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THE ANAMORPHS OF *GROVESIELLA ABIETICOLA*

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ABSTRACT

An ascomycete associated with the recently described coelomycete *Pitostroma abietinum* on dead twigs of *Abies alba* in Poland was identified as *Grovesiella abieticola*. Two characteristics of the *G. abieticola* from Poland differed from those of collections from North America. The ascospores of *G. abieticola* collected in North America were wider [(3.2–)3.5–4.2(–4.8) μm] than those from Europe [2.2–3.5(–4) μm], and the European populations do not produce cankers while the North American populations do. Single ascospore isolates of *G. abieticola* and single conidia isolates of *P. abietinum* produced identical colony morphologies and in single ascospore isolates the *Pitostroma*-state was formed in addition to the microconidial state. Thus, *P. abietinum* is the macroconidial state of *G. abieticola*. A description of the morphology of the micro- and macroconidial state of *G. abieticola* in culture is given.

Key Words: anamorph–teleomorph connection, *Grovesiella abieticola*, macroconidial state, microconidial state, morphology, *Pitostroma abietinum*

Pitostroma abietinum Kowalski & Sieber was newly described as a coelomycetous species occurring on twigs of *Abies alba* Mill. in Poland (Kowalski and Sieber, **1992**). Apothecia were observed adjacent to fructifications of *Pitostroma abietinum* on twigs of more recent collections from the same site. A study was designed to prove the hypothesis that *Pitostroma abietinum* is the anamorphic state of this discomycete.

MATERIALS AND METHODS

The discomycete was collected several times from *Abies alba* in Ojców National Park (grid reference 19°50′00″E, 50°12′30″N, topographical map no. 86.01.4 "Miechów" of Zjednoczenie Przedsiebiorstw Geodezyjno-Kartograficznych "GEOKART", 1:100 000), 350 m above sea level, 20 km north of Kraków, Poland. Two collections were deposited in the herbarium (ZT) of the Swiss Federal Institute of Technology, Zürich. POLAND. Ojców National Park, north of Kraków, *Abies alba*, 2 May 1991, *T. Kowalski* (ZT); Ojców National Park, north of Kraków, *Abies alba*, 25 May 1992, *T. Kowalski* (ZT).

The morphology of the following specimens of *Grovesiella abieticola* (Zeller & Goodding) Morelet & Gremmen have been compared with our collections: FRANCE. Lastic (Puy-de-Dôme), *Abies alba*, 11 July 1969, *C. Delatour, leg. Morelet, duplicate of C. N. R. F. 223* (ZT). CANADA. BRITISH COLUMBIA: Beaver Cove, Eve Creek Road, *Abies amabilis* (Dougl.) Forbes, 19 July 1963, *N. E. Alexander and D. G. Collis 20211* (DAVFP); Smithers, Smithers landing, *Abies lasiocarpa* (Hook.) Nutt., 8 June 1959, *D. G. Collis and R. H. Murfitt 11366* (DAVFP); Smithers, Smithers landing, Nilkitkwa River, *Abies lasiocarpa*, 25 May 1960, *E. G. Harvey and D. S. Ruth 12404* (DAVFP); Smithers, Hudson Bay Mt., *Abies lasiocarpa*, 17 Aug. 1960, *E. G. Harvey 13556* (DAVFP); Terrace, Spencer Lake, *Abies lasiocarpa*, 31 Aug. 1962, *A. G. Harvey 14781* (DAVFP); Smithers, Smithers landing, Nilkitkwa River trail, *Abies lasiocarpa*, 23 Aug. 1962, *A. K. D. Jardine 14792* (DAVFP); Ootsa Lake, *Abies lasiocarpa*, 7 Aug. 1960, *E. G. Harvey 14926* (DAVFP); Weissener Lake, *Abies lasiocarpa, J. A. Baranyay 19884* (DAVFP); Aleza Lake, Aleza Lake Forestry Station, West Branch Road, *Abies lasiocarpa*, 13 Aug. 1971, *A. T. Foster 19942* (DAVFP); Atlin, Atlin Lake, Warm Bay, *Abies lasiocarpa*, 23 June 1978, *R. L. Fiddick 21754* (DAVFP); New Denver, Wee Sandy Creek, *Abies lasiocarpa*, 27 Aug. 1981, *A. Renwick 22638* (DAVFP); 12 miles east of Hixon, *Abies lasiocarpa*, 31 July 1973, *H. S. Whitney 22815* (DAVFP). USA. OREGON: Mt. Hood Loop near Horse Thief Meadows, *Abies amabilis*, 20 May 1929, *L. N. Goodding 7247* and duplicate 4873 (NY, PARATYPE); Tillamook Co., Wilson River, *Abies grandis* (Dougl.) Lindl., 24 May 1928, *L. N. Goodding 4932* (NY, ISOTYPE); Tillamook Co., *Abies grandis*, 18 Oct. 1928, *L. N. Goodding 4934* (NY);

