

A new species of *Pestalosphaeria*, the teleomorph of *Pestalotiopsis neglecta*

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Three specimens of a new *Pestalosphaeria* species were obtained from a twig of *Quercus myrsinaefolia* from Kumamoto Pref., and from leaves of *Rhododendron hybridum* and *Ricinus communis* from Izu Peninsula, Shizuoka Pref. The collected materials were kept moist for 1 to 2 mo after collection. Monoascospore isolates from each specimen produced identical colonies with black slimy masses of conidia on them. Morphological characteristics of the conidia accorded well with those of *Pestalotiopsis neglecta* not only hitherto recorded but also formed on the same specimen. Hence, *Pestalosphaeria gubae* sp. nov. is proposed for the new species, as the teleomorph of *Pestalotiopsis neglecta*.

Key Words—anamorph; new species; *Pestalosphaeria gubae*; *Pestalotiopsis neglecta*.

As preliminarily reported in 1995 (Ishihara et al., 1995), an ascomycetous fungus which was assumed to be the teleomorph of *Pestalotiopsis neglecta* (Thüm.) Steyaert, was obtained from a twig of *Quercus myrsinaefolia* Blume, collected at Kumamoto Pref., Kyushu, which was kept moist for about 2 mo. In September 1999, several leaves of *Rhododendron hybridum* Hort. and *Ricinus communis* L., on which acervuli of *P. neglecta* were recognized, were collected at Ito, Shizuoka Pref., Honshu. Samples of the leaves were kept in Petri dishes under moist conditions for more than 1 mo. Perithecia of a Pyrenomycetes were produced separately on these two materials. A *Pestalotiopsis* anamorph was also produced on the colony derived from a monoascospore, and its morphological characteristics were identical with those of *P. neglecta*, which were produced on the same leaf materials and have been described in literature (Table 1; Fig. 1). The chief morphological characteristics of the present fungus are compared in Table 2 with those of known genera having the *Pestalotiopsis* and related anamorphs. Detailed comparison reveals that the present ascomycetous fungus can be accommodated well in the genus *Pestalosphaeria* M. E. Barr (Barr, 1975). The type species, *Pestalosphaeria concentrica* M. E. Barr, was found as the teleomorph of *Pestalotiopsis guepinii* var. *macrotricha* (Kleb.) B. C. Sutton (\equiv *Pestalotia macrotricha* Kleb.). Thereafter, seven species of *Pestalosphaeria* were added, each of which had a *Pestalotiopsis* anamorph (Table 3). Table 3 composes the morphological characteristics of the present *Pestalosphaeria* fungus having a *Pestalotiopsis* anamorph and the eight known species in this genus. The present

fungus was distinguished from the other species of *Pestalosphaeria* by its morphological characteristics in asci and ascospores and in its conidial state (anamorph). Hence, the present *Pestalosphaeria* is described as a new species, *P. gubae* Tak. Kobayashi, Ishihara et Yas. Ono. The species epithet *gubae* is dedicated to Prof. E. F. Guba, who published the world monograph of the genera *Pestalotia* and *Monochaetia* in 1961.

***Pestalosphaeria gubae* Tak. Kobayashi, Ishihara et Yas. Ono, sp. nov.** Figs. 1–3

Perithecia in foliis corticibusque immersis, solitariis vel gregariis, depresso-globosis, apice colliculato-papillatis et ostiolatis, 112–250 μm diam, 125–240 μm altis, glabris, pariete 7.5–10 μm crasso, membranaco fusco-brunneo vel atrato “textula porrecta” ex cellulis paralleto hyphoideis composito praedito; ascis unitunicatis, oblongo-cylindricis vel clavatis, breviter spicatis, annulo apicali jodo clareaerulescenti praeditis, octosporis, 75–88 \times 10–12.5 μm ; ascosporis monostichis vel irregulariter distichis, ellipsoideis vel subfusoides, pallide olivaceis vel brunneis, 2 (rarius 3)-septatis, 9.5–17.5 \times 5–7 μm , laevibus.

Anamorphosis: *Pestalotiopsis neglecta* (Thüm.) Steyaert

Holotypus: in foliis *Rhododendri hybridi* Hort. (Seiyoshakunage)—Ito, Shizuoka Pref., Sept. 29, 1999, Y. Ono, (TMF-FPH: 7584).

