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Distribution and New Species of Dictyostelids in Subalpine Zone of Mt. Paektu, Korea

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ABSTRACT: Fourteen different taxa of dictyostelid cellular slime molds were recovered from the alpine and subalpine zone in Mt. Paektu. In subalpine zone, six species were recovered; *Dictyostelium minutum, D. brefeldianum, D. crassicaule, D. capitatum, Polysphondylium solitarium* and *P. pallidum*. One of these species which were isolated from the soils of *Larix olgensis* community exhibited several distinctive features which differed from the published species. This species was designated as a new species, *Polysphondylium solitarium* Kang et Chang, sp. nov. When cultivated at 22~24℃ on low-nutrient agar media with *Escherichia coli, Polysphondylium solitarium* is distinguishable from other species by the following combination of features: (i) the sorocarps with vinaceous pigmentation; (ii) pseudoplasmodia radial, usually centralized and rarely subdivided; (iii) the various number of whorls; (iv) the spores with unconsolidated and nonconspicuous polar granules. Also, we confirmed this new species by analyzing ribosomal DNA (ITS1, ITS2 and 5.8s DNA) sequences of *P. solitarium* and *P. violaceum*.

Key Words: Cellular slime molds, Distribution, Mt. Paekdu, *Polysphondylium solitarium*

INTRODUCTION

A collection of 14 taxa of dictyostelid cellular slime molds inhabiting soils of the alpine and subalpine zone in the Mt. Paekdu were recovered in July, 1997 (Shim *et al.* 1998). Eleven of these belong to the genus *Dictyostelium* and the others belong to the genus *Polysphondylium*. Among these, two species had been unidentified. During the past two years, these species were subcultured and analyzed. As a result, one of them exhibited several distinctive features which differed from the published species and here was described as a new species, *Polysphondylium solitarium* Kang et Chang sp. nov.

Worldwide, sixty-five species of cellular slime molds were found to occupy various forest soils (Swanson *et al.* 1999). Ten of these belong to genus *Polysphondylium*. Since Choi and Kim (1981) introduced cellular slime molds for the first time in Korea, 33 species have been isolated from the forest soils in Korea including four new dictyostelids: *D. flavidum* (Hong and Chang 1992a), *D. floridum* (Hong and Chang 1992b), *D. valenstemmum* (Shim and Chang 1996), *D. caudabasis* (Shim and Chang 1998). Five polysphondylia have been isolated from forest soil in Korea (Shim 1999, Kang *et al.* 1998). The cosmopolitan *P. violaceum* Brefeld (1884), at least one member of the *P. pallidum* complex Olive (1901) and *P. tenuissimum* Hagiwara (1979) also were common components of the soil microflora of Korea. *P. candidum* has been isolated from the southern area of Korea. *P. pseudocandidum* was isolated from the Mt. Nam in Seoul.

P. pallidum and *P. violaceum* appear to be highly variable species. Therefore, Hagiwara introduced the term "*Polysphondylium pallidum* complex" as representing a series of variant species including *P. pallidum, P. album* and *P. tenuissimum*. But Vadell and Cavender (1998) reported four new species recovered from forest soils of Tikal, Guatemala, which belong to the genus *Polysphondylium*.

During studies of cellular slime molds over the past years, we frequently encountered taxonomical studies based on morphological and biochemical characteristics. However, these characteristics were often too ambiguous to differentiate one species from very similar ones. These ambiguous traits are likely to bring very different classification results and to take much time. In recent years, there has been an increased interest in utilizing molecular methods for systematics such as DNA sequencing (Baldwin 1992a, 1992b, Kim and Jassen 1994, Wojciechowchi 1993).

In this study, we isolated the dictyostelid cellular slime molds in subalpine zone in Mt. Paektu, North Korea and confirmed new species using morphological characteristics and ribosomal DNA sequencing.

METHODS

Isolation and characterization of cellular slime molds (CSM)

Soil samples were collected from the alpine and subalpine zone in the Mt. Paektu during July 1997. Mt. Paektu (2,749.6m), the biggest mountain in Northeast Asia, located on the border line of Korea and China, is characterized by an aspect with broad gentle mountain area and rich biota (Yim and Shim 1998). Clones were isolated by plating samples out on hay infusion agar according to the Cavender method (Cavender and Raper 1965a). Clones of taxonomic value were subcultured on nonnutrient agar using a streak of 24-h-pregrown *Escherichia coli* as food source. Subsequently, lactose-peptone media were used.

