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Dono Wahyuno · Makoto Kakishima · Yoshitaka Ono

Aeciospore-surface structures of *Phragmidium* species parasitic on roses

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Abstract Aeciospore-surface structures of *Phragmidium* species parasitic on roses were investigated by scanning electron microscopy. Seven kinds of structures were distinguished according to gross shape of ornamentations and their distribution on the aeciospore wall. The seven different structures were categorized into three major types: echinulate, annulate, and verrucose. The echinulate type was further classified into five types and designated in a numerical series, echinulate types 1–5. Application of the aeciospore-surface structures in the classification of *Phragmidium* species is discussed.

Key words Aeciospore · *Rosa* · Scanning electron microscopy · Taxonomy · Uredinales

Introduction

Morphological features of aeciospores have been suggested as important characters in the taxonomy of *Cronartium* (Hiratsuka 1971), *Puccinia* and *Uromyces* on Gramineae, Cyperaceae, and Juncaceae (Savile 1973), on Liliaceae, Gramineae, and Juncaceae (Holm 1984), and *Gymnosporangium* and *Roestelia* (Holm 1984). However, no systematic account on aeciospore-surface structures was published until Sato and Sato (1982) studied aeciospore-surface structures of 70 species in 39 genera of the Uredinales. They recognized eight major types, i.e., aciculate, annulate, coronate, echinulate, nailheaded, reticulate, tubulate, and verrucose, within which several subtypes were included. In the study of aeciospores of *Gymnosporangium* and *Roestelia*, Lee and Kakishima (1999) classified aeciospore-surface structures into ten groups, i.e., large coronate, small coronate, minutely coronate, mountain shaped, echinulate, small annulate, large annulate, tubulate, nailheaded, large verrucose, small verrucose, and verrucose with refractive granules. These studies indicated that the aeciospore-surface structures could be a good taxonomic character when employed concomitantly with other selected characters.

In the taxonomy of the genus *Phragmidium*, less attention was paid to morphological features of aeciospores than those of teliospores or urediniospores. However, Cummins (1931) showed that aeciospore wall thickness and wall sculptures as revealed by light microscopy could be employed to segregate some *Phragmidium* species on roses. Aeciospore-surface ornamentations and wall thickness at germ pores were considered important in distinguishing *P. tuberculatum* J. Müller from *P. mucronatum* Schlectendal by Wilson and Henderson (1966) and Laundon (1970).

Aeciospore-surface structures of a few *Phragmidium* species have been studied by scanning electron microscopy (Sato and Sato 1982; Bedland 1984; Preece and Hick 1990). However, no systematic study of aeciospore-surface structures of *Phragmidium* species was undertaken and, therefore, no thorough consideration of the aeciospore-surface structures as a taxonomic character has been carried out in the taxonomy of *Phragmidium*.

This study reports aeciospore-surface structures of all *Phragmidium* species recorded on the genus *Rosa* and discusses a possible application of the structural features in the classification of *Phragmidium* species parasitic on roses.

D. Wahyuno · M. Kakishima
Institute of Agriculture and Forestry, University of Tsukuba, Ibaraki, Japan

Y. Ono (✉)
Faculty of Education, Ibaraki University, Mito, Ibaraki 310-8512, Japan
Tel. +81-29-228-8240; Fax +81-29-228-8329
e-mail: onoy@mito.ipc.ibaraki.ac.jp

Contribution no. 166, Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, Japan

Materials and methods

Specimens examined

A total of 290 aecial specimens deposited in the Herbarium of Systematic Mycology, the Faculty of Education, Ibaraki University (IBA) and the Mycological Herbarium, the Institute of Agriculture and Forestry, the University of Tsukuba (TSH) and loaned from the following herbaria were studied: the Arthur Herbarium, Purdue University (PUR); the Mycological Herbarium, Faculty of Agriculture, Hokkaido University, Japan (SAPA); the Swedish Museum of Natural History, Stockholm, Sweden (S); the Herbarium of Mycology and Lichenology, the Institute of Microbiology, Academica Sinica (HMAS); and the Museum of Natural History, Wroclaw University, Poland (WRSL) (Table 1). The specimens represented *P. americanum* Dietel, *P. butleri* Sydow, *P. cinnamomeum* Durrieu, *P. fusiforme* Schröter, *P. montivagum* Arthur, *P. mucronatum* (Pers.) Schlectendal, *P. rosae-alpinae* (DC.) Winter, *P. rosae-arkansanae* Dietel, *P. rosae-californicae* Dietel, *P. rosae-lacerantis* Dietel, *P. rosae-moschatae* Dietel, *P. rosae-multiflorae* Dietel, *P. rosae-pimpinellifoliae* Dietel, *P. rosae-rugosae* Kasai, *P. rosae-setigerae* Dietel, *P. speciosum* (Fr.) Cooke, and *P. tuberculatum* J. Müller.

