

Spore Ornamentations of Selected *Badhamia* Species using a Scanning Electron Microscopy

TAKAMI HATANO

Department of Science Education,
Faculty of Education, Shitennoji University
3-2-1 Gakuenmae Habikino City, Osaka pref., 583-8501 Japan
and

HAROLD W. KELLER

Department of Biology, Graduate Studies & Research,
Central Missouri State University
Warrensburg, MO 64093-5018, USA

Abstract: The external spore ornamentation of 14 *Badhamia* species was examined using a scanning electron microscopy (SEM). The Genus *Badhamia* has two types of spores, non-clustered, free spores and clustered spores. The non-clustered spore type includes *Badhamia affinis*, *B. foliicola*, *B. gracilis*, *B. iowensis*, *B. lilacina*, *B. macrocarpa*, *B. obovata*, *B. panicea* and *B. rugulosa*. The clustered spore type includes *Badhamia capsulifera*, *B. crassipella*, *B. nitens*, *B. populina* and *B. utricularis*. Surface spore ornamentation was represented by spines, spinules, warts, and irregular ridges. *Badhamia affinis*, *B. capsulifera*, *B. crassipella*, *B. foliicola*, *B. iowensis*, *B. rugulosa* and *B. utricularis* have spines about 0.2-0.4 μ m in height and width. *Badhamia macrocarpa*, *B. nitens*, *B. panicea* and *B. populina* have spinules about 0.2-0.3 μ m in height and width. *Badhamia gracilis* has warts about 0.3 μ m in height and width. *Badhamia lilacina* and *B. obovata* have irregular ridges about 1 μ m in height and 0.2 μ m in width.

Keyword : Myxomycetes, *Badhamia*, spore, taxonomy, SEM

Introduction

The genus *Badhamia*, represented by over 40 species worldwide, belongs to the family Physaraceae. These species have various ornamentation patterns on the outer surface of the spore. 14 species were selected to illustrate the variation in external fine structure of spores using a SEM. The fine spore surface structure of following species of *Badhamia*. *affinis*, *B. capsulifera*, *B. crassipella*, *B. iowensis*, *B. lilacina*, *B. macrocarpa*, *B. obovata* and *B. rugulosa* have not been illustrated using a SEM.

Materials and Methods

14 species were used in this study represented by *Badhamia affinis* Rost., *B. capsulifera* (Bull.) Berk., *B. crassipella* Whitney & Keller, *B. foliicola* A. Lister, *B. gracilis* (Macbr.) Macbr., *B. iowensis* Macbr., *B. lilacina*, (Fries) Rost., *B. macrocarpa* (Ces.) Rost., *B. nitens* Berk., *B. obovata* (Peck) S. J. Smith, *B. panicea* (Fries) Rost., *B. populina* A. & G. Lister, *B. rugulosa* Brooks & Keller, and *B. utricularis* (Bull.) Berk. They were collected in the United States of America, Canada and Japan. Fruiting bodies were dried at room temperature. Sporangia were prepared for a SEM observation by dehydration in a graded ethyl alcohol series, placed into xylene, then mounted on a specimen stub with a double faced adhesive tape, and sealed with a silver paste. Spores were then coated to a 40nm thickness with platinum-palladium for about seven minutes in a vacuum chamber using an ion coater apparatus, IB-3 type of Eiko Engineering Co., LTD. The ion-coated spores were observed with a SEM, S-4000 Hitachi Co., LTD at 15kv accelerating voltage.

The authors followed the systematics of Martin and Alexopoulos (1969) and referred to the publications of Lister (1925), Macbride and Martin (1934), Farr (1976), Thind (1977), Lakhanpal and Mukerji (1981), Nannenga-Bremekamp (1991), Neubert *et al* (1993), Emoto (1977), Lado and Pando (1977) and Yamamoto (1998) to identify specimens.