Tillamook Co., *Abies grandis,* 16 Sept. 1929, *L. N. Goodding, 4924* (NY); Josephine Co., near Oregon Caves, *Abies grandis,* 18 June 1931, *L. N. Goodding 487* (NY); Silverton, Drake's Nursery, *Abies grandis,* 5 July 1983, *B. Long 22867* (DAVFP); Silverton, Drake's Nursery, *Abies procera* Rehd., 5 July 1983, *B. Long 22868* (DAVFP).

Dimensions of ascospores from selected herbarium specimens were measured and compared by statistical methods. Three sections were prepared from each of 10 randomly selected mature apothecia of each specimen, put in a drop of concentrated lactic acid on a microscope slide, covered with a cover slip, and squashed. Length and width of 30 randomly selected ascospores (only 10 in DAVFP 22815) were measured per specimen. Spore measurements were displayed graphically as scattergrams and box plots and were compared by analyses of variance (ANOVA) to detect significant differences among specimens.

A single-spore isolator constructed according to the description of Constantinescu (**1988**) was used to isolate single conidia of *Pitostroma abietinum* and single ascospores from the discomycete collected in Poland. This single-spore isolator was fitted on a 16× objective of a Zeiss microscope. Cultures derived from single conidia and ascospores were incubated on 2% (w/v) malt extract agar (MEA) at 20 C in the dark until fruiting bodies developed. Growth increment of cultures was recorded as the mean value of the longest and smallest diameter of cultures incubated for 10, 20, 30, and 50 days on 2% MEA in 90-mm-diam petri dishes at 20 C in the dark. Culture characteristics were noted after 30 days.

Fungal morphology was studied with a Zeiss stereomicroscope and with a Zeiss Axiophot using phase and differential interference contrast optics. Line drawings were prepared by means of a camera lucida. Dimensions of conidiophores and conidia were measured in both water and concentrated lactic acid.

RESULTS AND DISCUSSION

The disomycete from Poland was identified as *Grovesiella abieticola* based on the descriptions given by Morelet (**1969**) and Gremmen and Morelet (**1971**). Our identification was confirmed by M. Morelet (Nancy, France) from whom we also received a specimen from France. However, neither the Polish nor the French fungus corresponded to the original description of *G. abieticola* (Zeller and Goodding, **1930**) in two morphological characteristics: the width of the ascospores (FIG. 1) and the structure of the excipulum. Examination of type and paratype specimens of *G. abieticola* revealed that there was insufficient material to resolve these inconsistencies. Studies on some North American specimens of *G. abieticola* from the herbarium DAVFP yielded the necessary information. No differences could be detected between these and Polish or French specimens in the organization of tissues in the excipulum, but the width of ascospores of *G. abieticola* from North America was significantly different from that of *G. abieticola* from Europe (FIGS. 1–3). Ascospores of the collections from North American were wider [(3.2–)3.5–4.2(–4.8) μm, $\bar{x}$ = 3.9 μm] than those from Europe [2.2–3.5(–4) μm, $\bar{x}$ = 2.8 μm] ($F = 71.1$, $P \leq 0.0001$) but corresponded well with the range given by Zeller and Goodding (**1930**). Ascospores from different European collections [(37–)40–68 × 2.2–3.5(–4) μm, $\bar{x}$ = 54.7 × 2.8 μm] could not be differentiated. Ascospores from North American collections were significantly longer in collections on *Abies amabilis* [(46–)52–78(–82) × (3.2–)3.5–4.2(–4.8) μm, $\bar{x}$ = 66.9 × 3.9 μm] than on *A. lasiocarpa* [(38.4–)40–48 × 3.9 μm, $\bar{x}$ = 44 × 3.9 μm].

