Two new species of *Sphaerodes* from Spanish soils

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Abstract: The two new species, *Sphaerodes quadrangularis* and *Sphaerodes tenuissima,* isolated from soil in Spain, are described and illustrated. The former can be easily distinguished from the other species of the genus by the peculiar polygonal section of their ascospores. The most noteworthy feature of *Sphaerodes tenuissima* is the smooth-walled appearance of its ascospores, which is unusual in the genus. The new combinations *Sphaerodes ellipsospora*, *Sphaerodes levita,* and *Sphaerodes singaporensis* are proposed and a key to the accepted species of the genus is provided.

Taxonomic novelties: *Sphaerodes quadrangularis* D. García, Stchigel & Guarro sp. nov., *Sphaerodes tenuissima* D. García, Stchigel & Guarro sp. nov., *Sphaerodes ellipsospora* (Takada) D. García, Stchigel & Guarro comb. nov., *Sphaerodes levita* (Udagawa & Cain) D. García, Stchigel & Guarro comb. nov., *Sphaerodes singaporensis* (Morinaga, Minoura & Udagawa) D. García, Stchigel & Guarro comb. nov.
Key words: *Ceratostomataceae*, *Microthecium,* soil-borne fungi, *Sphaerodes*.

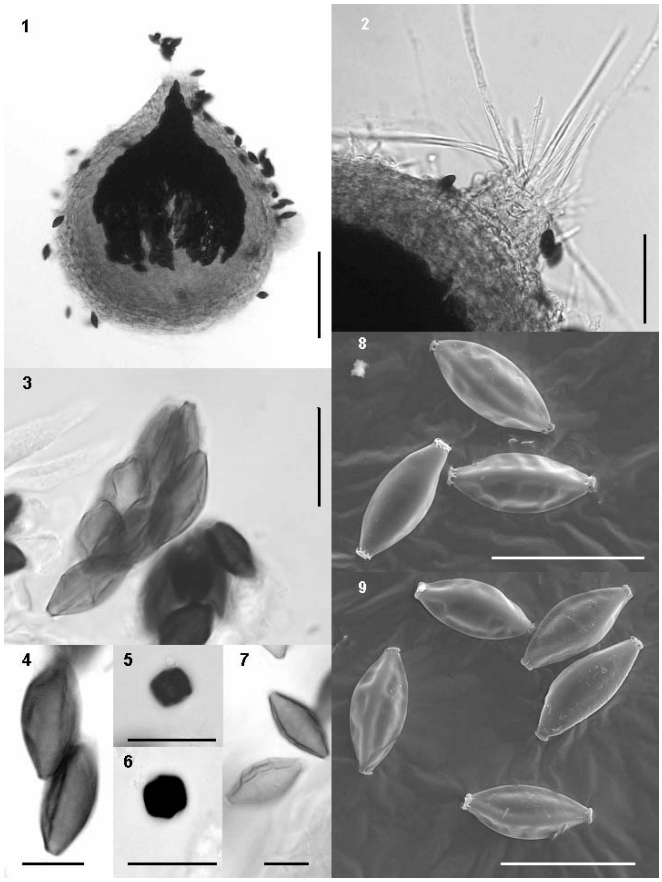
INTRODUCTION

The genera *Melanospora* Corda*, Microthecium* Corda, and *Sphaerodes* Clem. of the family *Ceratostomataceae* (*Hypocreales*) are morphologically very close to each other. They have been differentiated by the presence or not of ascomatal ostioles, the ornamentation of the ascospores, and the structure of the germ pores. Cannon & Hawksworth (1982) reviewed these genera and considered the smooth-walled ascospores with depressed germ pores as exclusive features of *Melanospora*. Consequently, *Microthecium* was considered as synonym. However, the species of *Microthecium* with reticulate ascospores were transferred to *Sphaerodes.* According to this point of view, the genus *Sphaerodes* would currently comprise eight species, characterized by non-ostiolate ascomata (if they are ostiolate they have a short neck surrounded by a crown of hyaline setae) and reticulate ascospores, umbonate at both ends, and with tuberculate germ pores, whose walls support a net-like growth (Cannon & Hawksworth 1982). Recently, Zang & Blackwell (2002) studied the molecular phylogeny of *Melanospora* and related genera; their results showed that *Melanospora* and *Sphaerodes* have a common phylogenetic origin, forming a basal clade within the *Hypocreales*. However, we agree with Eriksson *et al.* (2003) in that further studies are required to decide if both genera should be accepted as different or not. The reduced number of strains included in the analyses by Zang & Blackwell (2002) does not allow us to make a more general conclusion.