Specimens examined: *Badhamia affinis* (HWK 2774; Tarrant Co., Texas, USA, HTN 4173; Shimoina-gun, Nagano pref., Japan), *B. capsulifera* (TEB 2482; Cumberland Co., Kentucky, USA), *B. crassipella* (DTK 5252; Butte Co., California, USA), *B. foliicola* (DTK 5892; Butte Co., California, USA), *B. gracilis* (GWM 647; Johnson Co., Iowa, USA), *B. iowensis* (HWK 223; Johnson Co., Iowa, USA), *B. lilacina* (TEB 2485; Washtenaw Co., Michigan, USA), *B. macrocarpa* (GWM 326; Johnson Co., Iowa, USA), *B. nitens* (DTK 2634; Butte Co., California, USA), *B. obovata* (HWK 2205; Sumter Co., Florida, USA), *P. panicea* (THM 220; Johnson Co., Iowa, USA), *B. populina* (JS 98; Kapasiwin, Alberta, Canada), *B. rugulosa* (Eliasson 3719; Washington Co., Arkansas, USA), *B. utricularis* (DTK 4653; Butte Co., California, USA). Abbreviations of collectors are as follows: HWK=Harold W. Keller; HTN=Takami Hatano; DTK=Donald T. Kowalski; TEB=Travis E. Brooks; GWM=George W. Martin; THM=Thomas H. Macbride. Voucher specimens have been deposited in the Herbarium of the Department of Biology, the University of Texas at Arlington, Texas, USA.

Descriptions and Discussions

The conditions of the fine ornamentation on the surface of a spore using a SEM are as followings.
***Badhamia affinis* Rost.:** The spore is about 12µm in diameter with spines, 0.4µm in height and 0.2µm in width on the surface. Martin and Alexopoulos (1969) described spore ornamentation of *B. affinis* as “closely and densely spinulose” with a light microscopy (LM). The spore surface uniformly covered with closely spaced vertical projections flattened or truncate at the surface. This pattern of spore ornamentation would appear spinulose with a LM (Figs. 1,2).

***Badhamia iowensis* Macbr.:** The spore is about 10µm in diameter with spines, 0.2µm in height and width on the surface. Martin and Alexopoulos (1969) described the spores as “minutely verrucose, the warts clustered in patches, making darker areas”. Spore ornamentation uniformly covers the surface as short, vertical projections with truncate tops (Figs. 3,4).

***Badhamia rugulosa* Brooks & Keller:** The spore is about 10µm in diameter with spines, 0.3µm in height and 0.2µm in width on the surface. Keller and Brooks (1975) described spore ornamentation with a LM as “distinctly and uniformly verrucose”. This description is accurate using a LM, however, a SEM shows short, vertical, flat topped projections with overhanging edges uniformly covering the entire spore surface. These overhanging edges give the appearance of warts with a LM (Figs. 5,6).

***Badhamia foliicola* A. Lister:** The spore is about 10µm in diameter, spinulose, 0.2-0.3µm in height and width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as minutely warted. It is often difficult to make a clear distinction of spore ornamentation between spines, spinulose, and warts with oil immersion a LM at 1,000 times magnification. This is especially true when vertical projections are extremely short with flattened, truncate tops. Spore ornamentation uniformly covers the entire spore surface. It is difficult to interpret the use of spinulose and verrucose in the species descriptions of Martin and Alexopoulos (1969) since there is no glossary of terms in their book. However, if minutely warted is interchangeable with the term verrucose, represented by convex to broadly rounded protuberances, the type of surface ornamentation show here does not match the term verrucose. The type of spore ornamentation is quite common in the Myxomycetes (Figs. 7, 8).

***Badhamia macrocarpa* (Ces.) Rost.:** The spore is about 10µm in diameter, spinulose, 0.2µm in height and width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as “densely and somewhat irregularly verrucose” as seen with a LM. Higher magnifications show short, thick, flat-topped, vertical projections uniformly covering the entire spore surface similar to *B. panicea* (Figs. 9,10).

***Badhamia panicea* (Fries) Rost.:** The spore is about 9µm in diameter, spinulose, 0.2µm in height and width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as “minutely punctate” as seen with a LM. Punctate refers to small depressions or dots unlike the ornamentation seen here with a SEM of flat-topped, vertical projections uniformly covering the entire spore surface (Figs. 11,12).

