Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new pleosporalean family with *Tetraploa*-like anamorphs

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Abstract: A new pleosporalean family *Tetraplosphaeriaceae* is established to accommodate five new genera; 1) *Tetraplosphaeria* with small ascomata and anamorphs belonging to *Tetraploa* s. str., 2) *Triplosphaeria* characterised by hemispherical ascomata with rim-like side walls and anamorphs similar to *Tetraploa* but with three conidial setose appendages, 3) *Polyplosphaeria* with large ascomata surrounded by brown hypheae and anamorphs producing globose conidia with several setose appendages, 4) *Pseudotetraploa* an anamorphic genus, having obpyriform conidia with pseudepseota and four to eight setose appendages, and 5) *Quadricrura*, an anamorphic genus, having globose conidia with one or two long setose appendages at the apex and four to five short setoce appendages at the base. Fifteen new taxa in these genera mostly collected from bamboo are described and illustrated. They are linked by their *Tetraploa* s. l. anamorphs. To infer phylogenetic placement in the Pleosporales, analyses based on a combined dataset of small- and large-subunit nuclear ribosomal DNA (SSU+LSU nrDNA) was carried out. *Tetraplosphaeriaceae*, however, is basal to the main pleosporalean clade and therefore its relationship with other existing families was not completely resolved. To evaluate the validity of each taxon and to clarify the phylogenetic relationships within this family, further analyses using sequences from ITS-5.8S rDNA (ITS), transcription elongation factor 1-α (TEF), and β-tubulin (BT), were also conducted. Monophyly of the family and that of each genus were strongly supported by analyses based on a combined dataset of the three regions (ITS+TEF+BT). Our results also suggest that *Tetraplosphaeria* (anamorph: *Tetraploa* s. str.) is an ancestral lineage within this family. Taxonomic placement of the bambusicolous fungi in *Astrophytoidea*, *Kalmiusia*, *Katunotoma*, *Massarina*, *Ophiophaerella*, *Phaeosphaeria*, *Rousseia*, *Rousseosporis*, and *Versicolisporium*, are also discussed based on the SSU+LSU phylogeny.

Key words: Anamorphic fungi, Bambusoidea, bitunicate ascomycetes, Didymella, Dothideomycetes, evolution, Lophostoma, teleomorph.

Taxonomic novelties: *Tetraplosphaeriaceae* Kaz. Tanaka & K. Hiray., fam. nov., *Tetraplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Tetraplosphaeria* nagasakiiensis Kaz. Tanaka & K. Hiray., sp. nov., *Tetraplosphaeria* saasicoa Kaz. Tanaka & K. Hiray., sp. nov., *Tetraplosphaeria* tetraploa (Scheuer) Kaz. Tanaka & K. Hiray., comb. nov., *Tetraplosphaeria* yakushimensis Kaz. Tanaka, K. Hiray. & Hosoya, sp. nov., *Triplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Triplosphaeria* acuta Kaz. Tanaka & K. Hiray., sp. nov., *Triplosphaeria* cylindrica Kaz. Tanaka & K. Hiray., nom. nov., *Triplosphaeria* maxima Kaz. Tanaka & K. Hiray., sp. nov., *Triplosphaeria* yeozensia (I. Hino & Katum.) Kaz. Tanaka, K. Hiray. & Shirouzu., comb. nov., *Polyplosphaeria* fusca Kaz. Tanaka & K. Hiray., sp. nov., *Pseudotetraploa* Kaz. Tanaka & K. Hiray., sp. nov., *Pseudotetraploa* curvapendiculata (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Pseudotetraploa* javanica (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., nom. nov., *Quadricrura* longissima (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Quadricrura* meridionalis Kaz. Tanaka & K. Hiray., sp. nov., *Quadricrura* septentrionalis Kaz. Tanaka, K. Hiray. & Sat. Hatak., sp. nov.

INTRODUCTION

Bamboo is the vernacular or common term applied to small to large woody grasses ranging from 10 cm to 40 m in height. They are currently classified as a subfamily *Bambusoideae* within the extensive grass family *Poaceae* and comprise ca. 80–90 genera and 1 000–1 500 species. Indications are that major radiations of this extensive grass family occurred 40–50 million years ago within the Paleogene age. Bamboos are distributed all over the world except in Europe which has no native species, and are found at latitudes from 46°N to 47°S and from sea level to 4 000 m elevation. However, the major species richness is found in the Asian Pacific region (China: 626, India: 102, Japan: 84) and South America (Brazil: 134, Venezuela: 68, Colombia: 56) (Suzuki 1996, Scurlock et al. 2000, Das et al. 2008, Sungkawet et al. 2009). Approximately 1 500 commercial applications of bamboo — as fishing rods, flutes, paper, flooring materials, foods and energy feedstock — have been identified, and it is estimated that 2.5 billion people depend on or use bamboo materials valued at US$ 7 billion per annum (Scurlock et al. 2000, Bystriakova et al. 2003).

In addition to studies on economically important bambusicolous pathogenic fungi, such as *Ceratosphaeria phyllostachydis* and *Stereosrstratum corticoides* (Hyde et al. 2002b), a large number of studies on saprobic (Hyde et al. 2001, 2002c, Zhou & Hyde 2002) and endophytic fungi (Morakotkarn et al. 2007, 2008, Tanaka & Harada 2008, Tanaka et al. 2008) have also been conducted due to the diversity of fungal species on bamboo. According to Hyde et al. (2002b), more than 1 100 fungal species have been described or recorded worldwide from bamboo. In Japan, ca. 300 fungi are known from bamboo (Tanaka & Harada 2004), of which ca. 60 spp. belong to *Dothideomycetes* (Anonymous 2000). This number suggests that bamboo is a promising substrate for the study of *Dothideomycetes* diversity. Several *Dothideomycetes* with peculiar taxonomic features such as *Shiraira* (Amano 1983) and *Katumotoma* (Tanaka & Harada 2005b) have been reported from bamboo. However, phylogenetic information based on molecular data is poorly known for many bambusicolous fungi.

In our ongoing study of bambusicolous fungi in Japan (Shirouzu & Harada 2004, Tanaka & Harada 2004, 2005a, b, Tanaka et al. 2005, Hatakeyama et al. 2005, 2008, Sato et al. 2008), we...
encountered many undescribed Dothideomycetes resembling the genus Massarina. These fungi produced Tetraploa-like anamorphs in culture. The teleomorph-anamorph connection between Massarina and Tetraploa has been elucidated based on one example of M. tetraploa and T. arista on Carex (Scheuer 1991), but the molecular phylogenetic position of this species remains uncertain at the familial/generic level.

Massarina is a taxonomically heterogenous genus in the order Pleosporales, because Massarina s. l. contains many phylogenetically unrelated elements. Attempts to revise the genus have been undertaken by several authors (Bose 1961, Barr 1992, Aptroot 1998). In particular, Aptroot (1998) carried out taxonomic re-assessment of 160 species that had been placed in this genus previously and amended the generic concept of Massarina by accepting 43 species in the genus. Nevertheless, this study also pointed out that Massarina appears to be polyphyletic, because members of this genus have diverse anamorphs, like Tetraploa, Periconia, Tumularia, Ceratophoma, and others. Regarding this problem, Aptroot (1998) noted that the species accepted in Massarina may not form a monophyletic group; however, on the basis of morphological characteristics, no clear subdivision could be made. He further pointed out the need for examining the molecular and ultrastructural characteristics to gain a better understanding of the genus.

The current taxonomic concept of Massarina has been extensively amended based on its DNA sequence data (Liew et al. 2002, Belliveau & Bärlocher 2005, Kodsueb et al. 2007, Wang et al. 2007, Zhang et al. 2009b). Liew et al. (2002) revealed that five species of Massarina (e.g. M. corticola) possessing narrowly fusiform ascospores belong to the genus Lophiostoma, which is morphologically similar to Massarina, based on phylogenetic analyses of SSU and ITS sequences of nrDNA. They further suggested that other Massarina species with ascospores of similar morphology might have affinity with Lophiostoma (Liew et al. 2002). Following this suggestion, Hyde et al. (2002a) transferred 26 species of Massarina to Lophiostoma primarily based on their ascospore morphology. Massarina tetraploa, which produces the Tetraploa anamorph, was also transferred to Lophiostoma (Hyde et al. 2002a).

The phylogenetic position or the relationships of bambusicolous species with fungi from non-bamboo host plants have not been established. In this paper, phylogenetic analyses using 53 isolates of bambusicolous Dothideomycetes were carried out based on a combined dataset of small and large subunit nuclear ribosomal DNA (SSU+LSU), to infer their familial placement. These analyses include species placed in Astrosphaeriella, Kalmusia, Katumotoa, Massarina, Ophiopsalherella, Phaeosphaeria, Roussosella, Roussosellipsis, and Versicolourisporium. Special emphasis was paid to the taxonomy and phylogeny of Massarina s. l., which possess Tetraploa-like hypymocytous anamorphs. In order to assess their validity at familial, generic and specific levels, phylogeny of 29 isolates were analysed on the basis of their sequences from ITS-5.8S nrDNA (ITS), transcription elongation factor 1-α (TEF) and β-tubulin (BT), as well as SSU+LSU. We propose here a new family Tetraplophoreaacidae to encompass five new genera, Tetraplophorea, Triplosphaeria, Polyplosphaeria, Pseudotetraploa and Quadricrura. Fifteen new taxa in these genera are also described and illustrated.

MATERIALS AND METHODS

Morphological studies and fungal isolates

Measurements of all structures were taken from material mounted in water. India ink was added to water mounts to detect the gelatinous sheath and ascospore appendages. To observe the internal conidial structure, 5 % sodium hypochlorite solution (NaClO) was used for bleaching of strongly melanised spores as described in Eriksson (1989). The ascospore septum position was noted using the decimal system (Shoemaker 1984, Raja et al. 2008). To observe details of ascomal anatomy, ascomata were boiled in water for a few minutes and sectioned with a freezing microtome (HM 400R; MICROM, Germany). Light microscopy observations were conducted using an Olympus microscope (BX51) equipped with Nomarski interference differential contrast objectives. Specimens cited in this paper are maintained at the herbaria of Hiroasaki University (HHUF) and National Museum of Nature and Science (TNS), and some materials were borrowed from the herbaria of Yamaguchi University (YAM) and Karl-Franzens-Universität Graz (GZU).

Single ascospore cultures were obtained according to the methods of Tubaki (1978). Growth rate and colony characteristics were recorded from cultures grown on potato-dextrose agar (PDA, Difco) within 3 wk at 25 °C in the dark. Colours were designated according to Kornerup & Wanscher (1978). Induction of anamorph/teleomorph formation was attempted by culturing the isolates on rice straw agar (RSA; Tanaka & Harada 2003a) and/or incubating small colony pieces in sterilised water (Scheuer 1991, Hatakeyama et al. 2005). Fungal cultures newly obtained in this study were deposited at the CBS-KNAW Fungal Biodiversity Centre (Centraalbureau voor Schimmelcultures; CBS), the Japan Collection of Microorganisms (JCM), the Ministry of Agriculture, Forestry, and Fisheries, Japan (MAFF), and the National Biological Resources Center, Japan (NBRC) (Table 1).

DNA extraction and amplification

Mycelia were grown in malt extract broth (20 g malt extract, 1 000 mL distilled water). DNA from mycelia was extracted using the ISOPLANT Kit (Nippon Gene, Japan) according to the manufacturer’s instructions. Partial SSU (ca. 1 000–1 300 bp of the 3’ end) and LSU nrDNA (ca. 1 250 bp of the 3’ end) regions were determined for 53 isolates mostly obtained from bamboo to reveal their familial or generic positions; and complete internally transcribed spacers (ITS) region of nrDNA (ca. 500 bp), the intron sequence of the TEF gene (ca. 300 bp), and exons 1 to 6 with the respective introns of the BT gene (ca. 600 bp) were sequenced for 31 isolates to confirm their generic or species validities (Table 1). These regions were amplified by the polymerase chain reaction (PCR) using the primer pairs NS1–NS4 (White et al. 1990) and LROR–LR7 (Rehner & Samuels 1994) for SSU and LSU, respectively. Three primer sets, ITS1–IT5 (White et al. 1990), EF1-728F–EF1-986R (Carbone & Kohn 1999), and T1–BT2B (Glass & Donaldson 1995, O’Donnell & Cigelnik 1997) were used for the amplification of ITS, TEF and BT, respectively. Amplifications were conducted in 25 µL of PCR mixtures containing 1 µM of each primer, 0.125 U TaKaRa Ex Taq polymerase (TaKaRa Bio, Otsu, Japan), dNTP mixture (2.5 mM each stock), and Ex Taq reaction buffer (containing 2 mM Mg2+). PCR was carried out as follows: initial denaturation at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 1 min; annealing for 1 min at 48.8 °C for SSU nrDNA,
46.2 °C for LSU nrDNA, 61.5 °C for ITS, 57.2 °C for TEF, and 60 °C for BT; an extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The size of PCR products were verified using 7.5 % poly-acrylamide gels stained with ethidium bromide, and then sequenced directly at SORGENT Co., Ltd. (Korea).

