

SHORT COMMUNICATION

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Bambusicolous fungi in Japan (5): three species of *Tetraploa*

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Abstract Three species of *Tetraploa* collected from *Sasa*, or bamboos, are described and illustrated. Among them, *T. curviappendiculata* on *Sasa kurilensis* and *T. longissima* on *Pleioblastus chino* are compared with hitherto known species and described as new species. In the nomenclature, *T. javanica* is substituted for *T. biformis*, formerly reported from the dead bark of a broad-leaved tree in Japan, as a correct name.

Key words Bamboo · Hyphomycetes · *Sasa* · Taxonomy · *Tetraploa*

During investigation of microfungi that inhabit bamboos in Japan, numerous new or noteworthy fungi were encountered. Several of these have been described (Shirouzu and Harada 2004; Tanaka and Harada 2004, 2005; Tanaka et al. 2005). In this article, we report three species of *Tetraploa* Berk. & Broome.

The hyphomycete genus *Tetraploa* is characterized by conidia that consist of a main body and four appendages. Usually, species in this genus are known as saprophytes of herbaceous plants (Ellis 1949), but some species are found from rotten wood (Révay 1993), soil (Watanabe 2002), raindrops on leaves of *Acer* (Ando 1992), and liverworts (Ellis 1949). Eleven taxa are accepted in the genus (Arambarri et al. 1987; Ellis 1949; Matsushima and Matsushima 1996; Révay 1993; Rifai et al. 1988; Sharma 1978). Of these, only 1 species, *T. aristata* Berk. & Broome, is known to have a *Massarina* Sacc. teleomorph of pleosporalean ascomycete (Scheuer 1991). *Tetraploa aristata* and *T. ellisii* Cooke are

widely distributed and reported from many countries, including Japan (Shirai and Hara 1927; Tubaki 1971), but other species, such as *T. abortiva* Aramb. & Cabello and *T. setifera* Révay, are recorded only from the type localities (Révay 1993).

We followed the methods in our previous paper (Hatakeyama and Harada 2004), but as to sterilized plant agar (SPA) culture, stems of eulalia (*Miscanthus sinensis* Anderss.) were used instead of tree peony (*Paonia suffruticosa* Andr.). To stimulate sporulation, in addition to SPA, small agar pieces cut from colonies were incubated in Petri dishes (60 mm diameter) containing 10–15 ml sterilized distilled water (SDW) at 10°C under 12-h photoperiod.

All specimens, except for the type specimen of *Tetraploa biformis* K. Matsush. & Matsush., were kept at the Herbarium of Hirosaki University, Fungi (HHUF), and all isolates were deposited at culture collections (MAFF and JCM). The type specimen of *T. biformis* was borrowed from the Matsushima Fungus Collection (Kobe, Japan).

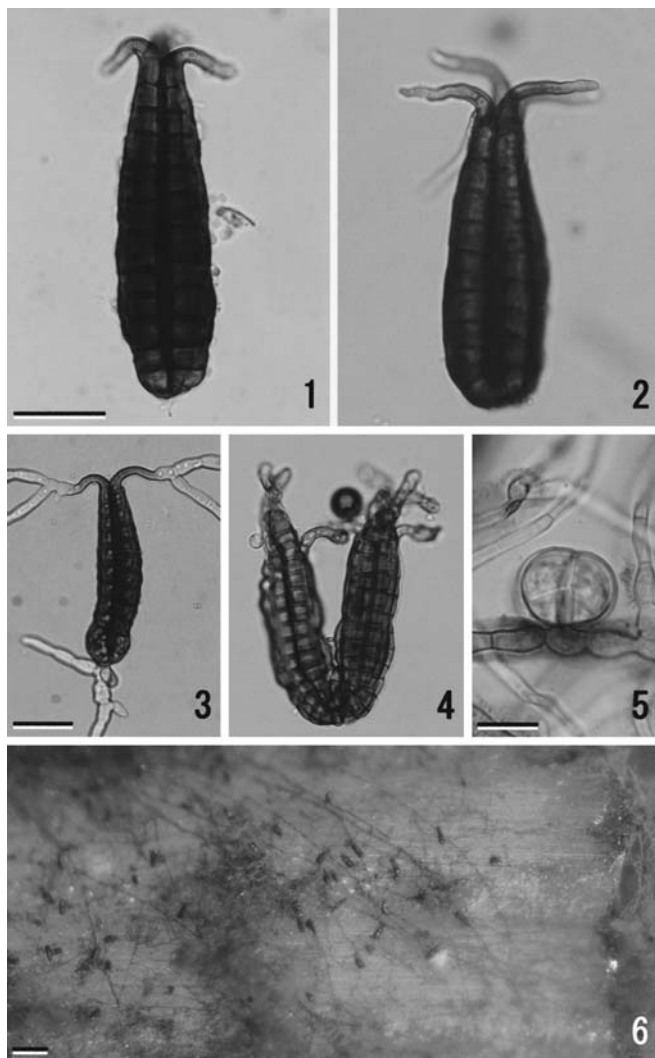
Tetraploa curviappendiculata Sat. Hatak., Kaz. Tanaka & Y. Harada, sp. nov.