Observed characteristics were aggregation patterns of myxamoebae, growth habits of sorocarps, branching patterns, color, length, tips, bases and size of sorophores, shapes, size, color, length/width ratio and polar granule of spore, phototropism, presence of microcyst and macrocyst, etc. These characteristics were observed and photographed with Olympus Vanox microscope and Seoul Selopt stereomicroscope.

Analysis of ribosomal DNA sequence

Genomic DNA was extracted from CSM according to Nellen *et al.* (1987). The extracted DNA was electrophoresed in 0.8% agarose gel to verify the quality. Polymerase chain reaction was applied to amplify the ribosomal DNA including 140 bps of 18S rDNA, full length of ITS1, 5.8S rDNA, ITS2 and 40 bps of 28S rDNA. The primers were designed according to the sequence of *Dictyostelium discoideum* (Ozaki *et al.* 1984). The primer sequences are: 18S Forward = CACACCGCCCGTCGCTCCTACCGATCG, 28S Reverse = TCCTCCGCTTACTGA TATGC. The 50 ml of PCR reaction mixture consisted of 34.5 ml sterile distilled water, 5 ml of 10x Taq polymerase buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM $MgCl_2$), 2 ml of 5 mM dNTP, 5 unit of Taq polymerase (TaKaRa Biochemicals, Japan), 2 ml each of 10 mM primer 18S Forward and 28S Reverse and about 5~10 ng genomic DNA. The thermal cycling was performed on a Perkin-Elmer Thermal Cycler with the following condition: 1 cycle of 5 min at 94℃ linked to 35 cycles of 1 min at 95℃ for denaturation, 1 min at 55℃ for annealing and 1 min at 72℃ for extension. Then they were reacted by Thermosequenase kit (Amersham Life Science, USA). The sequenced samples were separated by 4% Long Ranger gel (FMC, USA) and Automatic DNA Sequencer, Long ReadIR 4200 (LI-COR, INC.). Amplified sequences of CSM were aligned using the computer software CLUSTAL X (Thompson *et al.* 1997). The determination of ITS1, 5.8S rDNA and ITS2 was followed *D. discoideum* (Olsen and Sogin 1982, Ozaki *et al.* 1984). Additionally, the length of each region and GC contents were analyzed.

RESULTS

In the subalpine zone of Mt. Paektu, six species of dictyostelids were recovered (Table 1): *D. minutum*, *D. brefeldianum*, *D. crassicaule*,

Table 1. Dictyostelids in subalpine zone of Mt. Paektu

Site	BD8		BD9		BD10					
Altitude (m) Vegetation	1800 *Betula ermanii*		1820 *Veratrum grandiflorum*		1760 *Larix olgensis*					
	F[1]	D	F	D	F	D	RD[2]	SF	AF	IV
D. minutum	100	96	67	34	–	–	43	67	56	69
D. brefeldianum	17	1	33	14	83	90	30	100	44	68
D. crassicaule	17	2	50	47	–	–	13	67	22	39
P. pallidum	33	<1	–	–	33	<1	0	67	22	30
D. capitatum	–	–	8	5	17	6	3	67	8	27
P. solitarium	–	–	–	–	17	4	1	33	6	14
Total clones (No./g)	11,391		8,644		9,325					

[1]F(Frequency, %) = (the number of samples that a species occurred / total number of samples in a site) × 100
D(Density, %) = (the number of clones of a species / total number of clones of all species in a site) × 100
[2]RD(Relative density, %) = (the number of clones of a species / total number of clones) × 100
SF(Site frequency, %) = (the number of sites that a species occurred / total number of sites) × 100
AF(Average frequency, %) = (the number of samples that a species occurred / total number of samples) × 100
IV (Importance value) = (2RD + SF + AF)/3

D. capitatum, *P. solitarium* and *P. pallidum*. *D. minutum* and *D. brefeldianum* were dominant species which were recovered in most study sites. *P. solitarium* which was isolated from the soils of *Larix olgensis* community exhibited several distinctive features which differed from the published species. This species was designated as a new species, *Polysphondylium solitarium* Kang et Chang, sp. nov.