Phylogenetic analyses

Preliminary multiple alignments of sequences were conducted using MAFFT v. 6 (Katoh et al. 2005; http://align.bmr.kyushu-u.ac.jp/mafft sofware). Final alignments were manually adjusted using BioEdit v. 7.08 (HALL 1999). Alignment gaps and ambiguous positions were excluded from the analyses. Alignments used in this study were deposited in TreeBASE (S2505).

Two phylogenetic analyses, maximum-parsimony (MP) using a close-neighbour-interchange heuristic search with an initial tree by random addition sequence (100 replicates) and neighbour-joining (NJ) based on the Kimura 2-parameter substitution model, were carried out using MEGA v. 4 (Tamura et al. 2007). Characters were weighted equally and gaps were excluded. The bootstrap support (BS) values for nodes were computed from 1 000 replicates for both the MP and NJ analyses. In addition to these analyses, Bayesian analyses were done using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). MrModeltest v. 2.2 (Nylander 2004) in conjunction with PAUP 4.0b10 (Swoford 2003) was used to select substitution models for Bayesian analyses. On the basis of AIC (Akaike Information Criterion) of MrModeltest v. 2.2, a GTR+I+G model for the SSU+LSU nrDNA, ITS and BT, and a HKY+I+G model for TEF gene sequences were applied. Two runs with 10 chains of Markov chain Monte Carlo (MCMC) iterations were performed for 6 million and 1.2 million generations, keeping one tree every 100 generations, for a combined alignment of the SSU+LSU nrDNA sequences and the ITS+TEF+BT gene sequences, respectively. The first 5 million generations of the SSU + LSU and 200 000 generations of the ITS+TEF+BT were discarded as burn-in, and the remaining 20 002 trees were used to calculate 50 % majority rule trees and to determine the posterior probabilities (PP) for the individual branches.

RESULTS

Taxonomy

A new family, Tetraplosphaeriaceae typified by Tetraplosphaeria, is established in this paper. This family includes five new genera, 1) Tetraplosphaeria with small ascomata and anamorphs belonging to Tetraploa s. str., 2) Triposphaeria characterised by hemispherical ascomata with rim-like side walls and anamorphs similar to Tetraploa but with three conidial setose appendages, 3) Polylosphaeria with large-sized ascomata surrounded by brown hyphae and anamorphs producing globose conidia with several setose appendages, 4) Pseudotetraploa, an anamorphic genus, having obpyriform conidia with pseudosepta and four to eight setose appendages, and 5) Quadricrura, an anamorphic genus, having globose conidia with one or two long apical setose appendages and four to five short basal setose appendages. Fifteen new taxa of these genera are described below.

Tetraplosphaeriaceae Kaz. Tanaka & K. Hiray., fam. nov. MycoBank MB515253.

Etymology: In reference to the name of the type genus.

Ascomata immersed to superficial, globose to subglobose, glabrous or with brown hyphae. Beak short-papillate to cylindrical or absent, central. Ascomatal wall composed of hyaline to brown cells, sometimes with rim-like structure at the sides and poorly developed at the base. Pseudosporophyses cellular or trabeculae, septate, branched. Ascii fissitunicate, basal to somewhat lateral, cylindrical to clavate, short-stalked, with 8 ascospores. Ascospores narrowly fusiform to broadly cylindrical, straight or slightly curved, 1–3-septate, hyaline to pale brown, smooth, surrounded by an entire mucilaginous sheath or narrow appendage-like sheath. Anamorph Tetraploa-like. Conidiophores absent. Conidiogenous cells monoblastic. Conidia composed of 3–8 columns or internal hyphal structure, brown, mostly verrucose at the base, with more than 3–8 setose appendages.

Type genus: Tetraplosphaeria Kaz. Tanaka & K. Hiray., gen. nov.

Notes: Tetraplosphaeriaceae fits well in the Pleosporales on morphological grounds, but there is no suitable family to accommodate it in this order. The most common diagnostic features of this family are Massarina-like teleomorphs with almost hyaline 1(–3)-septate ascospores and/or Tetraploa-like anamorphs with several setose appendages.

Tetraplosphaeria Kaz. Tanaka & K. Hiray., gen. nov. MycoBank MB515254.

Anamorph: Tetraploa Berk. & Broome.

Etymology: In reference to the anamorphic state belonging to Tetraploa.

Ascomata immersed or erumpentia, globose or subglobose. Rostrum breviter papillatum vel cylindricum, interdum nullum. Pseudoparaphyses septatae, ramicanticae. Ascii fissitunicati, cylindrici vel clavati, ochotropi. Ascosporae anguste fusiformes vel late cylindricae, 1–3-septatae, hyalinae vel brunnea, cum vagina gelatinosa obtectae. Anamorphosis Tetraploa sensu lato. Conidiophora absenta. Cellulae conidiogenae monoblasticae. Conidia brunnea, cum plus quam 3–8 appendicibus.

Ascomata scattered to gregarious, immersed to superficial, globose to subglobose, glabrous or with brown hyphae. Beak short-papillate to cylindrical or absent, central. Ascornatal wall composed of hyaline to brown cells, sometimes with rim-like structure at the sides and poorly developed at the base. Pseudosporophyses cellular or trabeculae, septate, branched. Ascii fissitunicate, basal to somewhat lateral, cylindrical to clavate, short-stalked, with 8 ascospores. Ascospores narrowly fusiform to broadly cylindrical, straight or slightly curved, 1–3-septate, hyaline to pale brown, smooth, surrounded by an entire mucilaginous sheath or narrow appendage-like sheath. Anamorph Tetraploa-like. Conidiophores absent. Conidiogenous cells monoblastic. Conidia composed of 3–8 columns or internal hyphal structure, brown, mostly verrucose at the base, with more than 3–8 setose appendages.
| Taxon                          | SSU GenBank no. | LSU GenBank no. | ITS GenBank no. | TEF GenBank no. | BT GenBank no. | Strain no. | Herbarium no. | Collection no. | Original no. | Host no. |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|----------------|------------|---------------|----------------|--------------|---------|
| Astrosphaeria aggregata       |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 1517a |
| Kalamota bambusicola          |                 |                 |                 |                 |                |            |               |                 |              | 7 KT 1023   |
| Massarina arundinaria          |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2200   |
| Astrosphaeria aggregata       |                 |                 |                 |                 |                |            |               |                 |              | 12 KT 1466  |
| Kalamota bambusicola          |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2564   |
| Massarina arundinaria          |                 |                 |                 |                 |                |            |               |                 |              | 5 KT 1640   |
| Kalamota bambusicola          |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2124   |
| Massarina arundinaria          |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2301   |
| Ophiopsophaeria sp.            |                 |                 |                 |                 |                |            |               |                 |              | 12 KT 1466  |
| Pseudotetraploa curvicaudata   |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2678   |
| Pseudotetraploa javana          |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2678   |
| Quadricrura bicornis           |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 2694   |
| Quadricrura meridionalis       |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 3062   |
| Quadricrura septentrionalis    |                 |                 |                 |                 |                |            |               |                 |              | 9 KT 3062   |
| Roussoella hysterioides        |                 |                 |                 |                 |                |            |               |                 |              | 13 KT 1651  |

Table 1. Cultures and Genbank accession number of bambusicolous fungi used in this study.
Table 1. (Continued).

| Taxon                            | Host | Original no. | Herbarium no. | Strain no. | SSU   | LSU   | ITS no. | BT no. | TEF no. |
|----------------------------------|------|--------------|---------------|------------|-------|-------|---------|--------|---------|
| Roussoella hysterioides         | 9    | HH 26968     | HHUF 26968    | CBS 125434 | AB524481 | AB524622 | –       | –      | –       |
| Roussoella pustulans            | 9    | KT 1709      | HHUF 29220    | JOM 13127 = MAFF 239637 | AB524482 | AB524623 | –       | –      | –       |
| Roussoella sp.                  | 9    | KT 2303      | HHUF 30025    | NBRC 106245 | AB524483 | AB524624 | –       | –      | –       |
| Roussoellopsis tosaensis       | 3    | KT 1659      | HHUF 29234    | JOM 13128 = MAFF 239638 | AB524484 | AB524625 | –       | –      | –       |
| Roussoellopsis sp.              | 9    | KT 1710      | HHUF 30026    | NBRC 106246 | AB524485 | AB524626 | –       | –      | –       |
| Tetraploa aristata              | 1    | -            | CBS H-18781   | CBS 996.70 | AB524486 | AB524627 | AB524805 | AB524867 | AB524836 |
| Tetraploa sp. 1                 | 3    | KT 1684      | HHUF 29625    | JOM 14424 | AB524487 | AB524628 | –       | –      | –       |
| Tetraploa sp. 2                 | 6    | KT 2578      | HHUF 30027    | NBRC 106251 | AB524488 | AB524629 | –       | –      | –       |
| Tetraploasphaeria naganakensis  | 3    | KT 1682      | HHUF 29378    | JOM 13168 = MAFF 239678 | AB524489 | AB524630 | AB524806 | AB524868 | AB524837 |
| Tetraploasphaeria sascola       | 11   | KT 563       | HHUF 27566    | JOM 13167 = MAFF 239677 | AB524490 | AB524631 | AB524807 | AB524869 | AB524838 |
| Tetraploasphaeria yakuishimensis| 2    | KT 1906      | HHUF 29652    | CBS 125435 | AB524491 | AB524632 | AB524808 | AB524870 | AB524839 |
| Triploasphaeria acuta           | 10   | KT 1170      | HHUF 29387    | JOM 13171 = MAFF 239681 | AB524492 | AB524633 | AB524809 | AB524871 | AB524840 |
| Triploasphaeria cylindrica      | 9    | KT 1256      | HHUF 29381    | JOM 13169 = MAFF 239679 | AB524493 | AB524634 | –       | –      | –       |
| Triploasphaeria maxima          | 9    | KT 1800      | HHUF 29026    | JOM 14425 | AB524494 | AB524635 | AB524810 | AB524872 | AB524841 |
| Triploasphaeria yezoensis       | 9    | KT 2550      | HHUF 30028    | NBRC 106247 | AB524495 | AB524636 | AB524811 | AB524873 | AB524842 |
| Triploasphaeria sp.             | 9    | KT 1715      | HHUF 30029    | CBS 125436 | AB524497 | AB524638 | AB524813 | AB524875 | AB524844 |
| Triploasphaeria sp.             | 12   | KT 1732      | HHUF 30030    | CBS 125437 | AB524498 | AB524639 | AB524814 | AB524876 | AB524845 |
| Triploasphaeria sp.             | 9    | HC 4695      | HHUF 27481    | NBRC 106248 | AB524499 | AB524640 | AB524815 | AB524877 | AB524846 |
| Triploasphaeria sp.             | 9    | KT 2546      | HHUF 30031    | NBRC 106249 | AB524500 | AB524641 | AB524816 | AB524878 | AB524847 |
| Versicollosporium triseptatum   | 8    | SH 130       | HHUF 28815    | JOM 14775 | AB524501 | AB524608 | –       | –      | –       |

a 1. Alpinia formosa; 2. Arundo donax; 3. bamboo; 4. Chimonobambusa marmorea; 5. conifer; 6. gramineae; 7. Phyllostachys bambusoides; 8. Pleioblastus chinensis; 9. Sasa kurilensis; 10. Sasa nipponica; 11. Sasa senanensis; 12. Sasa sp.; 13. Sasa veitchii
Notes: A new genus *Tetraplosphaeria* is erected to accommodate four pleosporalean species having *Massarina*/*Lophiostoma*-like telemorphs and anamorphs belonging to *Tetraploa* *s. str.*. These species do not have olypate stromata around the ascomatal beak similar to the type species of *Massarina* (*M. eburnea*; Hyde 1995). Some species in *Tetraplosphaeria* have a well-developed beak similar to *Lophiostoma* species, but they do not have slit-like ostioles which is a characteristic feature of *Lophiostoma* (Holm & Holm 1988, Tanaka & Harada 2003a, Tanaka & Hosoya 2008).

*Tetraplosphaeria nagasakiensis* Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515259. Fig. 1.

Anamorph: *Tetraploa arista s. l.*

**Etymology:** In reference to the collection site.

Ascomata 280–330 × 290–350 µm, immersed or erumpent, globose or subglobose. Rostrum 75–150 × 85–110 µm, ostiolatum. Parietes ascomatis (17–30)–50–70 µm ad latus, ex cells 5–6-strati 5–13 × 2.5–5.5 µm compositus. Pseudoparaphyses 1–3 µm latae, septonatae, ramificantes et anastomosantes. Asci (82–)86–105–110 × 10.5–13.5 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae (27–29–35–37) × 5.5–6 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphism *Tetraplosphaeria* sensu stricto. Conidia in vitro (28–)32.5–42–(43) × 20–33 µm, brunnea, cum 4 appendicibus; appendices (70–)95–225–263 µm longae, 3–13-septatae.

Tetraplosphaeria sasicola Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515260. Fig. 2.

Anamorph: *Tetraploa ellisi s. l.*

**Etymology:** In reference to the host plant collection.

Ascomata 150–200 × 230–290 µm, immersed or erumpent, globose or subglobose. Rostrum 30–40 × 50–55 µm, ostiolatum. Parietes ascomatis 12–20 µm crassus ad latus, ex cells 3–6-strati 7–13 × 2–5 µm compositus. Pseudoparaphyses 1.5–2.5 µm latae, septonatae, ramificantes et anastomosantes. Asci (61–)65–89–100 × 9–11–13 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae 22.5–31.5–(34) × 3–5 µm, anguste fusiformes, 1–2-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphism *Tetraplosphaeria* sensu stricto. Conidia in vitro (32–)35–50–(52.5) × 20–30 µm, brunnea, cum 4 appendicibus; appendices (88–)113–190–200 µm longae, 9–15-septatae.