Perithecia on leaves and bark, embedded, solitary or in small group, without stroma, subglobular, 112–250 μm in diam and 125–240 μm in height, with a small papillate ostiole at the tip, without setae; perithecial wall

Table 1. Comparison of conidial morphology of *Pestalotiopsis* isolates from ascospores and from conidia.

Origin of isolate	Host	Length × width	Colored cell		Apical appendage	
			Color	Length	Number	Length
Ascospore ^{a)}	<i>Quercus</i>	18.5–24.5 × 4.5–7	Concolor	10.5–15.5	(2–) 3 (–4)	8–19.5
	<i>Rhododendron</i>	20–25 × 5–6.5	Concolor	12.5–15	(2–) 3 (–4)	10–17.5
	<i>Ricinus</i>	21–25 × 5.5–7	Concolor	14–15	(2–) 3 (–4)	10–17.5
Conidium ^{b)}	<i>Quercus</i>	18.5–24.5 × 5–6	Concolor	11.5–15.5	(2–) 3 (–4)	8–17.5
	<i>Rhododendron</i>	20–25 × 5.5–7	Concolor	14–16.5	(2–) 3 (–4)	7.5–15
	<i>Ricinus</i>	21–25 × 5.5–6.5	Concolor	12.5–16.5	(2–) 3 (–4)	10–17.5
<i>P. neglecta</i>	Various ^{c)}	20–26 × 5–7	Concolor	13–16	3	9–18
	Conifers ^{d)}	17–25 × 4.5–6.4	Concolor	11–17	(1–) 2–3 (–4)	5–19

a) *Pestalosphaeria*-state, b) *Pestalotiopsis*-state, c) Guba (1961), d) Suto and Kobayashi (1993).

dark brown to blackish, membranaceous, composed of parallel hyphal cells (textura porrecta), 7.5–10 µm in thickness; asci unitunicate, thin membrane, oblong-cylindric to oblong-clavate with short stipe, furnishing apical apparatus stained finely with iodine (Melzer reagent), containing 8 ascospores, 75–88 × 10–12.5 µm; ascospores irregularly mono- or bi-seriate, elliptic to subfusiform, pale olive to brown, 2 (rarely 3)-septated, 9.5–17.5 × 5–7 µm, smooth.

Anamorph: *Pestalotiopsis neglecta* (Thüm.) Steyaert
Conidia fusiform, 5-celled with apical and basal appendages, 17–25 × 4.5–7 µm in size without appendages, both end cells conical and hyaline, median 3 cells pale brown (concolor) and 10.5–16.5 µm in length, apical appendages 2–4, usually 3 at the tip of apical cell, 7.5–17.5 µm in length, basal appendage short needle like, 2.5–7.5 µm in length, sticky and blackish in mass.

Specimen examined: on leaves of *Rhododendron hybridum* (Seiyo-shakunage)—Ito, Shizuoka Pref., Sept. 29, 1999, by Y. Ono (TFM-FPH: 7584, Holotype). On bark of *Quercus myrsinaefolia* (Shirakashi)—Otsu, Kumamoto Pref., Oct. of 1994, by M. Ishihara (TFM-FPH: 7585). On leaves of *Ricinus communis* (Hima)—Ito, Shizuoka Pref., Sept. 29, 1999, by Y. Ono

(TFM-FPH: 7586).

Note: As noted above, the ascospore morphology of the present fungus, including the number of cells, color and surface structure, and the reaction of the ascus tip to iodine (Melzer reagent), are identical with those of the genus *Pestalosphaeria* (Table 2). In the number of cells of ascospores, the present fungus also accords with the genera *Discostroma* Clem. and *Griphosphaerioma* Höhn. However, *Griphosphaerioma* has only hyaline ascospores, its *Pestalotia* anamorph has 6-celled conidia with 4-colored cells, which distinguish it from the *Pestalotiopsis* anamorph of the present fungus, having 5-celled conidia with 3-colored cells. In the genus *Discostroma*, the anamorph belongs to the genus *Seimatosporium* Corda, of which conidia have an exogenous basal appendage and germ-tubes develop from each cell of conidia. In contrast, conidia of the genus *Pestalotiopsis* have an endogenous basal appendage and they germinate only from the lowest colored cell, except when wounded. Other genera shown in Table 2 could easily be distinguished from the present fungus by their number of cells and color of ascospores, reaction of ascus tip to iodine, and the difference of the anamorph. From these facts, the present fungus was classified into the genus

Table 2. Comparison of chief characters of the present fungus and the related genera in Amphisphaeriaceae.