***Badhamia gracilis* (Macbr.) Macbr.:** The spore is about 12µm in diameter with warts, 0.3µm in height and width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as “closely and irregularly warted, usually with cluster of dark warts, and with a very coarse network of 1-6 meshes to the hemisphere covering the surface” as seen with a LM. Irregular ridges are shown in Figure 13 and round protuberances considered as warts in Figure 14.

***Badhamia lilacina* (Fries) Rost.:** The spore is about 12µm in diameter with irregular ridges, 1µm in height, 0.2µm in width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as “covered with rough warts and ridges, subreticulate” as seen with a LM. The ridges as seen in profile with a SEM appear more like an incomplete, raised, episporic reticulum. These ridges sometimes form

an irregular coarse network with the edge of the ridges roughened by fine granular protuberances (Figs. 15, 16).

***Badhamia obovata* (Peck) S. J. Smith:** The spore is about 12µm in diameter with irregular ridges and many cauliflower shaped protuberances, 1µm in height, 0.2µm in width, on the edge of ridges on the surface of a spore. These protuberances are very similar to the ornamentation on the spore surface of *Didymium squamulosum* (Albert. & Schw.) Fries (Hatano 1986) (Figs. 17, 18).

***Badhamia capsulifera* (Bull.) Berk.:** The spore is about 11µm in diameter with spines, 0.4µm in height and 0.2µm in width on the surface. Spore morphology is described by Martin and Alexopoulos (1969) as “adhering in firm clusters, mostly of 6-20, broadly ovate, warted, or bluntly spiny on the exposed surface, elsewhere smooth or nearly so” as seen with a LM. Spines are clearly shown in profile on the exposed face of the individual spores under a SEM (Figs. 19, 20).

***Badhamia utricularis* (Bull.) Berk.:** The spore is about 12µm in diameter with spines, 0.4µm in height and 0.2µm in width on the surface. Martin and Alexopoulos described the spore ornamentation as “distinctly warted over entire surface but sometimes more strongly on one side” with a SEM. Spore ornamentation seen in profile with a SEM show flat-topped vertical projections more aptly described as spinulose (Fig. 21).

***Badhamia crassipella* Whitney & Keller:** The spore is about 12µm in diameter with spines, 0.4µm in height and 0.2µm in width on the surface. Clustered spores of this species were described by Whitney and Keller (1982) as “verrucose, in loosely adhering clusters of 4-40 spores, formed by fusion of the tips of the spore ornamentation of adjacent spores” as seen with a LM. Spore ornamentation shown here for the first time with a SEM, appears in profile as short, flat-topped vertical projections (Fig. 22).

***Badhamia populina* A. & G. Lister:** The spore is about 11µm in diameter, with spinules 0.3µm in height and 0.2µm in width on the surface. Martin and Alexopoulos (1969) described the spore ornamentation as “strongly warted on the exposed end, elsewhere minutely warted to nearly smooth” as seen with a LM. Figure 23 shows flat-topped vertical projections that are more spinulose than warted.

***Badhamia nitens* Berk.:** The spore is about 11-12µm in diameter, ellipsoid with spinules, 0.3µm in height and 0.2µm in width on the surface. Martin and Alexopoulos (1969) described spore ornamentation as “more coarsely warted on the exposed area, more finely warted elsewhere,” as seen with a LM (Fig. 24).

Conclusions

It was investigated the spore surface ornamentation of selected 14 species of Genus *Badhamia* using a SEM and made clear the fine surface ornamentation. Especially the fine spore surface ornamentation of following species of *Badhamia affinis*, *B. capsulifera*, *B. crassipella*, *B. iowensis*, *B. lilacina*, *B. macrocarpa*, *B. obovata*, and *B. rugulosa* have not been illustrated using a SEM and it was cleared in this paper for the first time. It includes two types of non-clustered spore and clustered spore. The non-clustered spore type includes nine species of *B. affinis*, *B. foliicola*, *B. gracilis*, *B. iowensis*, *B. lilacina*, *B.*

