
***Trematosphaeria*: taxonomic concepts, new species from Japan and key to species**

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Two new species, *Trematosphaeria crassiseptata* collected from dead twigs of an unknown plant near river, and *T. biappendiculata* collected from submerged twigs in ponds, are described and illustrated. *Trematosphaeria crassiseptata* is characterised by thick-walled, reddish-brown and 5-septate ascospores, while *T. biappendiculata* is distinguished by its ascospores with terminal appendages. In culture media, *T. crassiseptata* produced the teleomorph state, and *T. biappendiculata* produced a *Pleurophomopsis*-like microconidial state only. The differences between these new species and their related taxa are noted. A key of the 15 species accepted in the genus is given.

Key words: aquatic fungi, *Melanommataceae*, *Pleosporales*, taxonomy.**Introduction**

Trematosphaeria was established by Fuckel in 1870 (Boise, 1985). *Trematosphaeria pertusa* (Pers.: Fr.) Fuckel is the lectotype species (Clements and Shear, 1931). Most species of the genus are saprotrophs or hemibiotrophs of terrestrial woody plants (Boise, 1985), but three species: *T. confusa* (Garov.) Boise & D. Hawksw., *T. hydrela* (Rehm) Sacc. and *T. pertusa*, are reported from freshwater habitat (Shearer, 1993; Hyde, 1995), and three other species: *T. mangrovei* Kohlm., *T. lineolatispora* K.D. Hyde and *T. malaysiana* Alias, Mckeown, S.T. Moss & E.B.G. Jones, are known from marine habitats (Mckeown *et al.*, 2001).

Trematosphaeria is characterised by medium to large sized ascomata with peridia composed of small compressed cells, clavate to cylindrical bitunicate asci, a trabeculate hamathecium in a gelatinous matrix and reddish-

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brown ellipsoid to fusoid tranversely septate ascospores sometimes with paler end cells (Boise, 1985; Barr, 1990; Mckeown *et al.*, 2001).

Some species of *Astrosphaeriella* Syd. & P. Syd., like *A. striataspora* (K.D. Hyde) K.D. Hyde (1988, 1992a) and *A. pallidipolaris* C.Y. Chen & H.W. Hsieh (2004) resemble to some degree *Trematosphaeria* species. However, *Astrosphaeriella* is distinguished from the latter by the extremely slender ascospores and its monocotyledonous host, particularly on bamboos and palms (Hyde and Fröhlich, 1998).

No anamorph connection is known in *Trematosphaeria* (Boise, 1985). *Phoma* Sacc. (Arx and Müller, 1975) or *Aposphaeria* Sacc. (Samuels and Müller, 1978) have been reported as an anamorph state of the genus from cultural studies of *T. heterospora* (De Not.) G. Winter. However, *T. heterospora* is now placed in *Phaeosphaeria* I. Miyake (Boise, 1985) or *Lophiostoma* Ces & De Not. (Barr, 1992; Ahn and Shearer, 1999). Likewise, although the *Zalerion* R.T. Moss & Meyers state has been reported for an anamorph of *T. fallax* Mouton (as *T. clarkii* Sivan.; Fisher and Webster, 1992), the species is now treated as *Hadrospora fallax* (Mouton) Boise (Webster, 1993; Tanaka and Harada, 2003b).

Traditionally lignicellulosic fungi similar to *Leptosphaeria* Ces. & De Not., but with paler ascospore end cells have been placed in *Trematosphaeria* (Crane and Shearer, 1991; Crane *et al.*, 1992). More than 190 taxa have been described in the genus (Anonymous, 2004), but Boise (1984, 1985) accepted only five species in the genus mainly based on the trabeculate hamathecium which is an important criterion for melanommataceous fungi. This strict generic concept is accepted by many authors (e.g. Barr, 1990; Hyde and Goh, 1999) and the genus is assigned to the *Melanommataceae* in the *Pleosporales* (Kirk *et al.*, 2001). After the revision by Boise (1985), eight species have been added to the genus (Holm and Holm, 1988; Shoemaker and Babcock, 1989; Eriksson and Yue, 1990; Hyde, 1992b; Barr *et al.*, 1996; Barr and Mathiassen, 1998; Hyde and Fröhlich, 1998; Mckeown *et al.*, 2001), but some species do not have trabeculae. For example, the pseudoparaphyses of *T. lineolatispora* are described as cellular (Hyde, 1992b), and hamathecial tissues are lacking in *T. crustacea* (Rehm) O.E. Erikss. & J.Z. Yue (Eriksson and Yue, 1990). Recent molecular studies have indicated that the morphology of hamathecium (cellular pseudoparaphyses or trabeculae) appears to be of minor importance in phylogeny at the family level (Liew *et al.*, 2000; Lumbsch and Lindemuth, 2001). Evaluation of taxonomic importance of trabeculae at the generic level is, however, required.