Genus	Ascus tip ^{a)}	Ascospore			Anamorph	Literature
		No. of cell	Color	Surface view		
<i>Paradidymella</i>	Amyloid	2	Hyaline	Smooth	<i>Seimatosporium</i>	Barr (1975)
<i>Griphosphaerioma</i>	Non-amyloid	2–3 (–4)	Hyaline	Smooth	<i>Pestalotia</i> ^{d)}	Shoemaker (1968)
<i>Discostroma</i>	Amyloid	(1–) 3 (–7)	Hyaline & Brown	Smooth	<i>Seimatosporium</i>	Brockman (1976)
<i>Pestalosphaeria</i>	Amyloid	(2–) 3 (–4)	Brown	Smooth or LS ^{b)}	<i>Pestalotiopsis</i> ^{e)}	Barr (1975), Nag Raj (1985)
<i>Broomella</i>	Non-amyloid	4	Hyaline & Brown	Smooth	<i>Pestalotia</i> , <i>Truncatella</i>	Shoemaker & Muller (1963)
<i>Hymenoplella</i>	Amyloid	4	Brown	Smooth	<i>Monochaetia</i>	Shoemaker & Muller (1965)
<i>Blagiascospora</i>	Amyloid	4	Brown	Smooth or P ^{c)}	<i>Seiridium</i>	Shoemaker et al. (1966)
<i>Leptoeutypa</i>	Amyloid	4	Brown	Smooth or LS	<i>Hyalotiella</i>	Arx (1970), Papendorf (1967)
<i>Clathridium</i>	Amyloid	4–6	Hyaline	Smooth	<i>Seimatosporium</i>	Shoemaker & Muller (1964)
The present fungus	Amyloid	3 (–4)	Brown	Smooth	<i>Pestalotiopsis</i>	The present authors

a) Presence or absence of blue reaction to Melzer reagent, b) Longitudinally striate, c) Pitted entirely, d) 6-celled conidia with 4 colored cells, e) 5-celled conidia with 3 colored cells.

Table 3. Comparison of chief characteristics of the present fungus and known species of *Pestalospaeria*.

Species	Ascospore-state (<i>Pestalospaeria</i>)				Conidium-state (<i>Pestalotiopsis</i>)			
	Size	No. of cell	Surface view	Color	Species	Color	Size	
<i>P. accidenta</i>	13–17×5–7	(2–) 3 (–4)	Smooth	Concolor	<i>P. baarnensis</i>	Versicolor	20–27×6–7	a)
<i>P. austroamericana</i>	20–23×8.5–10	3 (–4)	Netted	Versicolor	<i>P. sp.</i>	Versicolor	28–30×10–11	b)
<i>P. concentrica</i>	13.5–20×7–10	3	Smooth & LS	Concolor	<i>P. macrotricha</i>	Versicolor	20–32×6–8	c)
<i>P. elaeidis</i>	12–21×4.5–6	3	Smooth	Concolor	<i>P. sp.</i>	Concolor	27–34×6–10	d, e)
<i>P. hansenii</i>	13–14.5×5–6	3 (–4)	Smooth	Concolor	<i>P. sp. (foedans)</i>	Versicolor	19–24×5.5–7	e)
<i>P. jingganngensis</i>	13–16.5×5–7	(2–) 3	Smooth	Concolor	<i>P. podocarp</i>	Versicolor	16.7–23.5×5.6–7.5	a)
<i>P. leucospermi</i>	12–15×5–7	3	Thick wall	Versicolor	<i>P. sp.</i>	Concolor	22–34×6.5–9	f)
<i>P. varia</i>	10–17×6–8	2 (–4)	Verrucose	Concolor	<i>P. besseyii</i>	Concolor	15–19×5–6	g)
The present fungus	9.5–17.5×5–7	3 (–4)	Smooth	Concolor	<i>P. neglecta</i>	Concolor	18.5–25×4.5–7	h)

a) Zhu et al. (1991), b) Nag Raj (1979), c) Barr (1975), d) Booth and Robertson (1961), e) Shoemaker and Simpson (1981), f) Samuels et al. (1987), g) Nag Raj (1985), h) The present authors.