Descriptions of species

Polysphondylium solitarium Kang et Chang sp. nov. (Korean name: 외돌려난가지팡이)

Sorocarpia phototropicis, erecta et aggregata, typi solitaria, raro coremiformis et raro cultum, nunc cultum ad 22~24℃ in agaro low-nutricio cum *E. coli* sunt. Sorophora gradatium attenuata ex basis ad apices, a dimidium 7mm et plerumque 1.3~15 mm longitrorsus. Sorophora cum ex 1 ad 23 verticillis non-regularibus longitrorsus, ex hyalinae ad vinei. Axes rectis lineis gradatum ex basis ad apices, dimidium longitrorsus: basis cum structura cellularis claviformis, frequenter dilatata, ex 15 ad 50 in diametro. Sori globosi, vineo color, a dimidium 100 μm et plerumque 70~160 μm in diametro et cum lateralis sori ex 40 ad 120 μm in diametro. Sporae ex hyalinae ad vinei, ex ovalis ad ellipticae, a dimidium 6.1×3.6 μm et plerumque 4.5~8.0×3~4 μm, cum nonconsolidatis granulis polaris et sub-polaris. Aggregationes tipi mucroides sunt. Macrocystii et microcystii in nullo substrato observati.

Sorocarps are erect to semierect, usually solitary and rarely gregarious or clustered, when cultured at 22~24℃ on low-nutrient agar with *E. coli*. Sorocarps are highly phototropic under unidirectional illumination. Sorocarps range from 1.3~15 mm (mean 7 mm) long tapering from bases to tips and are hyaline to vinaceous. Bases are round to expanded pyramidal or conical, 15~50 μm (mean 30 μm) in diameter. Whorls vary in number from 1~23 (mean 9~10), are spaced from 200~900 μm (mean 540 μm) or even more, irregularly distributed and sometimes rebranching. The terminal segment vary in length, 400~2000 μm. One to five branches are irregular in size, 170~400 μm (mean 274 μm),

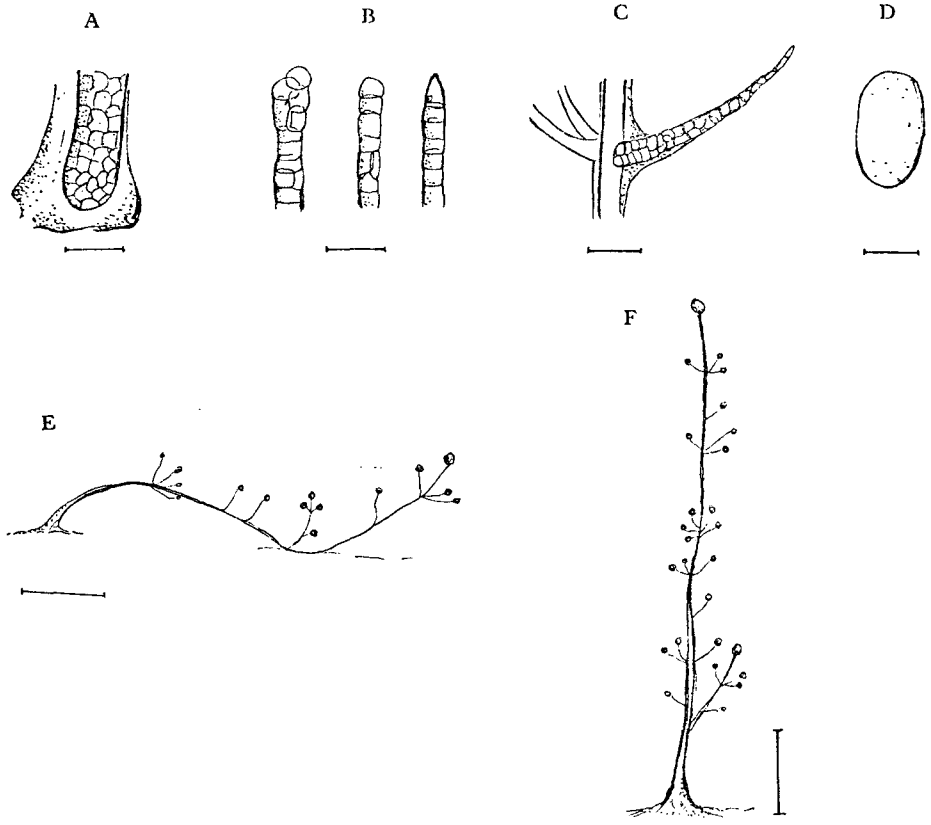

Fig. 1. *Polysphondylium solitarium*. A. Base. B. Tips. C. Branch. D. Spore E. Sorocarps under one-sided illumination F. Mature sorocarps formed under diffuse overhead illumination. Scale bar = 50 μm (A), 10 μm (B), 100 μm (C), 3 μm (D), 1mm (E, F)

also tapering markedly from base to tip. Sori are globose, with a mean diameter of 100 μm (70 ~160) and vinaceous. Lateral sori are also globose, with a mean diameter of 66 μm (40~ 120). Spores are hyaline to vinaceous, oblong to elliptical 4.5~8.0×3~4 μm (mean 6.1×3.6: length /width index 1.5~2.2), with nonconsolidated granules, which may not be consistently polar. Pseudoplasmodia radial, usually centralized and rarely subdivided, not migrating without sorophore formation (Fig. 1, 2). Microcyst and macrocyst are not observed.