Materials and methods

The methods of microscopic observation, single spore isolation and induction of anamorph / teleomorph formation are those described in Tanaka and Harada (2003a). Specimens cited in this paper were mainly maintained at the Herbarium of Hirosaki University, Fungi (HHUF), and some isotype or paratype materials were preserved at IMI. All culture strains were deposited at the culture collection of MAFF and JCM.

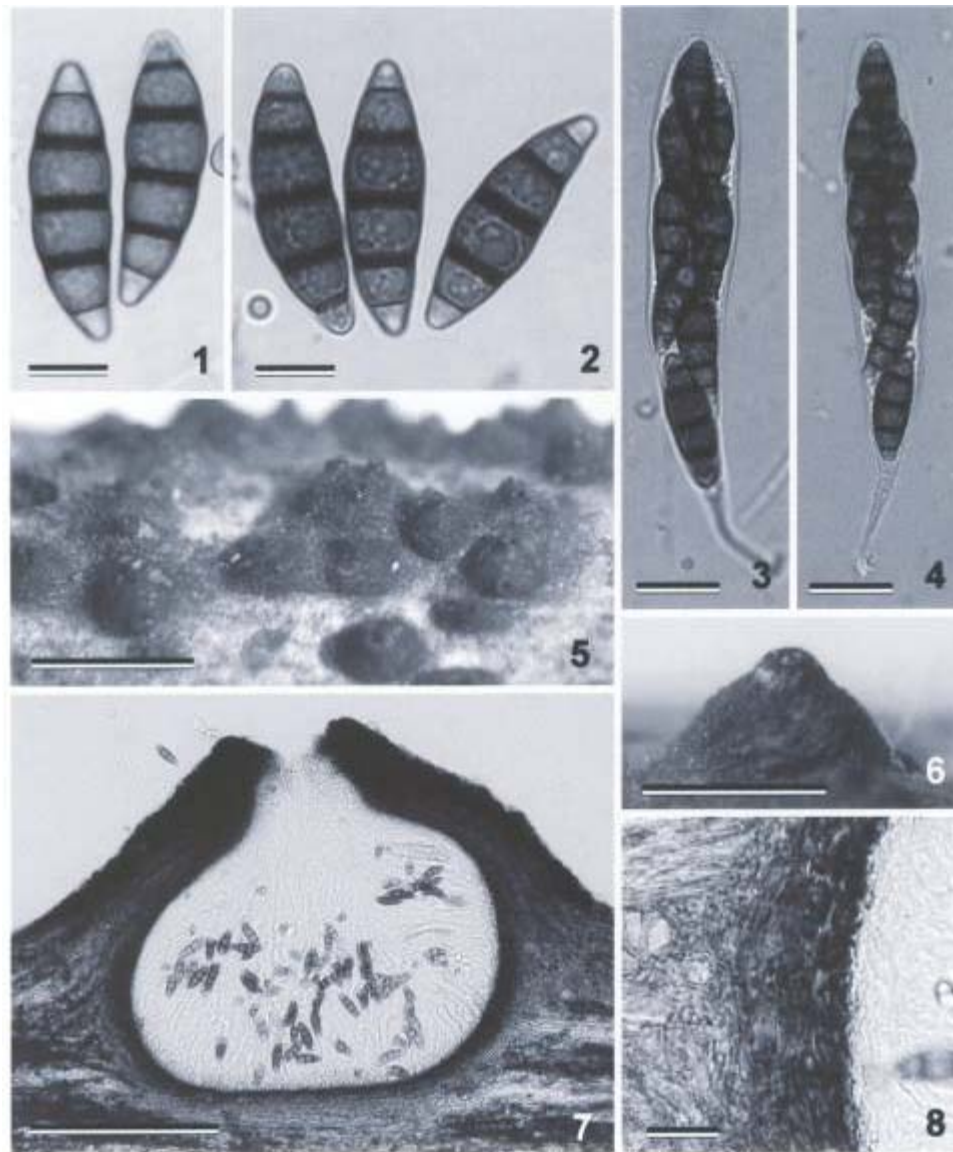
Taxonomy

***Trematosphaeria crassiseptata* Kaz. Tanaka, Y. Harada & M.E. Barr, sp. nov.**
(Figs. 1-8, 20-22)

Etymology: From the Latin prefix *crassi-* meaning “thick-” and *septatus* meaning “septate” in reference to the thick-walled septa of ascospores.