Table 1. Aeciospore-surface structure types of *Phragmidium* species on roses with their host range and geographic distributions

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. acicularis Lindl.	Alaska, U.S.A.	PUR7741, 7747, and 7749	E-1
	Canada	PUR44747 and 44749	E-1
	Canada	PUR53193	E-1
	Colorado, U.S.A.	PUR7925	E-1
	Japan	PURF11991, SAPA (3)	E-1
	Kansas, U.S.A.	PUR8113, 8132, and 8125	E-1
	Montana, U.S.A.	PUR7792, 55311, 7746, and 7836	E-1
	Russia	IBA7267, 6734, and 6750, SAPA	E-1
	U.S.A.	PUR7836	E-1
	Wyoming, U.S.A.	PUR61190 and 63222	E-1
	Unknown	PURF1587	E-1
R. alba L.	Maryland, U.S.A.	PUR8158	E-1
	Sweden	TSH-R1973	E-1
R. blanda Ait.	Canada	PUR65694 and 8484	E-1
	Iowa, U.S.A.	PUR8110 and 8481	E-1
	New York, U.S.A.	PUR8485	E-1
	Wisconsin, U.S.A.	PUR7660 and 44785	E-1
R. bourgeaniana Crép.	Canada	PUR44752	E-1
	Colorado, U.S.A.	PUR8571	E-1
	Montana, U.S.A.	PUR7799, 7800, and 7802	E-1
R. brunonii Lindl.	India	PURF18144	A
R. californica Cham. & Schlect.	California, U.S.A.	PUR8027, 53849, 8004, 8018, 62463, 8025, 8026, 8006, and 8010, S	E-1
	Nevada, U.S.A.	PUR8028	E-1
R. canina L.	Germany	PURF1560	E-3
	Sweden	PURF1563	E-3
R. carolina L.	Alabama, U.S.A.	PUR8530	E-1
	Delaware, U.S.A.	PUR7662	E-1
	Illinois, U.S.A.	PUR7718	E-1
	Indiana, U.S.A.	PUR8533, 8534, 8546, 8549, 8489, and 8539, S	E-1
	Massachusetts, U.S.A.	PUR44693 and 8491	E-1
	Missouri, U.S.A.	PUR47977	E-1
	Mississippi, U.S.A.	PUR44695 and 8493	E-1
	New York, U.S.A.	PUR7663, 7713, 7710, 8540, 8550, and 8536	E-1
	North Carolina, U.S.A.	PUR8552	E-1
	Vermont, U.S.A.	PUR8542	E-1
	West Virginia, U.S.A.	PUR44694	E-1
R. carolina L.	Wisconsin, U.S.A.	PUR7719	E-1
	Unknown	PUR7715	E-1
R. centifolia L.	Germany	PURF1503	E-1
R. cinnamomea L.	Germany	PURF1492	E-1
	Germany	PURF1552 and F1553	E-3
	Switzerland	PURF17039	E-3
R. damascena Miller	Alaska, U.S.A.	PUR8163	E-1
R. davurica Pall.	Russia	SAPA (4)	E-1
R. engelmannii Wats	Nebraska, U.S.A.	PUR8096	E-1

