

Yasunori Ono · Takao Kobayashi

Notes on new and noteworthy plant-inhabiting fungi from Japan (2): *Griphosphaerioma zelvicolae* sp. nov. with *Sarcostroma* anamorph isolated from bark of *Zelkova serrata*

Received: October 18, 2002 / Accepted: January 15, 2003

Abstract A species of *Griphosphaerioma* with a *Sarcostroma* anamorph was collected from the rough bark of *Zelkova serrata*. Based on comparison of morphology with *Griphosphaerioma kansensis*, the fungus was identified as *Griphosphaerioma zelvicolae* sp. nov. The *Sarcostroma* anamorph derived from a single ascospore of the fungus was morphologically identical to a species of *Sarcostroma* that was also found on the same bark material.

Key words Anamorph · Bark inhabitant · *Griphosphaerioma zelvicolae* · New species · *Sarcostroma zelvicolae* · Teleomorph · *Zelkova serrata*

Introduction

In the course of exploratory surveys of plant-inhabiting fungi as producers of secondary metabolites useful to the pharmaceutical industry, we encountered a number of new and noteworthy plant-inhabiting fungi (Ono and Kobayashi 2001).

In this article, *Griphosphaerioma* Höhn. (Amphisphaeriaceae) with a *Sarcostroma* Cooke anamorph is described and illustrated. The genus *Griphosphaerioma* was described by von Höhnel (1918). Shoemaker (1963) studied *Griphosphaerioma kansensis* (Ellis et Everh.) Shoemaker and its anamorph *Labridella cornu-cervae* Brenckle. In his study, he pointed out that “*Griphosphaerioma kansensis* does not have an amyloid ring in the ascus and differs thereby from *Griphosphaeria* Höhn. (= *Discostroma* Clem.) and *Clathridium* Berl. (= *Discostroma*).” Guba

(1961) established a connection between the teleomorph genus *Griphosphaerioma* and the anamorph genus *Labridella* Brenckle based on a culture study of *Pestalotia* (= *Labridella*) *cornu-cervae* (Brenckle) Guba. The genus *Sarcostroma* was newly added as a conidial state of the genus *Griphosphaerioma*.

Materials and methods

Collection, isolation, and optical microscope observation of specimens were carried out following the methods of Ono and Kobayashi (2001).

For scanning electron microscope (SEM) observation, 5-mm-diameter agar disks were cut from the colonies with a sterilized scalpel and fixed in 2% OsO₄ overnight. These disks were dehydrated in graded ethanol and *t*-butyl alcohol series. The disks were subsequently critical pointed dried (VFD-20; Vacuum Device, Ibaraki, Japan), coated with platinum-palladium in an ion sputter (Hitachi E-1030; Hitachi, Tokyo, Japan), and examined using a Hitachi S-4500 scanning electron microscope operating at 5 kV.

Colony characteristics and sporulation were observed at 23°C on a medium of potato dextrose agar (PDA; 39 g Nissui potato-dextrose agar, 1000 ml distilled water) and modified Weitzman and Silva-Hutner agar (WSH; 10 g oatmeal, 1 g KH₂PO₄, 1 g MgSO₄·7H₂O, 1 g NaNO₃, 20 g agar, 1000 ml distilled water). Color designation of colonies follows Kornerup and Wanscher (1978). The specimens cited in this article were deposited in the Herbarium of the Forestry and Forest Product Research Institute (TFM).