In the collection from France, one ascus contained the following ascospores: four moderate-sized (24–41 × 3–3.5 μm, one- to four-celled); two small, one-celled (8–12 × 1.8–2 μm); and two very small, one-celled (2 × 1.2 μm). Zeller and Goodding (**1930**) had described previously this phenomenon for *G. abieticola.* However, the ascospores found by these authors were much longer and wider than those we observed. The four large ascospores measured 70–80 × 4.5–6 μm and the four small ones 11–15 × 4–6 μm. Spore length is highly variable and is, therefore, a poor characteristic for classification within this group of fungi.

G. abieticola is reported to be associated with cankers on living branches and twigs of *Abies grandis* and *A. amabilis* (Zeller and Goodding, **1930**). This observation is supported by our examination of specimens collected by these authors and specimens from DAVFP. The cankers produced by *G. abieticola* in North America are characterized by a more or less elliptical area of depressed, necrotic bark usually combined with resin bleeding. The necrotic bark normally loosens like a scale in older cankers. Conversely, *G. abieticola* cankers have not been detected in Poland or in France (Gremmen and Morelet, **1971**).

Canker morphology and ascospore width of *G. abieticola* differed in collections of material from North America and Europe. However, a difference in only two characteristics was considered insufficient to split *G. abieticola* from the two continents into two separate species. Moreover,