Table 1. *Continued*

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. fendleri Crép.	Alaska, U.S.A.	PUR50575	E-1
	Colorado, U.S.A.	PUR7886 and 8559	E-1
	Montana, U.S.A.	PUR8098, 8556, and 8586	E-1
	Nebraska, U.S.A.	PUR8101 and 8102	E-1
	Utah, U.S.A.	PUR7866	E-1
	Wyoming, U.S.A.	PUR7904, SAPA	E-1
R. filifera Rydb.	California, U.S.A	PUR8070	E-1
R. foetida J. Herrmann	Wyoming, U.S.A.	PUR62725	E-1
R. foliosa Nutt.	Tennessee, U.S.A	PUR8497	E-1
R. gallica L.	Scandinavia	PURF1518	E-1
R. gymnocarpa Nutt.	California, U.S.A.	SAPA, PUR8045, 8048, 53854, 8043, 8047, 53852, and 8053	E-1
	Washington, U.S.A.	PUR8035, 8038, 8039, and 8044	E-1
	Oregon, U.S.A	PUR44776	E-1
R. lacerans Boiss	Iran	S (3)	V
R. macdaugalii Holz.	Idaho, U.S.A.	PUR7766 and 49821	E-1
	Montana, U.S.A.	PUR7773	E-1
R. macounii Greene	Colorado, U.S.A.	PUR8553 and 8554	E-1
	North Dakota, U.S.A.	PUR8528	E-1
	Utah, U.S.A.	PUR7872	E-1
R. macrophylla L.	Unknown	PURF10545	E-3
R. marretii Lév.	Russia	PURF12006, SAPA (3)	E-1
R. minutifolia Englm.	California, U.S.A	PUR8054	E-1
R. moschata Miller	India	PURF10548	E-4
	India	IBA4929	E-3
	India	PURF1584 and F10547	V
		PURF1573, F1574, and F17982	
R. multiflora Thunb.	Japan	IBA1897, 5696, 6592, 2148, 1786, and 4736, PURF1579, SAPA (6), TSH-R3759	E-2
R. nutkana Presl.	Alaska, U.S.A.	PUR7784, 48583, and 8065	E-1
	California, U.S.A.	PUR53855	E-1
	Canada	PUR44757 and 8057	E-1
	Montana, U.S.A.	PUR44755, 44756, and 7768	E-1
	Oregon, U.S.A.	PUR8068 and 65059	E-1
	Washington, U.S.A.	PUR8069, 8055, 8060, and 8062	E-1
	Wyoming, U.S.A.	PUR48847	E-1
	Unknown	PUR1473, 53856, and 53857	E-1
R. palustris Marsh.	Tennessee, U.S.A.	PUR44696	E-1
R. pecosensis Cockerell	New Mexico, U.S.A.	PUR8563	E-1
R. pendulina L.	Switzerland	PURF1558	E-1
	Austria	PURF1568	E-1
	Poland	WRSL	E-1
R. pimpinellifoliae L.	Austria	PURF1535	E-1
	Germany	PURF1532	E-1
	Wisconsin, U.S.A.	PUR8233	E-1
	Unknown	PURF1535	E-1
R. pisocarpa Gray	Canada	PUR58015	E-1
	California	PUR8076, 48209, and 53850	E-1
	Oregon, U.S.A.	PUR8603	E-1
	Washington, U.S.A.	PUR8074	E-1
R. pratincola Greene	Kansas, U.S.A.	PUR8113	E-1
R. rubiginosa L.	Massachusetts, U.S.A.	PUR 8227	E-1
	Nebraska, U.S.A.	PUR8228 and 8225	E-1
	New York, U.S.A.	PUR 8231	E-1
	Switzerland	PURF1558	E-3
R. rugosa Thunb.	Japan	IBA8132 and 1890, PURF12022, SAPA (11)	E-1
	Russia	IBA6182 and 7238, SAPA	E-1
	Wisconsin, U.S.A.	PUR8568	E-1
R. saundersiae Rolfe	India	PURF1575	E-3
R. sericea Lindl.	China	TSH-R1975, 1976, and 1977	A
R. sepia Koch	Switzerland	PURF1556	E-3
R. setigera Michx.	Indiana, U.S.A.	PUR7726	E-1
	Unknown	PUR7716	E-1
R. sikangensis Yu & Ku	China	HMAS 45217	E-5
R. spithamea Wats.	California, U.S.A.	PUR49614	E-1
R. subnuda J. Lunnel	North Dakota, U.S.A.	PUR8146	E-1

Table 1. *Continued*

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. suffulta Greene	Alaska, U.S.A.	PUR8131 and 8525	E-1
	Canada	PUR7750 and 8106	E-1
	Indiana, U.S.A.	PUR8510	E-1
	Iowa, U.S.A.	PUR8502, 8504, and PUR N37	E-1
	Kansas, U.S.A.	PUR8506, S	E-1
	Missouri, U.S.A.	PUR8137	E-1
	North Dakota, U.S.A.	PUR7760 and 8499	E-1
	South Dakota, U.S.A.	PUR8123 and 8513	E-1
R. virginiana Miller	Canada	PUR8522 and 8576	E-1
	Connecticut, U.S.A.	PUR8581	E-1
	Indiana, U.S.A.	PUR8582	E-1
	Maine, U.S.A.	PUR7680 and 7673	E-1
R. virginiana Miller	Massachusetts, U.S.A.	PUR7678	E-1
	Missouri, U.S.A.	PUR8585	E-1
	New York, U.S.A.	PUR7681 and 8584	E-1
R. webbiana Wallich ex Royle	India	PURF10549 and	E-4
		F15706	E-3
	Pakistan	IBA4937 and 4897	E-3
	Tibet, China	HMAS67833	A
R. wilmottiae Hemsl.	Tibet, China	HMAS67838	A
Rosa sp.	Alaska, U.S.A.	PUR7809 and 7790	E-1
	Arizona, U.S.A	PUR8084	E-1
	California, U.S.A	PUR8090, 8091, 8082, 8086, 8087, and 8597	E-1
	Canada	PUR8085	E-1
	Colorado, U.S.A.	PUR8601 and 49702	E-1
	Indiana, U.S.A.	PUR44699	E-1
	Michigan, U.S.A.	PUR8592	E-1
	Mississippi, U.S.A.	PUR8600 and 8602	E-1
	Missouri, U.S.A.	PUR51235	E-1
	Montana, U.S.A.	PUR8153	E-1
	Nebraska, U.S.A.	PUR7736 and 7689	E-1
	New York, U.S.A.	PUR7698	E-1
	Nevada, U.S.A.	PUR8030	E-1
	North Dakota, U.S.A.	PUR8148	E-1
	Pakistan	PURF1586	E-3
	India	PURF15878	E-1
	Oregon, U.S.A	PUR8088	E-1
	Russia	IBA7282	E-1
	South Dakota, U.S.A.	PUR7810	E-1
	Sweden	TSH-R1982 and 1984	E-1
	Washington, U.S.A.	PUR7969 and 8083	E-1
	Wisconsin, U.S.A.	PUR8591 and 8594	E-1
	Wyoming, U.S.A.	SAPA	E-1