Holotype. Strain BD10, isolated from soil of the subalpine forest (*Larix olgensis* community) of Mt. Paekdu, North Korea, by K. M. Kang, in July 1997.

Polysphondylium solitarium is distinguishable from other species by the following combination of features (Fig. 2). Firstly, the sorocarps have a vinaceous pigmentation, particularly in the sori, which may vary in color intensity according to culture condition. Secondly, pseudoplasmodia radial, usually centralized and rarely subdivided, subsequently making solitary sorocarps. Thirdly, the number of whorls varies from 1 to 23 and internode length between whorls is various. Fourthly, its polar granules of spores are usually unconsolidated and not conspicuous. The specific epithet is derived from the second characteristic. Aggregations are radial and of the *mucoroides* type and sorocarps are usually solitary and seldom gregarious or clustered.

DISCUSSION

Six species of dictyostelid cellular slime molds inhabiting soils of subalpine zone in the Mt. Paekdu were recovered. Mean total clones and species found were 9,787 No./g and 4, respectively. These values are higher than those in other subalpine zone. Since Brefeld's time, the genus *Polysphondylium* has been characterized among the Dictyosteliaceae by the whorled pattern of branches, robust and rapid growth, large size of aggregation, radial type of aggregation, the presence of unconsolidated polar granule in spore, the lack of response to cAMP as an acrasin, and acuminate sorophore tip (Vadell and Cavender 1998). But *P. violaceum* has several exceptions. This species usually fragments during late aggregation. Pseudoplasmodia develop radially and centralized, subsequently subdivided (Hagiwara 1989). And this species has spores with consolidated granules and clavate sorophore tip unlike other *Polysphondylia.*

P. solitarium is closer to *P. violaceum* and *P. acuminatum* in that they have vinaceous to violet sorocarps. The color of sorocarps allows a clear distinction to be made between these species and the other white *Polysphondylia. P. solitarium* is different from these two pigmented species by the following combination of features:

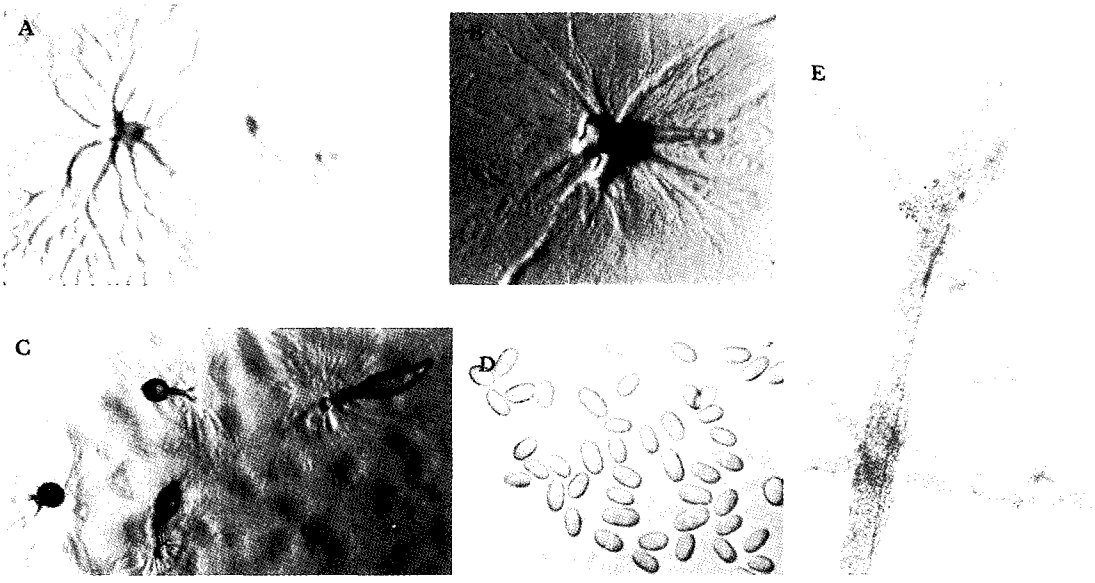

Fig. 2. *Polysphondylium solitarium* features important for identification.
A, B. Mucoroides type aggregation (×20) C. Solitary sorogen (×30)
D. Elliptical spores with nonconsolidated polar granules (×1000)
E. Irregular branches (×250)