During a continuous survey of soil ascomycetes from Spain, two interesting species of *Sphaerodes* were found. Their morphological characteristics differentiated them from all known species of the genus, and therefore they are described here as new. In addition, three new combinations are proposed and discussed.

MATERIAL AND METHODS

The soil samples were collected in different forays (1998–2001) in two Spanish nature reserves. One was in the Valle Tejerua, in the Muniellos Biosphere Reserve, in Asturias Province. It is at 680 m above sea level and surrounded by mountains of more than 1400 m. The annual temperature range is 6–10 ºC and the annual precipitation 1400–1700 mm. Oakwoods, composed mainly by *Quercus petraea* (Matthuschka) Liebl., *Quercus robur* L., and *Quercus* × *rosaceae* Bechst., are the predominant vegetation; the arboreal levels are represented also by *Corylus avellana* L., *Fagus sylvatica* L., *Ilex aquifolium* L., and *Populus* spp. The second area studied was the Umbria de Peña Apartada in Sierra de Espuña, Murcia Province, located at 700–1400 m above sea level. This region is a humidity island in the middle of the half-desert climate, the annual temperature ranges from 13–18 ºC and the annual precipitation ranges from 310–500 mm.



Figs 1–9. *Sphaerodes quadrangularis* (FMR 8049). 1. Ascoma. 2. Detail of the neck. 3. Mature asci. 4–9. Ascospores. 5, 6. Ascospores observed in transversal section. 8, 9. Ascospores (SEM). Scale bars: 1 = 100 µm, 2 = 50 µm, 3, 5, 6, 8, 9 = 20 µm, 4, 7 = 10 µm.

The vegetation is dominated by pinewoods composed mainly of *Pinus halepensis* Mill., *Pinus pinaster* Aiton, and *Pinus nigra* Link. In addition, *Quercus rotundifolia* Lam., *Quercus faginea* Tenore, and *Sorbus aria* Crantz are frequently interspersed with the pinewoods. The methods used for sampling, isolation and morphological study of the fungal isolates were described previously (García *et al.* 2002). Colour notations in parenthesis are from Kornerup & Wanscher (1984) (M. = Methuen). Reference strains included in the study were provided by NBRC (Biological Resource Centre, National Institute of Technology and Evaluation; Chiba, Japan).

TAXONOMY

Sphaerodes quadrangularis D. García, Stchigel & Guarro, **sp. nov.** MycoBank MB500012. Figs 1–11, 29.

Etymology: *quadrangularis* (L) quadrangular; referring to the most frequent shape of ascospores in transverse section.

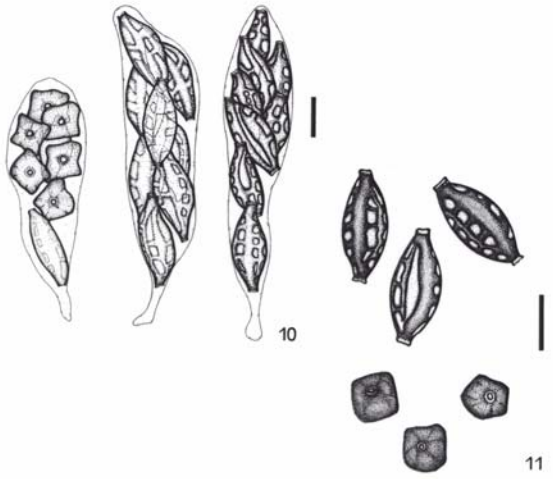
Mycelium ex hyphis hyalinis vel dilute flavo-brunneis, septatis, ramosis, anastomosantibus, laevibus, 2–6 µm diam compositum. Ascomata superficialia vel immersa, dispersa vel in catervis parvis aggregata, pyriformia vel subglobosa, ostiolata, flavo-brunnea, 190–220 µm longa, 135–180 µm diam. Collum conicum, (40–)50–60 µm longum, 65–90 µm latum ad basim, setosum. Peridium membranaceum, 5–10 stratis compositum, 40–55 µm crassum, textura angulari, setosum, setae rectae vel parum curvae, hyalinae vel dilute flavae, crassitunicatae, 82–150 µm longae, 6–10 µm latae ad basim, septatae. Asci 8-spori, ovoidei vel clavati, 70–90 × 20–22 µm, superne late rotundati, brevistipitati, tenuitunicati, evanescentes. Paraphysis nullis. Ascosporae unicellulares, irregulariter biseriatae, primum hyalinae, deinde brunneae vel atrobrunneae, crassitunicatae, fusiformes, 23–28(–30) × 10–12 µm, reticulatae, costis protrudentbus, e polo visae polygonales, utrinque umbonatae, foramine germinali praeditae. Status conidialis nullus.

