4.5 µm, without polar granules. Cell aggregations (Fig 1J) radiate in pattern, with conspicuous streams. Pseudoplasmodia do not migrate and usually produce single sorogens. Macrocyts (Fig 1N) 7.5–12.5 × 8.8–12.5 µm.



**FIGURE. 1** *Dictyostelium recurvibasicum* and *D. longosporum*. *D. recurvibasicum*: A, B, sorocarps; C, cell aggregation; D, spores; E, sorophore tip; F, G, H, sorophore base. *D. longosporum*: I, sorocarps; J, cell aggregation; K, sorophore tip; L, sorophore base; M, spores; N, macrocysts. Bars: A = 5 mm; B = 3 mm; C, J = 2 mm; D–H, K–N = 10  $\mu$ m; I = 10 mm.

*Cultures examined.* HMJAU MR055. Strain 0075–4 isolated in 2009 from mixed forest soil (855 m elevation) collected in the Wangqing National Nature Reserve, Jilin Province, China, in the same year.

*Known distribution.* Nepal and China.

*Commentary.* From the original report (Hagiwara 1983) of this species, the soil samples for dictyostelid isolation were collected in open forests of the temperate zone at relatively high elevations, at about 1900–2610 m. However, in the present study, the soil samples were collected at a lower elevation (about 855 m) in a middle-temperate zone, which shows that this species is affected less by elevation.



*Polysphondylium tenuissimum* H. Hagiw., Bull. Natn. Sci. Mus., Tokyo, Ser. B, 5: 69-72 (1979).

Sorocarps (Fig 2A) solitary or gregarious, not phototropic, erect or inclined, sometimes prostrate. Sorophores colorless, sinuous, 3.5–9.5 mm high, terminal segments of sorophores not elongate, gradually tapering from bases to tips, bases (Fig 2B) clavate or digitate, tips (Fig 2D) subulate; with 3–21 nodes, each whorl with 3–5 branches. Branches (Fig 2C) tapering from bases to tips, bases clavate, tips acuminate, usually 120–200 µm long. Sori white, globose, terminal sori 30–80 µm diam, lateral sori 20–50 µm diam. Spores (Fig 2E) hyaline, elliptical to oblong, mostly 5.0–7.5 × 2.5–3.7 µm, with polar granules. Cell aggregations radiate in pattern. Pseudoplasmodia do not migrate. Macrocyysts (Fig 2F) 6.3–10 µm diam.

*Cultures examined.* HMJAU MR064. Strain 0073–4 isolated in 2009 from a broadleaf forest soil (365 m elevation), collected in Wangqing National Nature Reserve, Jilin Province, China, in the same year.

*Known distribution.* U.S.A., Guatemala, Japan, and China.

*Commentary.* *Polysphondylium tenuissimum* is characterized by a large number of whorls, short branches, and small sori. Spores of this species, based on the isolate obtained in the present study, are slightly larger than the original report of 5.0–6.2 × 2.7–3.5 µm (Hagiwara 1989).

### New record for Jilin Province

*Dictyostelium brefeldianum* H. Hagiw., Bull. Natn. Sci. Mus., Tokyo, Ser. B, 10(1): 39 (1984).

Sorocarps (Fig 2G) solitary, usually unbranched, phototropic, prostrate. Sorophores colorless, sinuous, 0.6–4.5(10.5) mm long, tapering from bases to tips, usually consisting of one tier of cells except for the bases and tips, bases (Fig 2H) conical or round, tips (Fig 2I) capitate. Sori white, globose, 25–230(280) µm diam. Spores (Fig 2J) hyaline, oblong, mostly 5.3–7.3 × 3.0–4.0 µm, without polar granules, sometimes with irregular granules. Cell aggregations radiate. Pseudoplasmodia do not migrate, usually producing single sorogens. Myxamoebae (Fig 2K) irregular or triangular in the direction of movement.

*Cultures examined.* HMJAU MR043. Strain 0081–2 isolated in 2009 from mixed forest soil (861 m elevation) collected in the Wangqing National Nature Reserve, Jilin Province, China, in the same year.

*Known distribution.* U.S.A., Canada, Germany, England, France, Denmark, Switzerland, Japan, New Guinea, Nepal, Uganda, Ukraine, Korea, and China.