P. violaceum AATGATACGG TAAAGCCAAC GGATAAGATC CTATTGGGCA ACTAATATGG ATTTTAAAAG TTGTTTAAAT CTCATTGTTT AGAGGAAGGA GAAGTCGTAA

P. solitarium AATGATACGG TAAAGCCAAC GGATAAGGTC CTATTGGGCA ACCGGTATGG ATTTTAAAAG TTGTTTAAAT CTCATTGTTT AGAGGAAGGA GAAGTCGTAA

P. violaceum CAAGGTATCC GTAGGTGAAC CTGCGGATGG ATCATTTTTC AACTCGAAAA TTGATACAAA ATTGAAC-TT TGATCT-TA CTTGCAAAAG AAAGAACGGT

P. solitarium CAAGGTATCC GTAGGTGAAC CTGCGGATGG ATCATTTTTC AACTCGAAAC TTGATACAAA ATTGAACCTT TGATCTGTTT TTTTCTACAG AACA--GGT

P. violaceum TCTTTTTTGT TCCATCTGCA AT--CAAA- ACATTGTTTA C--TGATTT CTTTTTATAT ATTTTCATAT ATCTGAATCT ATTCAAT--- -ATAATCTC

P. solitarium TCTTTTTTGT TCCATCTGCA ATTTCCAGAC ACATTGTTTA CAAATGATTT CTTTCTTTAT TTTATAATA- -ACTGAATCT TTTCAATGTT TGATAATCTC

P. violaceum AAAT---- ------- ------T TTTTCTAGAA TGACAAACAA ACATTTAAAG ATGTTTATAA GCTTTAAATA ACTAAAGCAT AAACGGTGAA

P. solitarium AAAAAACACC TAATTTGTTT AGCAAATTAT TTTTCTAGTA TGTCAAACAA CCATT-AAAG ATGTTTA--A GCTTTAAATC ACTAAAGCAT AAACGGTGAA

P. violaceum TACCTCGACT CCTAAATCGA TGAAGACCGT AGCAAACTGC GATAATTCAC TTGAATTGCA GCCTACTGGG ATAGTTGAAA TGTTGAACGC ACATGATGGC

P. solitarium TACCTCGACT CCTAAATCGA TGAAGACCGT AGCAAACTGC GATAATTCAC TTGAATTGCA GCCTACTGGG ATAGTTGAAA TGTTGAACGC ACATGATGGC

P. violaceum ATTGGTCCTT TTGGATTAAG TGTCATACTT GGGTGAGAGC GATCTCTATA TTTAATATTA AA-ATTTTAT TAATTAAATT TAGGTT-- ---TAATGA

P. solitarium ATTGGCCCTT T-GGGTTAAG TGTCATACTT GGGTGAGAGC GATCTCTCT- -TTAATATCA AATATTCTAT TAGTTAAATT AGACTTGTCT AGAATTACAA

P. violaceum ACTAATTTTA --ATATGA-A GTTTATTTAT T--TAAACT- ------- ------- ---TTTTT ATAGTTGATC TACAGTAGTC TTTGCAGTGG

P. solitarium ACGAATTTTT TGATATAATA GTTTCTTTTT TAATAATCTC TTCGGAGTGT TTAGAAATTA AATTATTTTT ATAGTTGATC TATAGTAGTC TTTGCAGTGG

P. violaceum TATAAA-GAA TAGTTTTAAA ---CTAAA --CTTTAAT TAAACCAT-T ACTATATTAT ATGTTTATAC AATTTAAACT ATAATAATTA TTCAATTGAA

P. solitarium TATAATCGAC TTTACTCCAA GAAGCCTAAA AAACTTCAGT CGAACCATCT AATTTGATGA CTGTTTTCTT TACTTGAAAC ATTATCTACA TTCAATTGAA

P. violaceum AATGCGAATC TTTATTAAAG TTTATTCAAG TTGTA-TTTA CTAAATATTA TAA--TGGCT CATTTATAAT GTTAATTTAA TTAAA-ATT AAAGCTAA-T

P. solitarium AATGCGAATA AAGATTTTGA ATCGCTATAG GTTTAGTTTA CAAATTCTTT TTAGTTGGCT CTTT--AAT GTTAATTCGT CTAAAGAATT GGAGTGAGCT