Mycelium composed of hyaline to pale yellowish brown, septate, branched, smooth-walled hyphae, 2–6 µm diam. *Ascomata* superficial to immersed, scattered or aggregated in small groups, pyriform to subglobose, ostiolate, with a conical neck, yellowish brown to black due to mass of mature ascospores, 190–220 high µm, 135–180 µm diam. *Neck* short, conical (40–)50–60 µm long, 65–90 µm wide at the base, surrounded by an upright crown of setae, 45–200(–300) × 5–7 µm. *Peridium* membranaceous, 5–10-layered, 40–55 µm thick, translucent, pale yellow to yellowish brown, with *textura angularis,* composed by polygonal cells of 15–25 µm diam, setose; setae straight, hyaline to pale yellow, thick-walled, 82–150 × 6–10 µm. *Asci* 8-spored, ovoid to clavate, 70–90 × 20–22 µm, rounded at the apex, without apical structures, short stipitate, thin-walled and evanescent. *Paraphyses* absent. *Ascospores* 1-celled, irregularly biseriate, at first hyaline becoming brown to dark brown, thick-walled, fusiform, 23–28(–30) × 10–12 µm, reticulate, with 4–6 prominent longitudinal ribs, polygonal in transversal section, with a strongly umbonate germ pore at each end. *Anamorph* unknown.

Cultural characteristics: Colonies on PCA growing rapidly, attaining 50 mm diam after 14 d at room temperature (22–25 ºC), flat, consisting of submerged mycelium and sparse aerial hyphae, granulose and dark brown (M. 6F7) due to production of abundant ascomata; reverse brown (M. 6E5). Colonies on OA and MEA growing rapidly, attaining 85–90 mm diam after 14 d at room temperature, granulose and dark brown due the production of abundant ascomata (M. 6F7). At 15 ºC restricted growth on PCA, but growing rapidly on OA and MEA attaining 76–90 mm diam after 14 d in both media, ascomata abundantly produced. At 35 ºC no growth.

Specimen examined: **Spain**, Asturias, Muniellos Biosphere Reserve, Valle Tejerua, soil, 25 Jun. 1998, A.M. Stchigel & M. Calduch, **holotype** FMR 8049, culture ex-type CBS 112763.

Notes: *Sphaerodes quadrangularis* is morphologically similar to *S. fimicola* (E.C. Hansen) P.F. Cannon & D. Hawksw. and *S. micropertusa* Y. Horie, Udagawa & P.F. Cannon. The two latter species also have ostiolate ascomata and coarsely reticulate ascospores with strongly umbonate germ pores (Cannon & Hawksworth 1982, Horie *et al.* 1986). However, *S. quadrangularis* is easily distinguished by its polygonal section, which is circular in the other two species.

