

## FULL PAPER

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## ***Pleurovularia*, a new genus of hyphomycetes proposed for a parasite on leaves of *Microstegium* sp. (Poaceae)**

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**Abstract** The hyphomycete *Ovularia polliniae*, formerly excluded from *Ramularia* and allied genera and tentatively considered as related to *Beniowskia sphaeroidea*, was recently collected on leaves of the grass *Microstegium* sp. in Taiwan. Based on studies with light and transmission electron microscopy, *O. polliniae* was redescribed. Using type specimens, the taxonomic status of both fungal species was reevaluated. In *O. polliniae*, the conidiophores emerge directly through the outer cell wall of the host epidermis. They are either sparsely branched at the base or simple and composed of verruculose intercalary and terminal conidiogenous cells that produce conidia through minute, slightly darkened scars. The conidia are solitary, one-celled, obovoid, hyaline, smooth, and often have a large vacuole. By the combination of these characteristics, this fungus differs from similar species of *Beniowskia*, *Ramularia*, and *Ramulariopsis*. The new genus *Pleurovularia* and the new combination *Pleurovularia polliniae* are proposed to accommodate this parasite on *Microstegium*.

**Key words** Graminicolous fungus · Poaceae · *Ramularia* · Ultrastructure

### Introduction

*Ovularia* Sacc. is considered as a synonym of *Ramularia* Unger, and most species of *Ovularia* are now retained in *Ramularia* (Braun 1998). *Ovularia polliniae* Henn., however, parasitic on grasses of the genus *Microstegium* Nees in

East Asia, was excluded from *Ramularia* and allied genera and considered as a possible synonym of *Beniowskia sphaeroidea* (Kalchbr. & Cooke) E.W. Mason by Braun (1991). Because authentic material of the latter fungus had not been studied, a definite conclusion on its systematic status was not drawn. Using a recent collection of well-developed material of *Ovularia polliniae* on leaves of a species of *Microstegium* in Taiwan, the morphology and growth habit of this fungus were investigated with light and transmission electron microscopy (TEM). Authentic specimens of *O. polliniae* and *B. sphaeroidea* were restudied and compared with the Taiwanese material.

### Materials and methods

Specimens of *Ovularia polliniae* on leaves of sterile culms of a species of *Microstegium* (Poaceae, Panicoideae) (determined by H.-C. Kuo) were collected in Taiwan, Tao Yuan, Da Guan Shan, 23 May 2000, R. Kirschner 760 (TAI). The fungus was compared with the lectotype of *O. polliniae* on leaves of *Microstegium imberbe* (Nees ex Steud.) Tzelev (= *Pollinia imberbis* Nees ex Steud.), Japan, Prov. Tosa (Kochi), Katakasa-mura, June 1901, T. Yoshinaga 25 (B). Material on *Microstegium* sp. was collected in P.R. China, Yunnan Province, Mo Jiang, 1550m, 12 August 2001, R. Kirschner 1035 (HKAS). In addition, the following specimens of *Beniowskia* were studied: type of *Beniowskia graminis* Racib. on *Panicum nepalense* Steud. (PAD) without sporodochia; type of *Aegerita penniseti* Henn., on leaves of *Pennisetum japonicum* Trin., Japan, Prov. Tosa (Kochi), Engyoji, October 1907, T. Yoshinaga 14 (B); type of *Beniowskia sphaeroidea* (Kalchbr. & Cooke) E.W. Mason (= *Ceratium sphaeroideum* Kalchbr. & Cooke), on leaf of *Andropogon marginatus* Steud., South Africa, (without date), MacOwan (K); *B. sphaeroidea* (determined by R. Kirschner) on leaves of an unidentified grass (Poaceae), La Réunion, 21 March 2001, M. Göker & M. Hendrichs (TUB), *B. sphaeroidea* on leaves of *Pennisetum* Rich. sp., P.R. China, Yunnan Province, Jian Chuan, 2250m, 25 July,

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R. Kirschner 860 (HKAS); type of *Beniowskia penniseti* Wakef., on leaves of *Pennisetum purpureum* Schumach., Uganda, Kampala, 23 March 1915, T.D. Maitland ("Small") 251 (K).