Figs. 1–6, 18

Mycelia superficialia. Conidiophora micronematica, mononematica. Cellulae conidiogenae monoblasticae, incorporateae. Conidia ex uno corpore conidii et appendicibus quatuor composita, solitaria; corpus conidii 52–67(–75) × 15–22 μm, pallide brunneum vel brunneum, obclavatum vel anguste obpyriforme, ad basim rotundatum et verruculosum, ex 4–5 columnis compositum; columnae 7–13 μm crassae, 8–11-septatae, leviter constrictae ad septa; appendices 9–18(–22) × 2–5 μm, 0–1-septatae, inferiore pallide brunneae, superne hyalinae, laeves, eramosae, basi curvae.

Mycelium superficial. Conidiophores micronematous, mononematous. Conidiogenous cells monoblastic, integrated, usually indistinguishable from creeping hyphae. Conidia consist of 1 body and 4 appendages, solitary; the body 52–67(–75) × 15–22 μm (mean = 63.3 × 18.9 μm, *n* = 50), length/width ratio (L/W) = 2.7–4.4 (mean = 3.4, *n* = 50), pale brown to brown, obclavate to narrowly obpyriform,

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Figs. 1–6. *Tetraploa curviappendiculata*. **1, 2** Conidia; **3** Germinating conidium; **4** Aberrant conidium; **5** Conidium initial; **6** Conidia on sterilized plant agar (SPA) (**1** HHUF 28582; **2, 3, 5, 6** MAFF 239495 = JCM 12852; **4** HHUF 28589). Bars **1–4** 20 μm (**1, 2** same magnification; **3, 4** same magnification); **5** 10 μm ; **6** 100 μm

rounded and verruculate at the base, consisting of 4–5 columns of cells; columns 7–13 μm wide, 8–11-septate, slightly constricted at septa; appendages 9–18(–22) \times 2–5 μm (mean = 13.6 \times 3.3 μm , $n = 50$), 0–1-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, curved below.

Cultural characteristics: Conidia usually germinated from the apex of appendages on glucose agar [GA; 20 g glucose (Wako, Osaka, Japan), 20 g agar, and 1000 ml distilled water] at room temperature. Colonies on potato dextrose agar (PDA; Difco, Detroit, MI, USA) attaining a diameter of about 2.5 cm within 4 weeks at 20°C in the dark, velvety in appearance, Mouse Grey (5E3; Kornerup and Wanscher 1978) with whitish entire margin (1 mm); reverse Beaver (5F4); no pigment produced. Colonies on malt extract agar (MA; Difco) attaining a diameter of 1.5–2.0 cm

within same condition, plane in appearance, Dark Green (30F6) with entire margin; reverse similar; no pigment produced. On SPA, abundant conidia were produced on the eulalia stems at 20°C within 1 month. In SDW abundant sporulation occurred at 10°C within 2 weeks. The shape and size of conidia in vitro are quite identical with those found in nature (conidial body 60–70 \times 17–18 μm ; columns 8–9-septate; appendages 14–25 μm long).