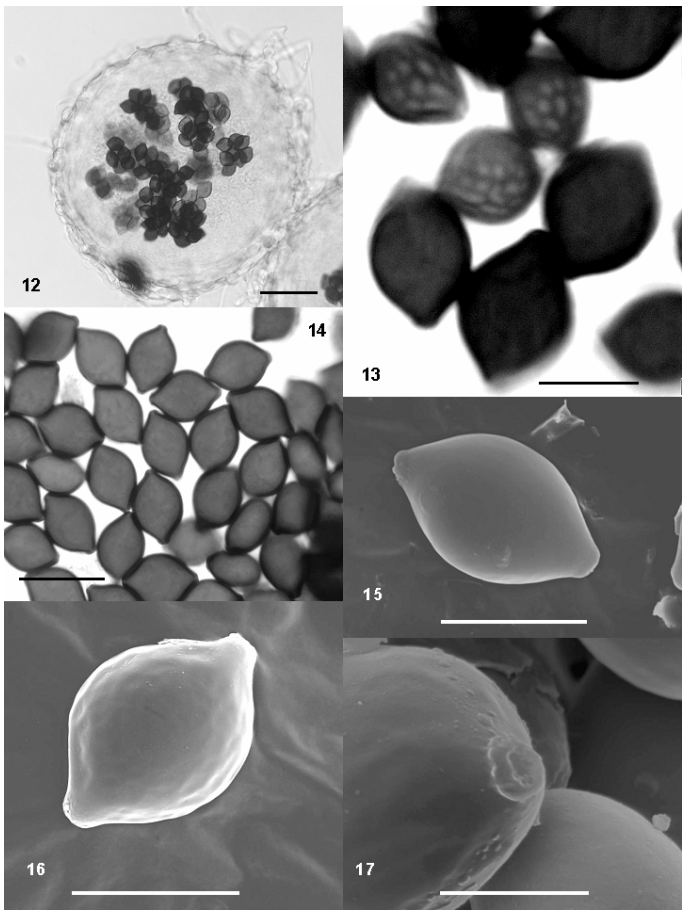


Figs 10–11. *Sphaerodes quadrangularis* (FMR 8049). 10. Asci. 11. Ascospores. Scale bars = 10 µm

Sphaerodes tenuissima D. García, Stchigel & Guarro, **sp. nov.** MycoBank MB500013. Figs 12–20, 32.

Etymology: *tenuis* (L) fine; referring to the inconspicuous ornamentation of the ascospores wall.

Mycelium ex hyphis hyalinis vel dilute flavo-brunneis, septatis, ramosis, anastomosantibus, laevibus, 2–8 µm diam compositum. Ascomata superficialia vel immersa, dispersa vel in catervis parvis aggregata, globosa vel subglobosa, non-ostiolata, pallide flavo-brunnea, 52–120 µm alta, 50–95 µm diam. Peridium membranaceum, e 3–5 stratis compositum, 10–20 µm crassum, textura angulari, glabrum vel leniter setosum; setae rectae vel parum curvae, 60–125 µm longae, 3–7 µm latae ad basim, hyalinae vel dilute flavo-brunneae, distanter septatae, crassitunicatae. Asci 8-spori, clavati, 40–55 x 20–25 µm, superne late rotundati, brevistipitati, tenuitunicati, evanescentes. Paraphysis nullis. Ascosporae unicellulares, irregulariter biseriatae, primum hyalinae, deinde brunneae vel atrobrunneae, crassitunicatae, leniter compressae, 19–23 × (12–)14–15 (–17) × 10–13 µm, citriformes, e latere visae ellipsoideae, tenuiter reticulatae, utringue umbonate, foramine germinali utrinque praeditae. Status conidialis nullus.



Figs 12–17. *Sphaerodes tenuissima* (FMR 8048). 12. Ascoma with asci. 13, 14. Ascospores. 15–17. Ascospores (SEM). 17. Detail of a pore of an ascospore. Scale bars: 12 = 50 µm, 13, 15, 16 = 10 µm, 14, = 20 µm, 17 = 5 µm.