Y. Ono (✉)
Lead Discovery Research Laboratories, Sankyo Co., Ltd.,
33 Miyukigaoka, Tsukuba, Ibaraki 305-0841, Japan
Tel. +81-298-56-3987; Fax +81-298-56-0572
e-mail: onoyas@tsuku.sankyo.co.jp

T. Kobayashi
Department of International Agricultural Development, Tokyo
University of Agriculture, Tokyo, Japan

Taxonomy

Teleomorph: *Griphosphaerioma zelvicolae* Yas. Ono et Tak. Kobayashi, sp. nov. Figs. 1 and 2

Ascomata immersa, erumpentia, solitaria vel jugata, obpyriformia, vertice papillata, brunnea vel fusca, 380–

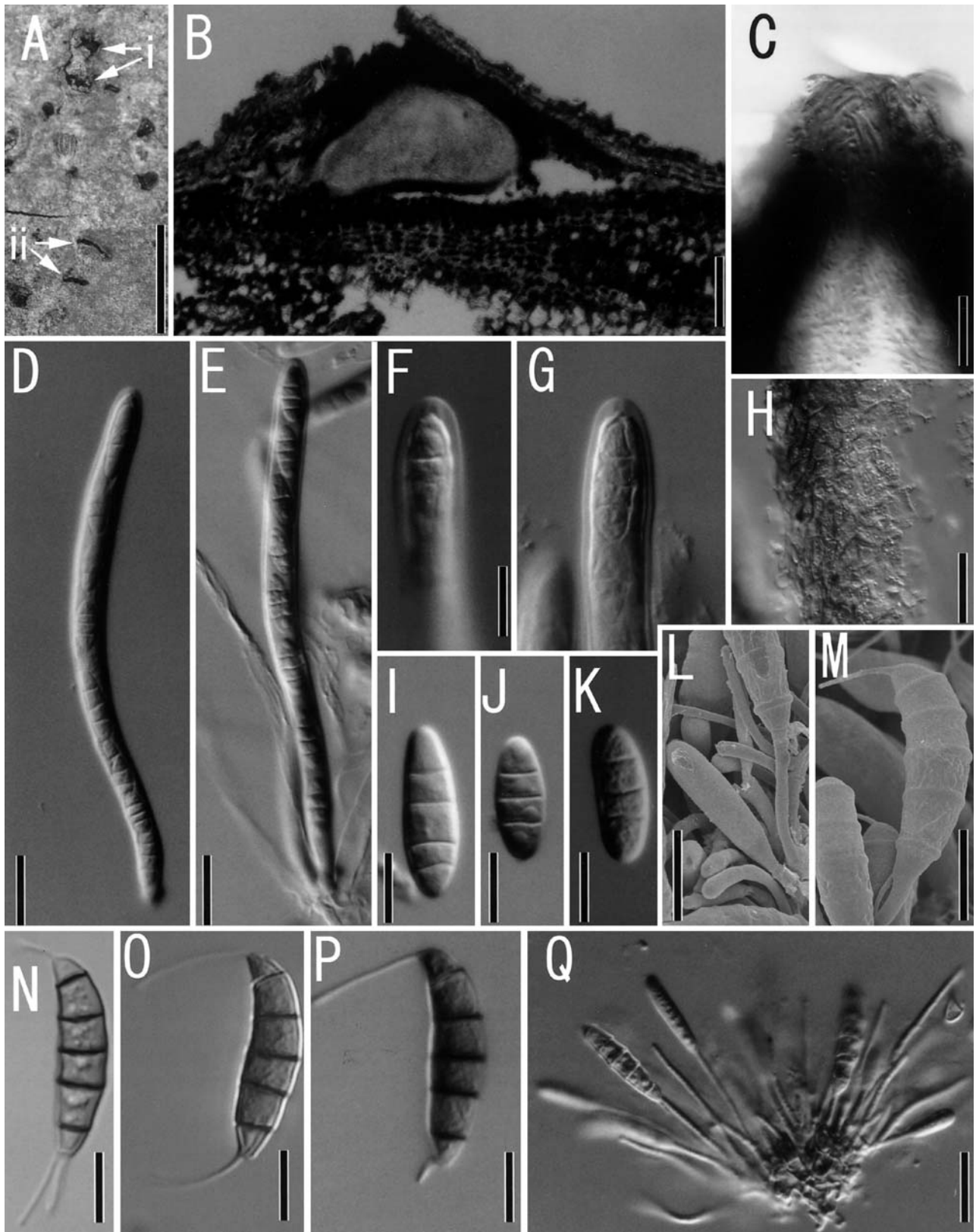
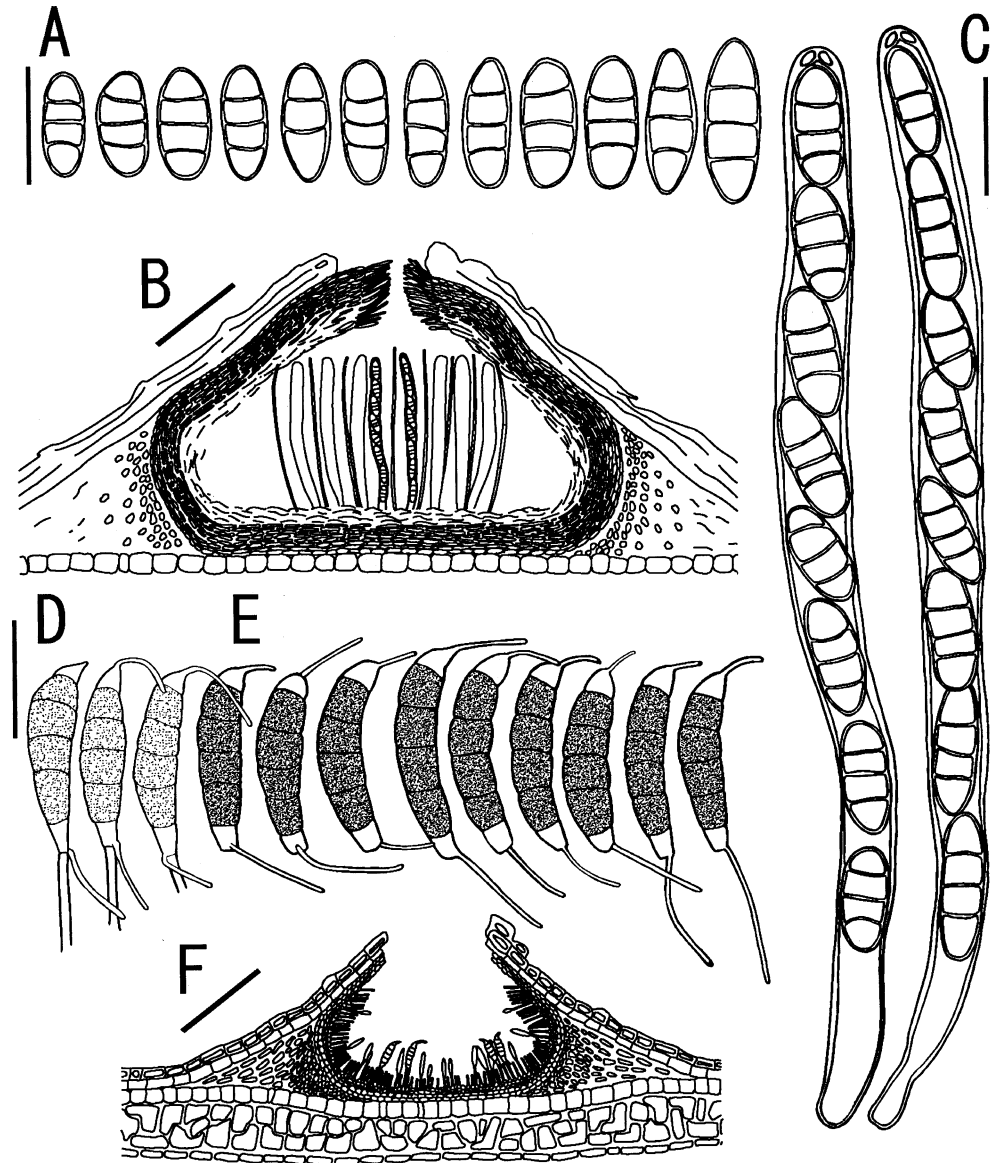