Infected leaves were sectioned with a razor blade by hand. The sections were either mounted in water, stained with 1% (w/v) phloxine and mounted in 10% (w/v) KOH, or stained and mounted in 0.1% (w/v) cotton blue in lactic acid solution. Measurements were made using mountings in 10% KOH, but there were no obvious differences concerning shapes and sizes in the other mounting media. For TEM, dried material of *O. polliniae* collected in Taiwan was processed as described in Kirschner and Oberwinkler (1999).

## Results

Well-developed *Ovularia polliniae* was collected in Taiwan and is newly recorded for Taiwan in this article. Comparisons of this specimen with the lectotype of *O. polliniae* revealed conspecificity. The conidiophores of the lectotype, however, were distorted and broken. The description of the

fungus given below is based on the material from Taiwan. Comparison of *O. polliniae* with *Beniowskia sphaeroidea* revealed that *O. polliniae* cannot be accommodated with *Beniowskia*.

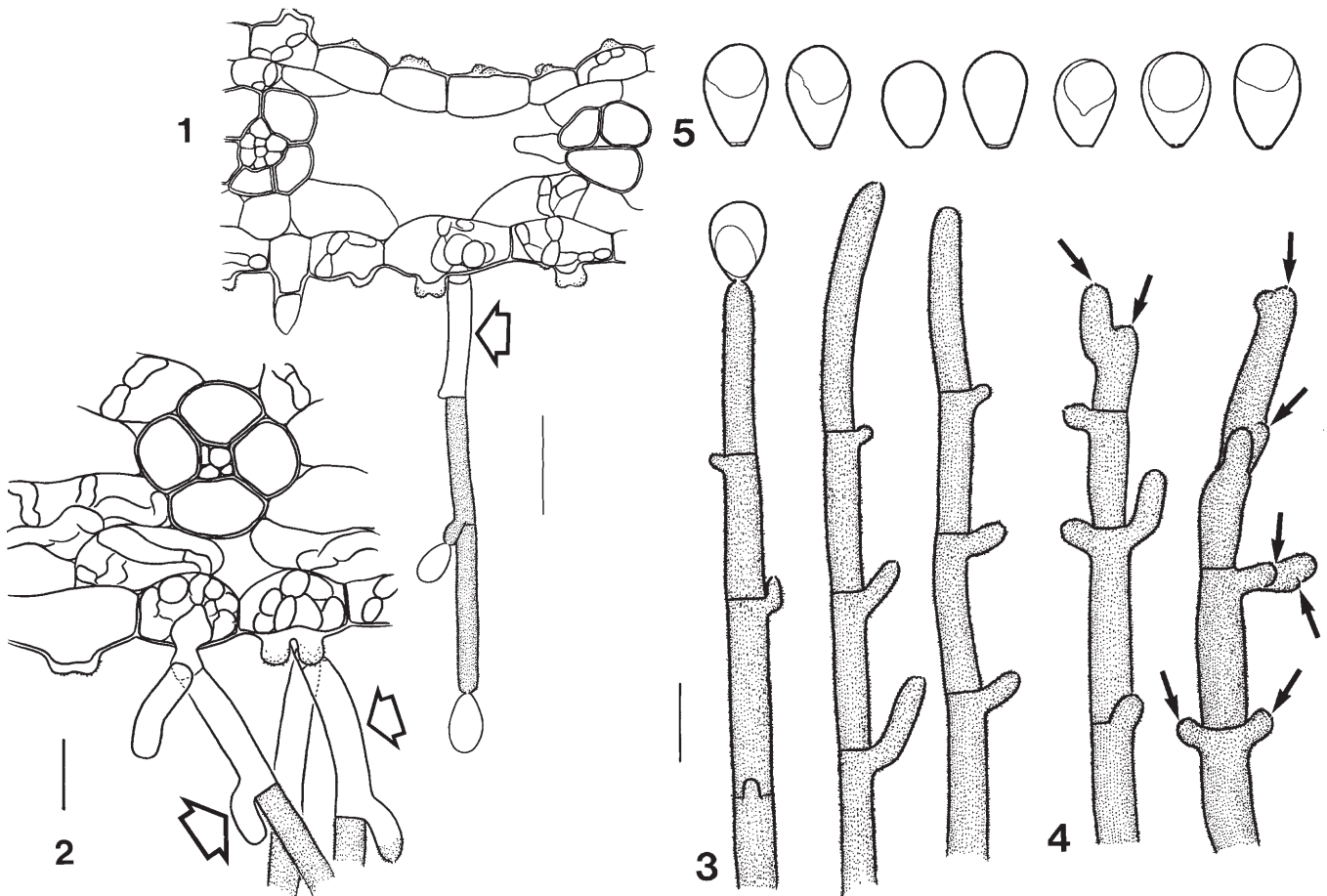
### *Pleurovularia* R. Kirschner & U. Braun, gen. nov.