macrocarpa, *B. obovata*, *B. panicea* and *B. rugulosa*. The clustered spore type includes five species of *B. capsulifera*, *B. crassipella*, *B. populina*, *B. utricularis* and *B. nitens*. Fine ornamentation on the surface of a spore was basically four types of spines, spinules, warts and irregular ridges. Seven species of *B. affinis*, *B. capsulifera*, *B. crassipella*, *B. foliicola*, *B. iowensis*, *B. rugulosa* and *B. utricularis* have spines about 0.2-0.4µm in height and width on the surface. Four species of *B. macrocarpa*, *B. nitens*, *B. panicea* and *B. populina* have spinules about 0.2-0.3µm in height and width on the surface. *Badhamia gracilis* has warts about 0.3µm in height and width on the surface. Two species of *B. lilacina* and *B. obovata* have irregular ridges about 0.2µm in width and 1µm in height on the surface.

Patterns of spore ornamentation in species of *Badhamia*, when described as warted, spinulose, or spiny with a LM, are basically a vertical, flat-topped projection with a thick base and truncate top. This type of spore ornamentation is one of the more common types seen in the Myxomycetes. More accurate species descriptions are possible using a SEM in conjunction with a LM observations.

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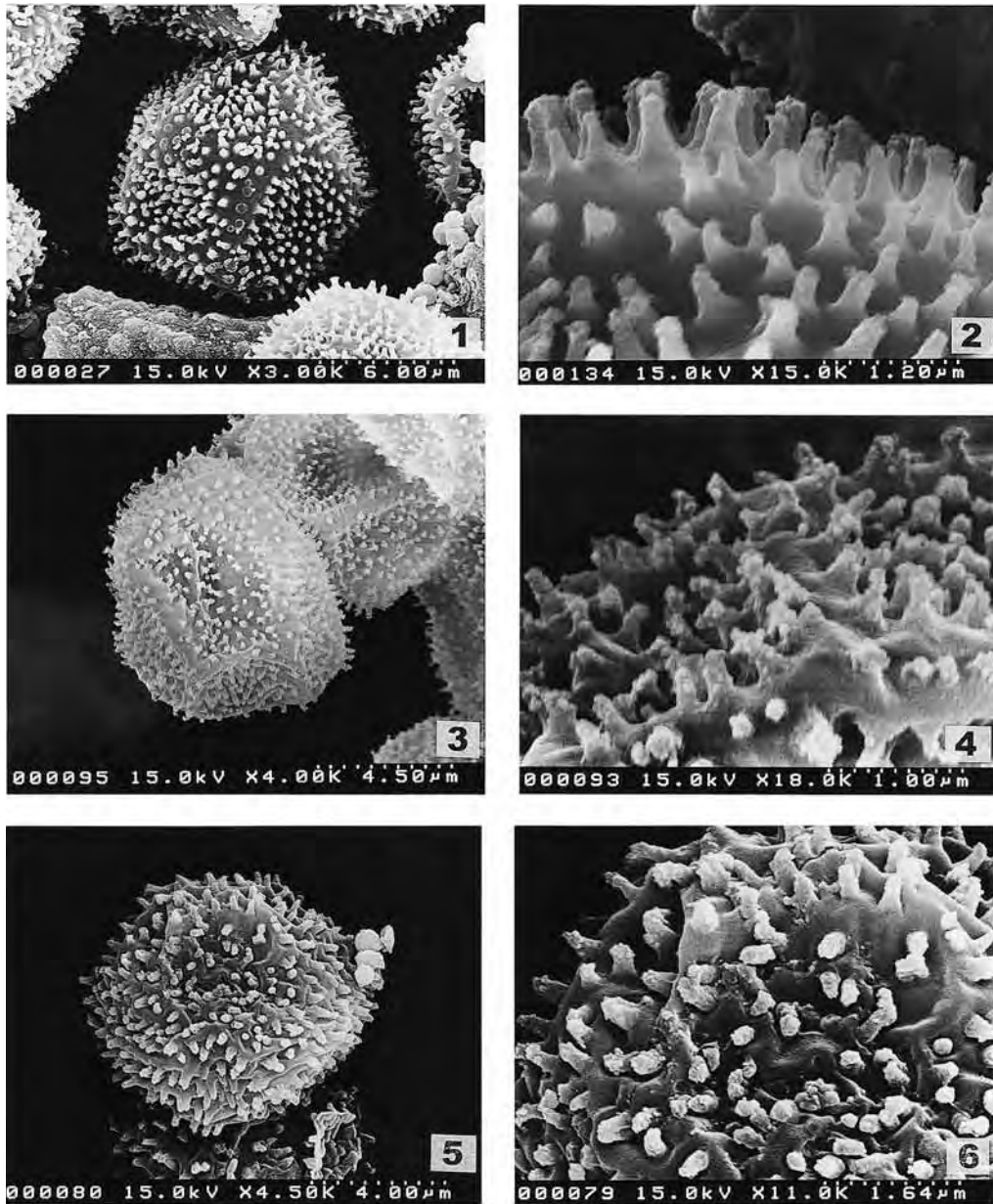
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Badhamia 属変形菌の代表種の胞子表面の 電子顕微鏡的微細構造