[a] HMAS, The Herbarium of Mycology and Lichenology, Institute of Microbiology, Academia Sinica, China; IBA, The Herbarium of Systematic Mycology, Faculty Education, Ibaraki University, Japan; PUR, The Arthur Herbarium, Purdue University, U.S.A.; SAPA, The Mycological Herbarium, Faculty of Agriculture, Hokkaido University, Japan; S, The Swedish Museum of Natural History, Sweden; TSH, The Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba, Japan; WRSL, The Museum of Natural History, Wroclaw University, Poland

[b] A, annulate; E-1, echinulate type 1; E-2, echinulate type 2; E-3, echinulate type 3; E-4, echinulate type 4; E-5, echinulate type 5; V, verrucose

Scanning electron microscopy

Sori were determined as aecia when they were associated with spermogonia on the herbarium specimens examined. Aeciospores were scraped from the aecia by a fine surgical knife and dusted on a small piece of double-adhesive tape on a specimen holder. The preparation was coated with platinum-palladium with an E-1030 Ion Sputter and examined under an S-4200 SEM (Hitachi, Ibaraki, Japan) operating at 15 kV. Observed surface structures were categorized according to Sato and Sato (1982) and Cummins and Hiratsuka (1983).

Results and discussion

Seven kinds of surface structures were revealed by scanning electron microscopy (SEM) and categorized into echinulate, annulate, or verrucose types (Figs. 1–3). The echinulate type was further classified into five types (Fig. 1). Each type is described as follows.

Echinulate type 1 (E-1)

Echinulate type 1 is characterized by even distribution of stout, pointed conical echinae (Figs. 1E-1, 2A). The apex may be weakly curved. The structure is essentially the same as the echinulate structure most commonly observed in urediniospores, and the process of echina development in the aeciospores is assumed to be the same as that in the urediniospores (Littlefield and Heath 1979). Length and density of echinae on the spores were fairly uniform within a specimen but varied among the specimens. The length ranged from 0.9 to 1.2 μm, and density ranged from 2 to 4 echinae per 10 μm². The spore surface structures of a majority of the specimens fell into this category (see Table 1). Holm et al. (1970) observed this type of echinulation in aeciospores from a specimen that they identified as *P. mucronatum*.

Echinulate type 2 (E-2)

Echinulate type 2 is essentially the same as type 1 except for the lack of echinae on the apical area of the spore wall (Figs. 1E-2, 2B). The echina size is decreased gradually toward the smooth area. The apical smooth area ranged from 10% to 30% of the total spore wall surface. As in type 1, the size and density of echinae were fairly uniform in the spores from a specimen but varied among the spores from different specimens. Echina length was ~1 μm on average, ranging from 0.3 to 1.5 μm; density ranged from 2 to 4 echinae/10 μm².

Type 2 was observed only in the spores produced on *R. multiflora* Thunb. in Japan (Table 1). Spores from 14 specimens on *R. multiflora* showed only this type of surface structure. The SEM photomicrograph published by Sato and Sato (1982) showed echinulate aeciospores from 1 of 2 specimens on *R. multiflora* they examined. They did not mention the presence of a smooth area on the spore wall (Table 2). Echinulate aeciospores with an apical smooth area have been reported in *Naohidemyces fujisanensis* S. Sato et al. (Sato et al. 1993). This type of surface ornamentation is not uncommon in urediniospores and has been reported in *Melampsoridium asiaticum* S. Kaneko & Hiratsuka, f. (Kaneko and Hiratsuka 1983), *Melampsora* spp. (Preece and Hick 1990), *Blastospora smilacis* Dietel (Ono et al. 1986), *Tranzschelia* species (López-Franco and Hennen 1990; Ono 1994), and *N. fujisanensis* (Sato et al. 1993).