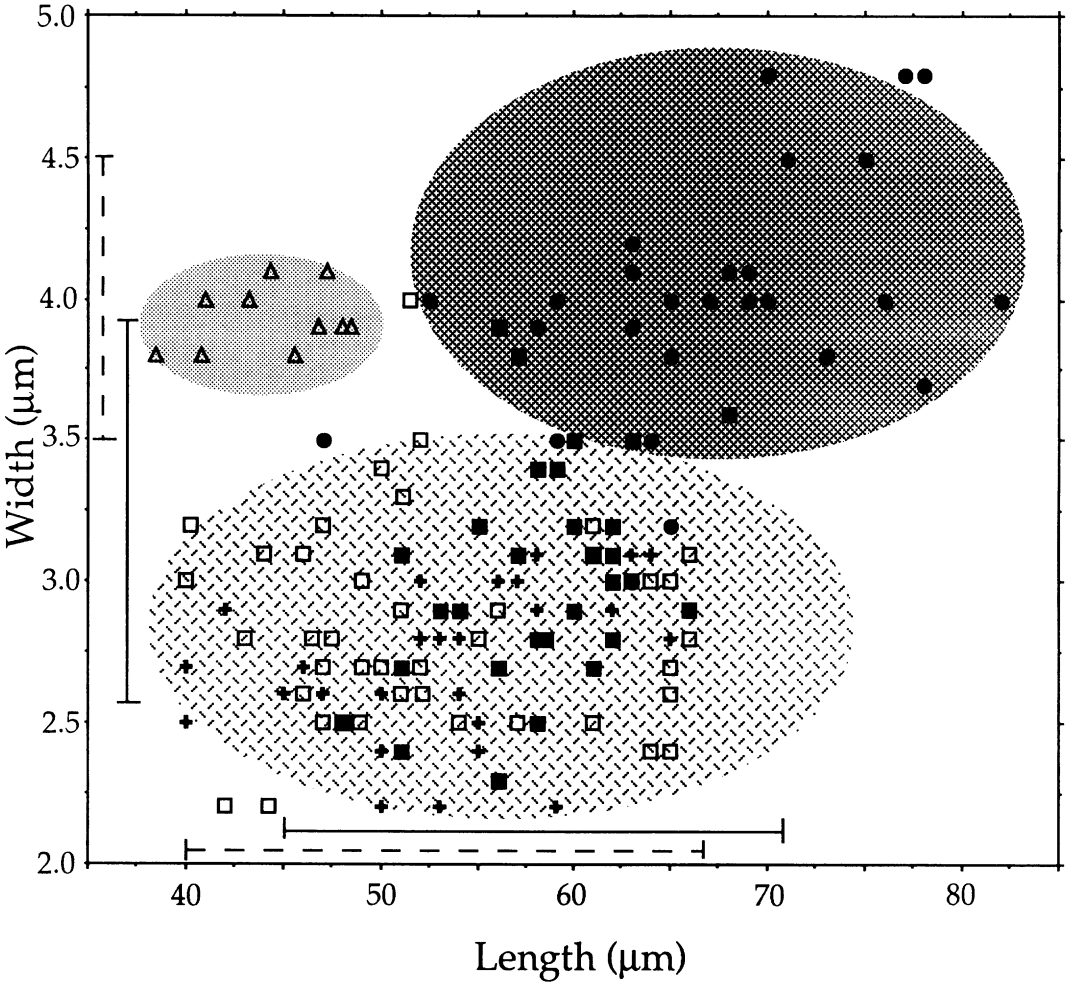


FIG. 1. Dimensions of ascospores of *Grovesiella abieticola*. European collections from *Abies alba* (▨); Poland, Ojców National Park, 12 May 1991 (■); Poland, Ojców National Park, 25 May 1992 (✚); France, Lastic (Puy-de-Dôme), 11 July 1969, duplicate of C. N. R. F. 223, ZT (□). North American collection from Canada, British Columbia, Beaver Cove, *Abies amabilis*, 19 July 1963, DAVFP 20211 (●, ▩). North American collection from Canada, British Columbia, Hixon, *Abies lasiocarpa*, 31 July 1973, DAVFP 22815 (△, ▩). |——| Range of length and width given by Gremmen and Morelet (**1971**) and |– – –| by Zeller and Goodding (**1930**).

the difference in symptomatology would have to be demonstrated by cross inoculation, i.e., inoculation of *G. abieticola* from North America on *Abies alba* and of *G. abieticola* from Europe on *Abies* spp. from North America.

Single ascospore isolates initially only produced the microconidial state, whereas single conidia isolates of *Pitostroma abietinum* produced only the long, cylindrical, multiseptate conidia typical of *P. abietinum*. After more than 4 months incubation some single ascospore isolates also produced the *P. abietinum* state. Thus, *P. abietinum* is the macroconidial state of *G. abieticola* from Europe. However, the microconidial state was not formed in cultures derived from single conidia isolates of *P. abietinum*.

Isolates of both single ascospores and single *P. abietinum* conidia produced morphologically identical cultures. The culture morphology can be summarized as follows (FIG. 4): colonies incline to sector; submerged mycelium dark brown, reverse of colonies black; center of colony usually covered by a brown to olivaceous, floccose aerial mycelium seemingly black in regions of abun-

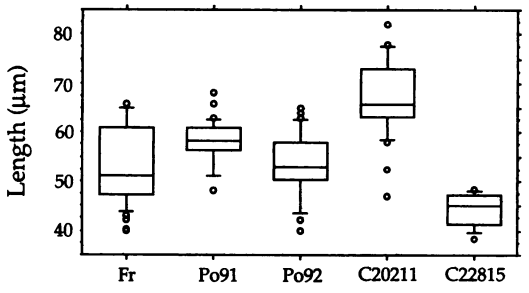


FIG. 2. Box plots with selected percentiles of the lengths of *Grovesiella abieticola* ascospores from different collections. The horizontal line in the interior of the box indicates the median value. The upper and the lower horizontal lines of the box represent the 75th and the 25th percentiles. The ends of the vertical bars indicate the 10th and the 90th percentiles. Points below or above these bars represent values of ascospores outside the 80% confidence interval. If the intervals of two boxes do not overlap, the two corresponding collections are significantly different with respect to length [or width (FIG. 3)] of the ascospores. Fr = France, Lastic (Puy-de-Dôme), *Abies alba,* 11 July 1969, duplicate of C. N. R. F. 223, ZT; Po91 = Poland, Ojców National Park, *Abies alba,* 12 May 1991; Po92 = Poland, Ojców National Park, *Abies alba,* 25 May 1992; C20211 = Canada, British Columbia, Beaver Cove, *Abies amabilis,* 19 July 1963, DAVFP 20211; C22815 = Canada, British Columbia, Hixon, *Abies lasiocarpa,* 31 July 1973, DAVFP 22815.