Mycelium composed of hyaline to pale yellowish brown, septate, branched, smooth-walled hyphae, 2–8 µm diam. *Ascomata* superficial to immersed, scattered or aggregated in small groups, globose to subglobose, non-ostiolate, pale yellowish brown to black due to ascospores mass, 52–120 µm high, 50–95 µm diam. *Peridium* membranaceous, 3–5-layered, 10–20 µm thick, translucent, pale yellow to yellowish brown, with *textura angularis,* composed by polygonal cells of 16–30 µm diam, glabrous to slightly setose, setae upright to slightly curved, 60–125 µm long, 3–7 µm diam at base, hyaline to pale yellowish brown, remote septate or non-septate, thick-walled. *Asci* 8-spored, clavate, 40–55 × 20–25 µm, rounded at the apex, without apical structures, short stipitate, thin-walled and evanescent. *Paraphyses* absent. *Ascospores* 1-celled, irregularly biseriate, at first hyaline becoming brown to dark brown, thick-walled, slightly bilaterally flattened, 19–23 × (12–)14–15 (–17) × 10–13 µm, citriform, ellipsoidal in lateral view, apparently smooth under light microscopy, finely reticulated under scanning electron microscopy; with terminal, strongly apiculate germ pores at each end. *Anamorph* unknown.

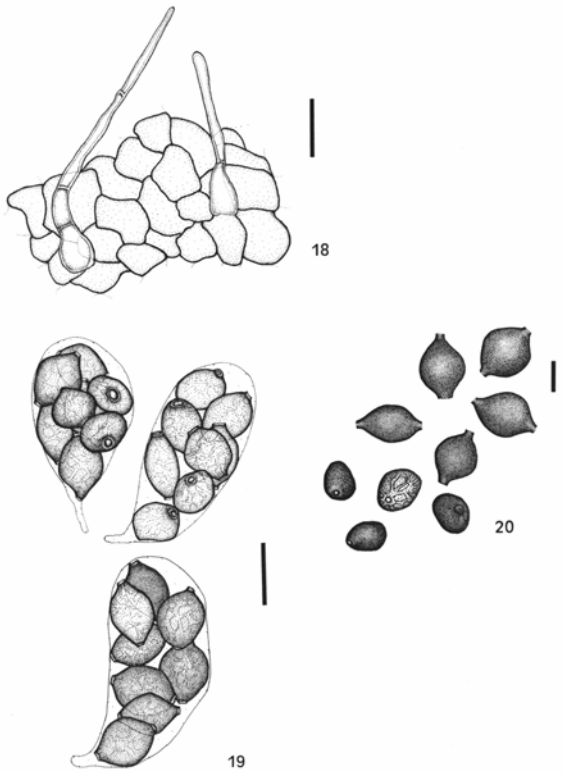
Cultural characteristics: Colonies on PCA growing rapidly, attaining 90 mm diam in 14 d at room temperature (22–25 ºC), consisting of submerged mycelium and aerial hyphae, granulose and brown (M. 6E6) due to production of abundant ascomata; reverse greyish brown (M. 6D4). Colonies on OA and MEA growing rapidly, attaining 90 mm diam in 14 d at room temperature, dark brown due to the production of abundant ascomata (M. 6E7). At 15 ºC growing rapidly on all media, attaining 65–90 mm diam in 14 d, with abundant aerial mycelia, ascomata abundantly produced. At 35 ºC no growth.

Specimen examined: **Spain**, Murcia, Sierra de Espuña Natural Reserve, Umbría de Peña Apartada, soil, 10 Oct. 2001, G. Sisó, **holotype** FMR 8048, culture ex-type CBS 112764.

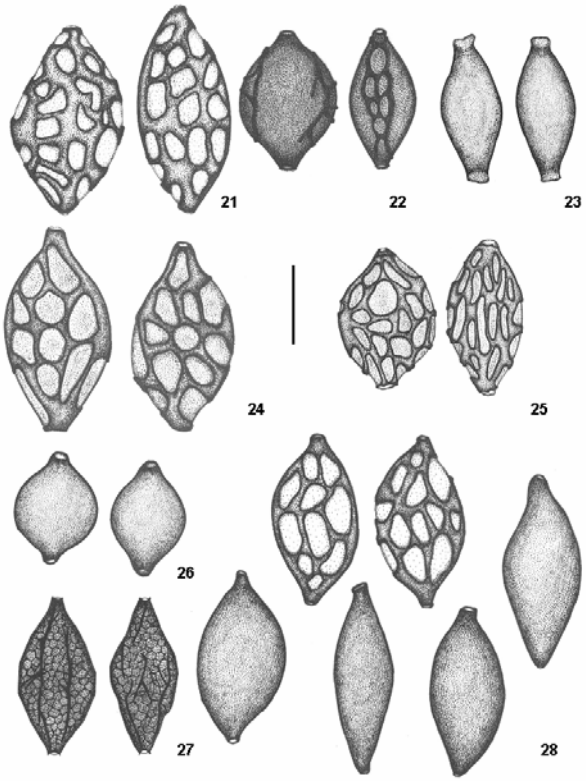
Additional cultures examined: *Sphaerodes compressa* NBRC 8627, NBRC 8659, and NBRC 30249.