Genus hyphomycetum. Mycelium in foliis vivis evolvens. Conidiophora macronematosa, mononematosa, hyalina, simplicia vel ad basim ramosa, verruculosa, per parietes exteriores cellularum epidermidis penetrantia; cellulae conidiogenae intercalares vel terminales, mono- vel polyblasticae, cicatricibus leviter incrassatis infuscatisque. Conidia hyalina, vacuolata.

Typus generis: *Pleurovularia polliniae* (Henn.) R. Kirschner & U. Braun.

Etymology: referring to the aseptate, noncatenate conidia of species formerly classified in *Ovularia* and to the pleurogenous conidium production.

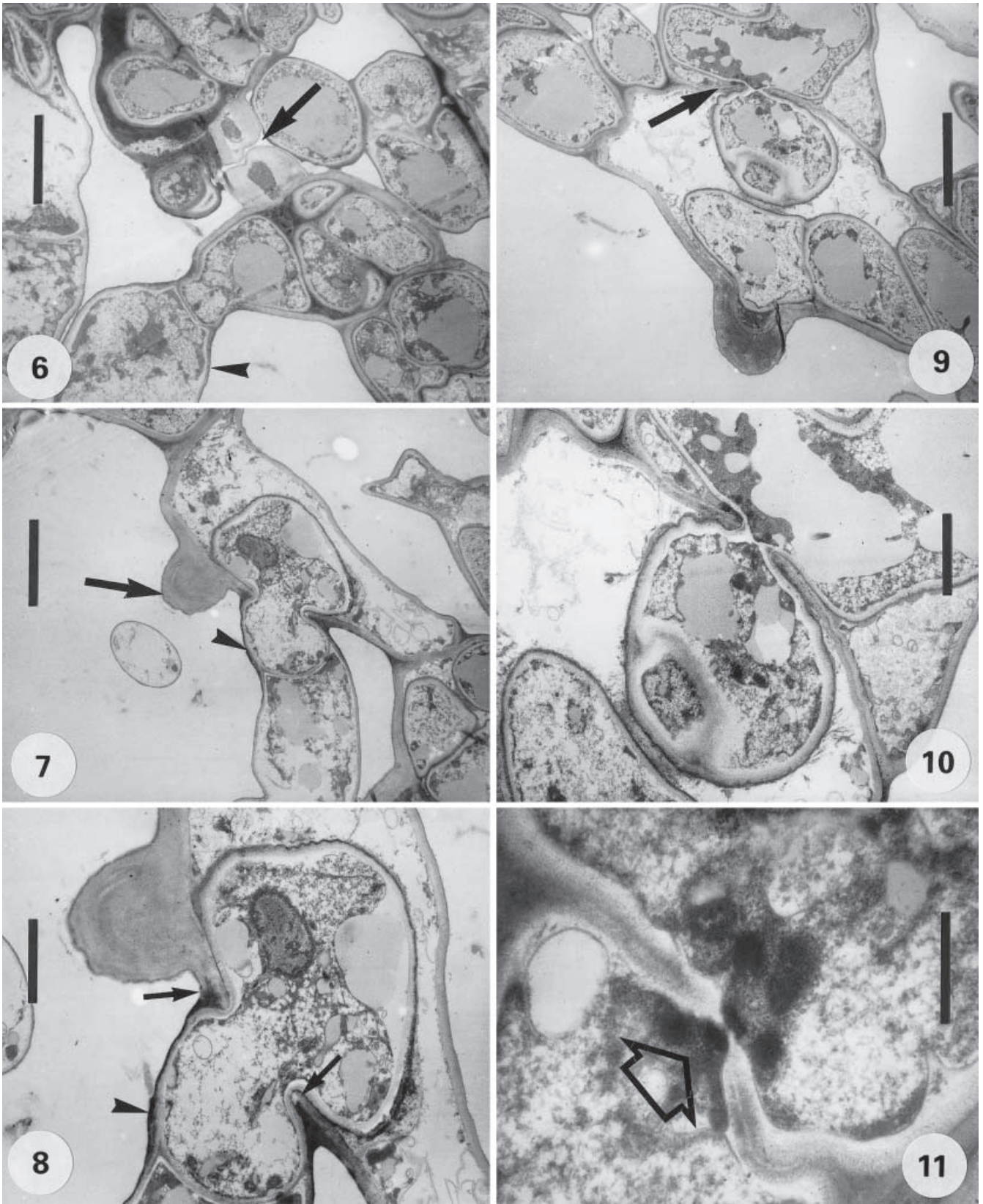
Phytoparasitic, conidiophores macronematous, mononematous, hyaline, simple or sparsely branched, verruculose at least in the distal part, emerging mainly through the outer wall of epidermal cells of the host, conidiogenous cells intercalary and terminal with slightly thickened, pig-