dant pycnidial conidiomata; color and morphology of the aerial mycelium of the periphery of sectors either like the center ones or white floccose with a black margin of submerged hyphae; the dark brown conidiomata with white to yellow-green conidial masses.

Two groups of isolates differing in growth rate were detected (FIG. 5). Two isolates derived from ascospores grew faster than isolates from either macroconidia or other ascospores. Mean culture

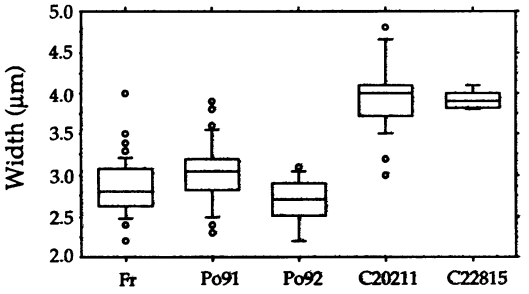


FIG. 3. Box plots displaying selected percentiles of the widths of *Grovesiella abieticola* ascospores from different collections. For explanation of abbreviations, see FIG. 2.

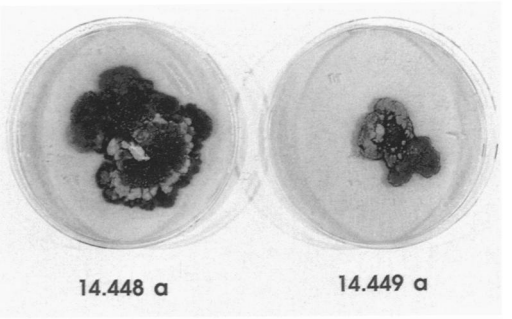


FIG. 4. Culture morphology of a single ascospore (left) and a single macroconidium (right) isolate on 2% MEA after 50 days at 20 C in the dark.

diameter of the faster growing isolates reached 33–36 mm after 30 days and 68–73 mm after 50 days, whereas slow growing isolates showed mean diameters of 9–16 mm after 30 days and 19–37 mm after 50 days. Growth of the faster growing isolates was within the range given by Gremmen and Morelet (**1971**) for two different formulations of potato dextrose agar (PDA): 22.8 mm and 45.6 mm, respectively, after 30 days under the same conditions as we used.

The morphology of the conidiomata in culture can be summarized as follows:

Conidiomata (micro- and macroconidial) mostly pycnidial (FIGS. 6, 10, 11), joined together in groups, rarely separate, dark brown, more or less spherical, unilocular, sometimes multilocular especially if conidiogenous region convoluted or apparently multilocular if two or more pycnidia adjacent, diameter of pycnidia 125–350 µm. *Ostiole* absent, dehiscence by an irregular rupture of the pycnidial wall. *Outer wall* comprised of *textura epidermoidea* and *textura angularis* consisting of cells with different amounts of melanization. *Inner wall* of small-celled, hyaline *textura angularis. Conidiophores* of microconidia hyaline, branched, septate, lining the locus (locules), 20–30 × 2–2.2 µm (FIGS. 7, 12). *Conidiogenous cells* of microconidia sympodial, hyaline, often with at least one small, indistinct, slightly protuberant conidiogenous locus, 8–12 × 1.8–2 µm (FIGS. 7, 13, 14). *Microconidia* holoblastic, hyaline, aseptate, smooth, cylindrical, apex obtuse, base truncate, 3–6 × 1.5–1.8 µm ($\bar{x}$ = 4 × 1.6 µm) (FIGS. 8, 15). Rate of germination of microconidia on 2% MEA was almost 100% and microconidia were able to form colonies. Thus, the microconidia of *G. abieticola* are not spermatia.