Fig. 1. *Griphosphaerioma zelkovicola* and its anamorph, *Sarcostroma zelkovicola* in nature (TFM: FPH-7595). **A** Perithecia (*i*, arrows) and conidiomata (*ii*, arrows) on rough bark of *Zelkova serrata*. **B–K** *Griphosphaerioma zelkovicola*: **B** cross section of a perithecium; **C** cross section through the neck with periphysis; **D,E** asci; **F,G** ascus with apical apparatus; **H** close-up of side wall of peridium; **I,K** ascospores, **L–Q** *Sarcostroma zelkovicola*: **L,M** conidia and conidiophores (SEM); **N–P** conidia; **Q** conidia and conidiophores. Bars **A** 2 mm; **B** 50 μ m; **C,F–P** 10 μ m; **D,E,Q** 20 μ m.

Fig. 2. *Griphosphaerioma zelkovicola* and its anamorph, *Sarcostroma zelkovicola* in nature (TFM: FPH-7595). **A–C** *Griphosphaerioma zelkovicola*: **A** ascospores; **B** perithecium; **C** asci and ascospores. **D–F** *Sarcostroma zelkovicola*: **D** immature conidia with conidiogenous cells; **E** mature conidia; **F** acervulus. Bars **A,C,D,E** 20 µm; **B,F** 50 µm



900 µm diametro, 240–700 µm longa. Asci unitunicati, cylindrici, octospori, (146–) 156–196 × 8–13 µm. Paraphyses filiformes, simplices, septatae. Ascosporae uniseriatae, ellipticae, transverse 3-septatae, raro 2-, 4-, vel 5-septatae, hyalinae, (13.5–) 16–32.5 (–40) × 6.5–12 (–14) µm.

Holotypus: On bark of *Zelkova serrata* (Thunb.) Makino (Japanese name: keyaki), Ulmaceae, Tsukuba, Ibaraki Prefecture, Oct. 27, 2000, Y. Ono and T. Kobayashi, P-747, TFM: FPH-7595 (ex-type: single-ascospore culture MAFF 238434).

Etymology: Refers to the host plant.

Perithecia immersed, erumpent, solitary or coupled, obpyriform, with a flattened base, papillate with periphysis, brown to blackish brown, without setae, 380–900 µm in diameter, 240–700 µm high. Perithecial wall in transverse section bilayered, 28–40 µm wide at the base; inner layer composed of thick-walled, elongated cells (*textura prismatica*), brown to blackish brown, 16–28 µm wide; inner

layer membranaceous, composed of parallel hyphal cells (*textura porrecta*), hyaline, 6–14 µm wide. Asci arising from a basal hymenium, unitunicate, cylindrical, thick-walled, short-stalked, apically round, with a nonamyloid apical apparatus, 8-spored, (146–) 156–196 × 8–13 µm, 173.5 × 10.3 µm on average. Paraphyses filiform, unbranched, thin-walled, flexuose, septate, hyaline, up to 240 µm long. Ascospores uniseriate in the ascus, ellipsoidal, transversely 3-septate, rarely 2-, 4-, or 5-septate, not constricted at the septa, hyaline, (13.5–) 16–32.5 (–40) × 6.5–12 (–14) µm, 24.4 × 9.3 µm on average.

Anamorph: *Sarcostroma zelkovicola* Yas. Ono et Tak. Kobayashi, sp. nov.