**Type species:** *Tetraplosphaeria sasicola* Kaz. Tanaka & K. Hiray., sp. nov.

**Notes:** This species is most similar to *Tetraplosphaeria yakushimensis* in having ascospores overlapping in size, but *T. nagasakiensis* differs from the latter in the dimension of conidia and the length of conidial appendages. The *Tetraploa* state of *T. nagasakiensis* shares some features with *Tetraploa arista* (Berkeley & Broome 1850, Ellis 1949), but has larger conidia (av. 37.4 × 27.2 µm vs. 31.8 × 20.6 µm) and considerably longer conidial appendages (av. 161.2 µm vs. 36 µm).

**Culture characteristics:** Colonies on PDA attaining a diam of 1.1–1.2 cm, dull-green (28E4; Komerup & Wanscher 1978); reverse almost black; no pigment produced. On RSA both teleomorphic and anamorphic states are produced. Ascospores are slightly smaller than those on the host, measuring 29–32 × 5 µm. Anamorph is *Tetraploa arista s. l.* *Conidiophores* absent. *Conidiophores* monoblastic. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, clearly verruculose, (28–)32.5–42–(43) × 20–33 µm (av. 37.4 × 27.2 µm, n = 30), LW 1.2–1.7 (av. 1.4, n = 30), 5–6-celled, composed of 4 columns and 4 setose appendages. Appendages (70–)95–225–263 µm long (av. 161.2 µm, n = 100), 2–3 µm wide at the apex, 5.5–8 µm at the base, with 3–13-septa at 10 to 25 µm intervals.

**Culture characteristics:** Colonies on PDA attaining 3.5–4 cm diam, velvety in appearance, grey (7C1), with entire margin; reverse Pompeian-red (9C7), and coral (9B7) pigment produced. On RSA, *Tetraploa* state similar to *T. ellisi* is formed on the surface of rice straw within 2 mo. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, slightly verruculose, (32–)35–50–(52.5) × 20–30 µm (av. 41.2 × 26 µm, n = 43), LW 1.3–1.9 (av. 1.6, n = 42), composed of 4 columns. The columns 12.5–15–(19.5) µm diam, 5–6-celled. Setose appendages 4, brown, (88–)113–190–200 µm long (av. 142.9 µm, n = 21), 2–4 µm wide at the apex, with 9–15-septa at 10 to 20 µm intervals. After the conidial state is formed, the ascomatal state is soon within 2 mo. Ascomatal appendages (80–)117–200–263 µm long (av. 161.2 µm, n = 100), 2–3 µm wide at the apex, 5.5–8 µm at the base, with 3–13-septa at 10 to 25 µm intervals.

**Notes:** Some species in *Tetraploa* state of *T. nagasakiensis* have ascospores slightly smaller than those on the host, measuring 29–32 × 5 µm. Anamorphic state similar in appearance to those on the host, but slightly larger, measuring 25–35 × 3.5–5.5 µm (av. 29.8 × 4.5 µm, n = 75), LW 5.9–7.5 (av. 6.6, n = 75), with a mid-septum submedian (0.50–0.53; av. 0.51, n = 55).
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Fig. 1. Tetraplosphaeria nagasakiensis. A. Ascomata on host surface. B–F. Ascospores; G. Ascoma in longitudinal section; H–I. Asci; J. Pseudoparaphyses; K. Ascomal wall; L. Ascomata on rice straw agar; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, P = 10 µm; G, L = 100 µm; H–K = 20 µm; M = 200 µm; N–O = 50 µm; Q = 1 cm. A–D, G–H, K from HHUF 29378 holotype; E–F, I–J, L–Q from culture KT 1682.
Fig. 2. Tetraplosphaeria sasicola. A. Ascomata on host surface; B–F. Ascospores; G. Ascoma in longitudinal section; H–I. Asci; J. Pseudoparaphyses; K. Conidia on rice straw agar; L–M. Conidia; N. Conidial body; O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, J, N = 10 µm; G, L–M = 50 µm; H–I = 20 µm; K = 100 µm; O = 1 cm. A–J from HHUF 27566 holotype; K–O from culture KT 563.

Specimen examined: Japan, Hokkaido, Yoichi, Sawamachi (140°46'E, 43°11'N), on culms of Sasa senanensis, 7 July 2001, K. Tanaka, HHUF 27566 holotype designated here, living culture KTC 563 (= JCM 13167 = MAFF 239677).

Notes: This species is characterised by the smallest asci and ascospores. The conidial morphology of this species resembles that of Tetraploa ellisii, but the latter species has more slender conidia (30–51 × 15–26 µm, L/W 1.9; Ellis 1949).

Tetraplosphaeria tetraploa (Scheuer) Kaz. Tanaka & K. Hiray., comb. nov. MycoBank MB515261. Fig. 3.
Basionym: Massarina tetraploa Scheuer, Mycol. Res. 95: 126. 1991.
≡ Lophiostoma tetraploa (Scheuer) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Diversity Res. Ser. (Hong Kong) 7: 108. 2002.
Anamorph: Tetraploa aristata s. 1.

Ascomata 180–200 µm high, 150–280 µm diam, scattered, immersed, globose to somewhat pyriform, glabrous to sometimes covered with sparse brown hyphae at sides. Beak 50–80(–100) µm long, 50–75 µm diam, central, papillate to short cylindrical, composed of subglobose to polygonal cells, with hyaline paraphyses. Ascomatal wall uniformly 6–12 µm thick, composed of 3–4 layers of
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Fig. 3. Tetraplosphaeria tetraploa. A. Ascomata on host surface; B–C. Ascomata in longitudinal section; D–G. Ascospores; H. Asci; I. Pseudoparaphyses; J. Conidia on malt extract agar; K–L. Conidia; M–N. Conidial bodies. Scale bars: A = 500 µm; B–C = 50 µm; D–G, M–N = 10 µm; H–I = 20 µm; J–L = 100 µm. A–I from GZU 36-91 holotype of Massarina tetraploa; J–N from GZU 32-91 (dried culture specimen of Tetraploa state).
polygonal brown cells (3.5–12.5 × 2.5–5 µm). Pseudoparaphyses cellular, 1.5–2.5 µm wide, branched and anastomosed, with septa at 8 to 15 µm intervals. Ascii (90–)95–128(–140) × 13–16(–19) µm (av. 109.4 × 14.2 µm, n = 50), numerous, basal, fissitunicate, cylindrical, with a short stipe of 5–15 µm long, with 8 biseriate ascospores. Ascospores (29–)32–41.5(−4) × 4–6(−7) µm (av. 37 × 5.2 µm, n = 50), L/W 6.4–8.1 (av. 7.1, n = 50), narrowly fusiform with acute ends, slightly curved, with a septum supramedian (0.44–0.49, av. 0.47, n = 45) and constricted, hyaline, smooth, with a sheath; sheath entire, narrow, 2–4 µm long at both ends, 1–1.5 µm thick at upper of the septum.

Culture characteristics: Not examined. According to Scheuer (1991) this fungus produces Tetrailoa aristata as anamorph. The anamorph on the dried culture specimen (GZU 32-91) examined in this study is as follows: Conidia 30–33 × 23–25 µm (av. 30.8 × 23.3 µm, n = 6), L/W = 1.3, solitary, short cylindrical, pale brown, verrucose, consist of 4 columns of 10–13 µm wide, 4-celled. Appendages 263–350 µm long (av. 295.8 µm, n = 6), 10–13 µm thick at the base, 2–3 µm at the apex, 17–22-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, straight.

Specimens examined: U.K., England, Exeter, Exminster marshes, on leaves of Carex acutiformis, 13 Nov. 1988, Ch. Scheuer, GZU 36-91 holotype of Massarina tetraploa; Dried culture specimen of conidial state grown on malt extract agar (derived from ex-type culture). GZU 32-91.

Notes: This species was originally described as a species of Massarina (Scheuer 1991), but later was transferred to the genus Lophiostoma (Hyde et al. 2002a). The original strain isolated by Scheuer (1991) from the holotype of Massarina tetraploa is no longer preserved (Scheuer, pers. comm.). There is one strain that is deposited as M. tetraploa in CBS (CBS 101683), but it is considered as a misidentified material because it produced a Phaeosphaeria-like teleomorph having 39–49 × 5.5–6.5 µm, yellowish, 3-septate ascospores on RSA. Therefore, M. tetraploa was not included in phylogenetic analyses in this study. However, morphological evidence obtained from the holotype and the dried culture specimen (anamorphic state) of M. tetraploa clearly indicates that it belongs to Tetraploa. This species can be distinguished from other species of this genus by the large-sized asci and ascospores. The anamorph of this species has been reported as Tetraploa aristata (Scheuer 1991), but the presence of several T. aristata-like anamorphs with sequence differences revealed in this study suggest that redefinition of T. aristata along with molecular evidence would be required for this anamorphic species.

Tetraploa yakushimensis Kaz. Tanaka, K. Hiray. & Hosoya, sp. nov. MycoBank MB515262. Fig. 4.

Anamorph: Tetraploa aristata s. l.

Etymology: In reference to the collection site.

Ascomata 135–180 × 150–250 µm, immensa, subglobosa. Rostrum 50 × 55–65 µm, ostiolum. Paries ascomata 15–20 µm crassus ad latus, ex cellulis 4–6-stratis 5–15 × 2.5–4 µm compositus. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci 85–110 × 10.5–13 µm, fissitunicati, clavati vel cylindrici, octospori. Ascospore 26.5–36.5 × 4–6 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis Tetraploa sensu stricto. Conidia in vitro 25–37(–40) × 20–30 µm, brunnea, cum 4 appendicibus; appendices (52–)62–142(–150) µm longae, 3–8-septatae.

Notes: This species is most similar to anamorphs of Tetraploa yakushimensis and Tetraploa aristata (CBS 996.70), both collected from non-bamboo species (Arundo donax and Alpinia formosa, respectively), clustered together (see phylogenetic section). Morphological comparison of these two strains could not be made, because the strain CBS 996.70 did not sporulate in any of the culture methods used. Sequence differences between the strains (e.g. 24/459 nucleotides in ITS) also suggest that they are different species.

Tetraploa sp. 1 (T. aristata s. l.). Fig. 5A–D.

Teleomorph: unknown.

Conidia short cylindrical, brown, verruculose, 26–31.5(−35) × 17.5–24 µm (av. 29.4 × 20.8 µm, n = 20), L/W 1.2–1.9, 3–5-celled, with 4 setose appendages of 100–175 µm long (av. 136.7 µm, n = 20).

Specimen examined: Japan, Kagooshima, Isil. Yakushima, near the mouth of Kurio-river, on culms of Arundo donax, 20 Oct. 2005. K. Tanaka & T. Hosoya, HHUF29652 holotype designated here (isotype TNS-F-12442), living culture KTC 1906 (= CBS 125436).

Notes: In terms of ascus and ascospore morphology, this species is quite close to Tetraploa yakushimensis, but it is distinct from the latter in its conidial morphology. Tetraploa yakushimensis and Tetraploa aristata (CBS 996.70), both collected from non-bamboo species (Arundo donax and Alpinia formosa, respectively), clustered together (see phylogenetic section). Morphological comparison of these two strains could not be made, because the strain CBS 996.70 did not sporulate in any of the culture methods used. Sequence differences between the strains (e.g. 24/459 nucleotides in ITS) also suggest that they are different species.
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*Tetraploa* sp. 2 (*T. ellisii* s. l.). Fig. 5E–H.

**Teleomorph:** unknown.

**Conidia** short cylindrical, broader at the base, brown, verruculose, 38–50 × 22–33 µm (av. 43.1 × 27.9 µm, n = 20), L/W 1.3–1.8, 4–5-celled, with 4 setose appendages of 142–330 µm long (av. 232 µm, n = 30).

*Specimen examined:* Japan, Okinawa, Isl. Iriomote, near Oomijya river, on culms of gramineae, 22 Nov. 2008, K. Tanaka & K. Hirayama, HHUF 30027, living culture KTC 2578 (= NBRC 106251).

**Notes:** This fungus has relatively large-sized conidia as compared with those of other *Tetraploa* species examined in this study. It is close to *Tetraploa ellisii* that was reported by Ellis (1949) and the anamorph of *Tetraplosphearia sasicola* in terms of conidial dimension, but differs from the latter in having longer appendages.

*Triplosphearia* Kaz. Tanaka & K. Hiray., gen. nov. MycoBank MB515255.

**Anamorph:** Undescribed *Tetraploa*-like state having conidia with three setose appendages.
Etymology: In reference to the anamorphic state of Tetraploa-like conidia with three setose appendages.

Ascomata immersa, subglobosa. Rostrum nullum vel breve. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes vel late fusiformes, 1-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis Tetraploa sensu lato. Conidiophora absentia. Conidiogenous cells monoblasticae. Conidia ovata vel obpyriformis, brunnea, cum 3 appendicibus.

Type species: Triplosphaeria maxima Kaz. Tanaka & K. Hiray., sp. nov.