Notes: *Sphaerodes tenuissima* is morphologically similar to *Microthecium ellipsosporum* Takada, *M. levitum* Udagawa & Cain, and *Melanospora singaporensis* Morinaga, Minoura & Udagawa. They all have smooth ascospores with strongly umbonate ends. *Microthecium ellipsosporum* can be easily differentiated from *S. tenuissima*, because *M. ellipsosporum* has fusiform ascospores (Takada 1973). *Microthecium levitum* and *M. singaporensis* have citriform ascospores, but they are smaller (10–17 × 8–12 µm and 16–18 × 6–7 µm, respectively) (Udagawa & Cain 1969, Morinaga *et al.* 1978) than those of *S. tenuissima*. The inconspicuous ornamentation of the ascospores of *S. tenuissima* is similar to *S. compressa* (Udagawa & Cain) P.F. Cannon & D. Hawksw. However, the ascospores of the latter are bilaterally flattened and their narrower sides are coarsely reticulate (Udagawa & Cain 1969, Cannon & Hawksworth 1982). In addition, *S. compressa* has a phialidic anamorph while in *S. tenuissima* the anamorph is absent.

Several species of *Ceratostomataceae*, such as *M. levitum*, *M. ellipsosporum*, and *M. singaporensis*, have smooth ascospores with tuberculate germ pores, which can be considered as intermediate between *Melanospora* and *Sphaerodes.* In spite of this, Cannon & Hawksworth (1982) have maintained them in their original genera. We agree with Zhang & Blackwell (2002) who on the basis of molecular studies concluded that in *Ceratostomataceae* ascospore shape and the structure of the germ pores are more informative characters than wall ornamentation. Therefore, the presence of reticulate walls is not considered a distinctive character of *Sphaerodes.* This is corroborated by the fact that within a given species, e.g. *S. perplexa,* both types of ascospores, smooth and reticulate, are present (Fig. 28). Accordingly, and emphasising the presence of tuberculate germ pores as a key feature in *Sphaerodes*, the following new combinations are proposed:



Figs 18–**20.** *Sphaerodes tenuissima* (FMR 8048). 18. Detail of peridium. 19. Asci with ascospores. 20. Ascospores. Scale bars 18, 19 = 20 µm, 20 = 10 µm.



Figs 21–**28.** Ascospores of *Sphaerodes*. 21. *S. beatonii.* 22. *S. compressa.* 23. *S. ellipsospora*. 24. *S. episphaeria*. 25. *S. fimicola*. 26. *S. levita*. 27. *S. micropertusa*. 28. *S. perplexa.* Scale bar = 10 µm.

Sphaerodes ellipsospora (Takada) D. García, Stchigel & Guarro, **comb. nov.** MycoBank MB500014. Fig. 23.
Basionym: *Microthecium ellipsosporum* Takada, Bull. N. S. Museum. 16: 527. 1973.

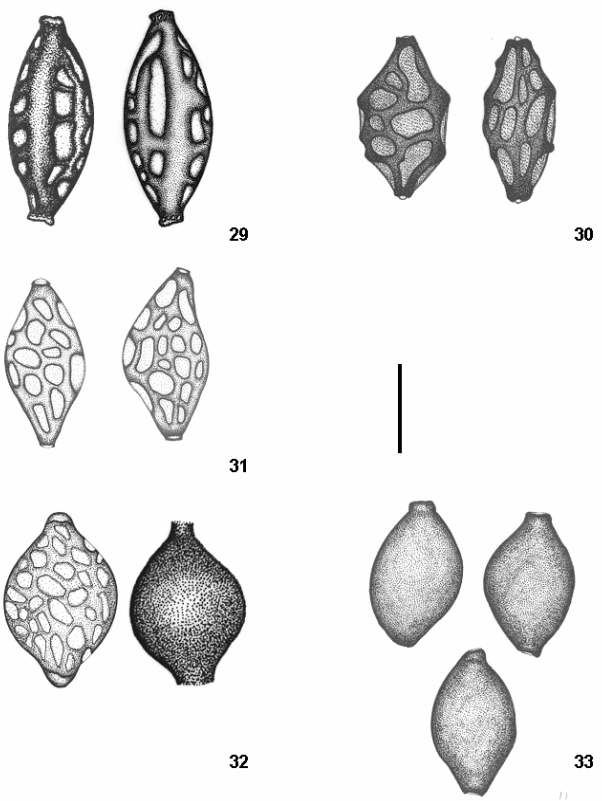
Culture examined: NBRC 31376 (culture ex-holotype).