Badhamia 属変形菌は Physaraceae に分類され、全世界で 40 種以上の生育が確認、報告されている。本属変形菌の胞子表面には、独特で特徴的な微細突起が存在し、分類学的に大きな特徴となっている。これらは系統分類学的に非常に重要な構造であるにもかかわらず、組織的な調査、研究は十分に行われていない。本研究では、アメリカ合衆国、カナダ、日本で採集された *Badhamia affinis* など 14 種について、その胞子表面の微細構造の精細な観察を走査型電子顕微鏡を用いて行った。このうち *B. capsulifera* など 8 種についてはこれまでに SEM による微細構造観察が実施されておらず、このたびはじめてその特徴的な微細構造が明らかにされた。この結果は以下のとおりである。

本属変形菌種の胞子直径は 9 μ m-12 μ m であった。*B. affinis* のように特徴的に胞子が団塊状の胞子塊をなさずに独立した胞子を形成する種、*B. capsulifera* のように団塊状をなす 2 型があることがわかった。また、胞子表面には棘状突起や疣状突起などの存在を確認した。*B. affinis* など 7 種は棘状突起、*B. macrocarpa* など 4 種は微細な棘状突起、*B. gracilis* は疣状突起、*B. lilacina* など 2 種は不規則な畝状隆起を有することが明らかにされた。これらの特徴は、変形菌を系統分類する上で極めて重要な特徴であり、今後の系統分類学的研究の進展に寄与できる。