Ascomata 300-500 μm alta, 400-530 μm diam, subglobosa, dispersa vel aggregata, erumpentia vel paene superficialia, cum ostiolo rotundato. *Rostrum* 90-120 μm longum, 100-130 μm diam, centrale, papillatum. *Paries ascomatis* ad latus 17-25 μm crassus, ex cellulis brunneis compressis 5-12.5 \times 2.5-4 μm compositus; ad basim 7-13 μm crassus; circum rostrum ex cellulis pachydermicis parvis nigris compositus. *Hamathecium* trabeculatum, 1.5-2.5 μm latum, ramificans et anastomosans, per spatia 8-15 μm longa septatum. *Asci* (98-)118-145(-160) \times 17.5-24.5 μm , clavati, fissitunicati, apice rotundati vel aliquando obtusi, tetra- vel octospori. *Ascospores* (27-)28.5-35(-38) \times 8-10.5(-11.5) μm , late fusiformes, 5-septatae, infirme constrictae ad septum primum submedianum, badiae, cum cellulis extremis flavidis praeditae, laeves, guttulate, cum tunica nec gelatinosa nec appendiculata.

Ascomata 300-500 μm high, 400-530 μm diam, subglobose, scattered to clustered, erumpent to superficial, with a rounded ostiole. *Beak* 90-120 μm long, 100-130 μm diam, central, papillate. *Ascomal wall* at sides 17-25 μm thick, composed of brown compressed cells of 5-12.5 \times 2.5-4 μm ; at the base 7-13 μm thick; around the beak composed of thick-walled small black cells. *Hamathecium* trabeculate, 1.5-2.5 μm thick, branched and anastomosed, with septa at 8-15 μm intervals. *Asci* (98-)118-145(-160) \times 17.5-24.5 μm (mean = 133 \times 20.8 μm , $n = 21$), clavate, fissitunicate, rounded to somewhat obtuse at the apex, with an apical chamber, short-stalked (12-45 μm long), (4-)8-spored. *Ascospores* (27-)28.5-35(-38) \times 8-10.5(-11.5) μm (mean = 31.8 \times 9.7 μm , $n = 100$), L/W 3.0-3.7 (mean = 3.3, $n = 100$), broadly fusiform with slightly rounded ends, slightly curved, 5-septate, with a primary septum near median (mostly submedian; 0.49-0.54, mean = 0.51, $n = 70$), weakly constricted at the primary septum, not constricted at other septa, widest at the third cell from the apex, brown to reddish brown at 4 central cells, pale yellow at both end cells, smooth, guttulate, without sheath or appendages.



Figs. 1-8. Micrographs of *Trematosphaeria crassiseptata*. **1, 2.** Ascospores. **3, 4.** Asci. **5, 6.** Ascomata on host surface. Note the rounded ostiole. **7.** Ascoma in longitudinal section. **8.** Ascomal wall at side. (1, 5-8 from HHUF 28601; 2-4 from culture MAFF 239516). Bars 1, 2 = 10 μm ; 3, 4, 8 = 20 μm ; 5 = 1 mm; 6 = 500 μm ; 7 = 200 μm .

Cultural characteristics: Colonies on potato dextrose agar (PDA; Difco, Detroit, MI, USA) 25-27 mm in diam. after 4 weeks at 20°C in the dark, Greenish-Grey (30C2; Kornerup and Wanscher, 1978) with white margin; reverse Dull-Green (25E3) with Yellowish-Grey (3C2) margin; no pigment

produced. On rice straw agar (RSA; Tanaka and Harada, 2003a), a teleomorph state formed on the surface of rice straws within 3 months. The ascospores are similar to those found from nature, (28.5-)31.5-37 × 9-11 μm (mean = 33.7 × 10.1 μm, *n* = 100), L/W 3.1-3.6 (mean = 3.3, *n* = 100), mostly with a submedian primary septum at 0.48-0.54 (mean = 0.52, *n* = 61).