Notes: A new genus Triplosphaeria is introduced here to place Massarina-like ascomycetes with Tetraploa-like anamorphs having three setose appendages. The ascomata of Triplosphaeria species are hemispherical with a flattened base and have rim-like regions composed of vertically oriented hyphoid cells at the side in longitudinal section. Morphology of anamorphs is superficially similar to that of Tetraploa, but conidia are composed of three columns and three setose appendages.

Triplosphaeria acuta Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515263. Fig. 6.

Etymology: In reference to the fusiform ascospores with acute ends.

Ascomata 135–230 × 540–750 µm, immersa, subglobosa. Rostrum nullum vel breve, ostiolatum. Paries ascomatis 85–180 µm crassus ad latus, ex cellulis 5–10 × 3.5–7.5 µm compositus. Pseudoparaphyses 1–2 µm latae, ramificantes et anastomosantes, septatae. Asci (62–)73–106 × 11–15 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae 25–35 × 4–6(–7) µm, anguste fusiformes, 1-septatae, hyalinae, strato mucoso 6–18 µm lato circumdatae. Anamorphosis Tetraploa sensu lato. Conidia in vitro (25–)31–50(–65) × 14–22 µm, brunnea, cum 3 appendicibus; appendices (37–)44–120(–130) µm longae, 3–8-septatae.

Ascomata 135–230 µm high, 540–750 µm diam (including the rim), with single locule of 230–400 µm diam, scattered to gregarious, immersed below the epidermis, subglobose, glabrous. Beak none or short, with hyaline sparse periphyses, ostiolate, filled with tips of pseudoparaphyses. Conidiophora at sides, composed of vertically oriented rectangular to subglobose hyphoid cells of 5–10 × 3.5–7.5 µm; near the epidermis, 25–38 µm thick, composed of polygonal to subglobose thick-walled cells of 3.5–10 µm diam; at the base flattened and poorly developed. Pseudoparaphyses narrowly cellular, numerous, 1–2 µm wide, guttulate, branched and anastomosed, septate, with slime coating. Asci (62–)73–106 × 11–15 µm (av. 86.1 × 12.6 µm, n =
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50), fissitunicate, numerous, basal and somewhat lateral, cylindrical to clavate, rounded at the apex, short-stalked (5–15 µm long), with 8 biseriate ascospores. Ascospores 25–35 × 4–6(–7) µm (av. 29.6 × 5.5 µm, n = 126), L/W 4.8–6.2 (av. 5.5, n = 126), narrowly fusiform with acute ends, mostly curved, with a septum usually submedian (0.49–0.53; av. 0.51, n = 113) and constricted, hyaline, smooth, with an inconspicuous entire sheath of 6–18 µm wide.

Culture characteristics: Colonies on PDA attaining 3–3.1 cm diam, velvety in appearance, dark green (30F4) with greyish green (25D6) entire margin (2 mm); reverse similar; no pigment produced. On RSA, Tetraploa-like anamorph having 3 appendages is found. Conidiophores absent. Conidiogenous cells monoblastic. Conidia consist of one conidial body and 3 or rarely 4 appendages, solitary. Conidial body (25–)31–50(–65) × 14–22 µm (av. 40.9 × 17.2 µm, n = 92), L/W = 1.8–3.3 (av. 2.4, n = 92), 3–4-pseudoseptate, pale brown, smooth, narrowly ovoid or ovoid. Selose appendages (37–) 44–120(–130) µm long (av. 90.3 µm, n = 70), 3–5 µm thick at the base, 2–3 µm at the apex, 3–8-septate, pale brown at the base and almost hyaline apex, smooth, unbranched, straight.

Fig. 6. Triplosphaeria acuta. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomatal wall at side; J. Asci; K. Pseudoparaphyses; L. Germinating ascospore; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. Breached conidia composed of three columns; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 500 µm; C–G, P = 10 µm; H, M = 100 µm; I–L, N–O, Q = 20 µm; R = 1 cm. A–L from HHUF 29387 holotype; M–R from culture KT 1170.
Note: This species is quite similar to *Triplosphaeria yezoensis* in its overall morphology, but has more slender ascospores with acute ends (L/W 5.5 vs. 4.4).

**Triplosphaeria cylindrica** Kaz. Tanaka & K. Hiray., nom. nov. MycoBank MB515264. Fig. 7.

≡ *Massarinia yezoensis* I. Hino & Katum., in Hino, Icon. Fung. Bambus. Jpn.: 188. 1961.

Ascomata 110–190 μm high, 450–1180 μm diam (including the rim), with single locule of 220–350 μm diam, scattered, immersed below the epidermis, subglobose, glabrous. *Beak* none to short, with hyaline, sparse periphyses, filled with tips of pseudoparaphyses. *Ascomatal wall* at sides, 100–350 μm wide and rim-like, composed of vertically orientated rectangular to cylindrical hyaline hyphoid cells of 5–15 × 2.5–5 μm; about 20 μm thick near the epidermis, composed of polygonal brown thick-walled cells of 3–10 μm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–3 μm wide, guttulate, branched and anastomosed, septate. *Asci* (70–)80–126 × 14.5–21.0–23.5 (μm (av. 98.2 × 17.9 μm, n = 82), fissitunicate, numerous, basal and lateral, cylindrical to clavate, rounded at the apex, short-stalked (4–25 μm long), with 8 biseriate ascospores. *Ascospores* (22–)25–31–33 × 6–10 μm (av. 28.2 × 8 μm, n = 153), L/W 3.0–4.4 (av. 3.5, n = 153), broadly fusiform to cylindrical with rounded ends, with a septum submedian (0.50–0.56; av. 0.53, n = 143) and strongly constricted, hyaline, smooth, with an entire sheath of 7–20 μm thick.

**Culture characteristics:** Colonies on PDA attaining 3.1 cm diam, velvety in appearance, brownish grey (6E2) with whitish entire margin of 2 mm; reverse similar to surface; no pigment produced. On RSA, a Tetraploa-like anamorph with 3 setose appendages is formed. *Conidiophores* absent. *Conidigenous cells* monoblastic. *Conidia* consist of one conidial body and 3 long appendages, solitary. Conidial body 29.5–40 × 14–23.5 (μm (av. 36.1 × 19.4 μm, n = 20), L/W = 1.4–2.3 (av. 1.9, n = 20), 2–4 pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Setose appendages 33–120 μm long (av. 73.4 μm, n = 26), 4–4.5 μm thick at the base, 2–3 μm at the apex, 3–9-septate, pale brown at the base and almost hyaline apex, smooth, unbranched, straight.

**Notes:** This species was originally described as *Massarina yezoensis* (Hino 1961), but is transferred to *Triplosphaeria* because of its hemispherical ascoma with a flattened base and rim-like side wall. The most distinctive feature of this species is the relatively wider ascospores (L/W 3.5) with rounded ends. A new name is introduced for this species because the epithet “yezoensis” has been applied for *Triplosphaeria yezoensis* (= *Didymella yezoensis* (Hino & Katumoto 1958)) in this study.

**Triplosphaeria maxima** Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515265. Fig. 8.

**Etymology:** In reference to the large-sized ascospores.

Ascomata 250–300 × 900–1000 μm diam, immersa, globose vel subglobosa. Rostrum nullum vel breve, osiolutum. Paries ascomatum 170–270 μm crassus ad latus, ex cellulis 5–13 × 3.5–8 μm compositus. Pseudoparaphyses 1–2.5 μm latae, ramificantes et Anastomosantes, septatae. *Asci* 95–133 × 14.5–21 μm, fissitunicati, clavati vel clavato-anguste fusiformes. *Ascospores* (32.5–)34–45–48.5 μm × (6–)7–9–(10) μm, anguste fusiformes, 1-septatae, hyalinae, strato mucoso 3–7 μm lato circumdatae. *Anamorphosis* Tetraploa sensu lato. Conidia in vitro 41–55 × 17–23–27.5 μm, brunnea, cum 3 appendicibus; appendices 12–66 μm longae, 1–6-septatae.

**Ascomata** 250–300 μm high, 900–1000 μm diam (including the rim), with single locule of 420–530 μm diam, scattered to sometimes clustered, immersed below the epidermis, globose to subglobous, glabrous. *Beak* none or short, with hyaline sparse periphyses-like hyphae, filled with pseudoparaphyses tips. *Ascomatal wall* at sides 170–270 μm wide and rim-like, composed of vertically orientated rectangular to polygonal 5–13 × 3.5–8 μm hyaline cells; near the epidermis composed of polygonal to subglobose brown cells of 3–10 μm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–2.5 μm wide, guttulate, branched and anastomosed, with thin septa at 7 to 20 μm intervals. *Asci* 95–133 × 14.5–21 μm (av. 113 × 18 μm, n = 50), fissitunicate, numerous, basal and somewhat lateral, clavate to cylindrical, rounded at the apex, short-stalked (7–25 μm long), with (4–)8 biseriate ascospores. *Ascospores* (32.5–)34–45–48.5 μm × (6–)7–9–(10) μm (av. 38.9 × 7.9 μm, n = 120), LW 4.2–5.9 (av. 5.0, n = 120), narrowly fusiform with acute ends, straight or slightly curved, 1-septate, submedian (0.50–0.54; av. 0.52, n = 94), constricted at the septum, hyaline, up to 4 guttules in each cell or without guttules, smooth, with an inconspicuous sheath of 3–7 μm wide.

**Culture characteristics:** Colonies on PDA attaining 2.6–2.8 cm diam, velvety in appearance, olive (2E4), with whitish entire margin of 2 mm; reverse dark green (29F6); no pigment produced. On RSA, a Tetraploa-like anamorph with 3 setose appendages is formed. *Conidiophores* absent. *Conidigenous cells* monoblastic. *Conidia* consist of a conidial body and 3 setose appendages, solitary. Conidial body 41–55 × 14–23 (μm (av. 48.3 × 19.4 μm, n = 61), L/W = 2.2–3.2 (av. 2.5, n = 61), 5–6 pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Appendages 12–66 μm long (av. 27.4 μm, n = 65), 3–4 μm wide, 1–6-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, slightly curved.

**Notes:** This fungus is clearly distinguishable from other species of *Triplosphaeria* by its largest asci and ascospores.

**Triplosphaeria yezoensis** (I. Hino & Katum.) Kaz. Tanaka, K. Hiray. & Shirouzu, comb. nov. MycoBank MB515266. Fig. 9.

*Basionym:* *Didymella yezoensis* I. Hino & Katum., Bull. Fac. Agr. Yamaguchi Univ. 9: 902. 1958.

Ascomata 140–160 μm high, 450–550 μm diam (including the rim), with single locule of 240–330 μm diam, scattered to sometimes clustered, immersed below the epidermis, globose to subglobous, glabrous. *Beak* none or short, with hyaline sparse periphyses-like hyphae, filled with pseudoparaphyses tips. *Ascomatal wall* at sides 170–270 μm wide and rim-like, composed of vertically orientated rectangular to polygonal 5–13 × 3.5–8 μm hyaline cells; near the epidermis composed of polygonal to subglobose brown cells of 3–10 μm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–2.5 μm wide, guttulate, branched and anastomosed, with thin septa at 7 to 20 μm intervals. *Asci* 95–133 × 14.5–21 μm (av. 113 × 18 μm, n = 50), fissitunicate, numerous, basal and somewhat lateral, clavate to cylindrical, rounded at the apex, short-stalked (7–25 μm long), with (4–8) biseriate ascospores. *Ascospores* (32.5–)34–45–48.5 μm × (6–7–9–10) μm (av. 38.9 × 7.9 μm, n = 120), LW 4.2–5.9 (av. 5.0, n = 120), narrowly fusiform with acute ends, straight or slightly curved, 1-septate, submedian (0.50–0.54; av. 0.52, n = 94), constricted at the septum, hyaline, up to 4 guttules in each cell or without guttules, smooth, with an inconspicuous sheath of 3–7 μm wide.
Fig. 7. *Triplosphaeria cylindrica*. A. Ascomata on host surface; B–E. Ascospores; F. Ascoma in longitudinal section; G. Ascomal wall at side; H. Pseudoparaphyses; I–J. Asci; K. Fissitunicate ascus with endoascus extending from ectoascus; L. Ascospores in India ink; M–N. Developing conidia; O. Conidial body; P–Q. Conidia; R. Breached conidium composed of three columns; S. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–E, O, R = 10 µm; F = 50 µm; G–L, N, P–Q = 20 µm; M = 100 µm; S = 1 cm. A–B, F, J from YAM 21797 holotype of Massarina yezoensis; C–D, I from HHUF 29626; E, G–H, K–L from HHUF 29381; M–R from culture KT 1256; S from culture KT 1800.
**Fig. 8.** *Triplosporea maxima*. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomal wall at side; J–K. Asci; L. Apex of ascus; M. Pseudoparaphyses; N. Conidia on agar piece immersed in water; O. Developing conidia; P–Q. Conidia; R. Conidial body; S. Breached conidium composed of three columns; T. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 500 μm; C, I–K, S = 20 μm; D–G, L–M, R = 10 μm; H, N–O = 100 μm; T = 1 cm. A–M from HHUF 29390 holotype; N–T from culture KT 870.
grouped, immersed below the epidermis, subglobose, glabrous. 