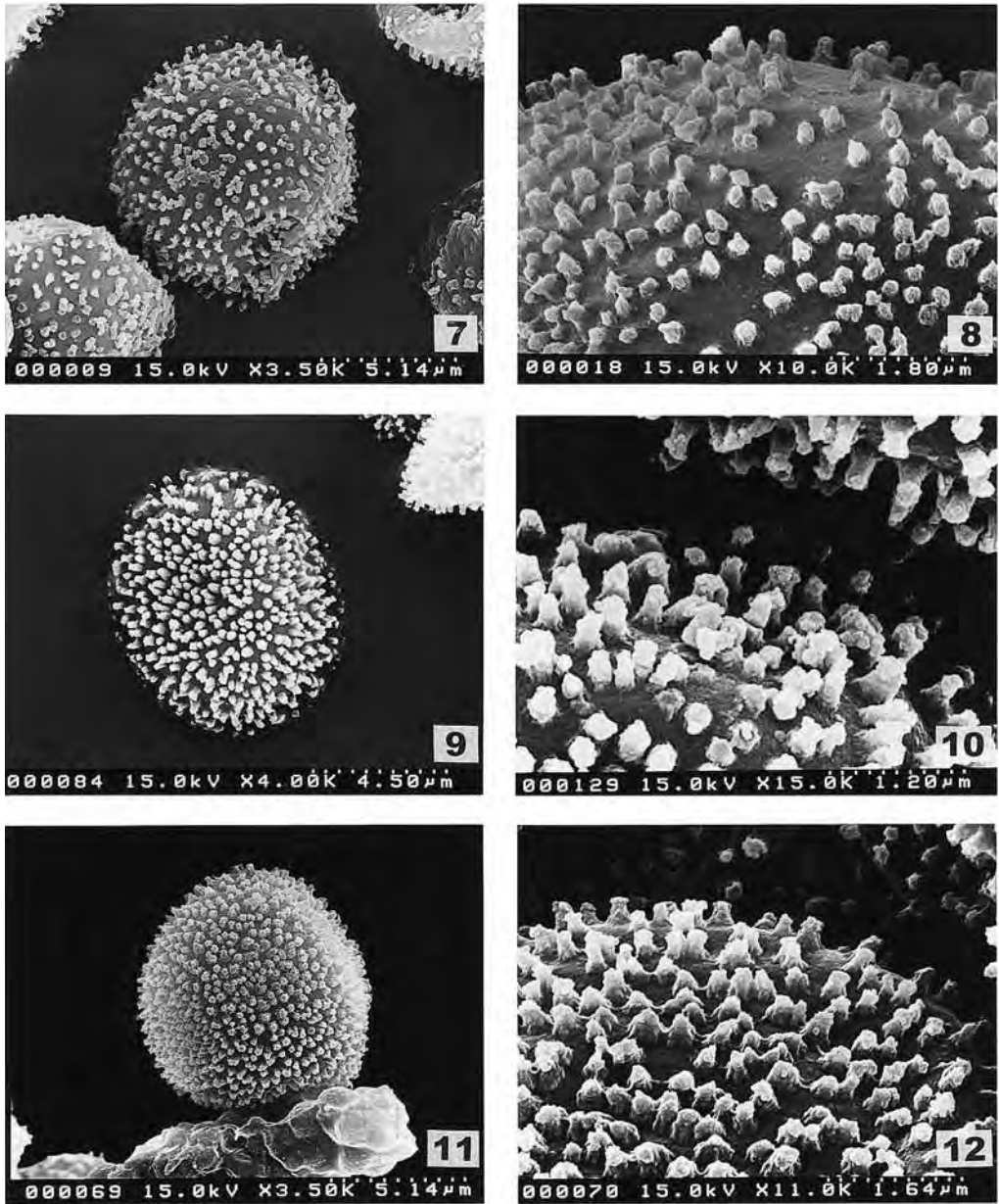
キーワード：変形菌、バダミア、胞子、植物分類学、走査型電子顕微鏡



Figs. 1-18. SEM aspects of spore surface ornamentations of non-clustered *Badhamia* species (1).