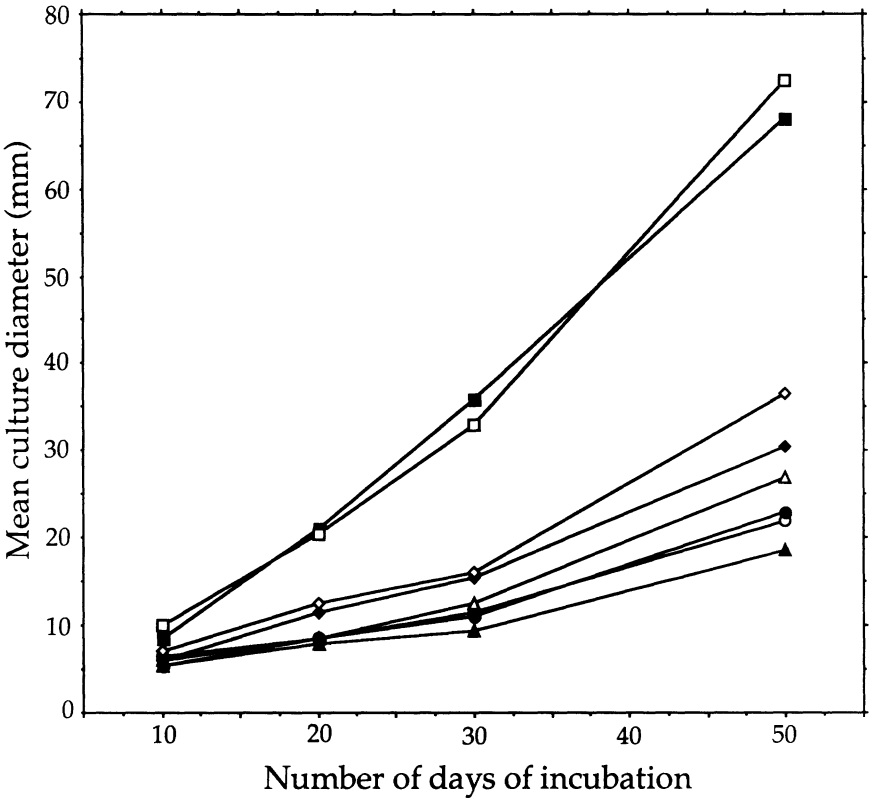


FIG. 5. Mean colony diameter and duration of incubation. □, ■, △, ▲, ◇, ◆ single ascospore isolates; ○, ● single macroconidia isolates.