Beak none or short, with hyaline sparse periphyses. Ascomatal wall at sides, 100–130 µm wide and rim-like, composed of vertically orientated, rectangular to subglobose, hyaline to pale brown, hyphoid cells of 5–15 × 5–7.5 µm; near the epidermis composed of polygonal to subglobose brown thick-walled cells of 2.5–7.5 µm diam; at the base flattened and poorly developed. Pseudoparaphyses narrowly cellular, numerous, 1–2 µm wide, guttulate, branched and anastomosed, septate, with slime coating. Asci (60–)72–119(–141) × 12–18.5 µm (av. 93.3 × 15.3 µm, n = 86), fissitunicate, numerous, basal and somewhat lateral, cylindrical to clavate, rounded at the apex, short-stalked (5–24 µm long), with 8 biseriate ascospores. Ascospores (22.5–)26–32(–35) × 5–8 µm (av. 29.1 × 6.6 µm, n = 109), L/W 3.6–5.3 (av. 4.4, n = 109), narrowly fusiform with acute ends, mostly curved, with a septum usually submedian (0.50–0.55; av. 0.52, n = 109) and constricted, hyaline, smooth, with an inconspicuous entire sheath of 2–8 µm thick.

Culture characteristics: On RSA, a Tetraploa-like anamorph with 3 setose appendages is formed. Conidiophores absent. Conidiogenous cells monoblastic. Conidia consist of a conidial body and 3 setose appendages, solitary, Conidial body 30–40(–45) × (13–)15–22 µm (av. 34.4 × 18 µm, n = 30), L/W = 1.7–2.2 (av. 1.9, n = 30), 3–4-pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Appendages (34–)40–75(–87) µm long (av. 51.6 µm, n = 40), 2.5–3 µm at the apex, 3–4.5 µm wide at the base, 2–9-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, slightly curved.

Fig. 9. Triplosphaeria yezoensis. A. Ascomata on host surface; B–F. Ascospores; G. Ascoma in longitudinal section; H. Ascospore in India ink; I. Apex of ascus; J. Pseudoparaphyses; K–M. Ascii; N. Conidia on rice straw agar; O–P. Conidia; Q. Conidial body; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, H–J, Q = 10 µm; G = 50 µm; K–M, O–P = 20 µm; N = 100 µm; R = 1 cm. A–B, G, I–K from YAM 21758 holotype of Didymella yezoensis; C–D, H, L from HHUF 30029; E–F, M from HHUF 30030; N–O from culture KT 1732; P–R from culture KT 1715.
Specimens examined: **Japan**, Hokkaido, Asahikawa, Kagura, on culms of *Sasa palmata*, 20 Sept. 1956, I. Hino, YAM 21758 holotype of *Didymella yezoensis*; Hokkaido, Yoichi, Sawamachi (140°46'E, 43°11'N), 6 June 2004, K. Tanaka, HHUF 30029, living culture KTC 1715 (= CBS 125436); Nagano, Sugadaira, Tsukuba Univ., on culms of *Sasa* sp., 28 June 2004, T. Shirouzu, HHUF 30030, living culture KTC 1732 (= CBS 125437).

**Notes**: Hino & Katumoto (1958) described this fungus as a species of *Didymella*, but the general characteristics of this fungus do not fit within the current concept of *Didymella* (Gruyter et al. 2009, Woudenberg et al. 2009). Due to the presence of hemispherical ascomata having rim-like side wall, and the morphology of the conidial state, it is transferred to the genus *Triplosphaeria*. This species is close to *Triplosphaeria acuta*, but differs from the latter in having relatively broader ascospores (L/W 5.5 vs. 4.4 µm) and slightly smaller conidia (av. 34.4 × 18 µm vs. 40.9 × 17.2 µm).

*Triplosphaeria* sp. (undescribed anamorphic state of *Triplosphaeria* sp.) Fig. 10.

**Conidiophores** absent. **Conidiogenous cells** monoblastic. **Conidia** (26–)31.5–46 × 14–23 µm (av. 38.4 × 18 µm, n = 61), LW 1.7–2.8 (av. 2.2, n = 61), brown, 3–5-pseudoiseptate, with 3 setose appendages. Appendages 36–90 µm long (av. 54 µm, n = 86), 2–8-septate.

**Culture characteristics**: **Conidia** produced on RSA are considerably larger than those on the host, 52–85 × 17–31 µm (av. 67.3 × 23.6 µm, n = 13), LW 1.9–3.7 (av. 2.9, n = 13), 6–8-pseudoiseptate, having 3 appendages of 51–120(–160) µm long (av. 78.5 µm, n = 14) with 4–12 septa.

Specimens examined: **Japan**, Aomori, Nakatsugaru, Nishimeya, Oosawa tril, on culms of *Sasa kurilensis*, 22 July 2002, S. Hatakeyama, HHUF 27481, living culture HC 4665 (= NBRC 106248); Hokkaido, Isl. Rishiri, Kutugata trail, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30031, living culture KTC 2546 (= NBRC 106249).

**Notes**: The conidia of *Triplosphaeria* sp. on the host plant (av. 38.4 × 18 µm) are similar to those of *Triplosphaeria maxima* produced under culture conditions (av. 48.3 × 19.3 µm), but *Triplosphaeria* sp. forms quite larger conidia in culture (av. 67.3 × 23.6 µm). The teleomorph of this fungus is unknown, but it obviously belongs to *Triplosphaeria* based on the anamorph morphology and molecular evidence. A new anamorph genus is needed to describe this species formally. However, we retain this species as *Triplosphaeria* sp. until further information is available, e.g. the possibility of collecting a teleomorph for this species.

**Polyplosphaeria** Kaz. Tanaka & K. Hiray., gen. nov. MycoBank MB515256.

**Anamorph**: Undescribed Tetraploa-like state producing conidia with three to eight setose appendages.

**Etymology**: In reference to the anamorphic state producing conidia with many setose appendages.

Ascomata erumpentia vel superficialia, globosa. Rostrum aliquantum papillatum. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, clavati, octospori. Ascospores anguste fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis Tetraploa sensu lato. Conidiophora absenta. Cellulae conidiigenae monoblasticae. Conidia globosa vel subglobosa, brunnea, cum 3–8 appendicibus.

Ascomata scattered to clustered, erumpent to superficial, globose, black to sometimes reddish-brown, with brown short hyphae at sides, mostly associated with reddish pigment. *Beak* slightly papillate, central, with hyaline periphyses. **Ascomatal wall** composed of rectangular to polygonal brown cells, sometimes poorly developed at the base. **Pseudoparaphyses** trabeal, numerous, tortuous, septate, branched and anastomosed, associated with gelatinous material. Asci fissitunicati, clavati, short-stalked, with 8 biseriate ascospores. Ascospores narrowly fusiform, slightly curved.
1–(3)-septate, constricted at the primary septum, hyaline to pale olive-brown, with an entire sheath. Anamorphs Tetraploa-like with 3 to 8 setose appendages. Conidiophores absent. Conidiogenous cells monoblastic. Conidia globose to subglobose, brown, with 3–8 appendices; appendices 92–200(–235) µm long (av. 147.6 µm, n = 56), 7–10 µm wide at the base, 2–3.5 µm wide at the apex, with 4–10 septa at 15 to 28 µm intervals. The teleomorph is similar to that found on the host, but the ascii and ascospores in culture are slightly larger. Ascii 120–155 × 17.5–23 µm (av. 135.4 × 20 µm, n = 56). Ascospores 39–54(–57) × 8.5–10.5 µm (av. 47.7 × 9.6 µm, n = 70), L/W 4.3–5.7 (av. 5.0, n = 70), with a submedian primary septum (0.50–0.53; av. 0.52 ± 0.03).

Specimens examined: Japan, Aomori, Sannohe, Gonohe, Asamizu (141°16'E, 40°28'N), on culms of Pleioblastus chino (CJ = MAF 23965); Shigok, Kanuma, Simosawa (139°42'2'E, 36°34'4N), on culms of Phyllostachys bambusoides, 20 Mar. 2003, N. Asama, MAF 23992, living culture KTC 1043 (= JCM 13176 = MAFF 239663); Shizuko, Syoutsu, Nagazumi, Minami-ishi, Fuji bamboo garden (138°53.1'N, 35°09.3'), on culms of Chimonobambusa marmorata, 8 Mar. 2004, K. Tanaka & Y. Harada, MAF 29405, living culture KTC 1640 (= JCM 13176 = MAFF 239668); Nagasaki, Nagayo, Nagasaki Siebold University (129°52.4'E, 32°42.2'), on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, MAF 29406, living culture KTC 1699 (= JCM 13177 = MAFF 239687); Aomori, Souma, Aina trail, on culms of Sasa kulinensis, 29 July 2006, K. Tanaka et al., MAF 30018, living culture KTC 2124 (= CBS 125425).

Notes: This species has a broad host preference within Bambusoideae because it has been associated with four bamboo genera in two subtribes; Arundinarini (Pleioblastus and Sasa) and Shibataeinae (Chimonobambusa and Phyllostachys). As discussed later, two distinct clades, K1043+1640 and K1616+2124, were found for this species in the tree. Ascomata in these specimens are “almost superficial without associated pigmentation” and “immersed to erumpent with reddish pigments”, respectively. Possibly, they may reflect the differences between the bamboo hosts, Arundinarini and Shibataeinae. Additional material will be helpful to evaluate the taxonomic significance of these variations.

**Pseudotetraploa** Kaz. Tanaka & K. Hiray., gen. nov. MycoBank MB515257.

**Teleomorph:** Unknown.

**Etyymology:** In reference to the Tetraploa-like conidial morphology.

**Notes:** This species is another representative of the genus with conidia similar to those of Tetraploa, although its macroscopic indistinguishable from the former. Mycelium superficial. Conidiophores absent. Conidiogenous cells monoblastic, indistinguishable from the teleomorph. Conidia obpyriformes vel anguste obpyriformes, brunnea vel atro brunnea, cum 3–6 appendicibus; appendices 92–200(–235) µm longae, 4–10-septatae.

**Ascomata** 180–420 µm high, 300–680 µm diam, scattered to obtectae. Anamorphosis fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa.

**Notes:** The characteristics of this new genus include globose ascomata surrounded by numerous brown hypheae, reddish pigment on the host surface around ascomata, clavate asci with fissitunicata dehiscence, and narrowly fusiform ascospores provided with an entire sheath. The anamorphic state of Polyplosphaeria produces almost globose conidia composed of numerous internal hypheae, thin peel-like outer wall, and three to eight setose appendages. These appearances of conidia are slightly similar to those of Piricauda (e.g. *P. cochinensis* and *P. longispera*), but Piricauda has been defined primarily based on monotectic conidiogenous cells and its muriform conidia (Mercado Sierra et al. 2005).

**Polylosphaeria fusca** Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515267. Fig. 11.
curved, as compared with those of *Tetraploa* (long and straight). There are several hyphomycetes with conidia resembling those of *Pseudotetraploa*, such as *Ceratosporella* (Kuthubutheen & Nawawi 1991), *Paratetraploa* (Wong et al. 2002), *Triposporium* (Rifai 1972), and *Tretospeira* (Pirozynski 1972, Ho et al. 2000), but they have macro- or semimacronematous conidiophores. *Kodonospora* (Ando 1993) shares some features with *Pseudotetraploa*, but this genus does not have well-developed appendages. The following three species previously described as *Tetraploa* (Hatakeyama et al. 2005) are transferred to *Pseudotetraploa*.
Molecular taxonomy of bambusicolous fungi: Tetraplospairaeeae, a new pleosporalean family with tetraploa-like anamorphs

Pseudotetraploa curviappendiculata (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov. MycoBank MB515268. Fig. 12A–E.

Basionym: Tetraploa curviappendiculata Sat. Hatak., Kaz. Tanaka & Y. Harada, Mycoscience 46: 196. 2005.

Specimens examined: Japan, Aomori, Hirosaki, Mt. Kudoji (140°25'E, 40°31'N), on culms of Sasa kurilensis, 9 May 2003, Y. Harada, HHUF 28582 holotype, living culture HC 4930 (= JCM 12852 = MAFF 239495); Aomori, Hirosaki, Matsukita (140°29'E, 40°33'N), on culms of Sasa kurilensis, 7 Dec. 2003, K. Tanaka & N. Asama, HHUF 28590, living culture HC 4932 (= MAFF 239496); Hokkaido, Isl. Rishiri, Shinrin-park, on culms of Sasa kurilensis, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30019, living culture KTC 2558 (= CBS 125426 = NBRC 106241).

Pseudotetraploa javanica (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., comb. nov. MycoBank MB515270. Fig. 12K–O.

Basionym: Tetraploa javanica Rifai, Zainuddin & Cholil, Reinwardtia 10: 420. 1988.

Specimens examined: Japan, Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of Pleioblastus chinensis, 2 Dec. 2003, K. Tanaka et al., HHUF 28590, living culture HC 4933 (= JCM 12855 = MAFF 239497).

Pseudotetraploa longissima (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov. MycoBank MB515269. Fig. 12F–J.

Basionym: Tetraploa longissima Sat. Hatak., Kaz. Tanaka & Y. Harada, Mycoscience 46: 196. 2005.

Specimens examined: Japan, Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of Pleioblastus chinensis, 2 Dec. 2003, K. Tanaka et al., HHUF 28590, living culture HC 4933 (= JCM 12855 = MAFF 239497).

