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

K. Tanaka[1*], K. Hirayama[1], H. Yonezawa[1], S. Hatakeyama[1], Y. Harada[1], T. Sano[1], T. Shirouzu[2] and T. Hosoya[3]

[1]*Faculty of Agriculture & Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan;* [2]*Fungus/Mushroom Resource and Research Center, Tottori University, Minami 4-101, Koyama, Tottori, Tottori 680-8553 Japan;* [3]*National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan*

Correspondence: Kazuaki Tanaka, k-tanaka@cc.hirosaki-u.ac.jp

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 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 setose appendages at the apex and four to five short setose 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 nrDNA (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 *Astrosphaeriella*, *Kalmusia*, *Katumotoa*, *Massarina*, *Ophiosphaerella*, *Phaeosphaeria*, *Roussoella*, *Roussoellopsis*, and *Versicolorisporium*, are also discussed based on the SSU+LSU phylogeny.

Key words: Anamorphic fungi, *Bambusoideae*, bitunicate ascomycetes, *Didymella*, *Dothideomycetes*, evolution, *Lophiostoma*, teleomorph.
Taxonomic novelties: *Tetraplosphaeriaceae* Kaz. Tanaka & K. Hiray., fam. nov., *Tetraplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Tetraplosphaeria nagasakiensis* Kaz. Tanaka & K. Hiray., sp. nov., *Tetraplosphaeria sasicola* 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 yezoensis* (I. Hino & Katum.) Kaz. Tanaka, K. Hiray. & Shirouzu, comb. nov., *Polyplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Polyplosphaeria fusca* Kaz. Tanaka & K. Hiray., sp. nov., *Pseudotetraploa* Kaz. Tanaka & K. Hiray., gen. nov., *Pseudotetraploa curviappendiculata* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Pseudotetraploa javanica* (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., comb. nov., *Pseudotetraploa longissima* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Quadricrura* Kaz. Tanaka, K. Hiray. & Sat. Hatak., gen. nov., *Quadricrura bicornis* Kaz. Tanaka, K. Hiray. & H. Yonez., sp. 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 grasses including *Bambusoideae* occurred 40–50 million years ago in 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, Sungkaew *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 *Stereostratum corticioides* (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 & Tanaka 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 *Shiraia* (Amano 1983) and *Katumotoa* (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 only on one example of *M. tetraploa* and *T. aristata* 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 *Masssarina* 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*, *Ophiosphaerella*, *Phaeosphaeria*, *Roussoella*, *Roussoellopsis*, and *Versicolorisporium*. Special emphasis was paid to the taxonomy and phylogeny of *Massarina s. l.*, which possess *Tetraploa*-like hyphomycetous 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 *Tetraplosphaeriaceae* to encompass five new genera, *Tetraplosphaeria*, *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 Hirosaki 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 ºC 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 5' end) and LSU nrDNA (*ca*. 1 250 bp of the 5' 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 LR0R–LR7 (Rehner & Samuels 1994) for SSU and LSU, respectively. Three primer sets, ITS1–ITS4 (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 Mg^{2+}). 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/software). 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 (Swofford 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) *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-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 immersa vel superficialia, globosa vel subglobosa. Rostrum breviter papillatum vel cylindricum, interdum nullum. Pseudoparaphyses septatae, ramificantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes vel late cylindricae, 1–3-septatae, hyalinae vel brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. 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. *Ascomatal wall* composed of hyaline to brown cells, sometimes with rim-like structure at the sides and poorly developed at the base. *Pseudoparaphyses* cellular or trabeculae, septate, branched. *Asci* 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.** Myco-Bank MB515254.

Anamorph: *Tetraploa* Berk. & Broome.

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

Ascomata immersa vel erumpentia, globosa vel subglobosa. Rostrum breviter papillatum vel cylindricum. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu stricto. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia breviter cylindricae vel obpyriformes, brunnea, cum 4 appendicibus.

Ascomata scattered to gregarious, immersed to erumpent, globose to subglobose, glabrous. *Beak* short-papillate to cylindrical, central, with periphyses. *Ascomatal wall* composed of nearly rectangular to polygonal thin-walled cells, sometimes poorly developed at the base. *Pseudoparaphyses* cellular, septate, branched. *Asci* fissitunicate, basal to somewhat lateral, cylindrical to clavate, short-stalked, with 8 ascospores. *Ascospores* narrowly fusiform, straight or slightly curved, with a septum and slightly constricted, hyaline, smooth, surrounded by a narrow mucilaginous appendage-like sheath. *Anamorph Tetraploa s. str. Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* composed of 4 columns, short-cylindrical, brown, verrucose at the base, euseptate, with 4 setose appendages at the apex.

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

Taxon	Host [a]	Original no.	Herbarium no.	Strain no.	GenBank no. SSU	LSU	ITS	BT	TEF
Astrosphaeriella aggregata	9	KT 767	HHUF 28232	MAFF 239485	AB524449	AB524590	–	–	–
Astrosphaeriella stellata	7	KT 984	HHUF 28233	MAFF 239486	AB524450	AB524591	–	–	–
	7	KT 998	HHUF 28494	MAFF 239487	AB524451	AB524592	–	–	–
Kalmusia scabrispora	7	KT 1023	HHUF 28608	JCM 12851 = MAFF 239517	AB524452	AB524593	–	–	–
	7	KT 2202	HHUF 30013	NBRC 106237	AB524453	AB524594	–	–	–
Katumotoa bambusicola	9	KT 1517a	HHUF 28661	JCM 13131 = MAFF 239641	AB524454	AB524595	–	–	–
Massarina arundinariae	7	KT 856	HHUF 27547	MAFF 239461	AB524455	AB524596	AB524786	AB524848	AB524817
	7	KT 2200	HHUF 30014	NBRC 106238	AB524456	AB524597	AB524787	AB524849	AB524818
	7	KT 1034	HHUF 30015	NBRC 106239	AB524457	AB524598	–	–	–
Ophiosphaerella sasicola	9	KT 1706	HHUF 29443	JCM 13134 = MAFF 239644	AB524458	AB524599	–	–	–
Phaeosphaeria brevispora	12	KT 1466	HHUF 28229	MAFF 239276	AB524459	AB524600	–	–	–
	9	KT 2313	HHUF 30016	NBRC 106240	AB524460	AB524601	–	–	–
Phaeosphaeria sp.	9	KT 2564	HHUF 30017	NBRC 106255	AB524461	AB524602	–	–	–
Polyplosphaeria fusca	7	KT 1043	HHUF 29392	JCM 13173 = MAFF 239683	AB524462	AB524603	AB524788	AB524850	AB524819
	8	KT 1616	HHUF 29399	JCM 13175 = MAFF 239685	AB524463	AB524604	AB524789	AB524851	AB524820
	4	KT 1640	HHUF 29405	JCM 13176 = MAFF 239686	AB524464	AB524605	AB524790	AB524852	AB524821
	3	KT 1686	HHUF 29406	JCM 13177 = MAFF 239687	AB524465	AB524606	–	–	–
	9	KT 2124	HHUF 30018	CBS 125425	AB524466	AB524607	AB524791	AB524853	AB524822
Pseudotetraploa curviappendiculata	9	HC 4930	HHUF 28582	JCM 12852 = MAFF 239495	AB524467	AB524608	AB524792	AB524854	AB524823
	9	HC 4932	HHUF 28590	MAFF 239496	AB524468	AB524609	AB524793	AB524855	AB524824
	9	KT 2558	HHUF 30019	CBS 125426 = NBRC 106241	AB524469	AB524610	AB524794	AB524856	AB524825
Pseudotetraploa javanica	8	HC 4934	HHUF 28596	JCM 12854 = MAFF 239498	AB524470	AB524611	AB524795	AB524857	AB524826
Pseudotetraploa longissima	8	HC 4933	HHUF 28580	JCM 12853 = MAFF 239497	AB524471	AB524612	AB524796	AB524858	AB524827
Quadricrura bicornis	5	yone 153	HHUF 30023	CBS 125427	AB524472	AB524613	AB524797	AB524859	AB524828
Quadricrura meridionalis	3	KT 2607	HHUF 30024	CBS 125684 = NBRC 106242	AB524473	AB524614	AB524798	AB524860	AB524829
Quadricrura septentrionalis	9	HC 4983	HHUF 28781	CBS 125429	AB524474	AB524615	AB524799	AB524861	AB524830
	9	HC 4984	HHUF 28782	CBS 125430	AB524475	AB524616	AB524800	AB524862	AB524831
	9	KT 920	HHUF 30020	CBS 125428	AB524476	AB524617	AB524801	AB524863	AB524832
	9	yone 44	HHUF 29747	CBS 125431	AB524477	AB524618	AB524802	AB524864	AB524833
	9	yone 176	HHUF 30021	CBS 125432 = NBRC 106243	AB524478	AB524619	AB524803	AB524865	AB524834
	9	yone 179	HHUF 30022	CBS 125433 = NBRC 106244	AB524479	AB524620	AB524804	AB524866	AB524835
Roussoella hysterioides	13	KT 1651	HHUF 29217	JCM 13126 = MAFF 239636	AB524480	AB524621	–	–	–

Table 1. (Continued).

Taxon	Host [a]	Original no.	Herbarium no.	Strain no.	GenBank no.				
					SSU	LSU	ITS	BT	TEF
Roussoella hysterioides	9	HH 26988	HHUF 26988	CBS 125434	AB524481	AB524622	–	–	–
Roussoella pustulans	9	KT 1709	HHUF 29229	JCM 13127 = MAFF 239637	AB524482	AB524623	–	–	–
Roussoella sp.	9	KT 2303	HHUF 30025	NBRC 106245	AB524483	AB524624	–	–	–
Roussoellopsis tosaensis	3	KT 1659	HHUF 29234	JCM 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	JCM 14424	AB524487	AB524628	–	–	–
Tetraploa sp. 2	6	KT 2578	HHUF 30027	NBRC 106251	AB524488	AB524629	–	–	–
Tetraplosphaeria nagasakiensis	3	KT 1682	HHUF 29378	JCM 13168 = MAFF 239678	AB524489	AB524630	AB524806	AB524868	AB524837
Tetraplosphaeria sasicola	11	KT 563	HHUF 27566	JCM 13167 = MAFF 239677	AB524490	AB524631	AB524807	AB524869	AB524838
Tetraplosphaeria yakushimensis	2	KT 1906	HHUF 29652	CBS 125435	AB524491	AB524632	AB524808	AB524870	AB524839
Trisplophaeria acuta	10	KT 1170	HHUF 29387	JCM 13171 = MAFF 239681	AB524492	AB524633	AB524809	AB524871	AB524840
Triplosphaeria cylindrica	9	KT 1256	HHUF 29381	JCM 13169 = MAFF 239679	AB524493	AB524634	–	–	–
	9	KT 1800	HHUF 29626	JCM 14425	AB524494	AB524635	AB524810	AB524872	AB524841
	9	KT 2550	HHUF 30028	NBRC 106247	AB524495	AB524636	AB524811	AB524873	AB524842
Triplosphaeria maxima	9	KT 870	HHUF 29390	JCM 13172 = MAFF 239682	AB524496	AB524637	AB524812	AB524874	AB524843
Triplosphaeria yezoensis	9	KT 1715	HHUF 30029	CBS 125436	AB524497	AB524638	AB524813	AB524875	AB524844
	12	KT 1732	HHUF 30030	CBS 125437	AB524498	AB524639	AB524814	AB524876	AB524845
Triplosphaeria sp.	9	HC 4665	HHUF 27481	NBRC 106248	AB524499	AB524640	AB524815	AB524877	AB524846
Triplosphaeria sp.	9	KT 2546	HHUF 30031	NBRC 106249	AB524500	AB524641	AB524816	AB524878	AB524847
Versicolorisporium triseptatum	8	SH 130	HHUF 28815	JCM 14775	AB524501	AB330081	–	–	–

[a] 1. *Alpinia formosa*; 2. *Arundo donax*; 3. bamboo; 4. *Chimonobambusa marmorea*; 5. conifer; 6. gramineae; 7. *Phyllostachys bambusoides*; 8. *Pleioblastus chino*; 9. *Sasa kurilensis*; 10. *Sasa nipponica*; 11. *Sasa senanensis*; 12. *Sasa* sp.,; 13. *Sasa veitchii*

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

Notes: A new genus *Tetraplosphaeria* is erected to accommodate four pleosporalean species having *Massarina/Lophiostoma*-like teleomorphs and anamorphs belonging to *Tetraploa s. str*. These species do not have clypeate 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 aristata s. l.*

Etymology: In reference to the collection site.