Etymology: “*curviappendiculata*” from Latin *curvi* and *appendiculatus*, referring to the appendages curved below.

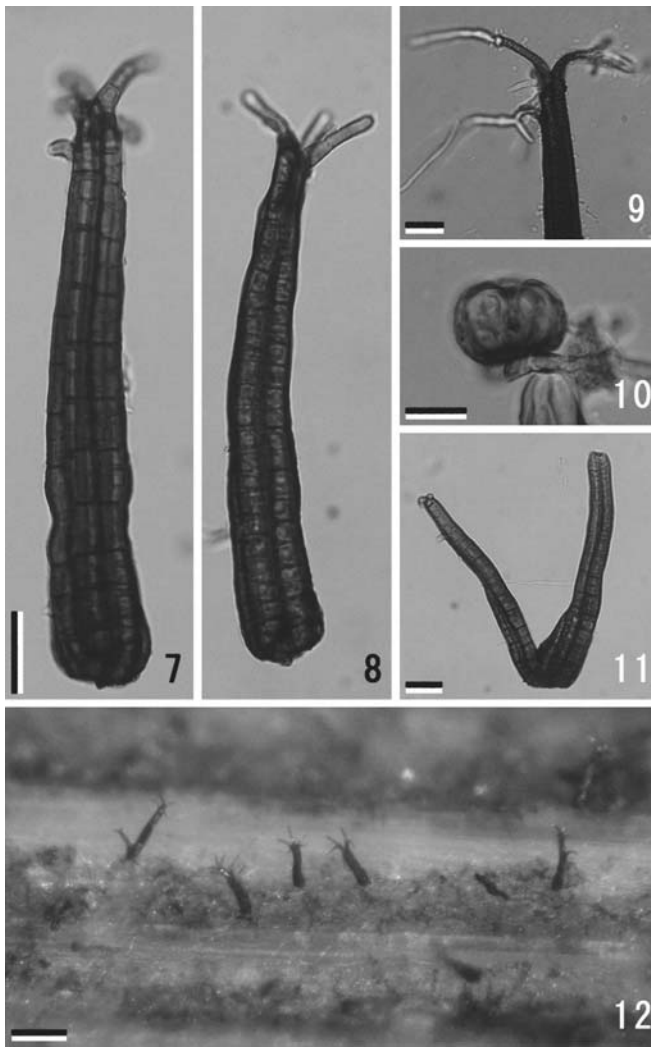
Holotype: HHUF 28582.

Materials examined: On culms of *Sasa kurilensis* (Rupr.) Makino & Shibata: Mt. Kudoji, Hirosaki, Aomori, 140°25' E, 40°31' N, May 9, 2003, Y. Harada (HHUF 28582; culture MAFF 239495 = JCM 12852); Aseishigawa Dam, Kuroishi, Aomori, 140°41' E, 40°34' N, May 2, 2003, K. Tanaka (HHUF 28583); Zatouishi, Hirosaki, Aomori, 140°26' E, 40°31' N, Nov. 8, 2003, K. Tanaka and T. Shirouzu (HHUF 28584); Kozawa, Hirosaki, Aomori, 140°26' E, 40°33' N, Oct. 25, 2002, Y. Harada (HHUF 28586); Dec. 21, 2002, Y. Harada and K. Tanaka (HHUF 28585); Akaiwa, Funadamari, Isl. Rebun, Hokkaido, 141°03.4' E, 45°23.2' N, June 2, 2004, K. Tanaka (HHUF 28588); Matsukitai, Hirosaki, Aomori, 140°29' E, 40°33' N, Nov. 29, 2003, K. Tanaka and N. Asama (HHUF 28589); Dec. 7, 2003, K. Tanaka and N. Asama (HHUF 28590; culture MAFF 239496); Notsukefuuren Park, Bekkai, Notsuke-gun, Hokkaido, 145°14' E, 43°31' N, Sept. 9, 2003, K. Tanaka and S. Hatakeyama (HHUF 28591). On culms of *Sasa senanensis* (Franch. & Sav.) Rehder: Tsukuba Univ. Sugadaira Montane Research Center, Nagano, 138°20' E, 36°31' N, Apr. 10, 2004, T. Shirouzu (HHUF 28587). Dried culture specimens: grown on culms of eulalia, from MAFF 239496 (HHUF 28598) and MAFF 239495 = JCM 12852 (HHUF 28599).