Conidiomata acervuloidea vel pycnidioidea, pycnidia, applanata, semiimmersa vel erumpentia, brunneola vel nigricans, 160–670 µm diametro; stromate basali “*textura angularis*” ex cellulis crassitunicatis brunneis vel fuscis

composito. Conidiophora ex cavitate conidiomatis omnifariam enascentia, simplicia vel basi ramosa, septata, laevia, hyalina. Cellulae conidiogenae holoblasticae, annulatae, laeves, cylindricae, hyalinae. Conidia fusiformia vel subcylindracea, leviter curvata, 5-septata, ad septa non vel vix constricta, (30.5–) 32.5–42.5 (–44.5) × 7–10 μm, apice utrinque appendiculata; cellula basalis obconica, ad basim truncata, hyalina vel subhyalina, 4–6.5 μm longa, ex appendice basali filiformi simplici solitaria exogena excentrica (1.5–) 4–20.5 (–28.5) μm longa praedita; cellulae quatuor medianae subcylindricae ver doliiformes, laeves, pallide brunneae vel olivaceae, (22.5–) 25.5–34.5 longae; cellula apicalis conica, apice rotundata, hyalina vel subhyalina, 3.5–5.5 μm longa, ex appendice apicali filiformi simplici solitaria polari (2–) 5.5–26.5 (–32) μm longa praedita.

Holotypus: On bark of *Zelkova serrata* (Thunb.) Makino (Japanese name: keyaki), Ulmaceae, Tsukuba, Ibaraki Prefecture, Oct. 27, 2000, Y. Ono and T. Kobayashi, P-747, TFM: FPH-7595.

Etymology: Refers to the host plant.

Anamorph in nature: Conidiomata acervuloid, pycnidiod, applanate, semiimmersed to erumpent, brown to black, dehiscing by a break in the overlying host tissue, 160–670 μm in diameter; basal stroma a *textura angularis* composed of thick-walled, brown to blackish-brown cells. Conidiophores lining the cavity of the conidiomata, simple or branched at the base, septate, smooth, hyaline. Conidiogenous cells holoblastic, annellidic, smooth, cylindrical, hyaline. Conidia fusiform to subcylindrical, slightly curved, 5-septate, not constricted or slightly constricted at the septa, (30.5–) 32.5–42.5 (–44.5) × 7–10 μm, 37.8 × 8.7 μm on average, bearing appendages at both ends; basal cells obconic with a truncate base, hyaline to subhyaline, 4–6.5 (average, 5.0) μm long; four median cells subcylindrical to doliiform, smooth, pale brown to olivaceous, (22.5–) 25.5–34.5 (average, 28.9) μm long; apical cells conical with a rounded apex, hyaline to subhyaline, 3.5–5.5 (average, 4.7) μm long. Appendages filiform, unbranched; basal appendage single, unbranched, excentric, (1.5–) 4–20.5 (–28.5) (average, 12.6) μm long; apical appendage single, subpolar, (2–) 5.5–26.5 (–32) (average, 14.7) μm long.

Culture characteristics (MAFF 238434): Colony on PDA attaining a diameter of 29 mm in 21 days at 23°C, plane, velvety to funiculose, Greyish Green (30E5) to Dull Green (30D3); margin indistinct, lacerate; conidiogenesis and soluble pigment not observed; exudate clear; reverse Dark Green (30F6), Dull Green (30D4) at the margin. The mycelium can grow at 5°–35°C but hardly grew at 35°C. Optimal growth temperature was at 15°–30°C. Conidial production was abundant at 25°–30°C. Colony on WSH attaining a diameter greater than 35 mm within 21 days at 23°C, zonate, velvety, Olive Brown (4E3) to Yellowish Grey (4B2); margin entire, Olive (3F4) to Yellowish Grey (3B2); conidiogenesis sparse, superficial; soluble pigment not observed; exudate clear; reverse Pale Yellow (4A3), Brownish Grey (4F2) at the margin. Mycelium superficial or immersed; hyphae branched, septate, pale to dark brown, smooth-walled, 1.5–2.5 μm wide. Conidiomata, conidio-

Table 1. Comparison of chief characters of the present fungus with the related genera in Amphispheeraceae *sensu* Kang et al.