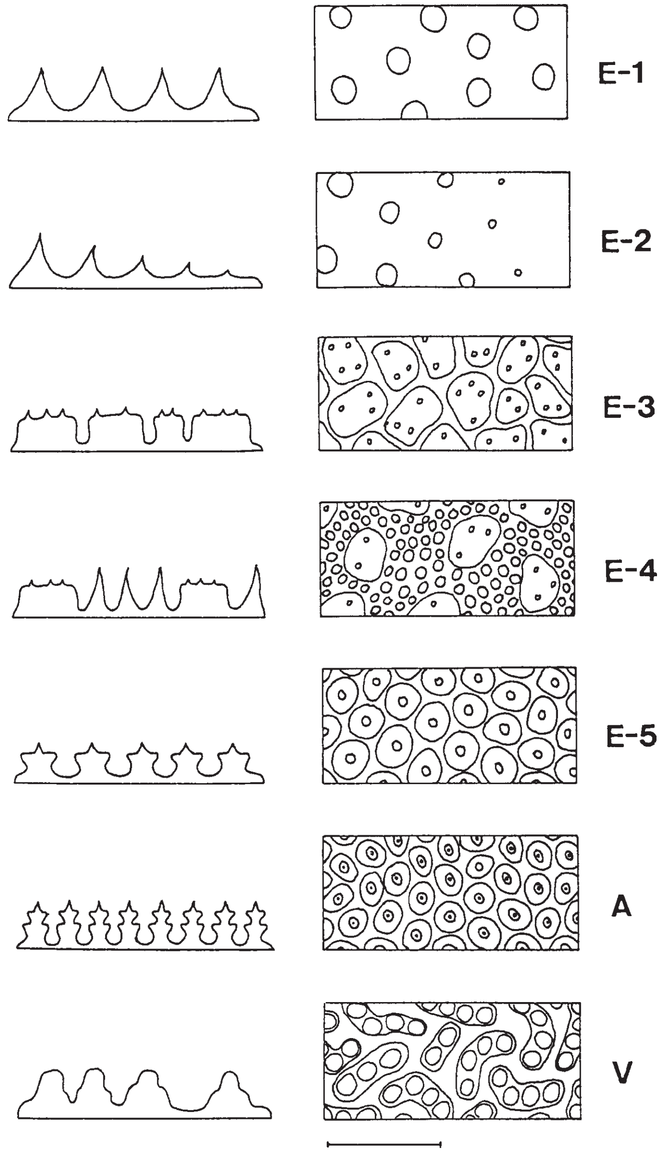

Fig. 1. Schematic presentation of aeciospore-surface structures of *Phragmidium* species on roses. *E-1*, echinulate type 1; *E-2*, echinulate type 2; *E-3*, echinulate type 3; *E-4*, echinulate type 4; *E-5*, echinulate type 5; *A*, annulate; *V*, verrucose. *Left*, side view; *right*, top view. *Bar* 2 μm

Echinulate type 3 (E-3)

This surface-structure type is characterized by one or more echinae arising on a plateau-shaped base (Figs. 1E-3, 2C,D). Number of echinae on each plateau-shaped base was mostly two or three. The echinae of this type were shorter (0.4–0.8 μm) than those in echinulate types 1 and 2 (0.9–1.2 μm). The plateau-shaped bases were various in shapes, mostly irregularly angular, and their length also varied from 1.4 to 5.0 μm. This type of surface structure was observed in the specimens on *R. canina* L., *R. cinnamomea* L., *R. macrophylla* Lind., *R. moschata* L., *R. rubiginosa* L., *R. saundersiae* Rolfe, *R. sepia* Koch., *R. webbiana* Wallich ex Royle, and *Rosa* sp. from Europe and the Himalayas (Table 1).

An SEM photomicrograph of aeciospores from a specimen identified as *P. tuberculatum* was published by Preece and Hick (1990). The surface structure shown in the photomicrograph is considered to be echinulate type 3, although the photomicrograph is out of focus and the authors did not describe the structure in detail. The nailheaded type observed in aeciospores of *Phakopsora meliosmae* Kusano (Sato and Sato 1982) resembles this type of surface structure. However, in the nailheaded type, one echina occurs on each peltate base, which is connected to adjacent peltate bases by buttresses (see Table 2).