Sphaerodes levita (Udagawa & Cain) D. García, Stchigel & Guarro, **comb. nov.** MycoBank MB500015. Fig. 26.
Basionym: *Microthecium levitum* Udagawa & Cain, Can. J. Bot. 47: 1917. 1969.

Culture examined: NBRC 8204 and NBRC 33029.

Sphaerodes singaporensis (Morinaga, Minoura & Udagawa) D. García, Stchigel & Guarro, **comb. nov.** MycoBank MB500016. Fig. 32.
Basionym: *Melanospora singaporensis* Morinaga, Minoura & Udagawa Trans. Mycol. Soc. Japan 19: 142. 1978.

Culture examined: NBRC 30865 (culture ex-holotype).



Figs 29–33. Ascospores of *Sphaerodes*. 29. *S. quadrangularis*. 30. *S. retispora* var. *retispora*. 31. *S. retispora* var. *inferior*. 32. *S. tenuissima.* 33. *S. singaporensis.* Scale bar = 10 µm.

Key the species of *Sphaerodes*

1. Ascomata ostiolate 2
1. Ascomata non-ostiolate 5

2. Ascospores coarsely reticulate 3
2. Ascospores smooth-walled, strongly umbonate at both ends, 15–18 × 9–11 µm ..... ***S. singaporensis*** (Fig. 33)

3. Ascospores fusiform 4
3. Ascospores citriform, 14–26 × 10–17 µm ..... ***S. fimicola*** (Fig. 25)

4. Ascospores delicately pitted, circular in 2 × transverse section, 18–22(–28) × 9–11(–13) × 8–9 µm. ..... ***S. micropertusa*** (Fig. 27)
4. Ascospores reticulate, polygonal in 2 × transvere section, 23–28 × 10–12 × 8–10 µm ..... ***S. quadrangularis*** (Fig. 29)

5. Ascospores longer than 20 µm 6
5. Ascospores shorter than 20 µm 9

6. Asci 8-spored 7
6. Asci 4-spored 8

7. Ascospores coarsely reticulate, 25–34 × 12–18 µm ..... ***S. episphaeria*** (Fig. 24)
7. Ascospores with indistinct reticulation, 19–23 × 12–17 µm ..... ***S. tenuissima*** (Fig. 32)

8. Ascospores (25–)28–34 × 14–16(–18) µm; coarsely reticulate ..... ***S. beatonii*** (Fig. 21)
8. Ascospores 22–28 × 12–15 × 9–11 µm; both types of ascospores, smooth and reticulate, present in a single strain ..... ***S. perplexa*** (Fig. 28)

9. Ascospores smooth, umbonate, and with a tuberculate germ pore at each end.....10
9. Ascospores reticulate.....11

10. Ascospores citriform, 10–17 × 8–12 × 9–10 µm; phialidic anamorph present .....***S. levita*** (Fig. 26)
10. Ascospores fusiform, 16–18 × 6–8 µm; anamorph absent .....***S. ellipsospora*** (Fig. 23)

11. Ascospores reticulate..... 12
11. Ascospores smooth and with a narrower sides reticulate ..... ***S. compressa*** (Fig. 22)

12. Ascospores prominently reticulated..... ***S. retispora*** var. ***retispora*** (Fig. 30)
12. Ascospores inconspicuously reticulated..... ***S. retispora*** var. ***inferior*** (Fig. 31)

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