Genus	Ascospores		Color	No. of septa	Anamorph	References
	Ascus tip ^a	Color				
<i>Amphisphaeria</i>	Amyloid	Concolor	Brown	1	<i>Bleptosporium</i>	Kang et al. (1999)
<i>Blotiascospora</i>	Amyloid	Concolor	Brown	3	<i>Seiridium</i>	Shoemaker et al. (1966)
<i>Broomella</i>	Nonamyloid	Versicolor	Hyaline and brown	3	<i>Pestalotiopsis</i> , <i>Truncatella</i>	Nag Raj (1993); Shoemaker and Müller (1963)
<i>Discostroma</i>	Amyloid	Concolor	Hyaline	(0–) 2 (–6)	<i>Seimatosporium</i> , <i>Sporocadus</i>	Brockmann (1976)
<i>Ellurema</i>	Amyloid	Concolor	Hyaline	3	<i>Hyalotiopsis</i>	Kang et al. (1999)
<i>Griphosphaerioma</i>	Nonamyloid	Concolor	Hyaline	1–3	<i>Labridella</i>	Kang et al. (1999)
<i>Leptotypha</i>	Amyloid	Concolor or versicolor	Brown	3	<i>Seiridium</i>	Dulyammode et al. (2001); Kang et al. (1999)
<i>Neobroomella</i>	Nonamyloid	Versicolor	Hyaline and brown	3	<i>Pestalotia</i>	Kang et al. (1999)
<i>Paracainiella</i>	Amyloid	Concolor	Brown	1	<i>Pestalotia</i> -like	Kang et al. (1999)
<i>Pestalotia</i>	Amyloid	Concolor or versicolor	Brown	(1–) 2 (–3)	<i>Pestalotiopsis</i>	Barr (1975); Nag Raj (1985)
Present fungus	Nonamyloid	Concolor	Hyaline	2–5	<i>Sarcostroma</i>	Present study

^a Reaction with or without blue to Melzer reagent

Table 2. Morphological comparison between *Griphosphaerioma zelkovicola* and *G. kansensis*

Species	Asci	Ascospores		Anamorph	References
	Length × Width (µm)	Length × Width (µm)	No. of septa		
<i>G. kansensis</i>	106–136 × 12–14	16–21 × 7–8	1–3	<i>Labridella</i>	Kang et al. (1999)
<i>G. kansensis</i>	75–120 × 8–10	18–22 × 6–7	1–3	<i>Labridella</i>	Shoemaker (1963)
Present fungus	162–190 × 11.5	15.5–30.5 × 7–8	2–5	<i>Sarcostroma</i>	Present study

Table 3. Comparison of conidial morphology between *Sarcostroma zelkovicola* and the six most similar species of *Sarcostroma*^a

Species	Length × Width (µm)	Wall ornamentation	No. of septa	Apical appendages (µm)	Basal appendages (µm)
<i>S. acaciae</i> ^a	13–17 × 6–6.5	Smooth	4	2–4	2–3
<i>S. brevilatum</i> ^a	13–20 × 5–8	Smooth	3	6–18	5–16
<i>S. coryneoides</i> ^a	32–37 × 9–11.5	Reticulose	4–5	4–8	2–7
<i>S. insidens</i> ^a	34–41 × 12–14	Reticulose	5	16–19	13–22
<i>S. mahinapuense</i> ^b	40–57 × 8.5–11	Smooth	5	8–19	11–21
<i>S. plagiochaetum</i> ^a	27–36 × 8–10	Verruculose	5	8–12	4–10
Present fungus ^c	30.5–44.5 × 7–10	Smooth	5	2–26.5	1.5–28.5

^aNag Raj (1993)^bGadgil and Dick (1999)^cPresent study

phores, and conidiogenous cells, same as those in nature. Conidia fusiform to subcylindrical, slightly curved, 5-septate, rarely 3- or 4-septate, not or slightly constricted at the septa, 28.5–35.5 × 6–8 µm, 32 × 7.2 µm on average, bearing appendages at both ends; basal cells obconic with a truncate base, hyaline, 4–6 (average, 5.2) µm long; four median cells subcylindrical to doliform, smooth, pale brown to olivaceous, 20–26.5 (average, 23.6) µm long; apical cells conical with a rounded apex, hyaline, 3–6 (average, 4.5) µm long. Appendages filiform, unbranched; basal appendage single, unbranched, excentric, 4–16.2 (average, 11.4) µm long; apical appendage single, subpolar, 4–24.3 (average, 11.8) µm long. The dimensions of the conidia were a little smaller than those found in nature.