Notes: This fungus resembles *Tretospeira ugandensis* (Hansf.) Piroz., the type species of *Tretospeira* Piroz., in conidial shape, but does not fit within the generic concept of *Tretospeira*; i.e., *Tetraploa* produces micronematous conidiophores with monoblastic conidiogenesis, whereas *Tretospeira* produces macronematous, erect conidiophores with enterotretic conidial succession (Ho et al. 2000; Pirozynski 1972).

Appendages of *T. curviappendiculata* are curved below. This feature is remarkable in *T. curviappendiculata* because most species of *Tetraploa* have erect appendages (Ellis 1949; Révay 1993). However, *T. curviappendiculata* is suitably classified in the genus *Tetraploa* by monoblastic and integrated conidiogenesis, conidial body composed of four columns, and appendages developed from each column independently. *Tetraploa curviappendiculata* is also distinguished from all other species in the longer and numerously septate conidial body.

Aberrant conidia having 6–10 columns or V-shaped are observed on some specimens (HHUF 28589–28591), but single-conidium cultures from these aberrant conidia produced typical conidia, which consist of 4–5 columns and curved appendages in vitro.



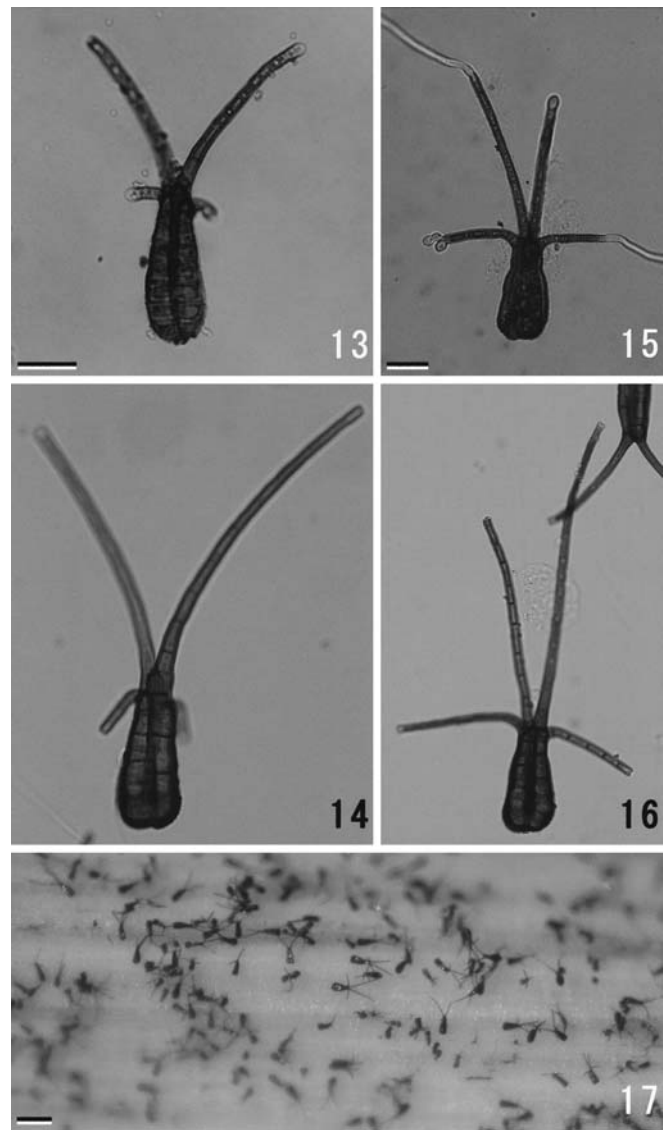
Figs. 7–12. *Tetraploa longissima*. **7, 8** Conidia; **9** Germinating conidium; **10** Conidium initial; **11** Aberrant conidium; **12** Conidia on SPA (**7** HHUF 28580; **8–12** MAFF 239497 = JCM 12853). Bars **7–9, 11** 20µm (**7, 8** same magnification); **10** 10µm; **12** 100µm