Ascomata 260–330 × 290–350 µm, immersa vel erumpentia, globosa vel subglobosa. Rostrum 75–150 × 85–110 µm, ostiolatum. Paries ascomatis 17–30 µm crassus ad latus, ex cellulis 5–6-stratis 5–13 x 2.5–5 µm compositus. Pseudoparaphyses 1–3 µm latae, septatae, ramificantes et anastomosantes. Asci (82–)86–105(–110) × 10.5–13.5 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae (27–)29–35(–37) × 3.5–6 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* 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.

Ascomata 260–330 µm high, 290–350 µm diam, scattered to gregarious, immersed in sheath or erumpent from bare culm, globose to subglobose, with sparse brown hyphae at sides. *Beak* 75–150 µm long, 85–110 µm diam, central, papillate to cylindrical, composed of dark brown, thick-walled cells, with numerous periphyses. *Ascomatal wall* at sides 17–30 µm thick, composed of 5–6 layers of rectanglar to polygonal brown cells of 5–13 × 2.5–5 µm, at the base 5–7.5 µm thick, composed of globose to polygonal cells of 2.5–7.5 µm diam. *Pseudoparaphyses* numerous, 1–3 µm thick, branched and anastomosed, with slime coating. *Asci* (82–)86–105(–110) × 10.5–13.5 µm (av. 95.4 × 11.9 µm, *n* = 50), fissitunicate, numerous, basal to somewhat lateral, clavate to cylindrical, short-stalked (*ca*. 10–20 µm long), 8-spored. *Ascospores* (27–)29–35(–37) × 3.5–6 µm (av. 32 × 4.4 µm, *n* = 100), L/W 5.9–8.5 (av. 7.3, *n* = 100), narrowly fusiform with acute ends, slightly curved, with a septum nearly median (0.48–0.52; av. 0.50, *n* = 100), hyaline, smooth, with bipolar elongated sheath of 1–3 µm long at both ends (but in india ink, an entire sheath 13–18 µm thick at sides is observed).

Culture characteristics: Colonies on PDA attaining a diam of 1.1–1.2 cm, dull-green (28E4; Kornerup & 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 aristata s. l. Conidiophores* absent. *Conidiogenous cells* 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), L/W 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.

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 aristata* (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).

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

Anamorph: *Tetraploa ellisii s. l.*

Etymology: In reference to the host plant of collection.

Ascomata 150–200 × 230–290 µm, immersa vel erumpentia, globosa vel subglobosa. Rostrum 30–40 × 50–55 µm, ostiolatum. Paries ascomatis 12–20 µm crassus ad latus, ex cellulis 3–6-stratis 7–13 × 2–5 µm compositus. Pseudoparaphyses 1.5–2.5 µm latae, septatae, ramificantes et anastomosantes. Asci (61–)65–89(–100) × 9–11(–13) µm, fissitunicati, clavati vel cylindrici, octospori. Ascosporae 22.5–31.5(–34) × 3–5 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* 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.

Ascomata 150–200 µm high, 230–290 µm diam, scattered, immersed below the epidermis, later erumpent, globose to subglobose with a flattened base, glabrous. *Beak* short-papillate, 30–40 µm high, 50–55 µm diam, central, composed of globose to polygonal dark brown thick-walled cells of 3–6 µm diam, with sparse short periphyses. *Ascomatal wall* 12–20 µm thick at sides, composed of 3–6 layers of rectangular to polygonal hyaline to pale brown cells of 7–13 × 2–5 µm; at the base much thinner, of compressed small hyaline cells. *Pseudoparaphyses* cellular, numerous, 1.5–2.5 µm thick, septate, branched. *Asci* (61–)65–89(–100) × 9–11(–13) µm (av. 76.6 × 9.9 µm, *n* = 50), fissitunicate, numerous, basal to somewhat lateral, clavate to cylindrical, short-stalked (*ca*. 5–15 µm long), with 8 ascospores triseriate to biseriate above and uniseriate below. *Ascospores* 22.5–31.5(–34) × 3–5 µm (av. 26.8 × 3.7 µm, *n* = 80), L/W 6.4–8.3 (av. 7.2, *n* = 80), narrowly fusiform, straight or slightly curved, with a submedian (0.49–0.54; av. 0.51, *n* = 51) septum and slightly constricted, hyaline, smooth, surrounded by a narrow mucilaginous sheath, 2–6 µm long at the apex, 1–3 µm long at the base, slightly wider at sides of septum. Senescent spores pale brown.

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. ellisii* 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), L/W 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 found. *Asci* 66–107 × 8–11 µm (av. 84.5 × 9.9 µm, *n* = 36). *Ascospores* 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), L/W 5.9–7.5 (av. 6.6, *n* = 75), with a mid-septum submedian (0.50–0.53; av. 0.51, *n* = 55).
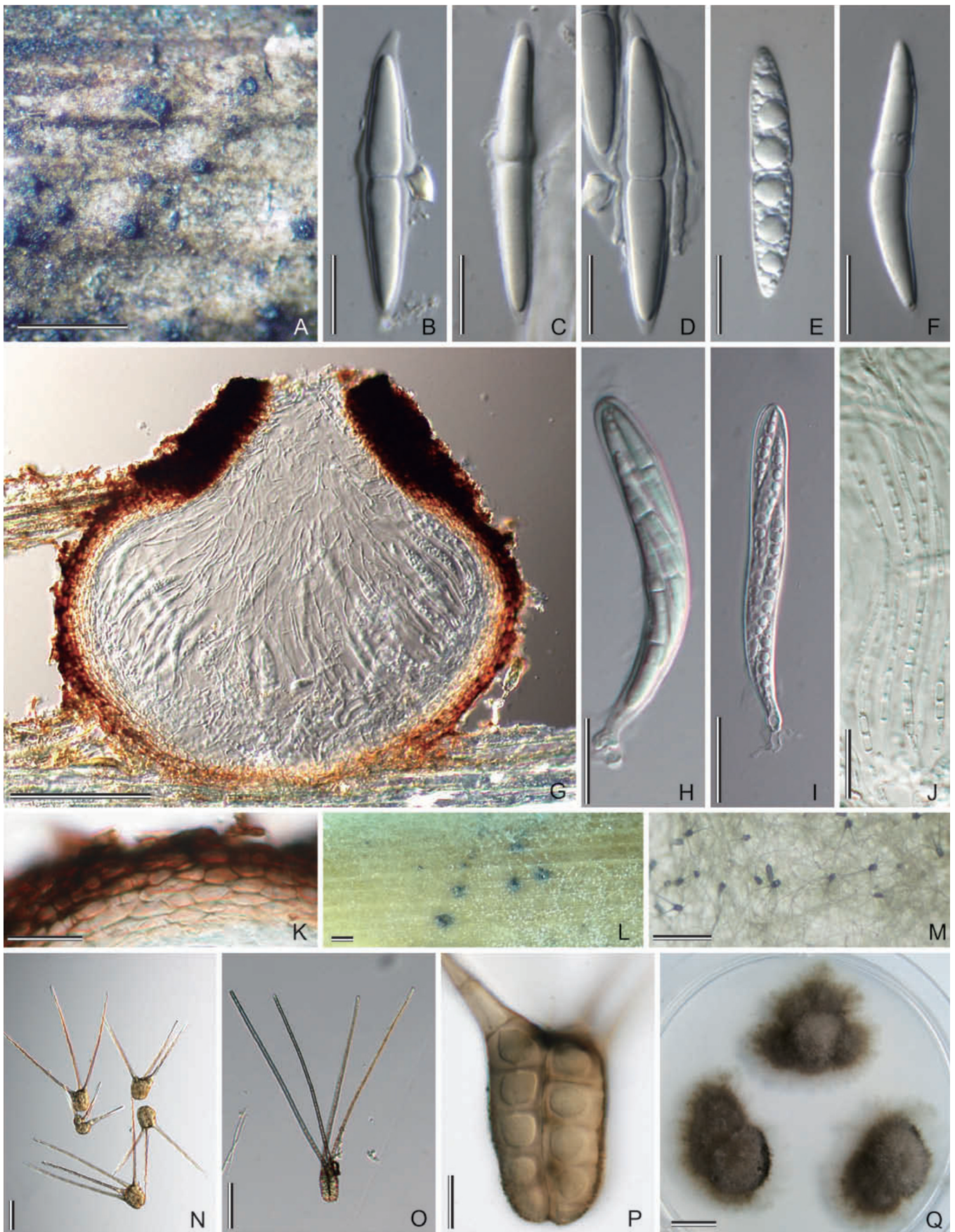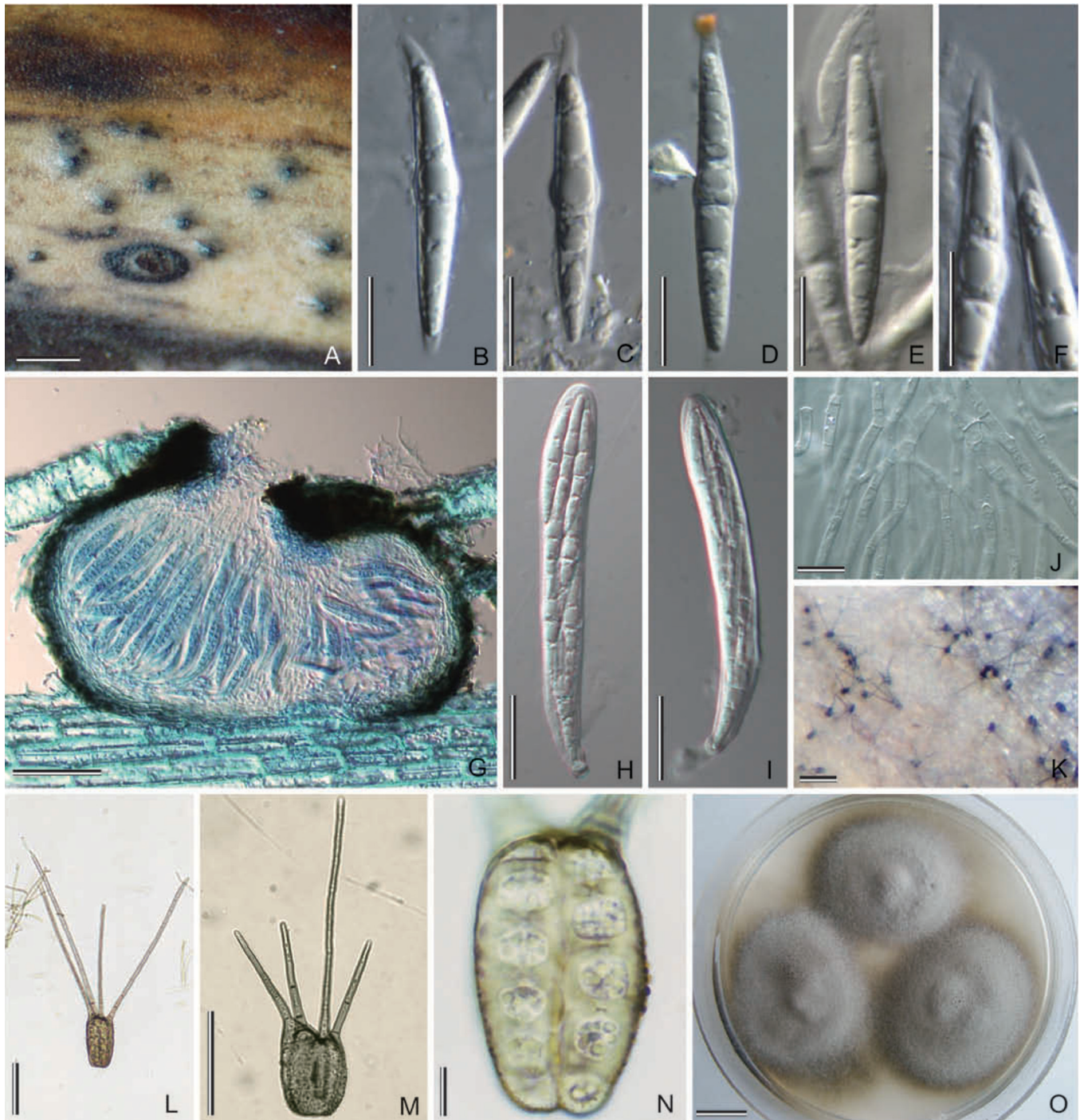
Fig. 1. *Tetraplosphaeria nagasakiensis*. A. Ascomata on host surface. B–F. Ascospores; G. Ascoma in longitudinal section; H–I. Asci; J. Pseudoparaphyses; K. Ascomal wall; L. Ascomata on rice straw agar; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. Colonies on PDA after 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. l.*