Additional specimens examined. Teleomorph: On bark of *Z. serrata*, Ushiku, Ibaraki Prefecture, May 9, 1999, T. Kobayashi, P-51, TFM: FPH-7598; *ibid.*, Choufu, Tokyo, Sept. 22, 2001, P-1054, TFM: FPH-7597 (culture MAFF 238205). Anamorph: On bark of *Z. serrata*, Tsukuba, Ibaraki Prefecture, April 15, 1999, Y. Ono, P-15, TFM: FPH-7596; *ibid.*, Ushiku, Ibaraki Prefecture, May 9, 1999, T. Kobayashi, P-51, TFM: FPH-7598; *ibid.*, Hiroshima, Hiroshima Prefecture, April 5, 2000, Y. Ono and T. Kobayashi, P-439 (culture MAFF 238436); *ibid.*, Choufu, Tokyo, Sept. 22, 2001, Y. Ono and T. Kobayashi, P-1054, TFM: FPH-7597.

Note: Kang et al. (1999) characterized *Griphosphaerioma* as having unitunicate, cylindrical asci with nonamyloid apical apparatus, and uniseriate, ellipsoidal, hyaline, 1-septate or rarely 2- to 3-septate ascospores. The characteristics of the present fungus are identical to those of the genus *Griphosphaerioma* (Table 1). In number of septa and color of the ascospores, the present fungus is similar to *Discostroma*. However, *Discostroma* are clearly different in that they have an amyloid apical apparatus. The present fungus is also similar to the genus *Broomella* Sacc. and

Neobroomella Petr. in its nonamyloid apical apparatus. However, it can be distinguished from *Broomella* and *Neobroomella* by its brown ascospores with appendages at each end. From these facts, the present fungus was classified in the genus *Griphosphaerioma*. A morphological comparison between the present fungus and *G. kansensis* is shown in Table 2. The present fungus can be distinguished from *G. kansensis* in its longer asci, 2- to 5-septate ascospores, and a *Sarcostroma* anamorph. The present fungus is the second species in the genus *Griphosphaerioma*.

According to Nag Raj, (1993), the anamorph of the present fungus is identical to *Sarcostroma*, having septate conidia with pigmented medium cells, paler or colorless end cells, one unbranched apical appendage, and a similar excentric basal appendage. Morphological comparison between the present anamorphous fungus and six most similar species of *Sarcostroma* is shown in Table 3. The present fungus can be distinguished from the six related species on basis of the following characteristics. The present fungus can be distinguished from *S. coryneoides* (Harkn.) Nag Raj, *S. insidens* (Zabriskie) Nag Raj, and *S. plagiochaetum* (Sacc.) Nag Raj in its smooth conidial wall. The present fungus is different from *S. acaciae* Nag Raj, *S. brevilatum* (H.J. Swart et D.A. Griffiths) Nag Raj, and *S. mahinapuense* Gadgil et M. Dick in conidial size and number of septa.

Determination of the teleomorph–anamorph connection is often carried out to clarify the taxonomy of many kinds of ascomycetes (Hughes 1976; Rossman et al. 1999). Samuels et al. (1987) noted that Amphispheeriaceae *sensu stricto* could be more precisely defined based on its *Pestalotiopsis*-like anamorphs (Table 1). In the genus *Griphosphaerioma*, the reported anamorph belongs to the genus *Labridella* (Kang et al. 1999; Guba 1961; Shoemaker 1963). The genus *Labridella* is a monotypic genus and characterized as having brown, euseptate conidia with one to five laterally branched

apical appendages (Nag Raj 1993). The anamorph of the present fungus is easily distinguished from the genus *Labridella* in its conidia with single basal and apical appendages.