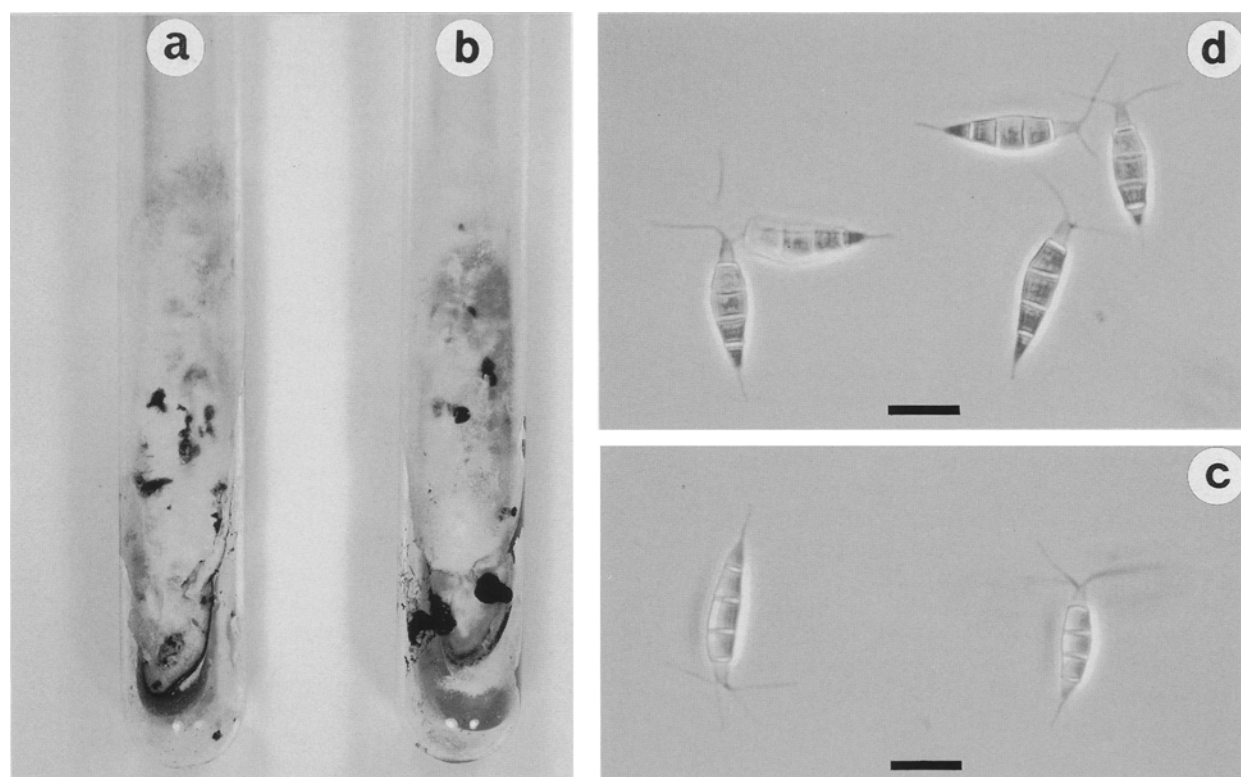


Fig. 1. PDA cultures from a monoascospore of *Pestalospaeria gubae* and from a monoconidium of *Pestalotiopsis neglecta*, and conidia produced by them. a: Monoascospore isolate, b: Monoconidium isolate, c: Conidia produced by monoascospore isolate (phase contrast), d: Conidia produced by monoconidium isolate (phase contrast). Scales: c, d=10 μm.

Pestalospaeria (Barr, 1975).

Eight species of the genus *Pestalospaeria* have been described since 1975, when Barr established this genus. All of them have the *Pestalotiopsis* anamorph. In the genus *Pestalotiopsis*, a difference in color character of the median three cells of conidia has been thought to be important for distinguishing species groups (Guba, 1961; Suto and Kobayashi, 1993). The genus *Pestalotiopsis* is divided into two groups by the color characters of the median three cells: concolor group has

three evenly pale brown cells, whereas the other versicolor group has two upper cells that are brown to chestnut brown cells, while the lowest cell is pale brown. Of the eight known *Pestalospaeria* species, *P. accidenta* P. L. Zhu, Q. X. Ge et T. Xu (Zhu et al., 1991), *P. austroamericana* Nag Raj et DiCosmo (Nag Raj, 1979), *P. concentrica* M. E. Barr (Barr, 1975), *P. hansenii* Shoemaker et J. A. Simpson (Shoemaker and Simpson, 1981) and *P. jingganngensis* P. L. Zhu, Q. X. Ge et T. Xu (Zhu et al., 1991) clearly differ from the present fungus in having