Fig. 12. Pseudotetraploa spp. A–E. P. curviappendiculata; F–J. P. javanica; A, F, K. Conidia; B, G, L. Conidial bodies; C, H, M. Weakly breached conidia; D, I, N. Strongly breached conidia (D, I. with four columns, I, with six columns); E, J, O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A, F, K = 50 µm; B–D, G–I, L–N = 20 µm; E, J, O = 1 cm. A–D from HHUF 28582 holotype; E from culture HC 4930; F–I from HHUF 28590 holotype; J form culture HC 4933; K–N from HHUF 28596; O from culture HC 4934.
Quadricrura Kaz. Tanaka, K. Hiray. & Sat. Hatak., gen. nov. MycoBank MB515258.

Teleomorph: Unknown.

Etymology: From Latin quadri meaning four and crura meaning leg, in reference to the conidial morphology with four leg-like short appendages.

Mycelium superficial. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia globosa vel subgloboa, brunnea vel atr brunnea, cum 1 vel 2 longiappendicibus et 4 vel 5 breviappendicibus.

Myelium superficial. Conidiophores absent. Conidiogenous cells monoblastic, indistinguishable from creeping hyphae. Conidia globose to subglobose, with thin peel on the outer wall of conidia, composed of numerous internal hyphae at the inside, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages of two forms, unbranched, smooth, brown at the base and mostly hyaline at the apex: long appendages usually single or 2, arising from apical part of conidia; short appendages mostly 4 to 5, arising from basal side part of conidia.

Type species: Quadricrura septentrionalis Kaz. Tanaka, K. Hiray. & Sat. Hatak.

Notes: This new genus is characterised by globose to subglobose conidia that are composed of internal hyphae and thin peel-like outer wall similar to the Polyplosphaeria anamorph. The presence of internal hyphae in conidia is known in the genus Piricaudilium (Holubová-Jechová 1988). Likewise, the peel-like outer wall of conidia is found in the genus Megacapitula (Chen & Tzean 1993). Quadricrura, however, differs from these genera in the morphology of setose appendages of conidia; one or two long appendages arising from the apical part and mostly four to five short appendages around the basal sides. Bioconiosporium (Ellis 1976, Narayan & Kamal 1986) and Pseudopetrakia (Ellis 1976) have conidia resembling those of Quadricrura to some degree, but produce setose appendages only on the apex of muriform conidia.

Quadricrura bicornis Kaz. Tanaka, K. Hiray. & H. Yonez., sp. nov. MycoBank MB515271. Fig. 13.

Etymology: From Latin bi meaning two and cornis meaning horned, referring to the two long setose appendages of conidia.
**Quadricrura meridionalis** Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515273. Fig. 14.

**Etymology:** In reference to the southern distribution of the taxon.

.pad_box[0.57,0.77,0.87,0.87]{

Notes: One of the most striking features of *Q. bicornis* is the presence of two pairs of long appendages at the conidial apex. The holotype of this fungus was collected from leaf litter of a conifer, but it is uncertain whether the conifer is a natural host of *Q. bicornis*. An additional specimen of this fungus on *Sasa kurilensis* (HHUF 30035) was also identified as *Q. bicornis* based on morphology, although there is no isolate and molecular evidence from this specimen. These two specimens were collected from the same locality, and the holotype was found around the base of a thicket of *Sasa kurilensis*.

**Quadricrura meridionalis** Kaz. Tanaka & K. Hiray., sp. nov. MycoBank MB515273. Fig. 14.

**Etymology:** In reference to the southern distribution of the taxon.

Mycelium superficialia. Conidiophores absent. Conidiogenous cells monoblastic, indistinguishable from creeping hyphae. Conidia 36–43.5(–56.5) × 41–75 µm (av. 48.8 × 57.5 µm, n = 22), subglobose, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages of two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendages 2, 170–295 µm long (av. 250.6 µm, n = 39), 10–13 µm wide at the base, 4–5 µm wide at the apex, 4–8-septate, arising from apical part of conidia; short appendages usually 4, 17.5–45.5 µm long (av. 30.6 µm, n = 39), 7–11.5 µm wide at the base, 4–5 µm wide at the apex, 0–2-septate, arising excentric from the conidal base.

**Culture characteristics:** The conidial state in culture condition is similar to that on the host, but the conidia are slightly larger (50–77.5 × 60–80 µm).

Specimens examined: Japan, Aomori, Shirakami, Chisan-dam, on leaf litter of a conifer, 21 July 2007, H. Yonezawa & K. Tanaka, HHUF 30023 holotype designated here, living culture yone153 (= CBS 125427); Aomori, Shirakami, Chisan-dam, on culms of *Sasa kurilensis*, 21 July 2007, H. Yonezawa & K. Tanaka, yone154 = HHUF 30035.
appendages. Appendages of two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendages usually single, rarely 2, 170–295 µm long (av. 236.3 µm, \(n = 15\)), 10–12 µm wide at the base, 3–4 µm wide at the apex, with 10 to 16 septa at 7.5 to 30 µm intervals, arising from the apical part of conidia; short appendages usually 4, rarely 5, 15–37.5 µm long (av. 24.9 µm, \(n = 27\)), 6–7 µm wide at the base, 3–4 µm wide at the apex, 0–2-septate, arising excentric from the conidial base.

**Culture characteristics:** On RSA, sporulation is observed on the surface of rice straw, but the conidial morphology is considerably different as compared with those on the host. The conidial body is larger, measuring 90–100 × 95–112 µm, and with 3–6 long appendages.

**Specimen examined:** Japan, Okinawa, Isl. Tonoguni, Irrida trail, on culms of bamboo, 23 Nov. 2008, K. Tanaka & K. Hirayama, HHUF 30024 holotype designated here, living culture KTC 2607 (NBRC 106242 = CBS 125684).

**Note:** It bears a slight resemblance to *Q. septentrionalis*, but can be separated on the basis of larger and subglobose conidia (av. 48.8 × 57.5 µm vs. 37.4 µm diam).

**Quadricrura septentrionalis** Kaz. Tanaka, K. Hiray. & Sat. Hatak., sp. nov. MycoBank MB515272. Fig. 15.

**Etymology:** In reference to the northern distribution of the taxon.

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**Mycelium superficial.** Conidiophores absent. Conidiogenous cells monoblastic, indistinguishable from creeping hyphae. Conidia 30–45(–52.5) µm, globose, brunnea vel atro brunnea, cum appendicibus; longiappendices unica, 115–210 µm longae, 6–12-septatae; breviappendices 4, 10–20 µm longae, 0–1-septatae.

**Culture characteristics:** Colonies on PDA attaining a diam of 2.9–3.2 cm, velvety in appearance, metal-grey (5E2) with 2 mm whitish entire margin, reverse clay (5D5); no pigment produced. On RSA, an anamorphic state is formed on the surface of rice straw. Conidia from culture are similar to those on natural specimen, but conidial body is slightly smaller (25–42.5 µm diam) and long appendage is longer (135–240 µm). In the culture HC 4984, a spermatial state is also produced; Spermogonia 80–150 µm, globose, black; Spermatia 2–2.5 × 1.5 µm, subglobose, hyaline.

**Specimens examined:** Japan, Aomori, Hirosaki, Serisawa-park, on culms of *Sasa kunlensis*, 3 May 2003, K. Tanaka & N. Asama, HHUF 28782 holotype designated.
Molecular Taxonomy of Bambusicolous Fungi: Tetraplosphaeriaceae, a New Pleosporalean Family with Tetraploa-like Anamorphs

Here, living culture HC 4984 (= CBS 125430); Aomori, Shimokita, Hotogekaura, on culms of S. kurilensis, 20 Oct. 2002; N. Asama, HHUF 30020, living culture KTC 920 (= CBS 125428); Aomori, Hiroaki, Serisawa-park, on culms of S. kurilensis, 7 Dec. 2002; K. Tanaka & N. Asama, HHUF 28781, living culture HC 4983 (= CBS 125429); Aomori, Zatoishi, Ogamisawa, on culms of S. kurilensis, 8 July 2006; K. Tanaka, HHUF 29747, living culture yone 44 = HC 5254 (= CBS 125431); Hokkaido, Isl. Rishiri, Kutsugata trail, on culms of S. kurilensis, 28 July 2007; K. Tanaka & G. Sato, HHUF 30021, living culture yone 176 (= CBS 125432 = NBRC 100243); Hokkaido, Isl. Rishiri, Oniwa-trail, on culms of S. kurilensis, 29 July 2007; K. Tanaka & G. Sato, living culture yone 179 (= CBS 125433 = NBRC 106244); Aomori, Hiroaki, Serisawa-park, on culms of S. kurilensis, 29 Nov. 2003; K. Tanaka & N. Asama, SH 91 = HHUF 28788; Iwate, Nishine, Mt. Iwate, on culms of S. kurilensis, 2 Dec. 2004; K. Tanaka, HHUF 29787, living culture KTC 920, 7 Dec. 2007; Aomori, Towada, Denbouzi, on culms of S. kurilensis, 8 Nov. 2003; K. Tanaka & T. Shirouzu, SH 35 = HHUF 28787; Hokkaido, Yoichi, Sawamachi (140°46'E, 43°11'N), on culms of S. kurilensis, 6 June 2004; K. Tanaka, SH 193 = HHUF 28790; Hokkaido, Sapporo, Manuyama (141°18.4'E, 43°02.4'N), on culms of S. kurilensis, 6 June 2004; K. Tanaka, SH 195 = HHUF 28792; Hokkaido, Sapporo, Botanical garden of Hokkaido Univ. (141°20.4'E, 43°03.4'N), on culms of Sasamorpha borealis var. borealis, 6 June 2004; K. Tanaka, SH 194 = HHUF 28791; Hokkaido, Notsuke, Bekki, Notsuketunen park (145°14'E, 43°31'N), on culms of Sasa nipponica, 8 Sept. 2003; K. Tanaka & S. Hatakeyama, SH 118 = HHUF 28783; Hokkaido, Akkeshi, Ootakita, Sattebetu-river (144°49.0'E, 43°08.7'N), on bamboo culms, 7 Sept. 2003; K. Tanaka & S. Hatakeyama, SH 88 = HHUF 28784; Hokkaido, Kamikawa, Shintono, Shinrui, Karikachi mountain pass (142°46.1'E, 43°07.6'N, 644m a.s.l.), on bamboo culms, 9 Sept. 2003; K. Tanaka & S. Hatakeyama, SH 92 = HHUF 28795; Aomori, Towada, Denbouzi (141°16.1'E, 40°34.2'N), on culms of Pleioblastus chino, 2 Dec. 2003; K. Tanaka et al., SH 87 = HHUF 28789.

Note: Quadricrura septentrionalis is frequently collected from various bamboos, particularly Sasa kurilensis, and might be widely distributed in northern Japan.

Phylogenetic analyses

SSU+LSU: Approximately 990–1 350 bp of SSU and 1 260–1 503 bp of LSU nDNA sequences were determined for 53 isolates of bambusicolous fungi. A combined dataset of SSU (893 bp) and LSU (985 bp) sequences were generated after excluding insertions of several species which correspond to positions 471–832 of Russoeolopsis tosaensis (GenBank AB524484) and positions 1 247–1 591 of Neotliosporina paspali (GenBank EU754073) in the SSU sequences. The combined dataset was aligned with sequences of 39 species belonging to Dothideomycetes (mainly Pleosporales) obtained from GenBank. Botryosphaeria dothidea, Spencermartinsia viticola (both belonging to Botryosphaeriaceae) and Dotheia insculpta (Dothideales) were used as the outgroup taxa. Of the 1 878 characters, 442 (23.5 %) were variable, of which 349 (18.6 %) were parsimony informative. An MP analysis yielded 31 equally most parsimonious trees with a tree length (TL) of 1 503 steps [consistency index (CI), retention index (RI) of 0.403 and 0.777, respectively]. A consensus tree was constructed from the 31 MP trees (Fig. 16). The trees obtained from NJ and Bayesian analysis had a similar topology to that of the MP tree on the whole, although the monophyly of Triplosphaeria was rejected in the Bayesian analysis. Bambusicolous fungi represented by 53 isolates comprising 32 species in 14 genera are scattered in nine clades. The new family Tetraplosphaeriaceae formed a monophyletic clade moderately or strongly supported by NJBS value (86 %) or Bayesian PP (1.00), but the monophyly was not well supported in MP analysis (54 %). Tetraplosphaeriaceae was positioned as a sister group to a clade composed of mainly pleosporalean families, such as Lophiostomataceae, Massarinaceae, Phaeosphaeriaceae, Pleomassaraceae and Pleosporaceae, but these relationships were not supported in the MP analysis (< 50 %) and not found in the Bayesian analysis. In the NJ analysis, Tetraplosphaeriaceae clustered with the Massarina arundinariae-Testudinaceae clade. ITS+TEF+BT: From 31 isolates of Tetraplosphaeriaceae species including the outgroup taxon (Massarina arundinariae), sequences of ca. 482–503 bp, 293–333 bp, 570–662 bp were obtained for the ITS, TEF and BT regions. The final alignment of the ITS region after eliminating gaps and ambiguous sites was composed of 459 bp. These included 131 variable sites (28.5 %) and 106 parsimony informative sites (23.1 %). The NJ tree using this alignment rejected the monophyly of Quadricrura and Triplosphaeria. In this analysis, the other three genera, Polyplosphaeria, Pseudotetraploa and Tetraplosphaeria, were supported with moderate or strong BS values (71–100 %; Fig. 17A). The data matrix of TEF comprised 281 aligned characters with 157 variable positions (55.9 %) and 141 parsimony-informative positions (50.2 %). Although the NJ tree generated from this dataset indicated that the four genera, Polyplosphaeria, Pseudotetraploa, Quadricrura and Triplosphaeria, form monophyletic clades, respectively (79–100 %), Tetraplosphaeria was separated into two clades (Fig. 17B). A dataset from BT sequences included 553 sites after truncating both ends and excluding ambiguous regions. Of these, 248 (44.8 %) and 228 (41.2 %) were variable and parsimony informative, respectively. The NJ tree based on this alignment showed five genera each in Tetraplosphaeriaceae as monophyletic clades. However, the BS value of Quadricrura was relatively low (67 %) and relationships between the genera were poorly resolved from the BT tree alone (Fig. 17C).