The present species, therefore, was treated as new and named *G. zelkovicola*, with a new anamorphic state *S. zelkovicola*. The present fungus was first reported by Ono and Kobayashi (2002) under the genus *Discostroma* but, after reexamination, it was reidentified as *G. zelkovicola*.

Acknowledgments We are grateful to Ms. E. Masuda, Ms. M. Nakaizumi, and Ms. M. Arai for their excellent technical assistance during the study. We are indebted to Dr. T. Hosoya, who reviewed the manuscript and provided advice and critical comments. We also thank Mr. M. Kitsuka and Mr. R. Enokita for their help in SEM.

References

- Barr ME (1975) *Pestalospaeria*, a new genus in the Amphisphaeriaceae. *Mycologia* 67:187–194
- Brockmann VI (1976) Untersuchungen über die Gattung *Discostroma* Clements. *Sydowia* 28:275–338
- Dulymamode R, Cannon PF, Sivanesan A, Peerally A (2001) Fungi from Mauritius: four new ascomycetes on native plants. *Mycol Res* 105:247–254
- Gadgil PD, Dick M (1999) Fungi silvicolae novaezelandiae: 2. *N Z J For Sci* 29:440–458
- Guba EF (1961) Monograph of *Monochaetia* and *Pestalotia*. Harvard University Press, London.
- Hughes SJ (1976) Sooty moulds. *Mycologia* 68:693–820
- Kang JC, Hyde KD, Kong YC (1999) Studies on Amphisphaeriales: the Amphisphaeriaceae (*sensu stricto*). *Mycol Res* 103:53–64
- Kornerup A, Wanscher JH (1978) Methuen handbook of colour, 3rd edn. Methuen, London
- Nag Raj TR (1985) Redisposals and redescrptions in the *Monochaetia* – *Seiridium*, *Pestalotia* – *Pestalotiopsis* complexes. II. *Pestalotiopsis besseyii* (Guba) comb. nov. and *Pestalospaeria varia* sp. nov. *Mycotaxon* 22:52–63
- Nag Raj TR (1993) Coelomycetous anamorph with appendage-bearing conidia. *Mycologue*, Waterloo, Ontario
- Ono Y, Kobayashi T (2001) Notes on new and noteworthy plant-inhabiting fungi from Japan (1). *Mycoscience* 42:439–446
- Ono Y, Kobayashi T (2002) A new species of *Discostroma* with *Seimatosporium*-anamorph isolated from bark of *Zelkova serrata*. In: 46th annual meeting of Mycological Society of Japan, Nagano, Japan, May 18–19, p 39
- Rossmann AY, Samuels GJ, Rogerson CT, Lowen R (1999) Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). *Stud Mycol* 42:1–248
- Samuels GJ, Müller E, Petrini O (1987) Studies in the Amphisphaeriaceae (*sensu lato*). 3. New species of *Monographella* and *Pestalospaeria*, and two new genera. *Mycotaxon* 28:473–499
- Shoemaker RA (1963) Generic correlations and concepts: *Griphosphaerioma* and *Labridella*. *Can J Bot* 41:1419–1423
- Shoemaker RA, Müller E (1963) Generic correlations and concepts: *Broomella* and *Pestalotia*. *Can J Bot* 41:1235–1243
- Shoemaker RA, Müller E, Morgan-Jones, G (1966) Fuckel's *Massaria marginata* and *Seiridium marginatum* Nees ex Steudel. *Can J Bot* 44:247–254
- von Höhnelt F (1918) Dritte vorläufige Mitteilung mycologischer Ergebnisse (Nr. 201–304). *Ber Dtsch Bot Ges* 36:309–317