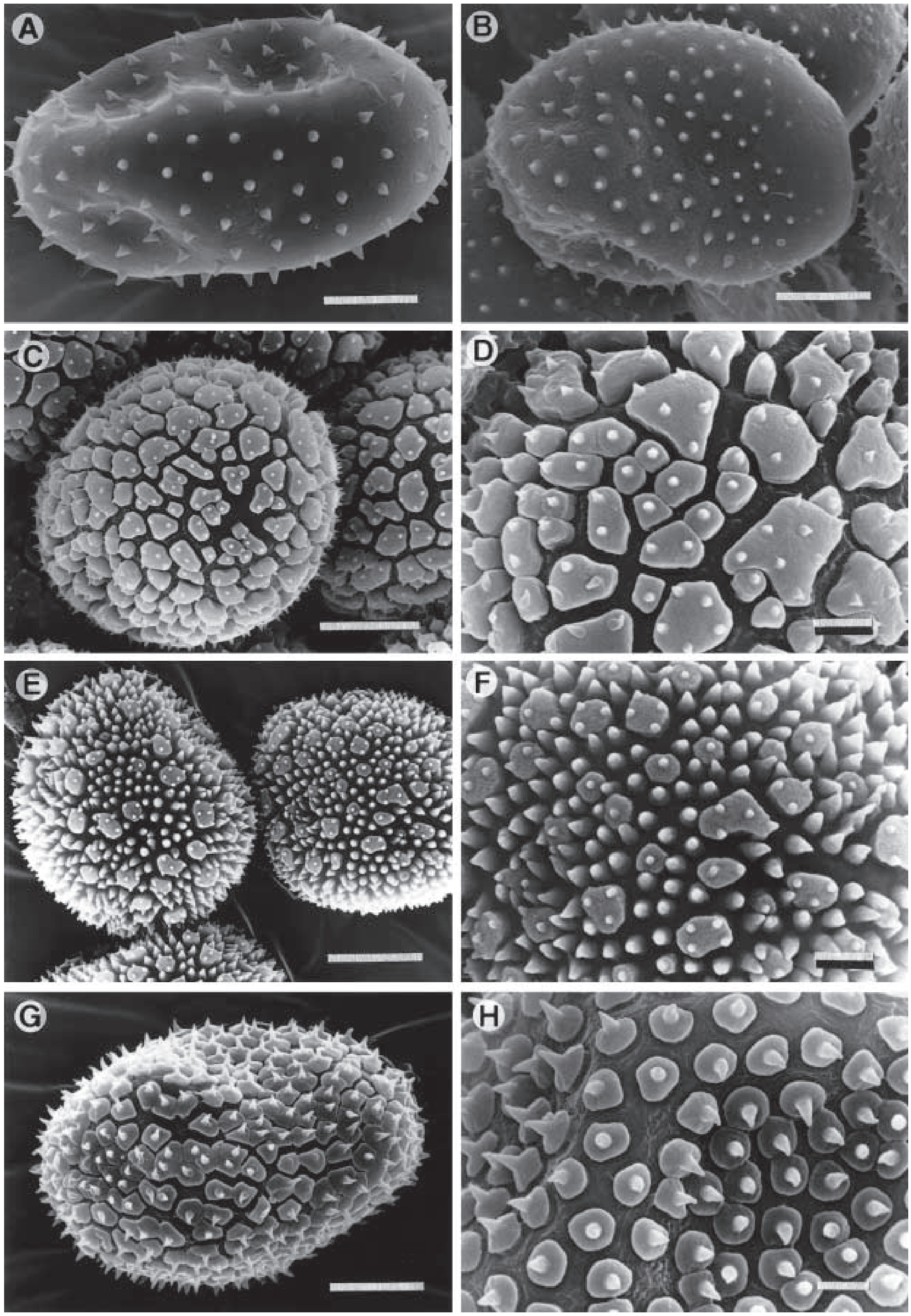

Fig. 2. Aeciospore-surface structures of *Phragmidium* species on roses. **A** Echinulate type 1 (E-1) on *Rosa* species (PUR44699). **B** Echinulate type 2 (E-2) on *R. multiflora* (IBA5696). **C**, **D** Echinulate type 3 (E-3) on *Rosa* sp. (PURF1586). **E**, **F** Echinulate type 4 (E-4) on *R. moschata* (PURF10548). **G**, **H** Echinulate type 5 (E-5) on *R. sikangensis* (HMAS45217). *Bars* **A–C**, **E**, **G** 5 μm; **D**, **F**, **H** 1.5 μm

Echinulate type 4 (E-4)

Type 4 is characterized by mixed distribution of echinae with or without a plateau-shaped base (Figs. 1E-4, 2E,F). The echinae without a plateau-shaped base are long and aciculate or subulate, whereas those arising on a plateau-shaped base are short and conical. The number of echinae formed on each plateau-shaped base varied from one to five. Length was 0.8–1.2 μm in the echinae without a plateau-shaped base and 0.4–0.7 μm in those arising on a plateau-shaped base.

This type of surface structure was observed in the specimens on *R. moschata* and *R. webbiana* from the Himalayas (Table 1) and has not been reported previously in the Uredinales (Table 2).