P. violaceum CAATATACAT TT-AAACACT GATTAAAGAA GTAACTTATG AACATTAAAC GATATGTTTA TCAT-ATTGG TTCAACAGTT ATATAT-TGT CGCCTCATCC

P. solitarium TTTTATATAT TCCAAACACT GATTAA-GAA GCAACTTATG AACATTAACG GTTTAGTTTA TCTTTACTGG TTCAACAGTT GTATATATTT CGCCTCATCC

P. violaceum AAGTAAGATT ACCCGCTGAA CTTAAGCATA TCAGTAAGCG GAGGA

P. solitarium AAGTAAGATT ACCCGCTGAA CTTAAGCATA TCAGTAAGCG GAGGA

Fig. 3. Sequence alignment of the ribosomal DNA of *P. violaceum* and *P. solitarium*. Conserved positions are indicated with asterisks, deleted nucleotides with dashes. 18S, 5.8S and 28S rDNA sequences (filled box) are separated by ITS1 and ITS2.

Table 2. The lengths and GC contents of ITS1, 5.8S rDNA and ITS2 region of *P. violaceum* and *P. solitarium*

Species	ITS1		5.8S DNA		ITS2		Total	
	length	GC ratio	length	GC ratio	length	GC ratio	length	GC ratio
P. violaceum	204	0.240	162	0.426	384	0.174	750	0.247
P. solitarium	236	0.270	161	0.441	436	0.269	833	0.301

(i) pseudoplasmodia develop radial and centralized, rarely subdivided, while radial and centralized, subsequently subdivided in the other two species, (ii) sorocarps are mostly solitary and rarely gregarious, while usually clustered and sometimes solitary in others, (iii) the polar granule of spore is not consolidated and non-conspicuous, while others have prominent consolidated granules, (iv) especially, *P. acuminatum* has regular branches in size, not exceeding 130 μm and acuminate sorophore under weak overhead illumination, but. *P. solitarium* have irregular branches in size, from 170 to 400 μm and its sorophores are not acuminate.

These morphological characterisics may be sufficient to differentiate *P. solitarium* from *P. violaceum* and *P. acuminatum*. However, when cultures are maintained over long periods by infrequent transfer, they often tend to become progressively atypical - in fact, the best proportioned sorocarps are often seen in primary isolation plates where the slime mold not only feeds upon indigenous bacteria but also competes with a diverse microflora (Raper 1984). Therefore, we compared ribosomal DNA sequencing of *P. solitarium* with that of *P. violaceum* which often shows quite different pictures on substrates of unlike composition and relatively closer to *P. solitarium* than *P. acuminatum* (Fig. 3, Table 2). *P. acuminatum* was isolated from soil of the semievergreen rain forest of Tikal, Guatemala and has not been isolated in any other area (Vadell and Cavender 1998).

According to the multiple sequence alignment by CLUSTAL X, sequence alignments of ITS1 and ITS2 between two species have too many ambiguous and gap sites. This difference is larger than sequence difference between *D. giganteum* and *D. dimigraformum* and between *P. pallidum* and *P. tenuissimum* which are similar to each other, respectively (Chang and Hong 1999). And length and GC ratio of ribosomal DNA (ITS1, ITS2 and 5.8s DNA) of *P. solitarium* are very different from those of *P. violaceum* (Table 2).

According to morphological characteristics, identification key to polysphondilia of Korea are as follows.

KEY TO THE POLYSPHONDYLIA OF KOREA

A1 Terminal and lateral sori are colorless or white
- B1 Sorocarp with terminal segment not elongated, solitary or clustered habit
  - C1 Sorocarps with 4~8 whorls, branches 80~630 μm long ··· *P. pallidum*
  - C2 Sorocarps with 3~30 or more whorls, 2~10 branches per whorl, branches < 20 μm long ··· *P. tenuissimum*
- B2 Sorocarp with elongated terminal segment (not creeping), solitary or clustered habit.
  - C1 Spores medial, branches short ··· *P. pseudo-candidum*
  - C2 Spores large, branches long ··· *P. candidum*

A2 Terminal and lateral sori are initially pigmented by violet
- B1 Pseudoplamodial usually subdivided but often centralized, sorocarps usually gregarious ··· *P. violaceum*
- B2 Pseudoplamodial usually centralized and rarely subdivided, sorocarps usually solitary, rarely gregarious ··· *P. solitarium*

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