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 periphyses. *Ascomatal wall* uniformly 6–12 μm thick, composed of 3–4 layers of
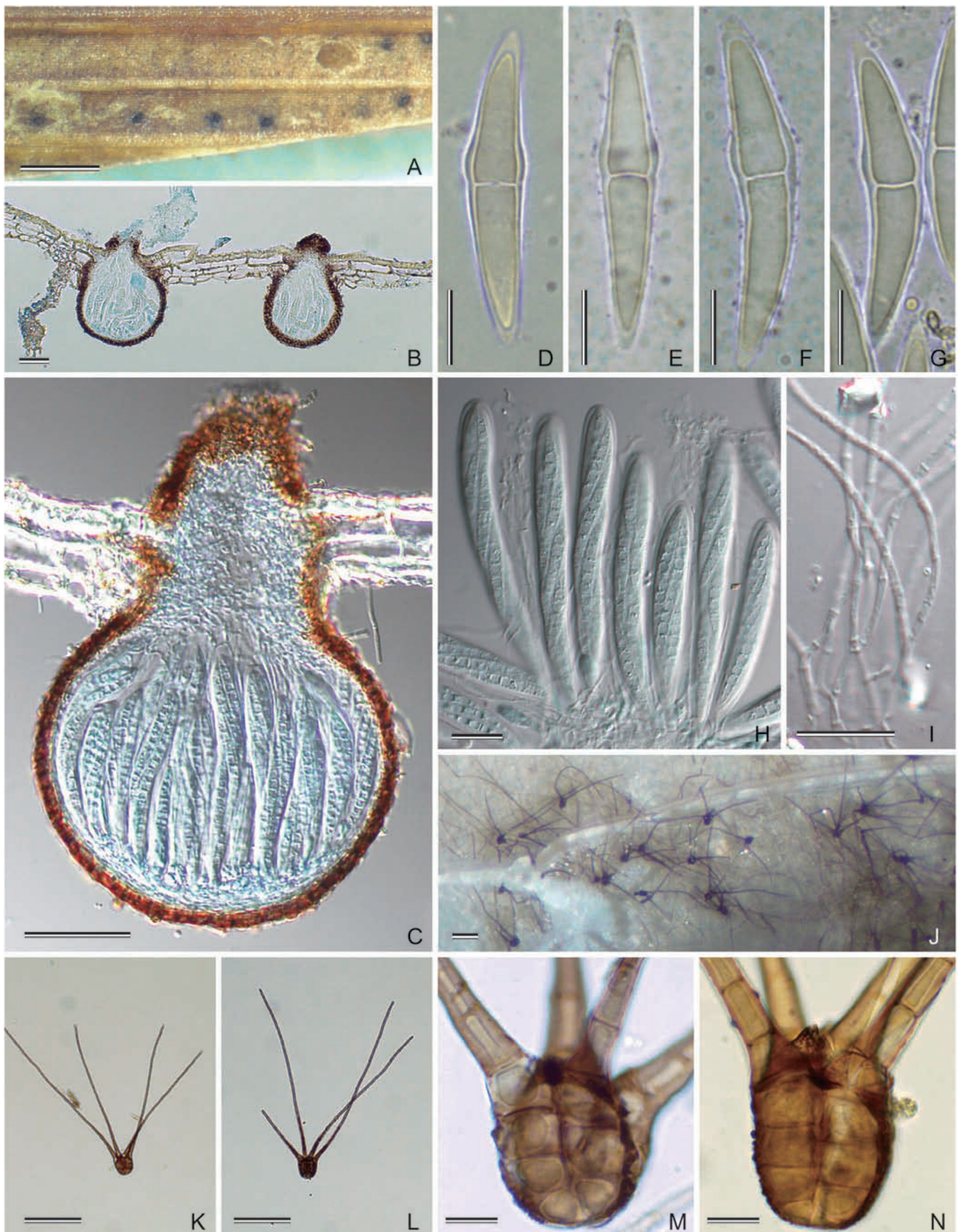
Fig. 3. *Tetraplosphaeria tetraploa*. A. Ascomata on host surface; B–C. Ascomata in longitudinal section; D–G. Ascospores; H. Asci; I. Pseudoparaphyses; J. Conidia on malt extract agar; K–L. Conidia; M–N. Conidial bodies. Scale bars: A = 500 μm; B–C = 50 μm; D–G, M–N = 10 μm; H–I = 20 μm; J–L = 100 μm. 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. *Asci* (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(–43) × 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 *Tetraploa 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, solitaly, 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 *Tetraplosphaeria*. 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.

Tetraplosphaeria 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, immersa, subglobosa. Rostrum 50 × 55–65 μm, ostiolatum. Paries ascomatis 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. Ascosporae 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.

Ascomata 135–180 μm high, 150–250 μm diam, scattered, immersed below the epidermis, subglobose, glabrous. *Beak* short-papillate, *ca*. 50 μm high, 55–65 μm diam, central, *Ascomatal wall* 15–20 μm thick at sides, composed of 4–6 layers of rectangular to polygonal hyaline to pale brown cells of 5–15 × 2.5–4 μm; at the base 10–20 μm thick. *Pseudoparaphyses* cellular, numerous, 1.5–4 μm wide, septate, branched, anastomosed. *Asci* 85–110 × 10.5–13 μm (av. 99.3 × 11.8 μm, *n* = 20), fissitunicate, numerous, basal and lateral, clavate to cylindrical, short-stalked (*ca*. 5–10 μm long), with 8 ascospores biseriate above and uniseriate below. *Ascospores* 26.5–36.5 × 4–6 μm (av. 30.6 × 4.5 μm, *n* = 31), L/W 5.1–8.3 (av. 6.9, *n* = 31), narrowly fusiform, straight or slightly curved, with a nearly median (0.47–0.51; av. 0.49, *n* = 30) septum and slightly constricted, hyaline, smooth, with a mucilaginous sheath. Sheath 3–10 μm long at the ends, 1–2 μm wide at the sides.

Culture characteristics: On RSA, both teleomorphic and anamorphic states are produced. *Ascospores* are similar to those on the host, measuring 29–37 × 4–5.5 μm (av. 32 × 4.7 μm, *n* = 20). *Anamorph* is *Tetraploa aristata s. l. Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, verruculose, 25–37(–40) × 20–30 μm (av. 31.4 × 24.9 μm, *n* = 50), L/W 1.1–1.5 (av. 1.3, *n* = 50), 4-celled, composed of 4 columns and 4 setose appendages. Appendages (52–)62–142(–150) μm long (av. 96.2 μm, *n* = 60), 2–3 μm wide at the apex, 7–8 μm at the base, with 3–8-septa at 8 to 16 μm intervals.

Specimen examined: **Japan,** Kagoshima, Isl. 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 125435).

Notes: In terms of ascus and ascospore morphology, this species is quite close to *Tetraplosphaeria nagasakiensis*, but it is distinct from the latter in its conidial morphology. *Tetraplosphaeria 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,** Kagoshima, Nagayo, Nagasaki Siebold University (129°52.4'E, 32°48.2'N), on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, HHUF 29625, living culture *KTC 1684* (= JCM 14424).

Notes: This species is most similar to anamorphs of *Tetraplosphaeria tetraploa* and *Tetraplosphaeria yakushimensis*, but has more slender conidia. The conidial morphologies of these three species match well with the description of *Tetraploa aristata* provided by Ellis (1949), but they may not be conspecific as discussed later.

Fig. 4. *Tetraplosphaeria yakushimensis*. A. Ascomata on host surface; B. Ascoma in longitudinal section; C–F. Ascospores; G–H, Asci; I. Ascus with an ocular chamber; J. Pseudoparaphyses; K. Conidia on agar piece immersed in water; L. Conidia; M. Conidia with verruculose ornamentation; N. Conidial body; O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 μm; B–E, I–J, M–N = 10 μm; F, L = 50 μm; G–H = 20 μm; K = 100 μm; O = 1 cm. A–J from HHUF 29652 holotype; K–O from culture KT 1906.

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 *Tetraplosphaeria sasicola* in terms of conidial dimension, but differs from the latter in having longer appendages.

Triplosphaeria Kaz. Tanaka & K. Hiray., **gen. nov.** Myco-Bank MB515255.

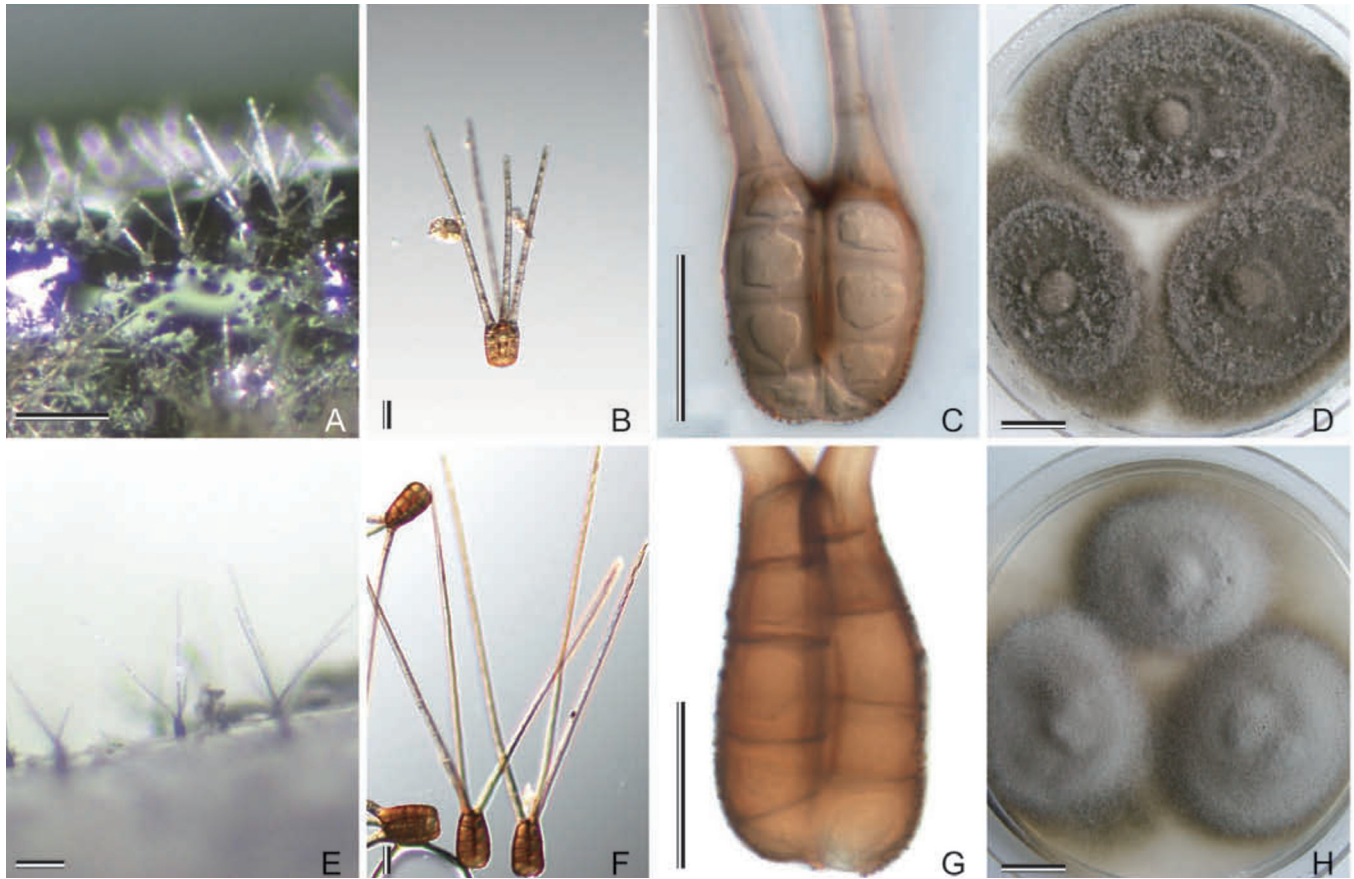*Anamorph*: Undescribed *Tetraploa*-like state having conidia with three setose appendages.