Anamorph: Unknown.

Habitat: Saprobic on dead twigs of an unknown plant near river.

Known distribution: Northern JAPAN.

Material examined: JAPAN, Toyohira-riv., riverbank, Sapporo, Hokkaido (141°21.5'E, 43°02.2'N), on an unknown plant (not submerged), 25 March 2001, K. Tanaka 474 (HHUF 28601, **holotype**; **isotype** IMI 392549). *Single ascospore culture*: isolated from HHUF 28601 (MAFF 239516 = JCM 12848). *Dry culture specimens* (grown on culms of *Oryza sativa* L.): from culture MAFF 23961 (HHUF 28602-28605, IMI 392550).

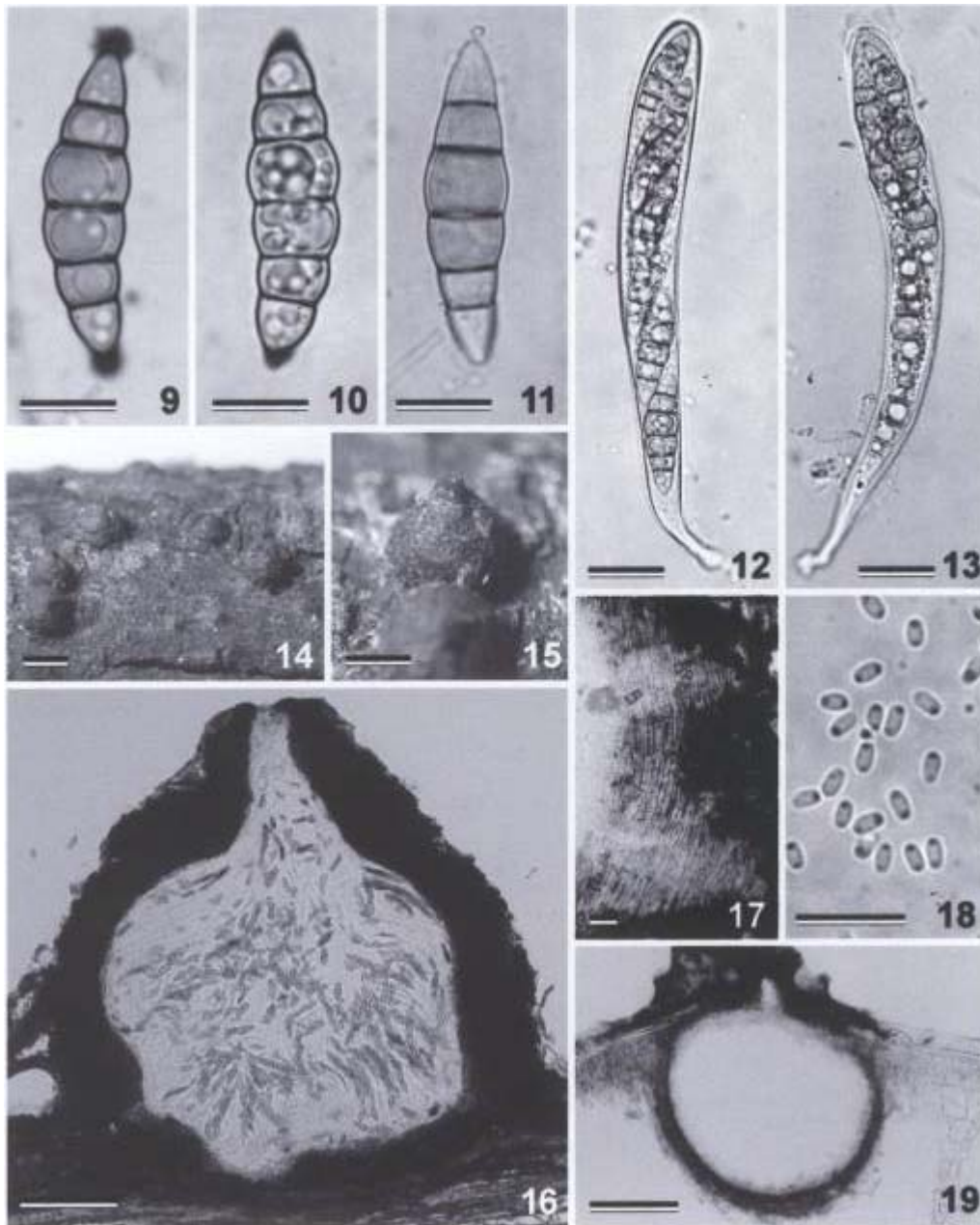
Notes: On the basis of the following morphological features, our collected fungus is best accommodated in *Trematosphaeria*: the large-sized carbonaceous ascomata, trabeculate hamathecium, clavate asci with fissitunicate dehiscence, and 5-septate reddish-brown ascospores with pale end cells. This species somewhat resembles *T. wegeliniana* L. Holm and K. Holm (1988), but the latter has ascomata with cristate beak and longer ascospores (36-50 × 8-10 μm). The ascospore dimensions of *T. crassiseptata* are identical to those of *T. confusa*, but the latter is different in having 3-septate ascospores (Boise, 1985).