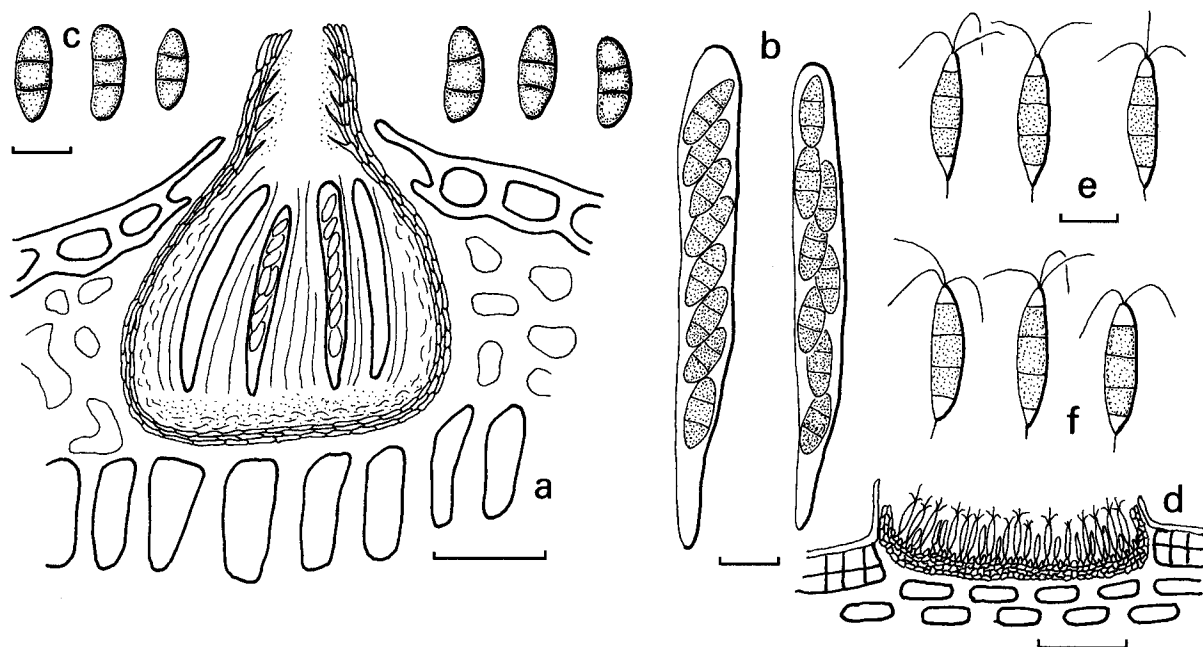


Fig. 2. *Pestalosphaeria gubae* and its anamorph, *Pestalotiopsis neglecta*. a-c: *Pestalosphaeria* state formed on *Rhododendron hybridum* leaf (a: Perithecium, b: Asci and ascospores, c: Ascospores). d-f: *Pestalotiopsis* state on plant material or on isolates (d: Acervulus on *R. hybridum* leaf, e: Conidia from ascospore-isolate, f: Conidia on *Pestalotiopsis*-isolate from *Rhododendron* leaf). Scales: a=50 μm , d=30 μm , b, c, e, f=10 μm .

conidia with versicolored median cells, as shown in Table 3.

The remaining 3 species, namely, *P. elaeidis* (C. Booth et J. S. Robertson) Aa (Booth and Robertson 1961; van der Aa, 1976), *P. leucospermi* Samuels, E. Mull. et Petrini (Samuels, 1987) and *P. varia* Nag Raj (Nag Raj, 1985), were described as having concolored median cells in their conidia. However, *P. elaeidis* has longer and more slender ascospores and much longer and thicker conidia than the present fungus. In *P. leucospermi*, the central cell of ascospore is darker than the pale-brown end cells. Moreover, conidia of *P. leucospermi* are much bigger than those of the present fungus. *Pestalosphaeria varia* is distinguishable from the present fungus by its mostly 2-celled and verrucose ascospores, and its 4-celled conidia with two median concolored cells.

The morphological characteristics of the conidial state (anamorph) of the present fungus agreed entirely with those of *Pestalotiopsis neglecta*, as shown in Table 1.

Therefore, the present species of *Pestalosphaeria*, which was thought to be the teleomorph of *P. neglecta*, was treated above as a new species, *P. gubae*.