In addition to the individual datasets of ITS, TEF and BT, a combined alignment of these regions (1 293 bp) was used for further analyses. The phylogenetic tree obtained from the Bayesian analysis is shown in Fig. 18. It was generally similar to the results form the individual analyses (Fig. 17) in terms of the arrangement of each genus. Other trees generated from MP and NJ analyses had essentially similar topologies, but monophyly of Tetraplosphaeria was rejected in the MP tree. Each genus was supported by strong statistical values of more than 96 % BS or 1.00 PP, except for the Tetraplosphaeria clade. Quadricrura and Polyplosphaeria together formed a well-supported single clade (1.00 PP and > 87 % BS), which was a sister group to Triplosphaeria, and the relationships of these three genera received strong support (1.00 PP and > 99 % BS). Pseudotetraploa was a sister taxon of the Quadricrura-Polyplasphaeria-Triplosphaeria clade. Tetraplosphaeria occurred at the most basal position in this family.
Fig. 16. Consensus tree of the 31 equally most parsimonious trees based on a combined dataset of SSU (893 bp) and LSU (985 bp) nrDNA sequences. MP and NJ bootstrap support greater than 50 % and Bayesian posterior probabilities above 0.90 are indicated at the nodes as MPBS/NJBS/PP. A small red circle is used for a clade with high statistical support (more than 90 % BS and 1.00 PP). The green branches represent lineages of bambusicolous fungi. TL = 1 503, CI = 0.403, RI = 0.777. Either two GenBank numbers (SSU+LSU) or the original isolate numbers are noted after the species names. An asterisk (“*”) indicates sequences obtained from two different strains of the same species. The tree was rooted to Botryosphaeria dothidea, Spencermartinsia viticola and Dothidea insculpta. Species of bambusicolous fungi are indicated in bold.
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Fig. 17. Neighbour-joining trees of the Tetraplospheariaeae based on the sequences from ITS (A: 459 bp), TEF (B: 281 bp), and BT (C: 553 bp). Bootstrap support greater than 50% are shown at the nodes. An original isolate number is noted after the species name. The tree is rooted to Massarina arundinariae.
Fig. 18. Phylogeny of Tetraplosphaeriaceae from Bayesian analysis based on a combined dataset (1 293 bp) of ITS, TEF, and BT. Bayesian posterior probabilities above 0.90 and MP and NJ bootstrap values greater than 50% are indicated at the nodes as PP/MPBS/NJBS. Hyphen ("-") indicates values lower than 0.90 (PP) or 50% (BS), and a node not present in an analysis is shown with "x". A small red circle is used for a clade with high statistical support (more than 1.00 PP and 90% BS). An original isolate number is noted after the species name. The tree was rooted to Massarina arundinariae. Abbreviations for species characterisation: Hos = host, B: bamboo, O: other plant; Con = conidial structure, int: with internal hyphae, col: with columns; Ape = number of conidial appendages, 1+4 or 2+4 indicates number of apical appendages + basal appendages; Tel = teleomorph formation, +: present, -: absent, ?: unknown; Mat = mating type, Ho: homothallic, He: heterothallic, ?: unknown.

| Species characterisation | Hos | Con | Ape | Tel | Mat |
|--------------------------|--|-----|-----|-----|-----|
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Quadricrura septentrionalis | B | Int | 1+4 | – | ? |
| Massarina arundinariae | KT 856 |
| Massarina arundinariae | KT 2200 |
DISCUSSION

Phylogenetic position of selected bambusicolous fungi

In this study, phylogenetic analyses of bambusicolous fungi were carried out based on SSU+ LSU sequences. Fifty-three isolates from bamboo comprising 32 species in 14 genera were found to cluster in nine clades. Notes on phylogenetic placements of species in the following nine genera except for members in Tetraploasphaeriaceae are described below.

Astrosphaeriella (Fig. 19A–B): This genus is characterised by the cone-shaped, large ascomata composed of carbonaceous firm peridium, with starlike flanges of ruptured host tissue around the base (Fig. 19A); the numerous trabeculate pseudoparaphyses in peridium, with starlike flanges of ruptured host tissue around the cone-shaped, large ascomata composed of carbonaceous firm peridium; thin ascomatal wall composed of small pseudoparenchymatous cells, cellular pseudoparaphyses, and fissitunicate ascospores, Katsumoto has been tentatively assigned to Phaeosphaeriaceae (Tanaka & Harada 2005b). However, Katsumoto did not group within Phaeosphaeria, and formed a clade with Ophiosphaerella sasicola, another bambusicolous fungus (Figs 16, 19I). This clade was sister to Massaria platani (Massariaceae) but the affinity of these taxa was insufficiently supported (<50 % BS).

Massaria (Fig. 19G–H): Several species in this genus (e.g. M. alpina, M. pustulata, M. bambusina) have been recorded from bamboo (Eriksson & Yue 1998, Tanaka & Harada 2003b), but there is no sequence data for most of them. In this study, M. arundinariae, which has been accepted as Massaria (Aproot 1998) was later transferred to Lophiotoma (Hyde et al. 2002a), was used for the analyses. All phylogenetic analyses revealed that placement of this taxon in either Massaria or Lophiotoma was not suitable (Fig. 16). The species grouped with the Verruculina-Testudinaceae clade, and were isolated from a core member of Pleosporales in the MP tree or were positioned as a sister group of Tetraploasphaeriaceae in the NJ tree. In the analyses of Pleosporales using sequences from nrDNA, TEF1 and RPB2 in this volume (Zhang et al. 2009a), this species is treated as a Lophiotrema. Phylogenetic re-evaluation of the generic placement of other Massaria species from bamboo would be required, because recent molecular studies on the genus suggest a considerable polyphyly of Massaria s. l. (Kodusev et al. 2007, Zhang et al. 2009b).

Ophiosphaerella (Fig. 19I): Ophiosphaerella sasicola deviated from the Phaeosphaeriaceae clade including Ophiosphaerella or Phaeosphaeria, genera that previously accommodated the species (Nagasawa & Otani 1977, Shoemaker & Babcock 1989). The multi-septated scolecospores (Fig. 19I) found in O. sasicola might suggest an affinity with species of Cochliobolus (Pleosporaceae), but this relationship was not supported (Fig. 16). Ophiosphaerella sasicola formed a monophyletic clade with K. bambusicola supported by strong statistical values (>96 % BS, 1.00 PP; Fig. 16), although there is no morphological similarity between the taxa. Most probably, a new genus should be established to accommodate this species.

Phaeosphaeria (Fig. 19J–K): Two species of Phaeosphaeria on bamboo, P. brevispora and Phaeosphaeria sp., were examined in our analyses, but they did not locate to Phaeosphaeria or Phaeosphaeriaceae. The separation of P. brevispora from the Phaeosphaeria clade might be due to morphological heterogeneity of this species among the genus, such as gregarious ascomata with clypeate and clavate asci with a relatively long stipe (Fig. 19J; Tanaka & Harada 2004). These morphological features of the species are similar to those of Kalmusia scabrispora (Fig. 19C–D), although the relationships between the taxa were not supported according to the molecular phylogeny in this study. While Phaeosphaeria sp. [Fig. 19K; the same species reported by Tanaka & Harada (2004) as Phaeosphaeria sp.] shares several characters with Phaeosphaeria on various monocots (Shoemaker & Babcock 1989). This might indicate that fungal species on bamboo are a peculiar lineage and do not belong to existing genera from other host plants, even though they have morphological similarities with the genera. Molecular phylogenetic studies of other Phaeosphaeria species described from bamboo (e.g. P. bambusae) should be conducted to confirm this expectation.

Roussoella (Fig. 19L–M): Roussoella is characterised by gregarious, clypeate ascomata, trabeculate pseudoparaphyses embedded in a gel matrix, bitunicate ascis without obvious
fissitunicate dehiscence, and brown, 1-septate ascospores with distinctive wall ornamentation (Fig. 19L; Hyde et al. 1999). This genus has traditionally been considered as a member of Amphisphaeriaceae (Xylariales) because of the misinterpretation of the asci as unitunicate with IKI ± apical rings (Aptroot 1995a), and the presence of heterogenous element in the genus, now treated as Arecophila (Hyde 1996). The genus, typified by R. hysterioides, is currently placed in Didymosphaeriaceae (Ju et al. 1996, Lumbsch & Huhndorf 2007), although the validity of this classification has not been assessed in previous phylogenetic studies (Kang et al. 1998, Verkley et al. 2004). Roussellopsis include more than 11 species (Hyde 1997, Hyde et al. 1999, Zhou et al. 2003) and most of them are known from bamboo. Four isolates of Roussellopsis used in our analyses did not cluster with members of Didymosphaeriaceae, such as Didymosphaeria futilis in the LSU tree (data not shown) or Verruculina enalia, and formed a strongly supported clade (99 % BS, 1.00 PP) with Roussellopsis and Arthopyriena salicis (Fig. 16). This result might suggest that Roussellopsis belongs to Arthopyrienaeae, but this relationship is not fully resolved because of the morphological differences between both taxa. Many of the characters found in Arthopyrienaeae, e.g. lichenised or non-lichenised nature, hemispherical ascomata with wall sometimes staining green by KOH, cellular pseudoparaphyses, fissitunicate asci, and mostly hyaline ascospores (Eriksson 1981, Cannon & Kirk 2007), are significantly different from those of Roussellopsis. Our results further suggest that Roussellopsis is not a monophyletic genus, but additional evidence would be necessary before taxonomic revisions of the genus can be proposed.
study (Fig. 16). The transfer of Roussoellopsis to an older genus Roussoella appears to be reasonable from the topology, but careful consideration must be given to the treatment. In this study, it was revealed for the first time that Roussoellopsis tosaensis has a Melanconiosis or Neomelanconium-like anamorph producing annellidic conidiogenous cells, and almost globose, black, 1-celled, thick-walled conidia (ca. 21–30 µm diam) surrounded by an entire gelatinous material (Fig. 19P). Differences found in anamorphs between Roussoellopsis and Roussoella having a Cytoplesia state (Fig. 19M; Hyde et al. 1996) indicate that they are not congeneric.

Versicolorisporium (Fig. 19O): It has been reported that this genus has a phylogenetic relatedness with Arthopyrenia based on the similarity of LSU sequences (Hatakeyama et al. 2008). In the result from our study using the SSU+LSU dataset, Versicolorisporium clustered as a sister taxon with the clade of Roussoellia-Roussoellopsis-Arthopyrenia (Fig. 16), although these relationships were supported only from the NJ analysis. Besides, the versicolous, 3-septate conidia of Versicolorisporium (Fig. 19Q) are quite different from those found in anamorphs of Roussoella or Roussoellopsis. Phylogenetic inference of this anamorphic genus could not be elucidated at this time, but it is probable that Versicolorisporium does not belong to the main existing families in Pleosporales.

Monophyly of Tetraploa and T. aristata

The anamorphic genus Tetraploa is a well-known dematiaceous hyphomycete. Tetraploa species mostly occur throughout the year on leaves or stems of monocotyledons including bamboo, and also on various dicotyledons (Ellis 1949). Sixteen taxa have been accepted in the genus until now (Ellis 1949, Sharma 1979, Arambbari et al. 1987, Rifai et al. 1988, Révay 1993, Matsushima & Matsuhashi 1996, Hatakeyama et al. 2005, Pratibha & Bhat 2008, Zhao et al. 2009). There have been no doubt regarding the monophyly of Tetraploa characterised by conidia that consist of a main body and four setose appendages and that are formed from a conidiogenous cell indistinguishable from creeping hyphae (Hatakeyama et al. 2005). However, our analyses revealed that the genus is composed of at least two lineages, i.e. Tetraploa s. str. and Pseudotetraploa (Figs 16–18). Several species previously described as Tetraploa might have phylogenetic affinities with Pseudotetraploa or might represent an additional lineage retaining a close relationship with Tetraploa. For example, T. opacta most likely belongs to Pseudotetraploa based on the original description and illustration of the species (Zhao et al. 2009). Tetraploa abortiva (Arambarri et al. 1987) and T. setiferia (Révay 1993, Markovskaja 2007) should probably be separated from Tetraploa s. str. owing to their unusual features such as conidial body composed of three columns or hyaline appendages. Results from our analyses indicate that the genus Tetraploa should be restricted to species with conidial features similar to that of T. aristata and T. ellisi.