Tetraploa longissima Sat. Hatak., Kaz. Tanaka & Y. Harada, sp. nov.

Figs. 7–12, 19

Mycelia superficialia. Conidiophora micronematica, mononematica. Cellulae conidiogenae monoblasticae, incorporatae. Conidia ex uno corpore conidii et appendicibus quatuor composita, solitaria; corpus conidii (98–)110–148(–155) × 18–25µm, pallide brunneum vel brunneum, obclavatum vel anguste obpyriforme, ad basim rotundatum et verruculosum, ex 4–6 columnis compositum; columnae 8–10µm crassae, 14–21-septatae, leviter constrictae ad septa; appendices 15–27 × 4–5µm, 2–4-septatae, inferiorem pallide brunneae, superne hyalinae, laeves, eramosae, basi curvae.

Mycelium superficial. Conidiophores micronematous, mononematous. Conidiogenous cells monoblastic, integrated, usually indistinguishable from creeping hyphae. Conidia consist of 1 body and 4 appendages, solitary; the

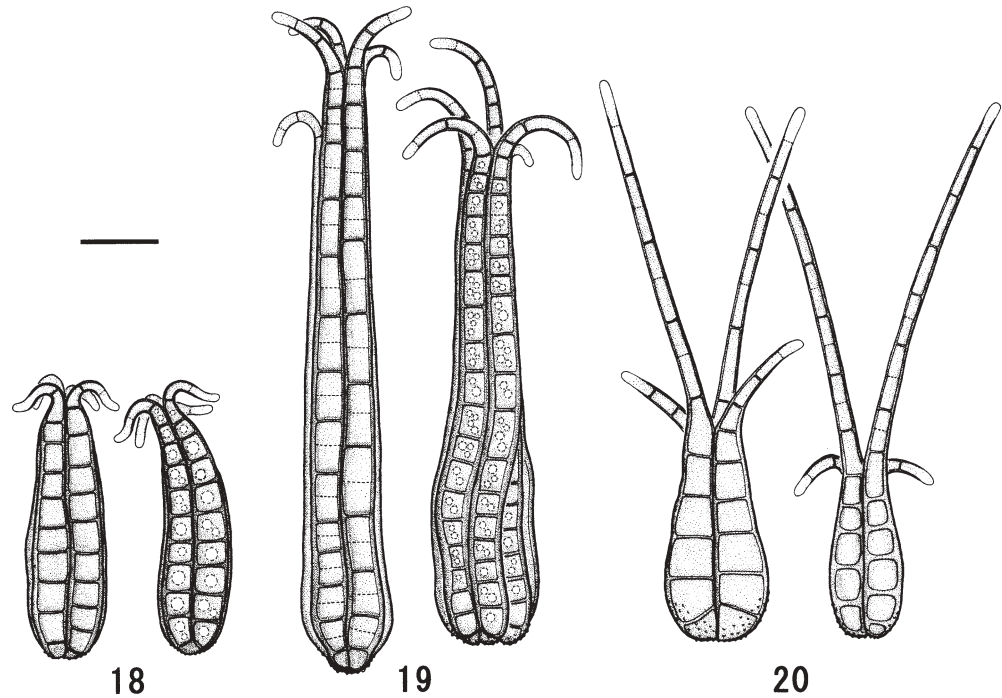


Figs. 13–17. *Tetraploa javanica*. **13, 14** Conidia; **15** Germinating conidium; **16** Conidium having longer appendages; **17** Conidia on SPA (**13** HHUF 26742; **14** MFC-6M196, holotype of *T. biformis*; **15–17** MAFF 239498 = JCM 12854). Bars **13–16** 20µm (**13, 14** same magnification; **15, 16** same magnification); **17** 100µm