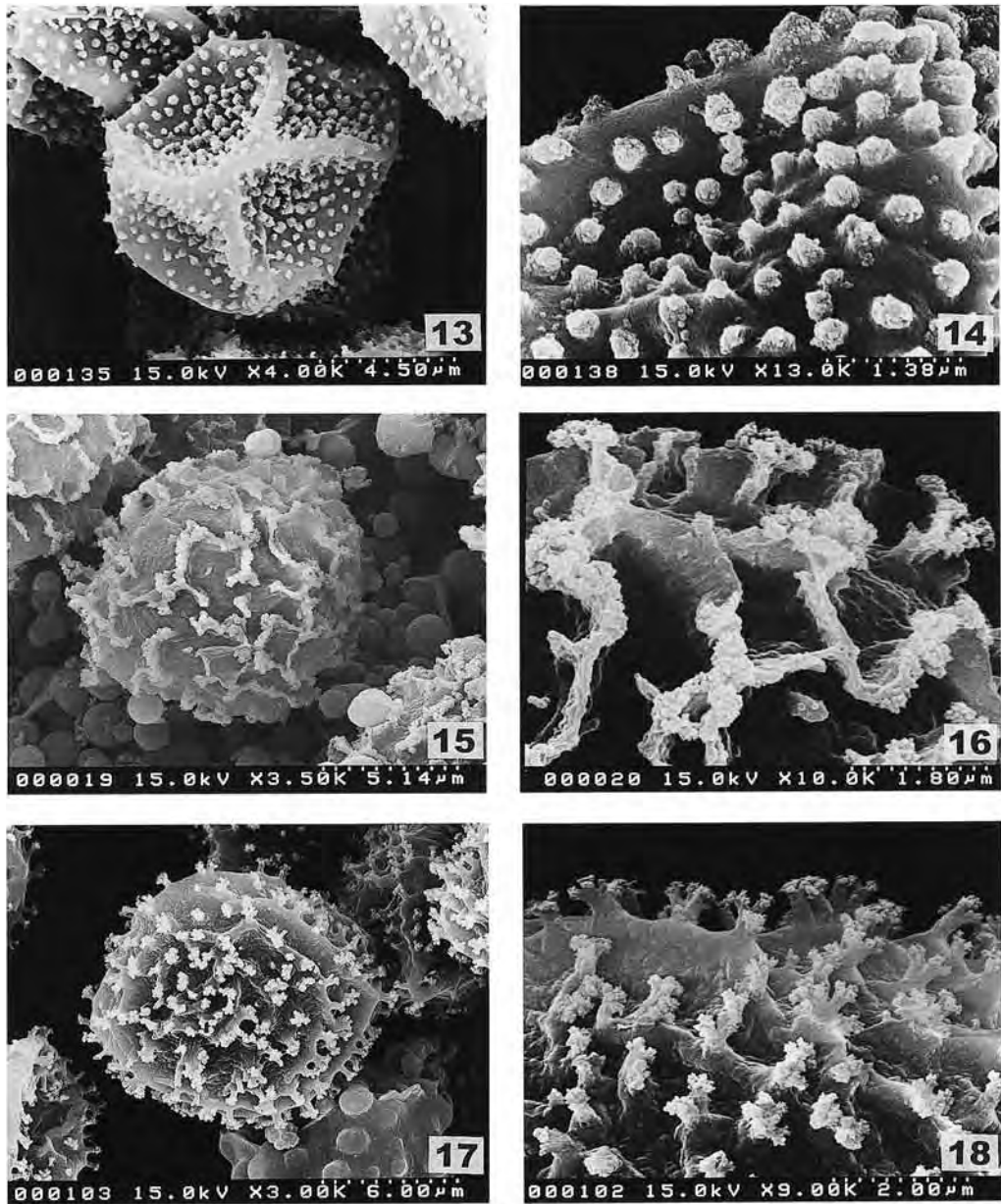
Figs. 1,2. *Badhamia affinis* (HWK 2774). 1. Spore, 2. Portion of spore. 3-4. *B. iowensis* (HWK 223). 3.

Spore, 4. Portion of spore. 5-6. *B. rugulosa* (Eliasson 3719). 5. Spore, 6. Portion of spore.