Interestingly, monophyly of T. aristata, the type species of the genus (Berkeley & Broome 1850), was also rejected in this study. Tetraploa aristata, the most well-known species in this genus, has been considered to have a wide geographical distribution (Ellis 1949). It has been recorded on more than 120 plant species (Farr & Rossman 2009), in particular on senescent culms of Gramineae (e.g. Pennisetum, Phragmites, Miscanthus) and Cyperaceae (e.g. Schoenoplectus) as a major saprophytic fungus (Wong & Hyde 2001). Moreover, there are several reports of the species as "facultative aquatic hyphomycete" (Kirk 1989, Descals & Moralezio 2001) or "terrestrial-aquatic hyphomycete" (Ando 1992, Goh & Hyde 1996), as an air-borne fungus (Sreeramulu & Ramalingam 1962, Tseng & Chen 1982, Green et al. 2006), and sometimes as a human pathogen causing keratomycosis or phaeohyphomycotic cysts (Markham et al. 1990). Traditionally, T. aristata has been believed to be a single species having high ecological diversity. However, the circumscription of T. aristata would be problematic because four isolates identified morphologically as T. aristata or Tetraploa cf. aristata (KT 1682, 1684, 1906, and CBS 996.70) showed low sequence similarities with each other (Fig. 16). Probably, this species-complex can likely be separated into several species based on minute morphological differences, e.g. dimension and degree of ornamentation of conidial body and length of setose appendages. Therefore, morphological re-assessment of T. aristata s. l. (Ellis 1949) based on the type specimen of T. aristata (Berkeley & Broome 1850) would be required. Among the 16 species in Tetraploa, only one species, T. aristata, is known to have a Massarina teleomorph of pleosporalean ascomycete (Scheuer 1991), but the identification of this anamorphic state should be re-evaluated in the future.

Generic placement of ascomycetes having Tetraploa anamorphs

Although the teleomorphic fungus of “T. aristata” found on Carex by Scheuer (1991) has been assigned to the genera Massarina (Scheuer 1991, Aptroot 1998) or Lophiostoma (Hyde et al. 2002a), our analyses revealed that these generic placements are inappropriate. These two genera are placed in Massarinaceae and Lohiostomataceae, respectively (Lumbsch & Huhndorf 2007). Massarinaceae seems to be poorly defined family in view of morphological aspects, but the type species of Massarina (M. eburnea) has phylogenetic relationships with Aquaticheirospora (Kosudse et al. 2007), Helminthosporium (Oliver et al. 2000), Saccharicola (Eriksson & Hawksworth 2003). On the other hand, Lophiostoma characterised by the slit-like ostiole of ascostoma is a well-defined genus because several taxa including the type species of this genus formed a family Lophiostomataceae as a sister group of Sporormiaceae (Fig. 16; see also Tanaka & Hosoya 2008). Because six isolates of Tetraploa s. str. with or without teleomorphs did not cluster with Massarinaeae or Lophiostomataceae (Fig. 16), a new genus, Tetraplosphaeria, was introduced for this lineage producing Tetraploa anamorphs as a common feature. In the protologue of M. tetraploa (anam.: T. aristata), Scheuer (1991) noted the morphological affinities of this species with several genera, such as Massarina, Keissleriella, Lophiostoma, Lophiotrema, and Massariosphaeria. All of them belong to a core group of Pleosporales, a clade with strong support values (97 % BS, 1.00 PP), ranging from Phaeosphaeria brevispora to Roussoella hyst erotides in Fig. 16. Tetraplosphaeria having Tetraploa anamorphs s. str. formed a single clade with four other genera (Triplosphaeria, Polyosphaeria, Pseudotetraploa and Quadricirura) having Tetraploa-like anamorphs, and this new lineage (Tetraplosphaeriaceae) deviated from a core group of Pleosporales, although it has characteristic features of the order, i.e. Pleospora-type centrum (Luttrell 1973). The five genera in Tetraplosphaeriaceae are clearly separated on the basis of their anamorphs (Figs 16–18). All these results suggest that morphology of anamorphs is a good predictor of phylogenetic relationships at the familial and genus levels, rather than their teleomorphs. Similar observations about the significance of anamorphic characters have been reported for Pleosporaceae in Dothideomycetes (Kosudse et al. 2006) and for Chaetosphaeriaceae in Sordariomycetes (Ráblová et al. 2008, 2009).
analyses of the combined dataset (ITS+TEF+BT; Fig. 18). The result (C), but most likely and reliable phylogenies were obtained from analyses using ITS, TEF, BT, and a combined dataset of these intergeneric relationships of five genera in an ancestral lineage within this family. Species in other existing families were poorly resolved, since the topologies were incongruent according to the different analyses. Further phylogenetic evidence from an additional dataset, such as sequences from the second largest RNA polymerase II subunit (RPB2) would provide useful information to understand the phylogenetic relatedness of the new family among the pleosporalean fungi (Schoch et al. 2006, Wang et al. 2007). To clarify intergeneric relationships of five genera in Tetraplosphaeriaceae, analyses using ITS, TEF, BT, and a combined dataset of these sequences were also conducted in this study. The branching patterns and monophyletic status of the five genera were slightly different according to each individual dataset and the intergeneric relationship could not be resolved in these analyses (Fig. 17A–C), but most likely and reliable phylogenies were obtained from analyses of the combined dataset (ITS+TEF+BT; Fig. 18). The result suggests that Tetraplosphaeria with anamorphs Tetraploa s. str. is an ancestral lineage within this family. Species in Tetraplosphaeria appear to have wide host selectivity, while species in the other four genera derived from this basal genus are restricted to bamboo as their host plants. Pseudotetraploa is the second basal lineage in this family and was strongly supported (1.00 PP and >99 % BS). Pseudotetraploa species produce conidia resembling those of Tetraploa in overall morphology, but conidia are composed of more than four columns with pseudosepta. In this genus, a teleomorph has not been found for any of the known species. Triposphaeria species produce conidia with pseudosepta similar to those of Pseudotetraploa but with a reduced number of conidial columns and setose appendages. Most species in Triposphaeria are likely to be heterothallic, because they form ascomata-like structures from single ascospore isolates but mature teleomorphs have to be heterothallic, because they form ascomata-like structures from single ascospore isolates but mature teleomorphs have never been observed under culture conditions. The monophyly of Polyposphaeria and Quadricrura, the most terminal lineages in this family, are also supported by their resemblance in their anamorphs. They have globose conidia composed of internal hyphal structure and more than four setose appendages, unlike the basal three genera having cylindrical conidia with several columns. Probably, the ability of teleomorph formation has been lost at least three times within this family, and anamorphs appear to have contributed greatly to their evolution.

It is interesting that there are several microfossil records of Tetraploa from the Palaeocene to the Holocene era (Saxena & Sarkar 1986, Kumaran et al. 2001, Antoine et al. 2006, Worobic et al. 2009). The oldest record of Tetraploa-like fossil from Devonian deposits has been reported as an acritarch genus Frasnacritetris (Taugourdeau 1968), but this is regarded as a contamination by a recent Tetraploa (Worobic et al. 2009). On account of the presence of Tetraploa fossils from the late Miocene accompanied by pollen grains of a bamboo (Graminoides bambusoides) and abundant freshwater phytoplanktons, it has been considered that the Tetraploa species could grow on G. bambusoides, a presumable origin of bamboo, in swamp forests (Worobic et al. 2009). A more complete fossil of Tetraploa as well as the other four genera in Tetraplosphaeriaceae would contribute to a better understanding of the evolutionary relationships within this family.

Relationships between genera in Tetraplosphaeriaceae

Tetraplosphaeriaceae was introduced to accommodate five new genera producing conidia with setose appendages. The monophyly of this family based on the SSU+LSU analyses was supported by NJ (86 % BS) and Bayesian (1.00 PP) trees, but not by the MP tree (54 % BS). Furthermore, the relationships of Tetraplosphaeriaceae with other existing families were poorly resolved, since the topologies were incongruent according to the different analyses. Further phylogenetic evidence from an additional dataset, such as sequences from the second largest RNA polymerase II subunit (RPB2) would provide useful information to understand the phylogenetic relatedness of the new family among the pleosporalean fungi (Schoch et al. 2006, Wang et al. 2007). To clarify intergeneric relationships of five genera in Tetraplosphaeriaceae, analyses using ITS, TEF, BT, and a combined dataset of these sequences were also conducted in this study. The branching patterns and monophyletic status of the five genera were slightly different according to each individual dataset and the intergeneric relationship could not be resolved in these analyses (Fig. 17A–C), but most likely and reliable phylogenies were obtained from analyses of the combined dataset (ITS+TEF+BT; Fig. 18). The result suggests that Tetraplosphaeria with anamorphs Tetraploa s. str. is an ancestral lineage within this family. Species in Tetraplosphaeria appear to have wide host selectivity, while species in the other four genera derived from this basal genus are restricted to bamboo as their host plants. Pseudotetraploa is the second basal lineage in this family and was strongly supported (1.00 PP and >99 % BS). Pseudotetraploa species produce conidia resembling those of Tetraploa in overall morphology, but conidia are composed of more than four columns with pseudosepta. In this genus, a teleomorph has not been found for any of the known species. Triposphaeria species produce conidia with pseudosepta similar to those of Pseudotetraploa but with a reduced number of conidial columns and setose appendages. Most species in Triposphaeria are likely to be heterothallic, because they form ascomata-like structures from single ascospore isolates but mature teleomorphs have never been observed under culture conditions. The monophyly of Polyposphaeria and Quadricrura, the most terminal lineages in this family, are also supported by their resemblance in their anamorphs. They have globose conidia composed of internal hyphal structure and more than four setose appendages, unlike the basal three genera having cylindrical conidia with several columns. Probably, the ability of teleomorph formation has been lost at least three times within this family, and anamorphs appear to have contributed greatly to their evolution.

It is interesting that there are several microfossil records of Tetraploa from the Palaeocene to the Holocene era (Saxena & Sarkar 1986, Kumaran et al. 2001, Antoine et al. 2006, Worobic et al. 2009). The oldest record of Tetraploa-like fossil from Devonian deposits has been reported as an acritarch genus Frasnacritetris (Taugourdeau 1968), but this is regarded as a contamination by a recent Tetraploa (Worobic et al. 2009). On account of the presence of Tetraploa fossils from the late Miocene accompanied by pollen grains of a bamboo (Graminoides bambusoides) and abundant freshwater phytoplanktons, it has been considered that the Tetraploa species could grow on G. bambusoides, a presumable origin of bamboo, in swamp forests (Worobic et al. 2009). A more complete fossil of Tetraploa as well as the other four genera in Tetraplosphaeriaceae would contribute to a better understanding of the evolutionary relationships within this family.

Outlook for further research

Tetraplosphaeriaceae was established for Massarina-like ascomycetes with conidial state similar to Tetraploa, morphologically most strongly supported by the common character of their anamorphs. Although application of an anamorphic phenotype for fungal classification is currently insufficient, our results suggest that anamorphs are good indicators of phylogenetic relationship at interfamilial or intergeneric levels. There are several anamorphic genera, e.g. Bioconiosporium (Ellis 1976, Narayan & Kamal 1986), Piricauda (Mercado Sierra et al. 2005) and Piricaudium (Holubová-Jechová 1988), having conidia similar to those of Tetraplosphaeriaceae. Their morphological resemblance, however, is possibly the result of convergence. The characteristic morphologies of Tetraploa, i.e. “tetraradiate” or “staurosporous” conidia and conidiogenous cells without conspicuous conidiophores, have been interpreted as a means of adaptation to small amounts of terrestrial water films. Namely, they need to possess water around the appended conidium for as long as possible to increase the possibility of germination, and they need to produce their conidia quickly and directly from conidiogenous cells without formation of conidiophores due to limitations of water resources on terrestrial host plants (Bandoni 1972, Ando 1992, Goh & Hyde 1996). There are many examples about the convergent evolution of anamorphic morphology resulting in adaptation to aquatic environments (Belliveau & Bárcišová 2005, Campbell et al. 2006, Tsui & Berbee 2006, Tsui et al. 2006). Therefore, molecular phylogenetic studies would be required to clarify the affinities between aforementioned dematiaceous hyphomycetes and Tetraplosphaeriaceae.

Bamboo is broadly divided into two tribes, Bambuseae (woody bamboos) and Olyreae (herbaceous bamboos). The former is a major group, which includes 67 genera in nine subtribes (Das et al. 2008). In this study, woody bamboos belonging to only four genera in two subtribes, Arundininarinae (Pleiochlastus and Sasa) and Shibataeinae (Chimonobambusa and Phyllostachys), were examined as host plants of bambusicolous fungi in Japan. Nevertheless, many novel fungal taxa were obtained from a limited area. It can be expected that there exists much more diverse Dothideomycetes on herbaceous bamboos and on the seven other subtribes of woody bamboos. In particular, we believe, a lineage referred to as “Neotropical woody bamboos” should receive more attention for taxonomic investigation of fungi. This bamboo group consisting of three subtribes, Arthrostylidiinae, Chusqueinae and Guaduinae, is distributed in Central and South America (Sungkaew et al. 2009), but our knowledge of bambusicolous fungi from these regions is still limited. Even though fundamental taxonomic studies are well advanced on this group, phylogenetic decisions based on molecular evidence would be required because bambusicolous fungi have the tendency to constitute an independent clade, deviating from existing families or genera on other host plants, even though they have morphological similarities with those known fungal groups, as was indicated in this study.
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