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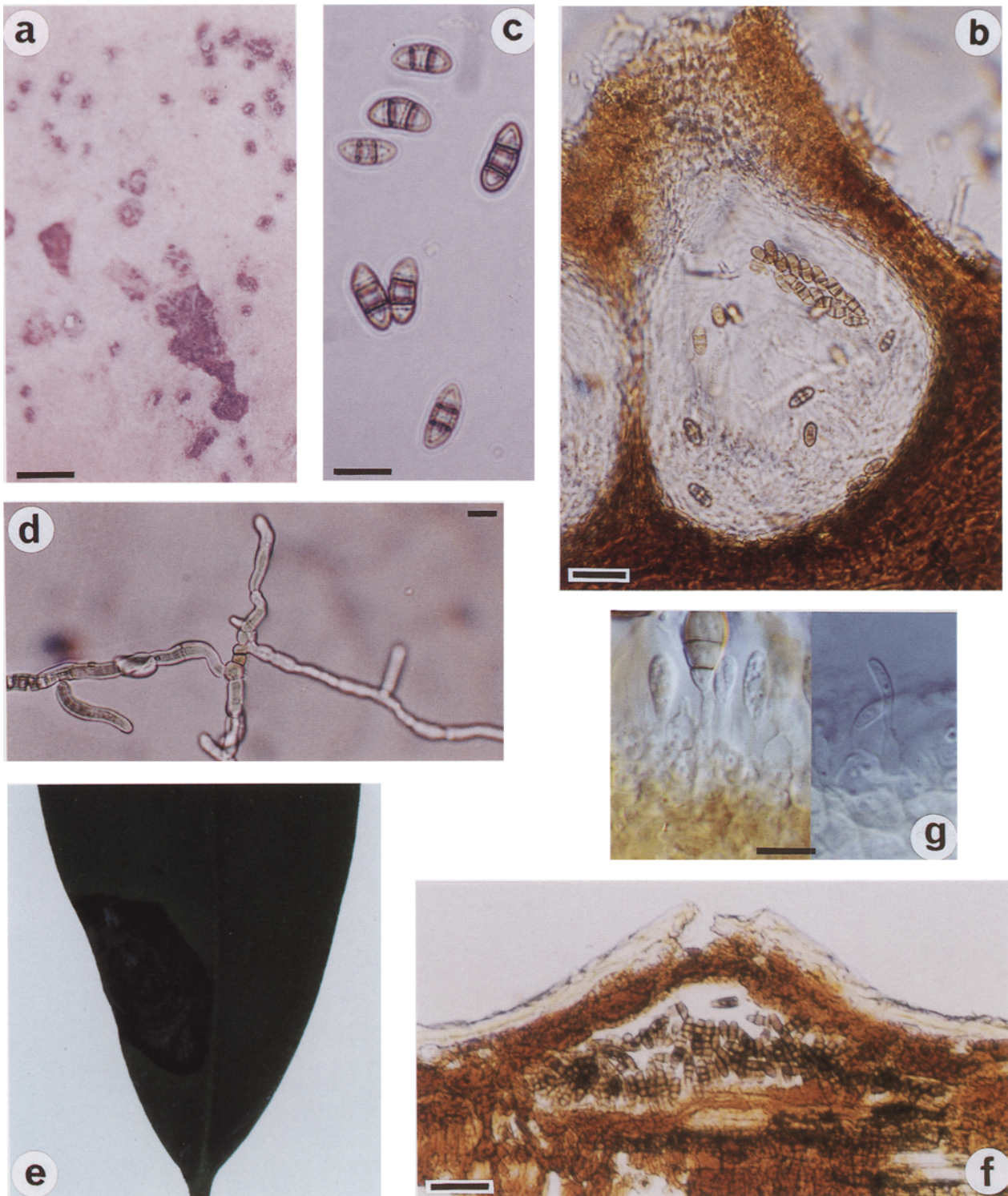


Fig. 3. *Pestalosphaeria gubae* and its anamorph *Pestalotiopsis neglecta*. a: Perithecia of *Pestalosphaeria gubae* produced on a diseased leaf of *Rhododendron hybridum* with many acervuli of *Pestalotiopsis neglecta*, kept in moist chamber for one month. b: Cross section of a perithecium of *Pestalosphaeria gubae*. c: Ascospore of *Pestalosphaeria gubae*. d: Germinating ascospore of *Pestalosphaeria gubae*. e: Leaf spot of *Rhododendron hybridum* caused by *Pestalotiopsis neglecta*. f: Cross section of acervulus of *Pestalotiopsis neglecta* on *Rhododendron hybridum*. g: Conidiogenous cells showing annellation. Scales: a=5 mm, b=30 μ m, c, d, g=10 μ m, f=20 μ m.

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