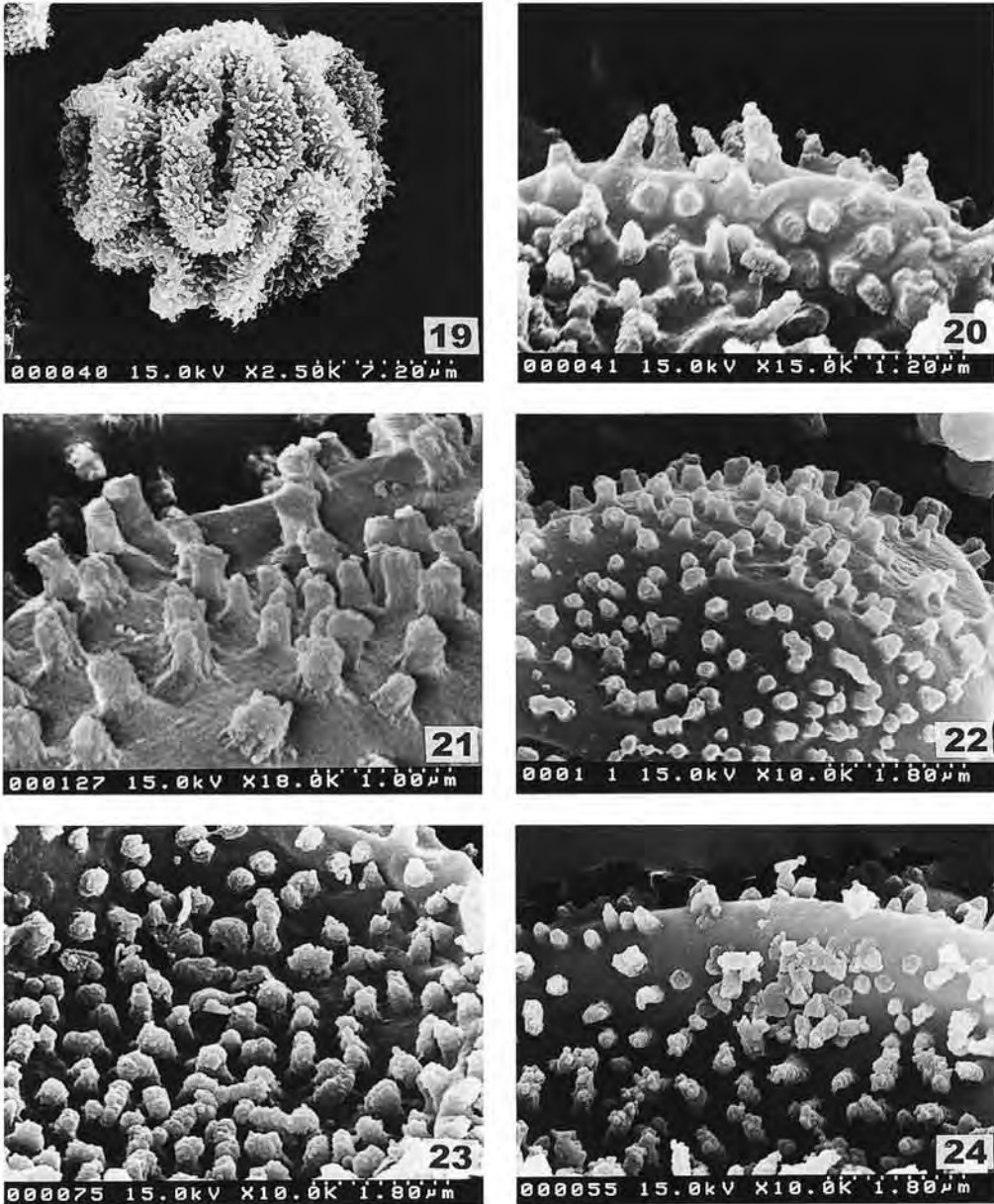
Figs. 1-18. SEM aspects of spore surface ornamentations of non-clustered *Badhamia* species (2).

7-8. *B. foliicola* (DTK 5892). 7. Spore, 8. Portion of spore. 9-10. *B. macrocarpa* (GWM 326). 9. Spore, 10. Portion of spore. 11-12. *B. panicea* (THM 220). 11. Spore, 12. Portion of spore.



Figs. 1-18. SEM aspects of spore surface ornamentations of non-clustered *Badhamia* species (3).

13-14. *B. gracilis* (GWM 647). 13. Spore, 14. Portion of spore. 15-16. *B. lilacina* (TEB 2485). 15. Spore, 16. Portion of spore. 17-18. *B. obovata* (HWK 2205). 17. Spore, 18. Portion of spore.



Figs. 19-24. SEM aspects of spore surface ornamentations of clustered *Badhamia* species(4).

19-20. *B. capsulifera* (TEB 2482). 19. Clustered spores, 20. Portion of spore. 21. *B. utricularis* (DTK 4653), Portion of spore. 22. *B. crassipella* (DTK 5252), Portion of spore. 23. *B. populina* (JS 98), Portion of spore. 24. *B. nitens* (DTK 2634), Portion of spore.