***Trematosphaeria biappendiculata* Kaz. Tanaka, Y. Harada & M.E. Barr, sp. nov.** (Figs. 9-19, 23-27)

Etymology: From the Latin prefix *bi-* meaning “two” and *appendiculatus* meaning “appendaged” in reference to the appendages at both ends of ascospores.

Ascomata 450-860 μm alta, 750-980 μm diam, globosa vel subglobosa, dispersa, erumpentia vel superficialia, cum ostiolo rotundato. *Rostrum* 150-250 μm longum, 170-210 μm diam, centrale vel excentricum, breviter papillatum vel cylindraceum, periphysatum. *Paries ascomatis* in latere 55-100 μm crassus, ex cellulis parallelis 15- vel 25-stratis rectangulatis brunneis 5-13 × 2.5-5 μm compositus; ad basim parum evolvens vel 12-25 μm crassus, ex cellulis globosis vel polygonis pallide brunneis compositus; circum rostrum ex cellulis globosis vel polygonis brunneis pachydermicis 2.5-8 μm diam compositus. *Hamathecium* trabeculatum, 1-1.5 μm latum, ramificans et anastomosans, matricem gelatinosam ostendens. *Asci* 112.5-152.5 × 15.5-19.5 μm, clavati, fissitunicati, cum camera apicali, stipitati, octospori. *Ascosporae* 30-38(-41) × 9-11(-12) μm, fusiformes, 5 (raro 7) -septatae, valde constrictae ad septum primum submedianum, ad septa alia infirme constrictae, luteo-brunneae vel olivaceae, cum cellulis extremis pallidis praeditae, laeves, guttulate, apice utrinque appendiculate.

Ascomata 450-860 μm high, 750-980 μm diam, globose to subglobose, scattered, erumpent or superficial by weathering of the host epidermis, with a rounded ostiole. *Beak* 150-250 μm long, 170-210 μm diam, central to eccentric, short papillate to cylindrical, with hyaline periphyses. *Ascomal wall* at sides 55-100 μm thick, rim-like, composed of parallel rows (15-25 layers) of rectangular



Figs. 9-19. Micrographs of *Trematosphaeria biappendiculata*. **9-11.** Ascospores (in blue ink except for Fig. 11). Note the bipolar appendages. **12, 13.** Asci. **14, 15.** Ascomata on host surface. Note the rounded ostiole. **16.** Ascoma in longitudinal section. **17.** Ascomal wall at side. **18.** Microconidia. **19.** Longitudinal section of conidioma. (9, 10, 17 from HHUF 28026; 11, 13 from HHUF 28017; 12 from HHUF 28028; 14-16 from HHUF 28036; 18, 19 from culture MAFF 239296). Bars: 9-11, 18 = 10 μ m; 12, 13, 17 = 20 μ m; 14 = 1 mm; 15 = 500 μ m; 16 = 200 μ m; 19 = 100 μ m.