Fig. 5. *Tetraploa* spp. A–D. *Tetraploa* sp. 1 (culture KT 1684); E–H. *Tetraploa* sp. 2 (HHUF 30027); A, E. Conidia on agar piece immersed in water; B, F. Conidia; C, G. Conidial bodies; D, H. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A, E = 200 µm; B–C, F–G = 20 µm; D, H = 1 cm.

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 fusiformis, 1-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia ovata vel obpyriformis, brunnea, cum 3 appendicibus.

Ascomata scattered to gregarious, immersed below the epidermis, subglobose, with single locule, glabrous. *Beak* none to short, with hyaline sparse periphyses. *Ascomatal wall* rim-like at sides, composed of vertically orientated rectangular to cylindrical hyaline hyphoid cells, flattened and poorly developed at the base. *Pseudoparaphyses* narrowly cellular, numerous, branched and anastomosed, septate. *Asci* fissitunicate, basal and lateral, cylindrical to clavate, rounded at the apex, short-stalked, with 8 ascospores. *Ascospores* narrowly fusiform to broadly fusiform with rounded ends, 1-septate, constricted at the septum, hyaline, smooth, with an entire sheath. *Anamorph* *Tetraploa*-like with 3 setose appendages. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* composed of 3 columns with pseudosepta, ovoid to obpyriform, brown, almost smooth, verrucose at the base, with 3 setose appendages at the apex.

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. *Ascomatal wall* at sides, 85–180 µm wide and rim-like, composed of vertically orientated rectangular to subglobose hyaline hyphoid cells of 5–10 × 3.5–7.5 µm; near the epidermis, 25–38 µm thick, composed of polygonal to subglobose brown 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* =
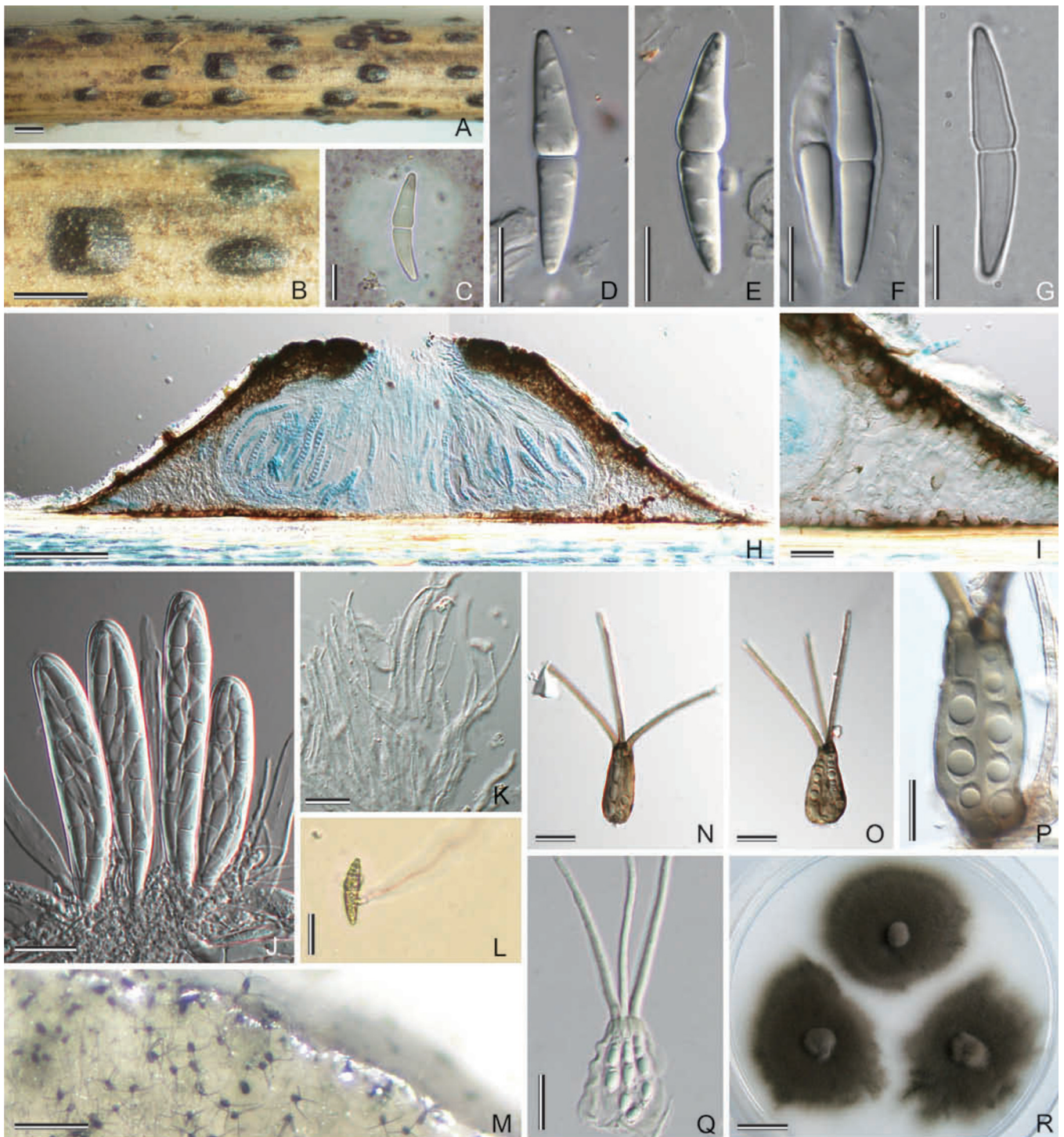
Fig. 6. *Triplosphaeria acuta*. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomal wall at side; J. Asci; K. Pseudoparaphyses; L. Germinating ascospore; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. 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.

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 ovate or ovate. Setose 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.

Specimens examined: **Japan,** Hokkaido, Akkeshi, Ariake, Small stream (144°52.0'E, 43°01.2'N), on submerged culms of bamboo (*Sasa nipponica*?), 3 June 2003, K. Tanaka & S. Hatakeyama, HHUF 29387 **holotype** designated here, living culture *KTC 1170* (= JCM 13171 = MAFF 239681); Hokkaido, Akkeshi, Ootakita, Sattebetsu-river (144°49.0'E, 43°08.1'N), on submerged culms of bamboo (*Sasa nipponica*?), 3 June 2003, K. Tanaka & S. Hatakeyama, KT 1218 = HHUF 29388.

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.

≡ *Massarina 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(–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. *Conidiogenous 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.

Specimens examined: **Japan**, Hokkaido, Oiwaki, on culms of *Sasa kurilensis*, 16 Sept. 1956, I. Hino, YAM 21797 **holotype** of *Massarina yezoensis*; Aomori, Souma, Jinba-dake (1049m a.s.l.), 14 June 2003, Y. Harada, HHUF 29381, living culture *KTC 1256* (= JCM 13169 = MAFF 239679); Aomori, Mt. Iwaki, 9 July 2005, K. Tanaka, HHUF 29626, living culture *KTC 1800* (= JCM 14425); Hokkaido, Isl. Rishiri, Afutoromanai trail, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30028, living culture *KTC 2550* (= NBRC 106247).

Notes: This species was originally described as *Massarina yezoensis* (Hino 1961), but is transferred to *Triplosphaeria* because of its hemispherical ascomata 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, immersa, globosa vel subglobosa. Rostrum nullum vel breve, ostiolatum. Paries ascomatis 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 cylindrici, octospori. Ascosporae (32.5–)34–45(–48.5) × (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 subglobose, 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) × (6–)7–9(–10) μm (av. 38.9 × 7.9 μm, *n* = 120), L/W 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. *Conidiogenous cells* monoblastic. *Conidia* consist of a conidial body and 3 setose appendages, solitary. Conidial body 41–55 × 17–23(–27.5) μm (av. 48.3 × 19.3 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.

Specimen examined: **Japan,** Aomori, Nishimeya, Ookawa, on culms of *Sasa kurikensis*, 23 July 2002, S. Hatakeyama, HHUF29330 **holotype** designated here, living culture *KTC 870* (= JCM 13172 = MAFF 239682).