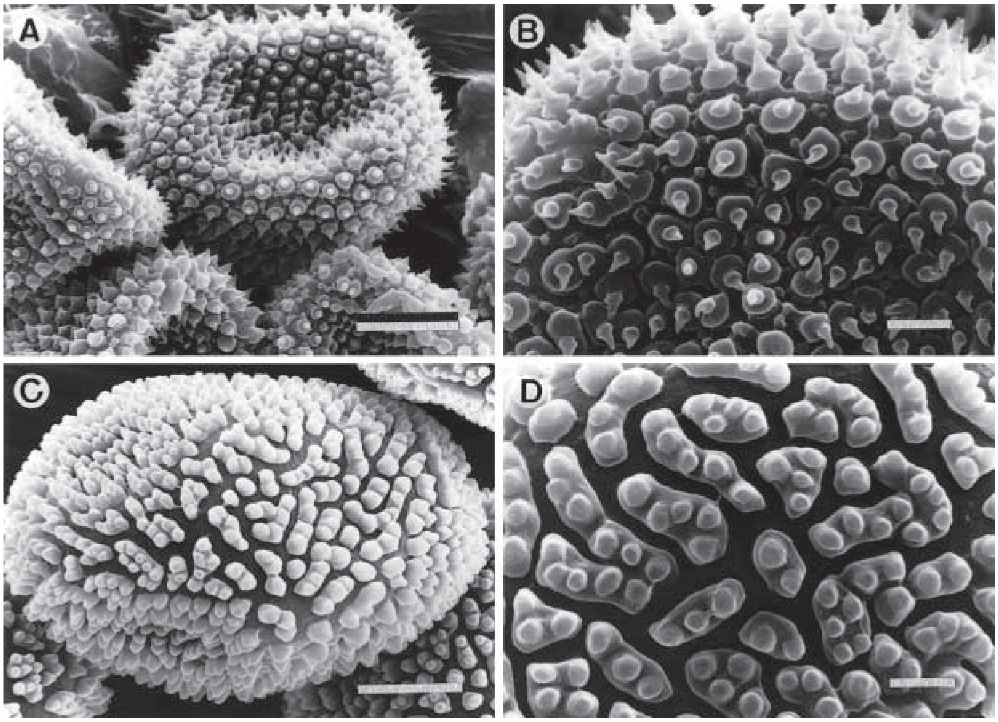

Fig. 3. Aeciospore-surface structures of *Phragmidium* species on roses. **A**, **B** Annulate type (A) on *R. sericea* (TSH-R1976). **C**, **D** Verrucose type (V) on *R. moschata* (PURF17982). *Bars* **A**, **C** 5 µm; **B**, **D** 1.5 µm

Table 2. Correspondence among different classifications of aeciospore-surface structures

Present study[a]	Sato and Sato (1982)	Lee and Kakishima (1999)[b]
–	Aciculate	–
Annulate	Annulate	Large and small annulate
–	Coronate	Coronate (large coronate, small coronate, minutely coronate) and mountain shape
Echinulate (type 1–5)	Echinulate	Echinulate
–	Nailheaded	Nailheaded
–	Reticulate	–
–	Tubulate	Tubulate
Verrucose	Verrucose	Verrucose (large verrucose, small verrucose, verrucose with refractive granules)

–, not observed
[a] Aeciospore-surface structures of *Phragmidium*
[b] Aeciospore-surface structures of *Gymnosporangium* and *Roestelia*

Echinulate type 5 (E-5)

Type 5 is characterized by an echina arising from a mushroom-shaped base (Figs. 1E-5, 2G,H). The echinae were straight or weakly curved, sharp pointed, and ~0.9 µm long. The mushroom-shaped bases appeared more or less rounded on a spore surface area where they were separated, whereas they were variously shaped on a spore area where they were densely packed. This type of surface structure was observed in one specimen on *R. sikangensis* Yu & Ku from China (Table 1).

Sato and Sato (1982) described an echina produced on a broad moundlike base on the aeciospore surface as a variant of the echinulate type. In contrast, the echina in echinulate type 5 is unique in having the constriction at the lower half of the base, thus giving the base a mushroom-like appearance (Table 2). This type of aeciospore-surface structure was unknown in species of *Phragmidium*.

Annulate type (A)

The annulate type of surface structure is characterized by a two-layered torulose base on which an echina is produced (Figs. 1A, 3A,B). The echinae were conical, straight, or slightly curved at the apex and ~1 µm long. The height of the two-layered bases was 1.5–2.7 µm. The density of the ornamentations varied from 7 to 11 per 10 μm^2 depending on the specimens examined. This type of surface structure was observed in specimens on *R. brunonii* Lindl., *R. sericea* Lindl., *R. webbiana*, and *R. wilmottiae* Hemsl. (Table 1).