brown $5\text{-}13 \times 2.5\text{-}5 \mu\text{m}$ cells; at the base poorly developed or $12\text{-}25 \mu\text{m}$ thick, composed of globose to polygonal pale brown cells; around the beak composed of globose to polygonal brown thick-walled cells of $2.5\text{-}8 \mu\text{m}$ diam. *Hamathecium* trabeculate, $1\text{-}1.5 \mu\text{m}$ thick, branched and anastomosed, in gel matrix. *Asci* $112.5\text{-}152.5 \times 15.5\text{-}19.5 \mu\text{m}$ (mean = $135.2 \times 17.4 \mu\text{m}$, $n = 22$), peripheral, clavate, fissitunicate, with an apical chamber, short-stalked ($10\text{-}15 \mu\text{m}$ long), with 8 biseriate ascospores. *Ascospores* $30\text{-}38(-41) \times 9\text{-}11(-12) \mu\text{m}$ (mean = $33.8 \times 9.8 \mu\text{m}$, $n = 180$), L/W $3.2\text{-}3.8$ (mean = 3.4 , $n = 180$), fusiform with acute or narrowly rounded ends, mostly curved, 5-(rarely 7-)septate, with a primary septum submedian ($0.50\text{-}0.53$; mean = 0.52 , $n = 166$) and strongly constricted, slightly constricted at other septa, the third cell from the apex enlarged, pale yellowish to olivaceous brown except pale end cells, smooth, guttulate, with appendages at both ends. *Appendages* globose to conical, $0.5\text{-}3 \mu\text{m}$ diam, staining with blue ink. Ascospores germinating from both end cells.

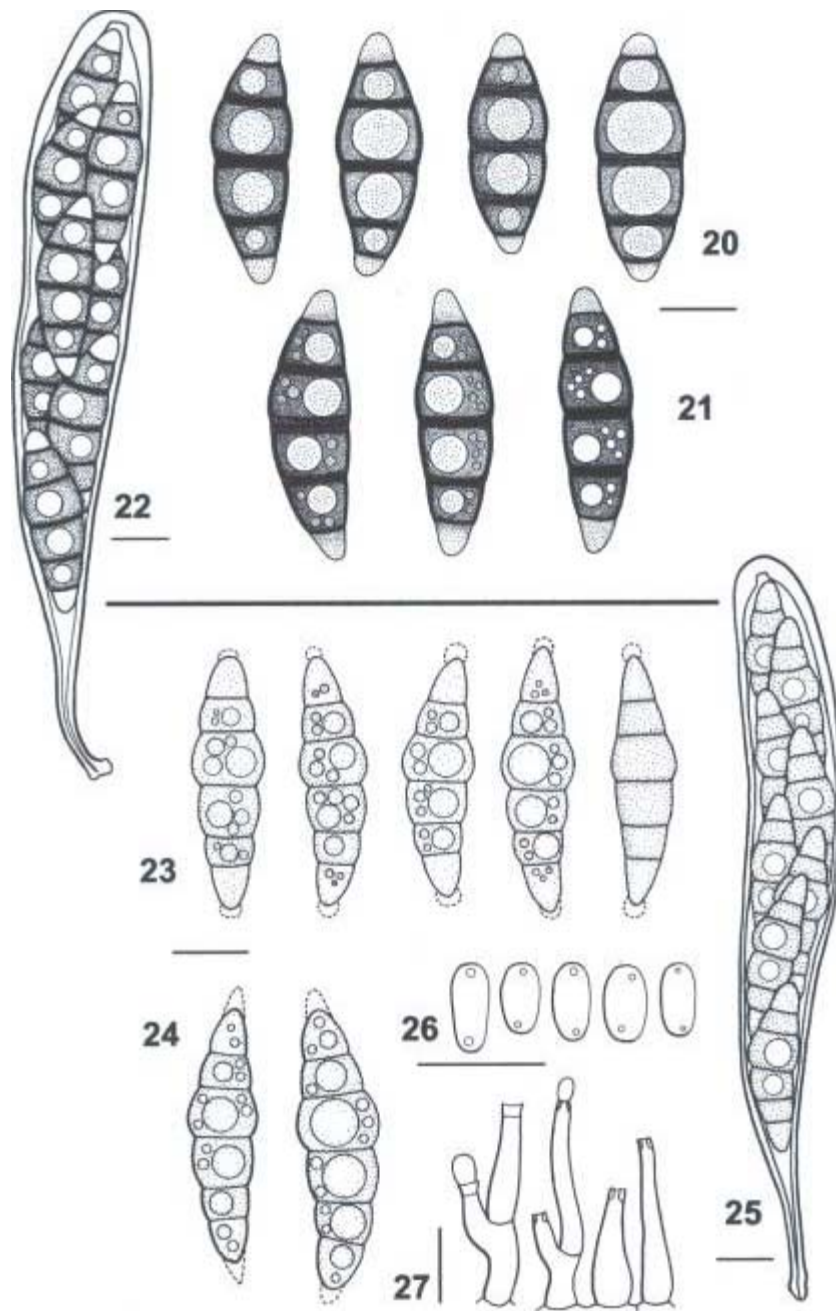
Cultural characteristics: Colonies on PDA $12\text{-}14 \text{ mm}$ in diam. after 4 weeks at 20°C in the dark, Olive (3E3); reverse Olive-Brown (4F5) to almost black; yellowish pigment slightly produced. On RSA, a *Pleurophomopsis* Petr.-like microconidial state formed on the surface of rice straws within 2 months. Conidiomata $125\text{-}190 \mu\text{m}$ high, $135\text{-}200 \mu\text{m}$ diam., erumpent to superficial, globose, uniloculate, with $3 \mu\text{m}$ thick brown sparse hyphae at sides. Beak short papillate, central, $60\text{-}120 \mu\text{m}$ long, $50\text{-}85 \mu\text{m}$ wide. Conidiomatal wall $12\text{-}25 \mu\text{m}$ thick at sides, composed of polygonal to globose cells of $2.5\text{-}5 \mu\text{m}$ diam. Conidiophores $6\text{-}25 \times 2\text{-}3 \mu\text{m}$, cylindrical, branched, 0 to 1-septate, formed from all round of the locular cavity. Conidiogenous cells phialidic. Conidia $2.5\text{-}3.5 \times 1.5\text{-}2 \mu\text{m}$, subglobose to ellipsoid, aseptate, hyaline, smooth.