Note: 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 2–3

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.
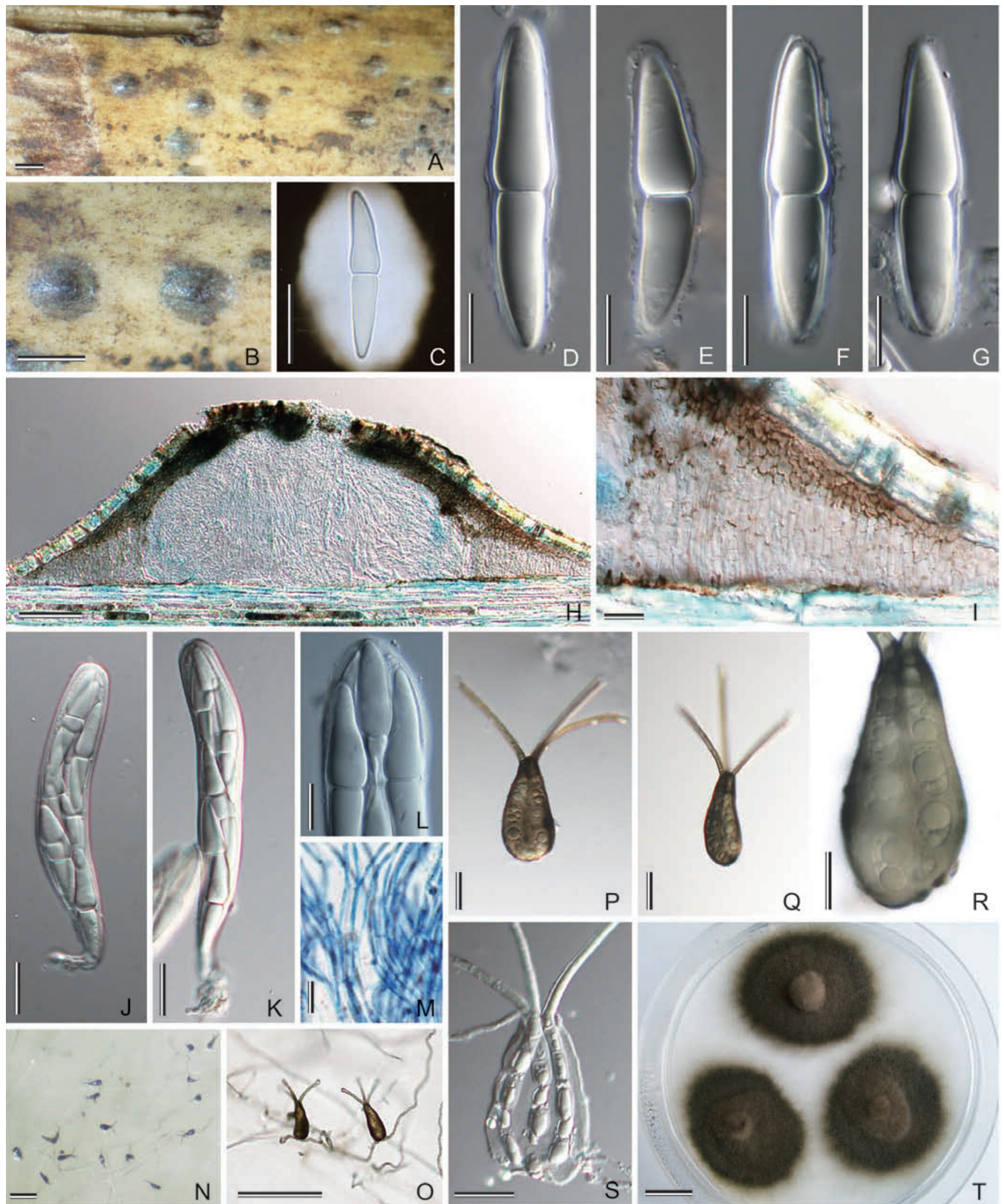
Fig. 8. *Triplosphaeria maxima*. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomal wall at side; J–K. Asci; L. Apex of ascus; M. Pseudoparaphyses; N. Conidia on agar piece immersed in water; O. Developing conidia; P–Q. Conidia; R. 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, P–Q, 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.
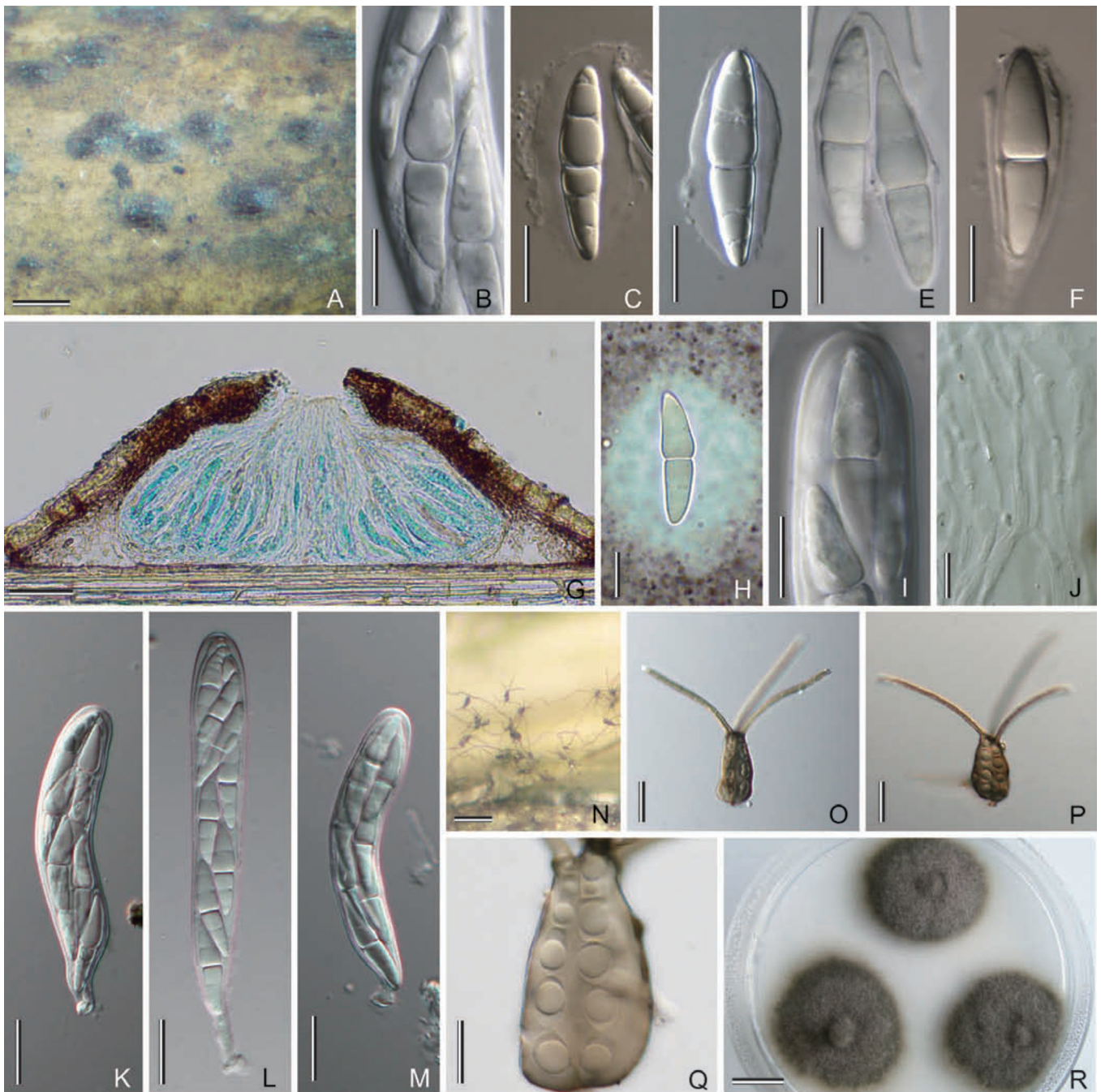
Fig. 9. *Triplosphaeria yezoensis*. A. Ascomata on host surface; B–F. Ascospores; G. Ascoma in longitudinal section; H. Ascospore in India ink; I. Apex of ascus; J. Pseudoparaphyses; K–M. Asci; N. Conidia on rice straw agar; O–P. Conidia; Q. Conidial body; R. Colonies on PDA after 45 d at 25 °C in the 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.

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.
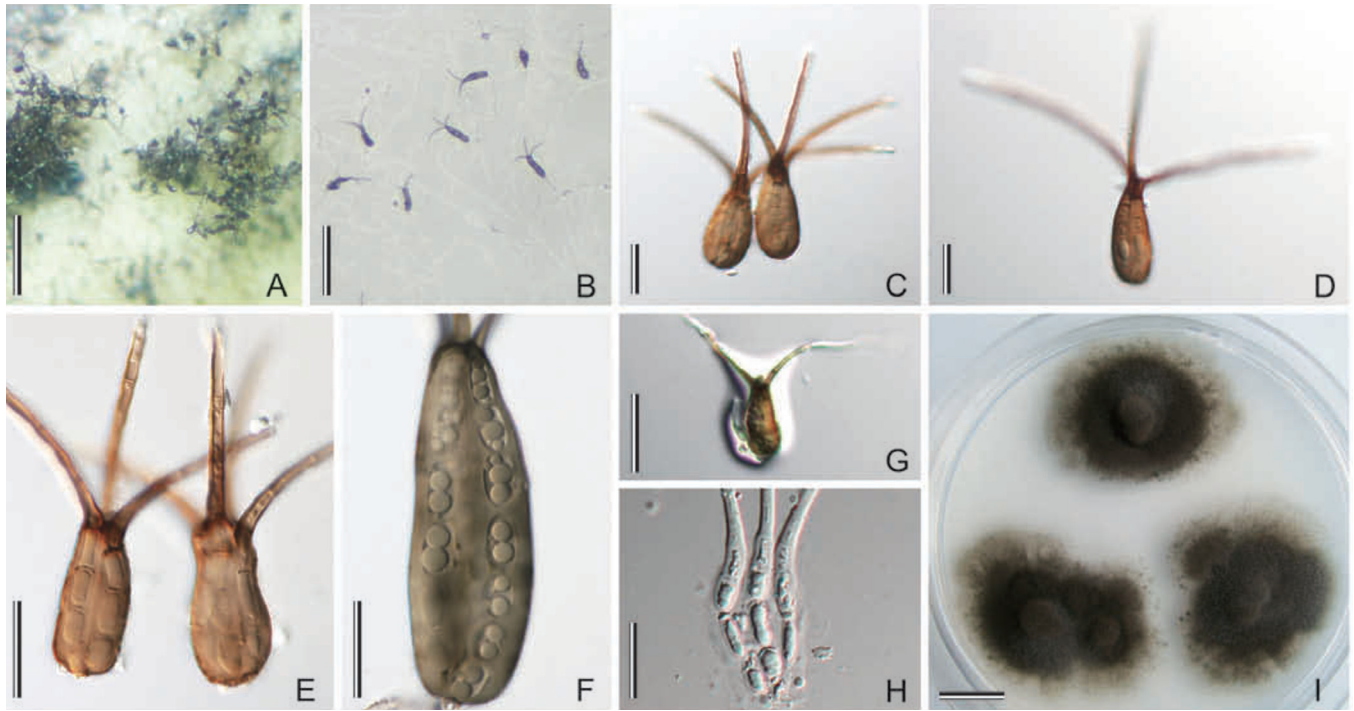
Fig. 10. *Triplosphaeria* sp. A. Conidia on host surface; B. Conidia on agar piece immersed in water; C–E. Conidia; F. Conidial body; G. Germinating conidium; H. Breached conidium composed of three columns; I. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–F, H = 20 µm; G = 50 µm; I = 1 cm. A, D from HHUF 27481; B, F from culture KT 2546; C, E, G–H from HHUF 30031; I from culture HC 4665.

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), L/W 1.7–2.8 (av. 2.2, *n* = 61), brown, 3–5-pseudoseptate, 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), L/W 1.9–3.7 (av. 2.9, *n* = 13), 6–8-pseudoseptate, having 3 appendages 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. Ascosporae anguste fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. Cellulae conidiogenae 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* trabecular, numerous, tortuous, septate, branched and anastomosed, associated with gelatinous material. *Asci* fissitunicate, clavate, 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, with thin peel-like outer wall of conidia, composed of numerous internal hyphae at the inside, brown, almost smooth, verrucose at the base. Appendages brown, straight.

Type species: *Polyplosphaeria fusca* Kaz. Tanaka & K. Hiray., sp. nov.

Notes: The characteristics of this new genus include globose ascomata surrounded by numerous brown hyphae, reddish pigment on the host surface around ascomata, clavate asci with fissitunicate dehiscence, and narrowly fusiform ascospores provided with an entire sheath. The anamorphic state of *Polyplosphaeria* produces almost globose conidia composed of numerous internal hyphae, 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. longispora*), but *Piricauda* has been defined primarily based on monotretic conidiogenous cells and its muriform conidia (Mercado Sierra *et al.* 2005).

Polyplosphaeria fusca Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515267. Fig. 11.

Etymology: From the Latin *fuscus*, in reference to the coloured ascospores.

Ascomata 180–420 × 300–680 μm, erumpentia vel superficialia, globosa. Rostrum 50–90 × 75 μm, ostiolatum. Paries ascomatis 20–50 μm crassus ad latus, ex cellulis 4–7-stratis 2.5–12.5 × 2.5–5 μm compositus. Pseudoparaphyses 1–2 μm latae, septatae, ramificantes et anastomosantes. Asci (84–) 92.5–135 × 17–23 μm, fissitunicati, clavati, octospori. Ascosporae 36.5–49(–57) × 7–10 μm, anguste fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidia in vitro 43–100(–125) μm diam, globosa vel subglobosa, brunnea, cum 3–8 appendicibus; appendices 92–200(–235) μm longae, 4–10-septatae.