body (98–)110–148(–155) × 18–25µm (mean = 138.4 × 21.8µm, $n = 50$), L/W = 4.8–7.2 (mean = 6.3, $n = 50$), pale brown to brown, obclavate to narrowly obpyriform, rounded and verruculate at the base, consisting of 4–6 columns of cells; columns 8–10µm wide, 14–21-septate, slightly constricted at septa, rarely separate and make V-shaped conidia; appendages 15–27 × 4–5µm (mean = 23.6 × 4.7µm, $n = 50$), 2–4-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, curved to below.

Cultural characteristics: Conidia usually germinated from the apex of appendages on GA at room temperature. Colonies on PDA attaining a diameter of about 3.7cm within 4 weeks at 20°C in the dark, velvety in appearance, Celadon Green (30D3) with whitish entire margin (2mm);

Figs. 18–20. Conidia of *Tetraploa* species. **18** *T. curviappendiculata* (left, HHUF 28582; right, MAFF 239495 = JCM 12852); **19** *T. longissima* (left, HHUF 28580; right, MAFF 239497 = JCM 12853); **20** *T. javanica* (left, HHUF 28596; right, MAFF 239498 = JCM 12854). Bar, **18–20** 20µm



reverse Soot Brown (5F5); no pigment produced. Colonies on MA attaining a diameter of about 2.0cm within same condition, plane in appearance, Dark Green (30F5) with entire margin; reverse similar; no pigment produced. On SPA, abundant conidia were produced on the eulalia stems at 20°C within 1 month. In SDW, no conidia were produced at 10°C within 1 month. The size of conidia in vitro is shorter than that found in nature (conidial body 90–125 × 20–27µm; columns 18–23-septate; appendages 17–33µm long).

Etymology: “*longissima*” referring to the longest conidia of this species in the genus.

Holotype: HHUF 28580.

Materials examined: On culms of *Pleiblastus chino* (Franch. & Sav.) Makino: Asamizu, Gonohe, Sannohe-Gun, Aomori, 141°18.0' E, 40°28.1' N, Dec. 2, 2003, K. Tanaka, N. Nakagawara, and S. Hatakeyama (HHUF 28580; culture MAFF 239497 = JCM 12853). Dried culture specimen: grown on culms of eulalia, from MAFF 239497 = JCM 12853 (HHUF 28581).

Notes: This species is somewhat similar to *T. curviappendiculata* in conidial shape. However, the conidial length and number of septa of this species are almost twice as those of the latter. Moreover, in SDW, *T. curviappendiculata* produced abundant conidia within 2 weeks, whereas *T. longissima* did not do so within 1 month.

Tetraploa javanica Rifai, Zainuddin & Cholil, Reinwardtia 10: 420, 1988.
= *Tetraploa bififormis* K. Matsush. & Matsush., Mats. Mycol. Mem. 9: 38, 1996.

Mycelium superficial. Conidiophores micronematous, mononematous. Conidiogenous cells monoblastic, inte-

grated, usually indistinguishable from creeping hyphae. Conidia consist of 1 body and 2 forms of appendages, solitary; the body 38–65 × 17–30µm (mean = 52.9 × 21.4µm, $n = 80$), L/W = 1.5–3.3 (mean = 2.5, $n = 80$), pale brown, ovoid, rounded and verruculate at the base, consisting of 4–5 columns of cells; columns 7–15µm wide, 3–8-septate; appendages dimorphic, pale brown at the base and tapering toward almost hyaline apex, smooth, unbranched [long appendages 45–120(–177) × 4–8µm (mean = 78.2 × 5.6µm, $n = 80$) (2–5µm wide at the apex), 3–11-septate, straight; short appendages 8–35(–50) × 3–5µm (mean = 20.6 × 4.4µm, $n = 80$), 0–3-septate, curved to below].