Anamorph: *Pleurophomopsis* Petr.-like.

Habitat: Saprobic on submerged wood in freshwater.

Known distribution: Northern JAPAN.

Material examined: JAPAN, Serisawa-park (pond), Matsukitai, Hirosaki, Aomori ($140^\circ29.43'\text{E}$, $40^\circ33.06'\text{N}$), on submerged twigs, 7 December 2002, KT & N. Asama 975 (HHUF 28017), 26 April 2003, KT & NA 1095 (HHUF 28018); Mohei-pond, Aoki, Hirosaki, Aomori ($140^\circ26.25'\text{E}$, $40^\circ34.12'\text{N}$), on submerged twigs, 3 May 2003, KT & NA 1117-1123 (HHUF 28019-28025), KT & NA 1124 [HHUF 28026 **holotype**; **isotype** IMI 391844], KT & NA 1125 (HHUF 28027), KT & NA 1126 (HHUF 28028), 28 June 2003, KT & NA 1264-1266 (HHUF 28298-28300), 19 July 2003, KT & NA 1325 (HHUF 28301), KT & NA 1326 (HHUF 28302), 22 August 2003, KT & NA 1358-1359 (HHUF 28303-28305), 27 September 2003, KT & NA 1489a (HHUF 28306), KT & NA 1490a (HHUF 28307), KT & NA 1490b (IMI 391845), KT & NA 1491-1494 (HHUF 28308-28311), 25 October 2003, KT & NA 1528-1530 (HHUF 28315-28317), 29 November 2003, KT & NA 1596-1598 (HHUF 28315-28317). *Single ascospore cultures*: isolated from HHUF 28017 (MAFF 239296 = JCM 12850), isolated from HHUF 28026 (MAFF 239297 = JCM 12849). *Dry culture specimens of microconidial state* (grown on culms of *Oryza sativa*): from culture MAFF 239296 (HHUF 28318-28323), from culture MAFF 239297 (HHUF 28320).



Figs. 20-22. *Trematosphaeria crassiseptata*. **20, 21.** Ascospores (20 from HHUF 28601; 21 from culture MAFF 239516). **22.** Ascus (from culture MAFF 239516). **Figs. 23-27.** *Trematosphaeria biappendiculata*. **23, 24.** Ascospores (23 from HHUF 28017; 24 from HHUF 28026). **25.** Ascus (from HHUF 28017). **26.** Microconidia (from culture MAFF 239296). **27.** Conidiophores (from culture MAFF 239296). Bars: 20-25 = 10 μ m; 26, 27 = 5 μ m.

Notes: We assign this species with some reservation to *Trematosphaeria*. The morphological characters of ascomata, hamathecium, and asci of this species fit well within the generic concept of *Trematosphaeria*, whereas the nature of ascospores is slightly discordant in that those are constricted at all septa, form terminal appendages, and in particular have pale yellowish to olivaceous-brown pigmentation. Usually, the ascospores of *Trematosphaeria* species described to date are constricted at the primary septum only, lack any appendages, and form reddish brown pigmentation.