Ascomata 180–420 μm high, 300–680 μm diam, scattered to clustered, erumpent to superficial, globose, black to sometimes reddish-brown, with short brown hyphae at sides, mostly associated with reddish pigment. *Beak* 50–90 μm long, 75 μm diam, slightly papillate, central, with hyaline periphyses, composed of subglobose to polygonal slightly thickened cells of 2–5 μm diam. *Ascomatal wall* at sides 20–50 μm thick, composed of 4–7 layers of (irregular to paralell rows) rectangular to polygonal brown cells of 2.5–12.5 × 2.5–5 μm diam, sometimes poorly developed at the base. *Pseudoparaphyses* trabecular, numerous, tortuous, 1–2 μm wide, septate, branched and anastomosed, associated with gelatinous material. *Asci* (84–) 92.5–135 × 17–23 μm (av. 107.9 × 20.1 μm, $n = 32$), fissitunicate, clavate, short-stalked (10–30 μm long), with 8 biseriate ascospores. *Ascospores* 36.5–49(–57) × 7–10 μm (av. 43.8 × 8.4 μm, $n = 111$), L/W 4.5–5.8 (av. 5.2, $n = 111$), narrowly fusiform, slightly curved, with a submedian primary septum (0.49–0.53; av. 0.51, $n = 106$), constricted at the primary septum, 1(–3)-septate, hyaline to pale olive-brown, with a sheath up to 12 μm wide. At germination ascospores become 3- to 5-septate and produce germ tubes from both end cells.

Culture characteristics: Colonies on PDA attaining 1.9–2 cm diam, velvety in appearance, dark green (28F8), with whitish entire margin of 2 mm; reverse raw-sienna (6D7); mellon (5A6) pigment

produced. On RSA, a *Tetraploa*-like anamorph with 3 to 8 setose appendages and a teleomorph are observed. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* 43–100(–125) μm diam (av. 71.2 μm, $n = 58$), globose to subglobose, brown, almost smooth, verrucose at the base. Appendages 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 asci and ascospores in culture are slightly larger. Asci 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 $n = 69$), 1–3-septate.

Specimens examined: **Japan,** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al.*, HHUF 29399 **holotype** designated here, living culture *KTC 1616* (= JCM 13175 = MAFF 239685); Tochigi, Kanuma, Simosawa (139°42.2'E, 36°34.4'N), on culms of *Phyllostachys bambusoides*, 20 Mar. 2003, N. Asama, HHUF 29392, living culture *KTC 1043* (= JCM 13173 = MAFF 239683); Shizuoka, Syuntou, Nagaizumi, Minami-isshiki, Fuji bamboo garden (138°53.1'N, 35°09.3'N), on culms of *Chimonobambusa marmorea*, 8 Mar. 2004, K. Tanaka & Y. Harada, HHUF 29405, living culture *KTC 1640* (= JCM 13176 = MAFF 239686); Nagasaki, Nagayo, Nagasaki Siebold University (129°52.4'E, 32°48.2'N), on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, HHUF 29406, living culture *KTC 1686* (= JCM 13177 = MAFF 239687); Aomori, Souma, Ainai trail, on culms of *Sasa kurilensis*, 29 July 2006, K. Tanaka *et al.*, HHUF 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; *Arundinariinae* (*Pleioblastus* and *Sasa*) and *Shibataeinae* (*Chimonobambusa* and *Phyllostachys*). As discussed later, two distinct clades, KT1043+1640 and KT1616+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, *Arundinariinae* 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.

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

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia obpyriformes vel anguste obpyriformes, brunnea vel atro brunnea, cum 4 (raro 6 vel 8) appendicibus.

Mycelium superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* composed of 4 to 8 columns, obpyriform to long obpyriform, brown to dark brown, almost smooth, verrucose at the base, pseudoseptate, with setose appendages at the apical part. Appendages mostly 4, rarely 6 to 8, curved or straight.

Type species: *Pseudotetraploa curviappendiculata* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov.

Notes: An anamorphic genus *Pseudotetraploa* is established for species with conidia similar to those of *Tetraploa*. The conidial body of *Pseudotetraploa* is obpyriform to long obpyriform rather than short cylindrical, and has pseudosepta rather than eusepta. In general, setose appendages of *Pseudotetraploa* are short and

Fig. 11. *Polyplosphaeria fusca.* A. Ascomata on host surface; B–C. Ascomata in longitudinal section; D–G. Ascospores; H. Ascospore in India ink; I. Germinating ascospore; J–K. Asci; L. Ascomal wall; M. Pseudoparaphyses; N. Conidia on rice straw agar; O–P. Conidia; Q. Conidial body with peel-like wall; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 1 000 µm; B, N = 200 µm; C–F, M = 10 µm; G–H, J–L, Q = 20 µm; I, O–P = 50 µm; R = 1 cm. A–C, H–J, L from HHUF 29399 holotype; D, N, P, R from culture KT 1616; E from HHUF 29405; F–G, K, M from HHUF 30018; O from culture KT 1043; Q from culture KT 2124.

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*.

Fig. 12. *Pseudotetraploa* spp. A–E. *P. curviappendiculata*; F–J. *P. longissima*; K–O. *P. javanica*; A, F, K. Conidia; B, G, L. Conidial bodies; C, H, M. Weakly breached conidia; D, I, N. Strongly breached conidia (D, N. 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 28580 holotype; J form culture HC 4933; K–N from HHUF 28596; O from culture HC 4934.

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, Matsukitai (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 longissima (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515270. Fig. 12F–J.

Basionym: *Tetraploa longissima* Sat. Hatak., Kaz. Tanaka & Y. Harada, Mycoscience 46: 198. 2005.

Specimen examined: **Japan.** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al.*, HHUF 28580 **holotype**, living culture *HC 4933* (= JCM 12853 = MAFF 239497).

Pseudotetraploa javanica (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515269. Fig. 12K–O.
Basionym: *Tetraploa javanica* Rifai, Zainuddin & Cholil, Reinwardtia 10: 420. 1988.

Specimen examined: **Japan,** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al.*, HHUF 28596, living culture *HC 4934* (= JCM 12854 = MAFF 239498).
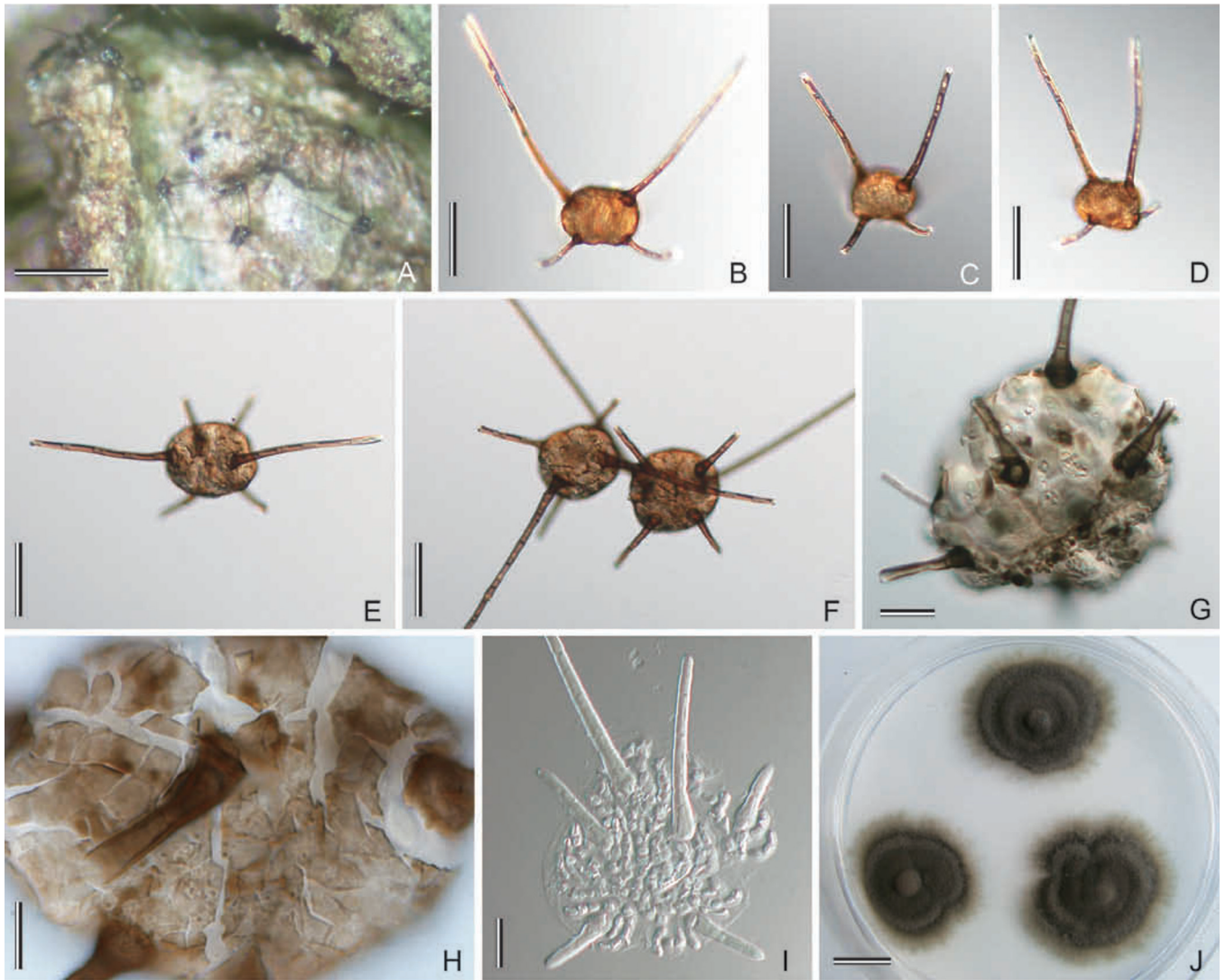
Fig. 13. *Quadricrura bicornis*. A. Conidia on host surface; B–F. Conidia; G. Base of conidium with warty surface; H. Conidial body with peel-like wall; I. Breached conidium with internal hyphal structure; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 200 μm; B–H = 10 μm; I = 20 μm; J = 1 cm. A–F, H–I from HHUF 30023 holotype; G, J from culture (yone 153).

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.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia globosa vel subglobosa, brunnea vel atro brunnea, cum 1 vel 2 longiappendicibus et 4 vel 5 breviappendicibus.

Mycelium superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form 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 almost 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.

Fig. 14. *Quadricrura meridionalis*. A. Conidia on host surface; B. Conidia on rice straw agar; C. Germinating conidium; D–G. Conidia; H. Base of conidium with warty surface; I. Conidial body; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–G, I = 50 µm; H = 20 µm; J = 1 cm. A, C–H from HHUF 30024 holotype; B, I–J from culture KT 2607.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 32.5–60 × 40–65 µm, subglobosa, brunnea vel atro brunnea, cum appendicibus; longiappendices 2, 65–175(–200) µm longae, 10–13-septatae; breviappendices 4, 17.5–45.5 µm longae, 0–2-septatae.

Mycelium superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* 32.5–60 × 40–65 µm (av. 40.6 × 48.8 µm, *n* = 32), 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, 65–175(–200) µm long (av. 130.6 µm, *n* = 36), 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 conidial 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 *yone 153* (= CBS 125427); Aomori, Shirakami, Chisan-dam, on culms of *Sasa kurilensis*, 21 July 2007, H. Yonezawa & K. Tanaka, yone154 = HHUF 30035.

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.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 36–43.5(–56.5) × 41–75 µm, subglobosa, brunnea vel atro brunnea, cum appendicibus; longiappendices 1 vel 2, 170–295 µm longae, 10–16-septatae; breviappendices 4 vel 5, 15–37.5 µm longae, 0–2-septatae.