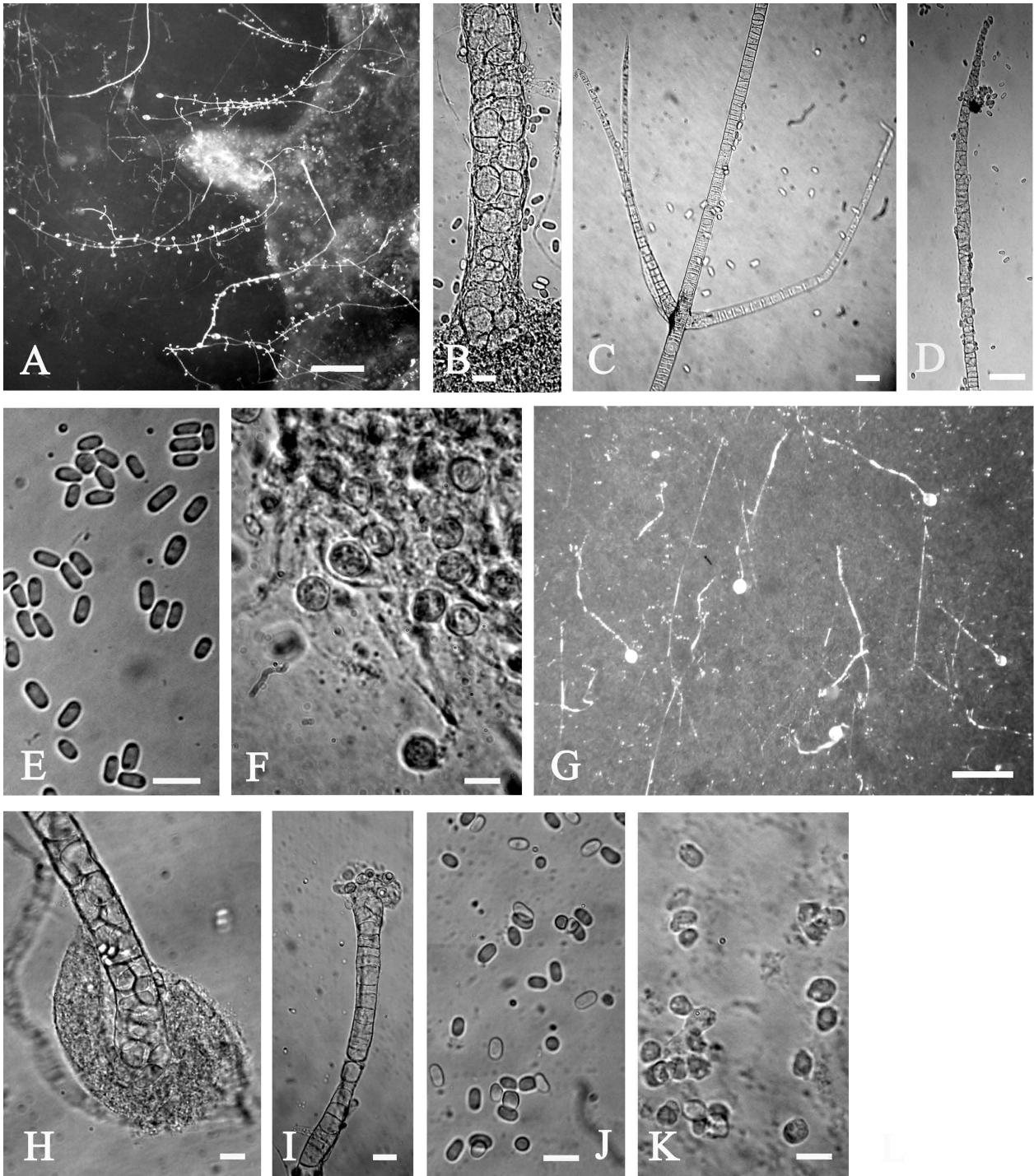
*Commentary.* *Dictyostelium brefeldianum* is a medium-sized cosmopolitan species that is often prostrate and strongly phototropic. This study extends its worldwide distribution in China.

## Discussion

The characteristics of sorophore bases is essential for the taxonomy of dictyostelids. Normally, the shape of the sorophore bases of dictyostelids is conical, round, clavate, acuminate, or digitate (Hagiwara 1989). In this study, the new species *Dictyostelium recurvibasicum* has recurved bases of the sorophores, which is different from all types of sorophore bases reported previously.

Wangqing National Nature Reserve is 40 km from the Russian border, and 18 km from the North Korea border. The average elevation is 806 m. The average temperature is 3.9 C/y. The average precipitation is 580 mm/y. As mentioned in the introduction, five forestry farms were investigated within Wangqing National Nature Reserve in the present study. Six isolates represented four species were obtained in these soil samples. *Dictyostelium recurvibasicum* is the second new dictyostelid species isolated from Wangqing, Jilin Province, China.

As in previous studies (He & Li, 2008a; Liu & Li, 2014) of Jilin Province, we found the same situation to exist that had been noted previously for studies carried out in China—the absence of members of genus *Acytostelium* and a low frequency of occurrence of species of *Polysphondylium*. This appears to be the result of certain environmental factors, possibly drastic changes in temperature and precipitation, which inhibit their occurrence (Vadell *et al.* 2011). Wangqing National Nature Reserve has long winters and short summers which leads to lower temperatures and precipitation in winter, with both being higher in summer, and both subject to rapid and drastic seasonal differences.



**FIGURE. 2** *Polysphondylium tenuissimum* (A–F) and *Dictyostelium brefeldianum* (G–K): A, G, sorocarps; B, H, sorophore base; C, sorophore branch; D, I, sorophore tip; E, J, spores; F, macrocysts; K, myxamoebae. Bars: A = 2 mm; B, C, D, E, F, H, I, J, K = 10  $\mu$ m; G = 3 mm.

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