Cultural characteristics: Conidia usually germinated from the apex of appendages on GA at room temperature. Colonies on PDA, attaining a diameter of about 2.7cm within 4 weeks at 20°C in the dark, velvety in appearance, Dull Green (30E3) with whitish entire margin (1mm); reverse Dark Green (30F4); no pigment produced. Colonies on MA, attaining a diameter of about 4.5cm within same condition, velvety in appearance, Greenish Grey (30E2) with entire margin; reverse Dark Green (30F4); no pigment produced. On SPA, abundant conidia were produced on eulalia stems at 20°C within 1 month. In SDW, a few conidia were produced at 10°C within 1 month. Although the shape of conidia in vitro is quite identical with that found in nature, conidia with longer appendages were also observed in vitro (long appendages were up to 150µm long, short ones up to 60µm long).

Materials examined: On dead bark of broad-leaved tree: Tottori, March, 1996 (MFC-6M196, holotype of *Tetraploa bififormis*). On culms of *Sasa* sp.: Kyoto Prefectural Botanical Garden, Kyoto, 135°45' E, 35°2' N, May 25, 2001, Y. Harada (HHUF 26742). On culms of *Phyllostachys bambusoides* Siebold & Zucc.: Tsuru country club,

Figs. 13–17, 20

Utsunomiya, Tochigi, 139°47' E, 36°37' N, Aug. 29, 2003, N. Asama and K. Oota (HHUF 28592). On dead stems of an unknown herbaceous plant: Sattebetu River, Akkeshi, Akkeshi-gun, Hokkaido, 144°49.0' E, 43°08.1' N, Sept. 9, 2003, K. Tanaka (HHUF 28593). On culms of *Bambusa multiplex* (Lour.) Raeusch. var. *gracillima* (Makino ex E.G. Camus) S. Suzuki: Fuji bamboo Garden, Gotenba, Shizuoka, 138°53' E, 35°9' N, Mar. 8, 2004, K. Tanaka and Y. Harada (HHUF 28594). On culms of *Sasa veitchii* (Carrière) Rehder: Sankeien, Yokohama, Kanagawa, 139°39' E, 35°24' N, Mar. 9, 2004, K. Tanaka and Y. Harada (HHUF 28595). On culms of *Pleioblastus chino* (Franch. & Sav.) Makino: Asamizu, Gonohe, Sannohe-gun, Aomori, 141°18.0' E, 40°28.1' N, Dec. 2, 2003, K. Tanaka, N. Nakagawara, and S. Hatakeyama (HHUF 28596; culture MAFF 239498 = JCM 12854). Dried culture specimen: grown on culms of eulalia, from MAFF 239498 = JCM 12854 (HHUF 28600).

Notes: Our results on the examined materials including the type specimen of *T. biformis* (Matsushima and Matsushima 1996) fit well with the original description of *T. javanica* (Rifai et al. 1988), which is characterized by the conidia with two long and two short appendages. However, the length of long appendages in our materials [45–120 (–177) µm long] was slightly shorter than that found in the original description of *T. javanica* (up to 215 µm long; Rifai et al. 1988). We concluded in this article that the length of appendages is not suitable as a taxonomic criterion for *Tetraploa* species because it tends to change depending on cultural conditions. Therefore, we consider *T. biformis* to be a synonym of *T. javanica*.

Since *T. javanica* was first recorded on *Bambusa glaucescens* Hedge in Java, which was introduced from Japan to Indonesia, the fungus has been considered to distribute also in Japan (Rifai et al. 1988). In this study, *T. javanica* was found from *Sasa* Makino & Shibata, *Phyllostachys* Siebold & Zucc., and *Pleioblastus* Nakai as well as *Bambusa* Schreb.

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