Mycelium superficial. *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
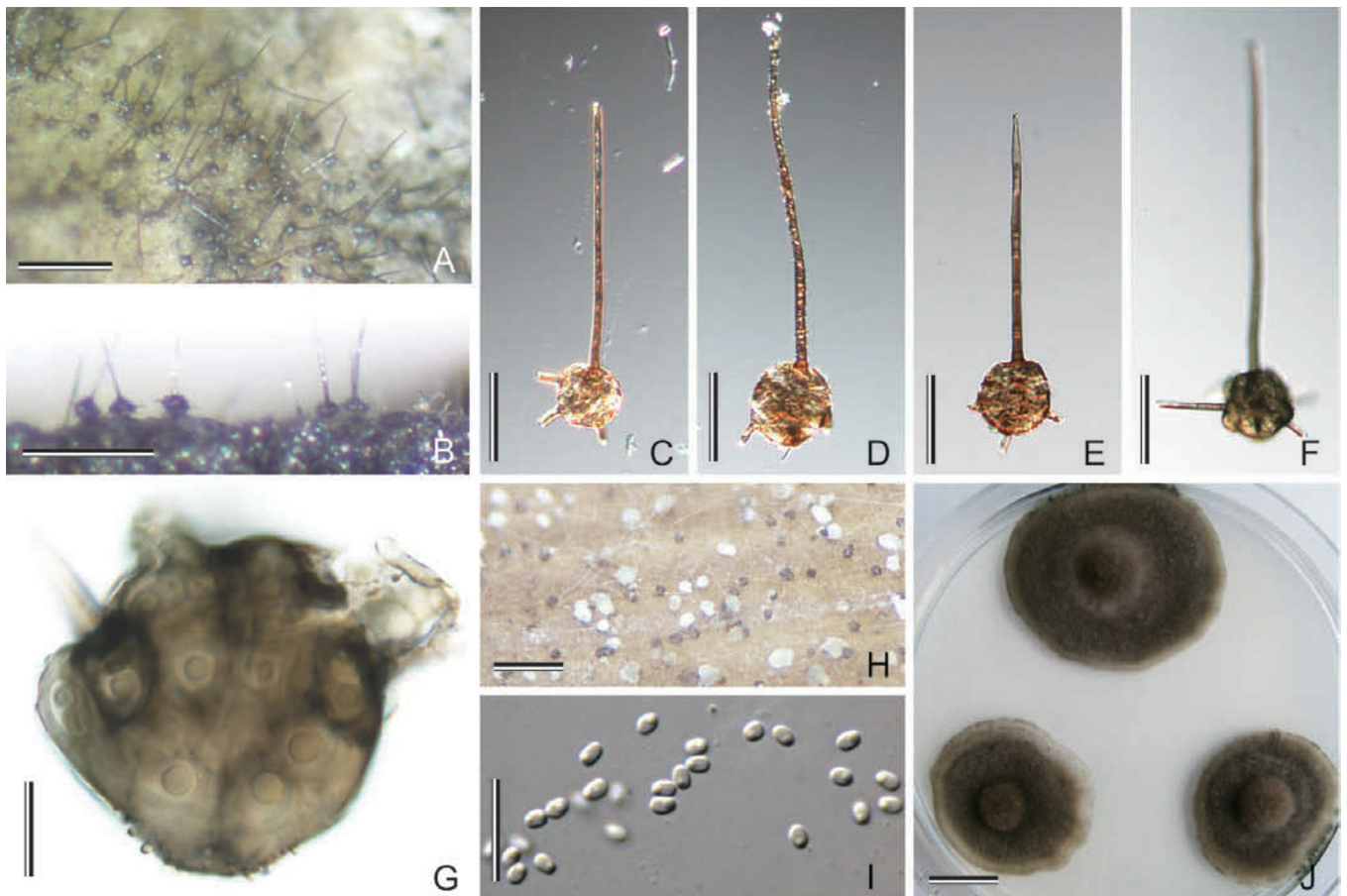
Fig. 15. *Quadricrura septentrionalis*. A. Conidia on host surface; B. Conidia on agar piece immersed in water; C–F. Conidia; G. Conidial body composed of internal hyphoid structure; H. Spermogonia on rice straw agar; I. Spermatia; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–F = 50 µm; G, I = 10 µm; H = 500 µm; J = 1 cm. A, C from HHUF 30021; B, F–J from culture HC 4984; D–E from HHUF 28782 holotype.

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. Yonaguni, Irinda 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.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 30–45(–52.5) µm, globosa, brunnea vel atro brunnea, cum appendicibus; longiappendices unica, 115–210 µm longae, 6–12-septatae; breviappendices 4, 10–20 µm longae, 0–1-septatae.

Mycelium superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* 30–45(–52.5) µm (av. 37.4 µm, *n* = 50) diam, globose, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendage single, 115–210 µm long (av. 159.7 µm, *n* = 50), 5–6 µm wide at the base, 3–4 µm wide at the apex, 6–12-septate, arising from apical part of conidia; short appendages usually 4, 10–20 µm long (av. 14.7 µm, *n* = 50), 3–4 µm wide at the base and apex, 0–1-septate, arising excentric from the conidial base.

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 kurilensis*, 3 May 2003, K. Tanaka & N. Asama, HHUF 28782 **holotype** designated