**Figs. 1, 2.** Section through leaves of *Microstegium* sp. with conidiophores of *Pleurovularia polliniae* emerging through the outer wall of the lower epidermis and cuticle (R. Kirschner 760). The basal part (arrows) appears smooth. **1** Simple conidiophore. Bar 20  $\mu$ m. **2** Bases of two conidiophores showing basal branching. Bar 10  $\mu$ m

**Figs. 3–5.** Conidia and apical parts of conidiophores of *Pleurovularia polliniae* (inverted into an upright position) (R. Kirschner 760). Bar 10  $\mu$ m. **3** Most common type of conidiophores with apparently monoblastic conidiogenous cells. **4** Rare type of conidiophores showing several conidiogenous loci per conidiogenous cell (arrows). **5** Conidia





**Figs. 6–11.** Transmission electron microscopy (TEM) microphotographs of *Pleurovularia pollinae* within and emerging from the tissues of *Microstegium* sp. (R. Kirschner 760). **6** Base of a conidiophore (arrowhead) emerging through the outer wall of an epidermal cell close to a stoma (arrow). Bar 4 $\mu$ m. **7** Base of a conidiophore (arrowhead) emerging through the outer wall of an epidermal cell. Note the papilla of the epidermal cell (arrow). Bar 4 $\mu$ m. **8** The same structures as in Fig.

**7** at higher magnification. The cell wall of the host was ruptured or lysed (arrows) by the emerging conidiophore (arrowhead). Bar 1.5 $\mu$ m. **9** A hypha proliferating from one plant cell to another (arrow). Bar 4 $\mu$ m. **10** As in Fig. 9 at higher magnification. The hypha has perforated the plant cell wall. Bar 1.5 $\mu$ m. **11** Longitudinal section through a hyphal septum with a simple pore (arrow). Bar 0.5 $\mu$ m

mented scars, mono- or polyblastic, producing hyaline conidia with vacuole.

*Pleurovularia pollinae* (Henn.) R. Kirschner & U. Braun, comb. nov. Figs. 1–13

Basionym: *Ovularia pollinae* Henn., Bot. Jahrb. Syst. 38:165, 1905.

Synonym: *Ramularia pollinae* (Henn.) U. Braun, Int. J. Mycol. Lichenol. 3:282, 1988.