In terms of ascospore measurements, *T. biappendiculata* is close to *T. lineolatispora*, but the latter has striate ornamentation in ascospore surface and is a marine species (Hyde, 1992b). It is also similar to *T. pachycarpa* (Sacc. & El. Marchal) Shoemaker & C.E. Babcock, but the latter has median (0.50) primary septum and graminicolous habit (Shoemaker and Babcock, 1989; Scheuer and Nogrsek, 1993; Table 1).

We could find *T. biappendiculata* from two ponds in northern Japan, in every month except for winter (January to March). The occurrence of this species was rather common in spring (April to June) and it was collected together with other freshwater ascomycetes such as *Lophiostoma bipolare* (K.D. Hyde) Liew, Aptroot & K.D. Hyde or *Massariosphaeria typhicola* (P. Karst.) Leuchtm. on the same substrata.

Key to *Trematosphaeria* species

- | | | |
|----|---|---------------------------------------|
| 1. | Ascospores 1-septate | 2 |
| 1. | Ascospores more than 1-septate | 3 |
| 2. | Ascospores small, 20-30 × 6-8 μm | <i>T. vicina</i> ⁸⁾ |
| 2. | Ascospores larger, 75-85 × 24-26 μm | <i>T. crustacea</i> ⁴⁾ |
| 3. | Ascospores 1 to 3-septate | 4 |
| 3. | Ascospores 3 to 7-septate | 9 |
| 4. | Ascospores 20-25 × 4-6 μm, with striate surface | <i>T. malaysiana</i> ¹⁰⁾ |
| 4. | Ascospores not striate | 5 |
| 5. | Ascospores slender, mostly less than 10 μm wide | 6 |
| 5. | Ascospores broad, mostly more than 10 μm wide | 8 |
| 6. | Ascospores large, (30-)34-45(-52) × (8-)8.5-11 μm | <i>T. confusa</i> ²⁾ |
| 6. | Ascospores smaller | 7 |
| 7. | Ascospores (21-)22-32(-34) × 6-10 μm; on various wood | <i>T. pertusa</i> ²⁾ |
| 7. | Ascospores 30 × 6 μm; on <i>Tephrosia virginiana</i> | <i>T. tephrosiae</i> ^{1) 7)} |

8. Ascospores 30-41(-45) × 10-13(-16.5) µm; asci long, 220 × 22 µm *T. mangrovei*²⁾
8. Ascospores (34-)45-49 × 13-16.5 µm; asci shorter, 110 × 36 µm *T. cariosa*²⁾
9. Ascospores large, more than 70 µm long 10
9. Ascospores smaller, mostly less than 50 µm long 11
10. Ascospores fusiform, (71-)80-88(-92) × (12-)15-16.5(-18) µm; on wood *T. hydrela*²⁾
10. Ascospores clavate to broadly fusiform, 72-94 × 14-18.5 µm; on palms *T. abuensis*⁹⁾
11. Ascomata with a cristate beak; ascospores 36-50 × 8-10 µm *T. wegeliniana*³⁾
11. Ascomata without a cristate beak 12
12. Ascospores 34-48 × 7-10 µm, with striate surface *T. lineolatispora*⁵⁾
12. Ascospores surface without striate ornamentation 13
13. Ascospores 40-52 × 7.5-10 µm, with an entire sheath *T. pachycarpa*⁶⁾
13. Ascospores mostly less than 40 µm long 14
14. Ascospores (27-)28.5-35(-38) × 8-10.5(-11.5) µm, without sheath or appendages
..... *T. crassiseptata*
14. Ascospores 30-38(-41) × 9-11(-12) µm, with appendages at both ends. *T. biappendiculata*

*Data from ¹⁾ Cooke and Ellis (1878); ²⁾ Boise (1985); ³⁾ Holm and Holm (1988); ⁴⁾ Eriksson and Yue (1990); ⁵⁾ Hyde (1992b); ⁶⁾ Scheuer and Nogrsek (1993); ⁷⁾ Barr *et al.* (1996); ⁸⁾ Barr and Mathiassen (1998); ⁹⁾ Hyde and Fröhlich (1998); ¹⁰⁾ Mckeown *et al.* (2001).

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