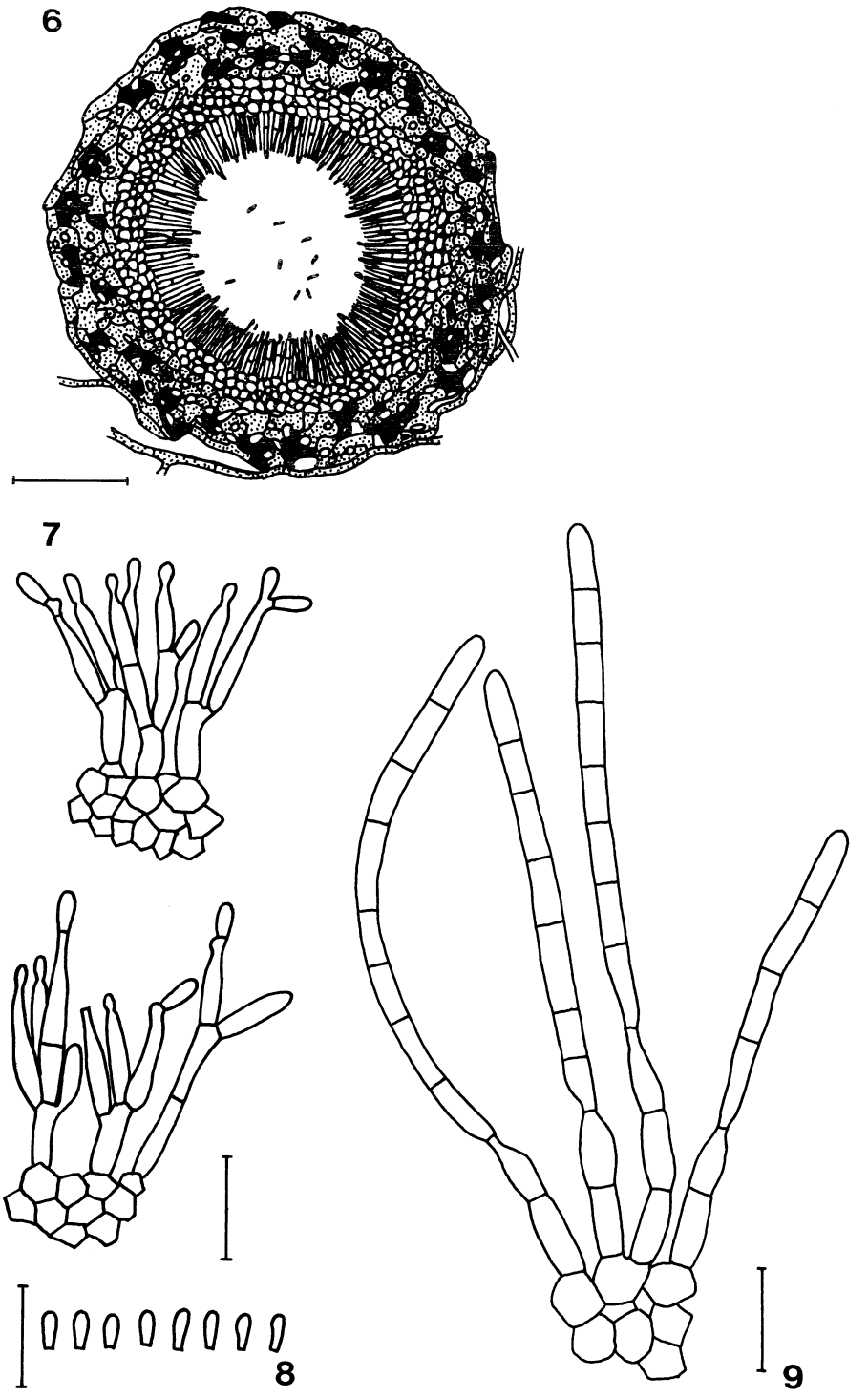
Conidiophores of macroconidia hyaline, branched only at the base, septate, lining the locus (locules), 14–23 × 2.5–3 μm (FIG. 9). *Conidiogenous cells* of macroconidia determinate, straight to slightly curved, hyaline, smooth, 8–9 × 2.5–3.3 μm (FIG. 9). *Macroconidia* holoblastic, euseptate, hyaline, filiform, straight to slightly curved, smooth, apex obtuse, base truncate, mostly four- or eight-celled, eight-celled conidia 33–68 × 2.2–3 μm ($\bar{x}$ = 50 × 2.5 μm), four-celled conidia 27–42 × 2.2–2.5 μm ($\bar{x}$ = 37.2 × 2.5 μm).

Macroconidia forming conidiomata in culture were different from those found on the host (Kowalski and Sieber, **1992**). Conidiomata were mostly pycnidial in culture, whereas they were stromatic on the host. In addition, *textura porrecta* was not present in pycnidia formed in culture in contrast to conidiomata produced on the host.