Leaf spots elongated, confluent, centre ochre, with olive or dark brown margin within an outer, yellow or orange-yellow margin, spots on the lower surface appearing white by aggregated conidiophores. Conidiophores hypophyllous, hyaline, rarely light brown at the base, straight, filiform, each filum composed of 2–5 intercalary and 1 terminal conidiogenous cells (Figs. 1–4), mononematous, macronematous, simple or scarcely branched at the base, aggregated in a loose, brushlike manner, arising from intracellular mycelium with simple septal pores seen with TEM, penetrating through the outer epidermal wall and cuticle, apparently not through the stomata (Figs. 1, 2, 6–10), densely verruculose except for the basal part, 70–140 × 3.5–7 μm. Conidiogenous cells intercalary and terminal (Figs. 1–4), hyaline, 16–36 × 3.5–7 μm, intercalary cells with short papillate protuberances arising just below the septum, mostly verruculose, mostly apparently monoblastic (Fig. 3), but sometimes also polyblastic (Fig. 4), with minute hyaline tubes or slightly thickened and darkened conidial scars. Cell wall of the conidiophores composed of an electron-transparent inner layer, a medium electron-opaque middle layer, and a thin conspicuously electron-opaque outer layer (Figs. 12, 13), ornamented with minute warts mainly built up by the middle wall layer (Fig. 13), but with the outer wall layer slightly thicker than in the smooth areas of the conidiophore. Conidia (Fig. 5) hyaline, smooth, often with an apical vacuole, obovoid, thin-walled, solitary, with or with-

out a basal, thickened, hyaline or darkened scar, 11–14 × 7–10 μm.

*Beniowskia sphaeroidea* (Kalchbr. & Cooke) E.W. Mason, Mycol. Pap. 31:26, 1928 (type, K) Figs. 14–18

≡ *Ceratium sphaeroideum* Kalchbr. & Cooke, Grevillea 9: 22, 1880, n.v.

= *Beniowskia graminis* Racib., Parasitische Algen und Pilze Javas. II. Theil 37, 1900, type in PAD without sporodochia

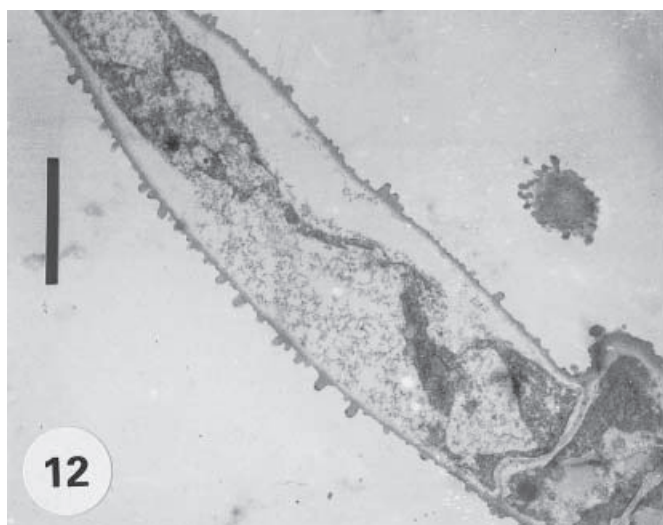
= *Aegerita penniseti* Henn., Hedwigia 43:153, 1904 (type, B)

= *Beniowskia penniseti* Wakef., Kew Bull. Misc. Inform. 75, 1920, n.v. (type, K)

The following description is mainly based on the material in B: sporodochia white, 300–900 μm in diam. (Fig. 14), basal and central plectenchyma embedded within the leaf tissue and erumpent through the epidermis, composed of hyaline, parallel, closely aggregated rows of cells 10–25 μm long and 4–6 μm wide (Fig. 15). Free hyphae radiating from this basal plectenchyma, hyaline, 3–4 μm in diameter, with many anastomoses, terminally coiled or straight (Fig. 16). Conidiogenous cells intercalary, smooth, 10–26 × 3.5–4 μm, with flat, 1–2 μm wide scars (Figs. 16, 17). Conidia solitary, in some cases anastomosing with each other or with hyphae, hyaline, smooth, globose, 7–11 μm in diameter with flat basal scar (Fig. 18).