This type of surface structure resembles echinulate type 5 in that an echina is produced on a mushroom-like base. However, in this type of surface structure, the echina is produced on an additional small base, the lower half of which is also constricted, formed on a large mushroom-like base (Table 2).

The annulate type as defined by Sato and Sato (1982) was characterized by two or more annulations (or stacked layers) in the processes. The surface structure of aeciospores of *Aecidium balanse* Cornu ex Pat. (Punithalingam and Jones 1971) and *Coleosporium* *phellonderi* Komarov (Hiratsuka and Kaneko 1975) represents the annulate type. Annulate-type ornamentation was also characterized by a peltate or flat apex (Sato and Sato 1982). In contrast, the annulate type we described here has a pointed apex, as already described.

Verrucose type (V)

The verrucose type is characterized by one or more verrucae produced on a distinct base on the spore wall (Figs. 1V, 3C,D). The verrucae are short and cylindrical with an obtuse apex and ~1 μm long. The base was rounded, triangular, broadly ellipsoidal, or curved beltlike in shape depending on the number and arrangement of verrucae produced on the base. SEM photomicrographs of aeciospores and urediniospores of *P. fragariae* (DC.) Rabh. published by Preece and Hick (1990) appear to be the same as the verrucose type we describe here. However, details of the structures were not determined because the photomicrographs were out of focus. This type of surface structure was observed in the specimens on *R. lacerans* Boiss and on *R. moschata* (Table 1).

Application of aeciospore-surface structures as a taxonomic character

Classification and identification of *Phragmidium* species have relied heavily on selected morphological characteristics in the telial-uredinial stage and putative host specificity (Wahyuno et al. 2001). The reasons for this taxonomic practice are (1) the telial-uredinial stage is often observed both in the field and in herbarium specimens; (2) morphological features of teliospores and their pedicels are diverse, characteristic, and easy to observe; (3) a few unique features are observed in urediniospores; and (4) different sets of morphological characteristics are often associated with different hosts or host groups.

Nevertheless, ambiguities have not been excluded with the species circumscriptions and identifications by the selected telial-uredinial characters. To overcome this taxonomic difficulty, search for new taxonomic characters has been urgently needed; the aeciospore-surface structures have became the most important candidate for taxonomic characters in the classification of *Phragmidium* as indicated by the successful application of the aeciospore-surface structure in the classification of *Coleosporium* (Kaneko 1981) or *Gymnosporangium* (Lee and Kakishima 1999), among others.

In the SEM study of aeciospores of all the currently accepted species of *Phragmidium* species on *Rosa*, seven different surface structures have been revealed. Although aeciospores from a majority of specimens on a broad spectrum of host species collected across the world exhibited the echinulate type 1 of surface structure, the other six types of surface structure seemed to be restricted to specimens on certain host species and with a narrow geographic distribution. Thus, echinulate type 2 is specific to the specimens on *R. multiflora* in Japan; echinulate type 3 is restricted to the specimens on *R. canina*, *R. cinnamomea*, *R. macrophylla*, *R. moschata*, *R. rubiginosa*, *R. saundersiae*, *R. sepia*, and *R. webbiana* in Europe and the Himalayas; echinulate type 4 is restricted to *R. moschata* and *R. webbiana* in the Himalayas; and echinulate type 5 is specific to specimens on *R. sikangensis* in southwestern China. The annulate type, which has not been reported previously, was observed on *R. brunonii*, *R. sericea*, *R. webbiana*, and *R. wilmottiae* from China and India. The verrucose type is observed from specimens on *R. lacerans* and *R. moschata* in Iran and India.

Among the seven specimen groups circumscribed by the aeciospore-surface structures, the first group with the echinulate type 1 aeciospores is highly likely to contain two or more species. However, from the host relationship and the geographic distribution, the second group with echinulate type 2 aeciospores is highly likely to be *P. rosae-multiflorae*. No rust species other than *P. rosae-multiflorae* is known on *R. multiflora*. Similarly, the fourth group with the echinulate type 4 is predicted to be *P. rosae-moschatae*. Only *P. rosae-moschatae* has been known to occur on both *R. moschata* and *R. webbiana* within the Himalayan region. *Rosa lacerans* is the only host for *P. rosae-lacerantis* in the Himalayas and, thus, the verrucose-type aeciospores observed in this study are likely to be those of *P. rosae-lacerantis*. These predictions of the three species by the aeciospore-surface structures can be justified only after the structure differences are proven to be correlated or associated with other morphological differences or host specificities. Covariations among different morphological features in different spore stages in the species of *Phragmidium* under discussion will prove the aeciospore-surface structures to be a good taxonomic character.

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