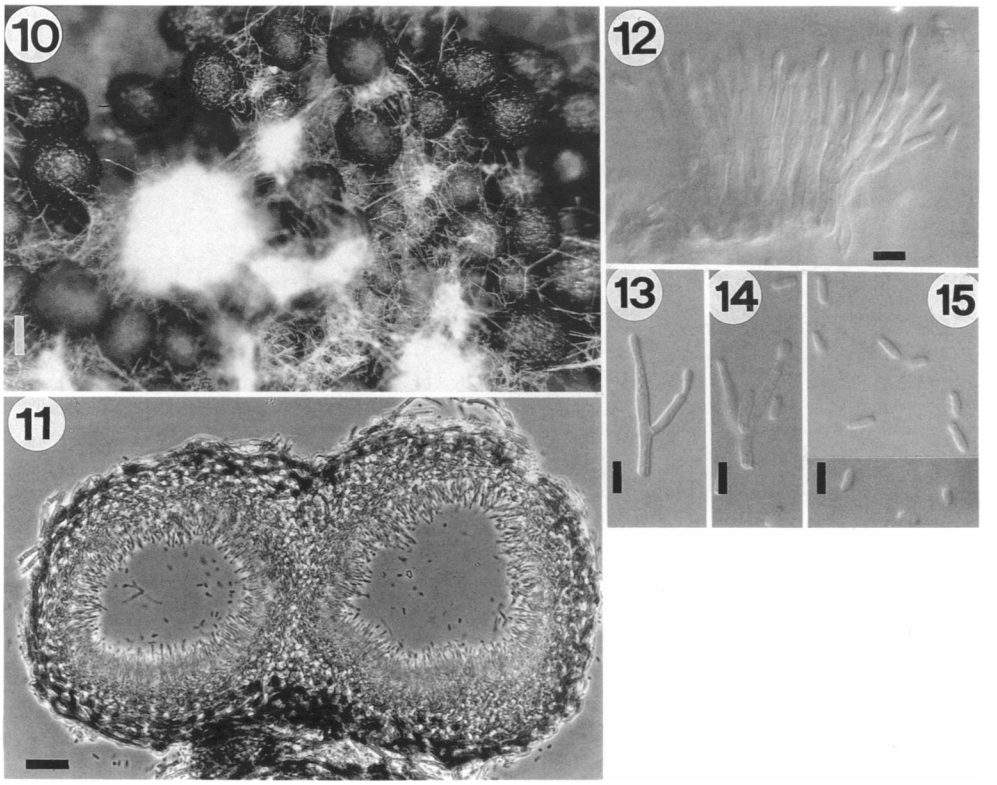
Whereas Gremmen and Morelet (**1971**) found the microconidial state of *G. abieticola* in culture, they were not able to induce the macroconidial state to form in pure culture although they incubated some cultures for more than 2 yr. They rarely observed the microconidial state on the host whereas we never did. Gremmen and Morelet (**1971**) stated that microconidia formed in culture were 3.9–6.6 × 1.3–1.6 μm which corresponds well to our measurements.

Neither the microconidial nor the macroconidial state have been observed in collections of *G. abieticola* from North America so far (Zeller and Gooding, **1930**; Seaver, **1951**). Our observations of herbarium specimens from North America led to the same conclusion. However, it would be premature to exclude the existence of conidial states of *G. abieticola* from North America because single spore isolates of the latter have never been examined and the micro- and macroconidial states of *G. abieticola* from Europe are also very rarely found in the field.

An endophyte [reported as *Agyriellopsis caeruleo-atra* von Höhnel in Sieber-Canavesi and Sieber (**1987**) and Sieber (**1989**)] from twigs and needles of *Abies alba* in Switzerland had pro-



FIGS. 6–9. Micro- and macroconidial state of *Grovesiella abieticola* in culture. 6. Conidioma with microconidia, scale bar = 50 μm. 7. Conidiophores with conidiogenous cells forming microconidia, scale bar = 10 μm. 8. Microconidia, scale bar = 10 μm. 9. Macroconidiophore with macroconidia, scale bar = 10 μm.



FIGS. 10–15. Microconidial state of *Grovesiella abieticola* in culture. 10. Pycnidia on the colony surface, scale bar = 100 μm. 11. Section of two pycnidia grown together, scale bar = 25 μm. 12–14. Conidiophores and conidiogenous cells, scale bars = 5 μm. 15. Conidia, scale bars = 5 μm.

duced the same morphology and cultural characteristics as single ascospore isolates of *G. abieticola*. Thus, this endophyte most probably is conspecific with *G. abieticola*. Apothecia of *G. abieticola* have not been reported from Switzerland but its presence as an endophyte demonstrates its occurrence in that country.

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