## Discussion

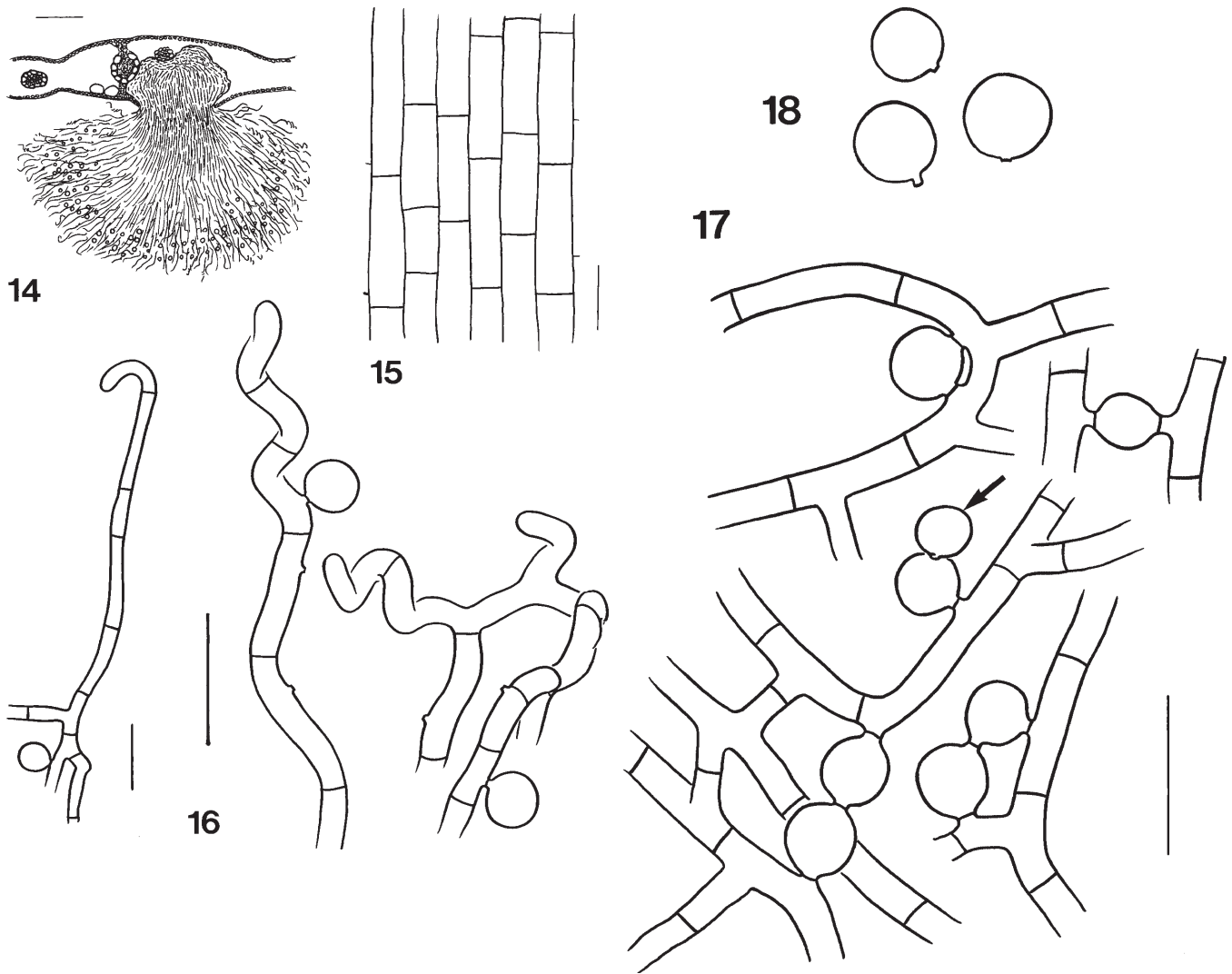
The hyaline conidiophores with thickened, darkened conidiogenous scars of *Pleurovularia pollinae* are similar to those described for species of *Ramularia* and *Ramulariopsis* Speg. (Braun 1998). With respect to the aseptate, non-



**Figs. 12, 13.** TEM photographs of conidiophores of *Pleurovularia pollinae* (R. Kirschner 760). **12** Longitudinal section showing the dense verruculose ornamentation. Bar 3 μm. **13** Section through a conidiophore showing the cell wall ornaments that are composed of material of

the middle wall layer and outer wall layer (arrows). The electron-transparent inner wall layer is not involved in the construction of the ornaments. Bar 0.25 μm





**Figs. 14–18.** *Beniowskia sphaeroidea* (type of *Aegerita penniseti* in B, except for Fig. 15; type of *Beniowskia penniseti* in K). **14** Habit sketch of a sporodochium erumpent through the leaf surface. *Bar* 100  $\mu\text{m}$ . **15** Longitudinal section through the basal plectenchyma of a sporodochium. *Bar* 10  $\mu\text{m}$ . **16** Conidiophores with conidia, denticles,

and terminally coiled ends. *Bars* 20  $\mu\text{m}$ . **17** Conidiophores with anastomoses, conidiogenous cells, and conidia fused with adjacent hyphae or conidia. In one case, an apparently free conidium was attached to another one (*arrow*) (by primary growing out from the other conidium or possibly by secondary fusing). **18** Conidia. *Bar* **17**, **18** 20  $\mu\text{m}$

catenate conidia, *P. pollinae* is similar to species that were formerly classified in *Ovularia* but are now retained in *Ramularia* (Braun 1998). Species with one-celled conidia and species with septate conidia are presently accepted within *Ramularia* as well as graminicolous species and parasites on dicots (Braun 1998). The conidiogenous cells of species of *Ramularia*, however, are exclusively terminal (Braun 1998). Furthermore, a vacuole is not present in conidia of *Ramularia*. Among phytopathogenic *Ramularia*-like hyphomycetes, *P. pollinae* is most similar to species of *Ramulariopsis* Speg. according to the intercalary conidiogenous cells. This genus comprises four species with smooth conidiophores emerging through the stomata of the dicotyledonous hosts and with septate, catenate conidia (Braun 1998), whereas the conidiophores of *P. pollinae* are verruculose and not found to emerge through stomata, and the conidia are one-celled and solitary.

Other hyaline graminicolous hyphomycetes are also reported to emerge directly through the outer wall of cells of the host epidermis and rarely or not through the stomata, e.g., species of *Mastigosporium* Riess, *Rhynchosporium* Heinsen, *Rhynchosporina* Arx, and *Spermospora* R. Sprague. In these genera, however, the conidiophore is restricted to a single conidiogenous cell (Braun 1995) and can be considered as micronematous or semimacronematous, in contrast to the distinctly macronematous conidiophores of *P. pollinae*.

Macronematous hyaline conidiophores also develop in members of *Beniowskia*. In this genus, however, conidiophores are aggregated in sporodochia with a basal plectenchyma, often anastomosing, smooth, with denticles scattered over the length of the conidiogenous cells. Therefore, *Beniowskia* and *Pleurovularia* are distinct genera.

The probable synonymy of *B. sphaeroidea* with *B. graminis* is difficult to clarify. We found many small, young conidia but no hyphal coils in the two collections preserved in K and the one in HKAS, and the opposite situation, namely, a considerably higher proportion of large, old conidia and the presence of hyphal coils in the two collections preserved in B and TUB. The production of conidia and the development of coiled hyphae might, therefore, take place at different times in the living sporodochium.

The occasional development of conidia on lateral, discrete conidiogenous cells and on other conidia reported for *B. graminis* by Cole and Samson (1979) could not be verified using the material mentioned above. Mason (1928) pointed out that *B. graminis* differs from *B. sphaeroidea* only by the presence of coiled hyphae in *B. graminis* and doubted that the distinction between both species could be maintained. Hughes (1958) considered *B. sphaeroidea* as a synonym of *B. graminis*. Because the type of *B. graminis* in PAD does not contain any material of *Beniowskia*, we cannot draw a final conclusion on the probable synonymy of *B. graminis* and *B. sphaeroidea*. We, however, preliminarily adopt the synonymy proposed by Hughes (1958), until additional material authenticated by Raciborski is discovered or more is known about the life cycle in *Beniowskia*.

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