here, living culture *HC 4984* (= CBS 125430); Aomori, Shimokita, Hotokegaura, on culms of *S. kurilensis*, 20 Oct. 2002, N. Asama, HHUF 30020, living culture *KTC 920* (= CBS 125428); Aomori, Hirosaki, 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 tail, on culms of *S. kurilensis*, 28 July 2007, K. Tanaka & G. Sato, HHUF 30021, living culture *yone 176* (= CBS 125432 = NBRC 106243); Hokkaido, Isl. Rishiri, Oniwaki trail, on culms of *S. kurilensis*, 29 July 2007, K. Tanaka & G. Sato, living culture *yone 179* (= CBS 125433 = NBRC 106244); Aomori, Hirosaki, 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*, 19 Oct. 2003, K. Tanaka, SH 89 = HHUF 28786; Aomori, Hirosaki, Zatoishi, 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, Maruyama (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, Bekkai, Notsukefuren park (145°14'E, 43°31'N), on culms of *Sasa niopponica*, 8 Sept. 2003, K. Tanaka & S. Hatakeyama, SH 118 = HHUF 28783; Hokkaido, Akkeshi, Ootakita, Sattebetu-river (144°49.0'E, 43°08.1'N), on bamboo culms, 7 Sept. 2003, K. Tanaka & S. Hatakeyama, SH 88 = HHUF 28784; Hokkaido, Kamikawa, Shintoku, Shinnai, 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 28785; 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 1 260–1 290 bp of LSU nrDNA sequences were determined for 53 isolates of bamboo 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 *Roussoellopsis tosaensis* (GenBank AB524484) and positions 1 247–1 591 of *Neottiosporina 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 *Botryosphaeriales*) and *Dothidea 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*, *Pleomassariaceae* 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 of 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-Polyplosphaeria-Triplosphaeria* clade. *Tetraplosphaeria* occurred at the most basal position in this family.
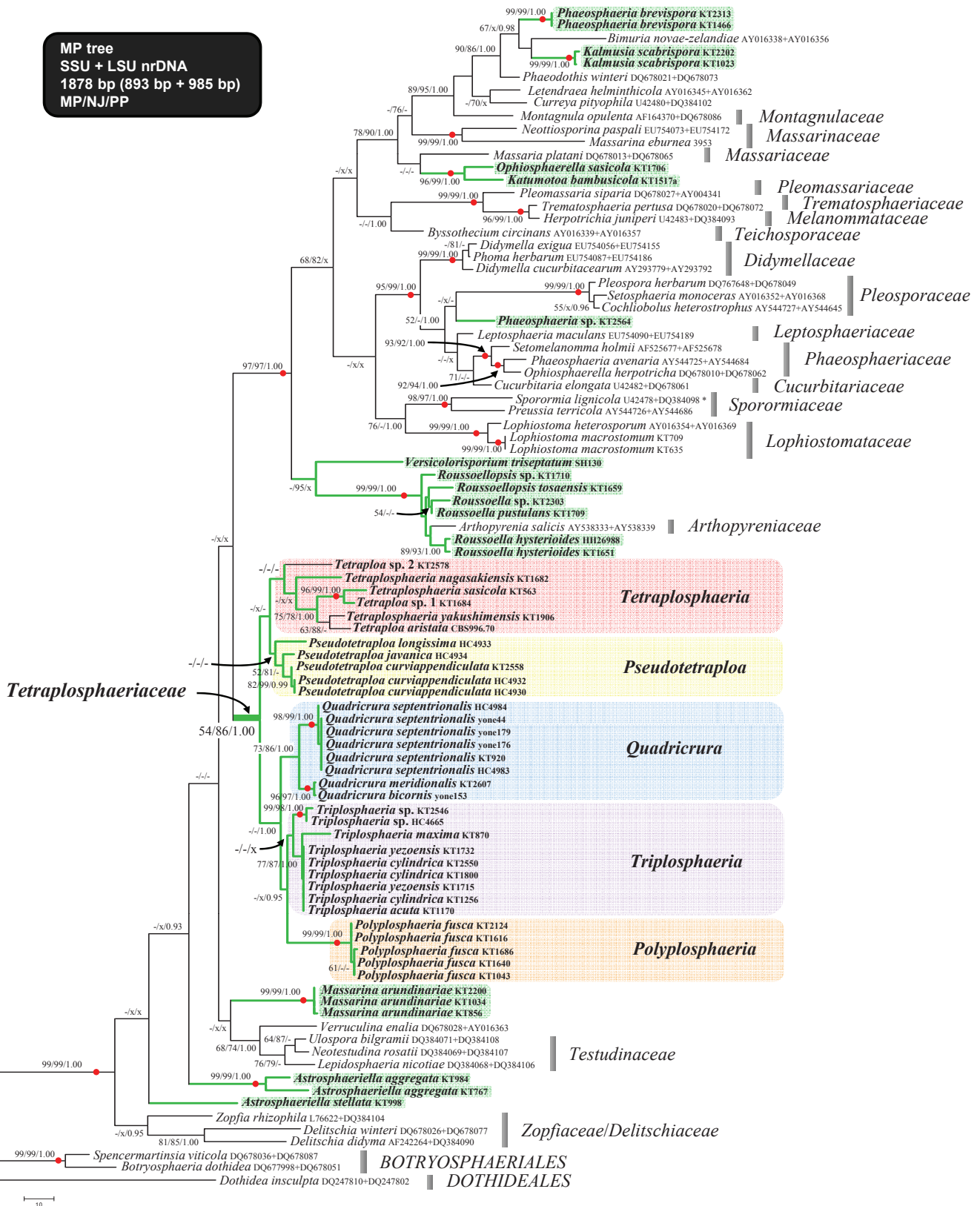
Fig. 16. Consensus tree of the 31 equally most parsimonious trees based on a combined dataset of SSU (893 bp) and LSU (985 bp) nrDNA sequences. MP and NJ bootstrap support greater than 50 % and Bayesian posterior probabilities above 0.90 are indicated at the nodes as MPBS/NJBS/PP. Hyphen ("-") indicates a value lower than 50 % (BS) or 0.90 (PP), 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 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**.
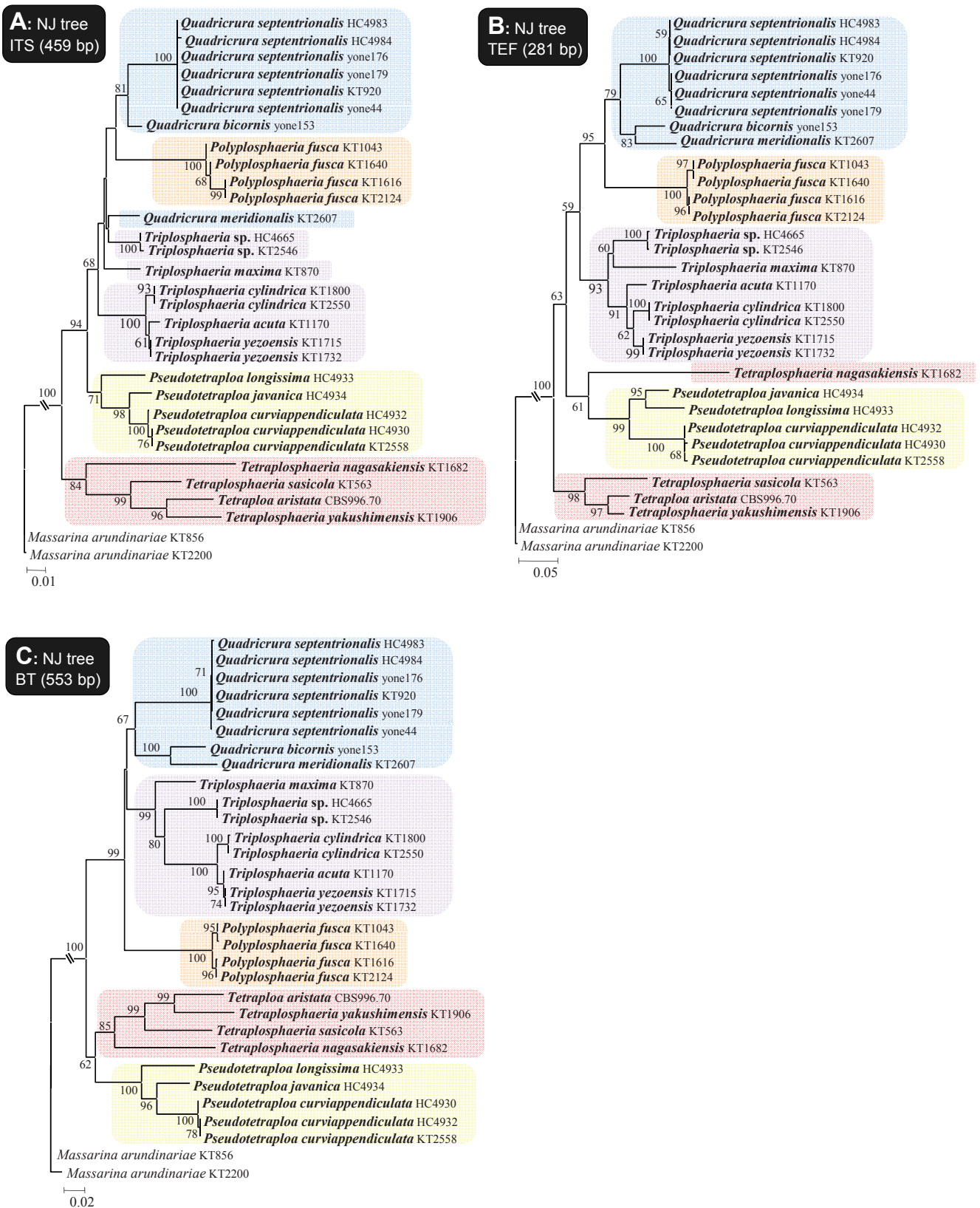
Fig. 17. Neighbour-joining trees of the *Tetraplosphaeriaceae* based on the sequences from ITS (A: 459 bp), TEF (B: 281 bp), and BT (C: 553 bp). Bootstrap support greater than 50 % are shown at the nodes. An original isolate number is noted after the species name. The tree is rooted to *Massarina arundinariae*.
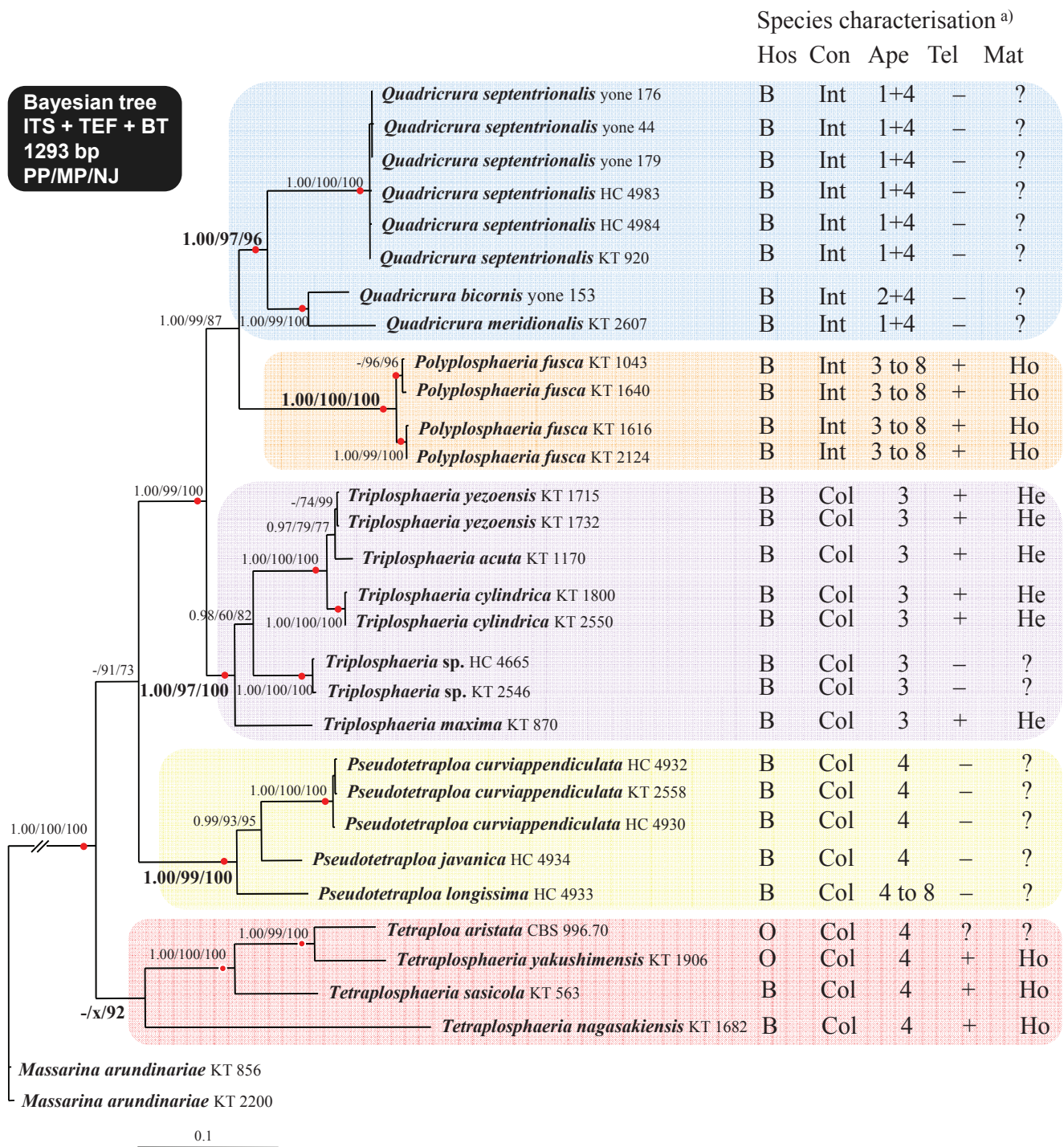
Fig. 18. Phylogeny of *Tetraplosphaeriaceae* from Bayesian analysis based on a combined dataset (1 293 bp) of ITS, TEF, and BT. Bayesian posterior probabilities above 0.90 and MP and NJ bootstrap values greater than 50 % are indicated at the nodes as PP/MPBS/NJBS. Hyphen ("-") indicates values lower 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*. [a] 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.

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 *Tetraplosphaeriaceae* 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 gel matrix; the bitunicate cylindrical-clavate asci; and the narrowly fusiform ascospores (Barr 1990, Hyde & Fröhlich 1998, Fröhlich & Hyde 2000). Currently, 47 taxa are accepted in *Astrosphaeriella* (Wang *et al*. 2004, Jagadeesh Ram *et al*. 2005, Tanaka & Harada 2005a, Chen & Huang 2006), and most of them are recorded on bamboo. *Astrosphaeriella* has been provisionally placed in *Melanommataceae*, *Pleosporales* (Lumbsch & Huhndorf 2007), although the molecular phylogeny of this genus has not been revealed to date. The result from our study (Fig. 16) suggests that *Astrosphaeriella* is not a member of *Melanommataceae*, because *Astrosphaeriella stellata*, the type of the genus, deviated from *Herpotrichia juniperi*, a representative species of the *Melanommataceae* (Zhang *et al*. 2008), and was located at the basal position of *Pleosporales*. Monophyly of *Astrosphaeriella* was not supported. Tanaka & Harada (2005a) transferred *Melanopsamma aggregata* (Hino & Katumoto 1955) to *Astrosphaeriella* according to the broad generic concept of *Astrosphaeriella* proposed by Hyde *et al*. (2000) to accept *Massarina*-like species having a slit-like ostiole at the ascomata. However, this classification was not supported by our results, because *A. aggregata* with a slit-like ostiole (Fig. 19B) did not form a clade with *A. stellata* (Fig. 16). Chen & Hsieh (2004) recognised three elements in this genus: 1) typical *Astrosphaeriella* species (*e.g. A. stellata*), 2) *Trematosphaeria*-like species with striate ascospores (*e.g. A. africana*), and 3) *Massarina*-like species with immersed ascomata (*e.g. A. bakeriana*); they proposed a strict generic concept excluding *Massarina*-like species. The phylogeny obtained from our study support their opinion.

Kalmusia (Fig. 19C–D): One species of the genus, *Kalmusia scabrispora* (Tanaka *et al*. 2005), was used for phylogenetic analyses. This fungus was originally described as a species of *Leptosphaeria* by Teng (1934) and was later transferred to *Massariosphaeria* by Shoemaker & Babcock (1989). The phylogenetic tree based on the SSU+LSU nrDNA sequences in this study did not accept these two classifications, although we could analyse only SSU sequences for the type species of the latter genus (*M. phaeospora*). It is uncertain whether the species belong to *Kalmusia* from a molecular perspective, because there are no sequence data available for other *Kalmusia* species. The genus *Kalmusia*, typified by *K. ebuli*, has been assigned to the *Montagnulaceae* (Barr 2001), and the clypeate ascomata (Fig. 19C) and asci with a long stipe (Fig. 19D) of *K. scabrispora* fit well in the family.

Katumotoa (Fig. 19E–F): The monotypic genus *Katumotoa*, based on *K. bambusicola*, is characterised by apiosporous ascospores provided with bipolar enlarged sheath (Fig. 19E–F). Based on morphological features of the species, such as immersed perithecioid ascomata, thin ascomatal wall composed of small

pseudoparenchymatous cells, cellular pseudoparaphyses, and fissitunicate asci, *Katumotoa* has been tentatively assigned to *Phaeosphaeriaceae* (Tanaka & Harada 2005b). However, *Katumotoa* 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).

Massarina (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 *Massarina* (Aptroot 1998) but was later transferred to *Lophiostoma* (Hyde *et al*. 2002a), was used for the analyses. All phylogenetic analyses revealed that placement of this taxon in either *Massarina* or *Lophiostoma* was not suitable (Fig. 16). The species grouped with the *Verruculina*-*Testudinaceae* clade, and they were isolated from a core member of *Pleosporales* in the MP tree or were positioned as a sister group of *Tetraplosphaeriaceae* 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 *Massarina* species from bamboo would be required, because recent molecular studies on the genus suggest a considerable polyphyly of *Massarina s. l.* (Kodsueb *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 clypei 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 asci without obvious
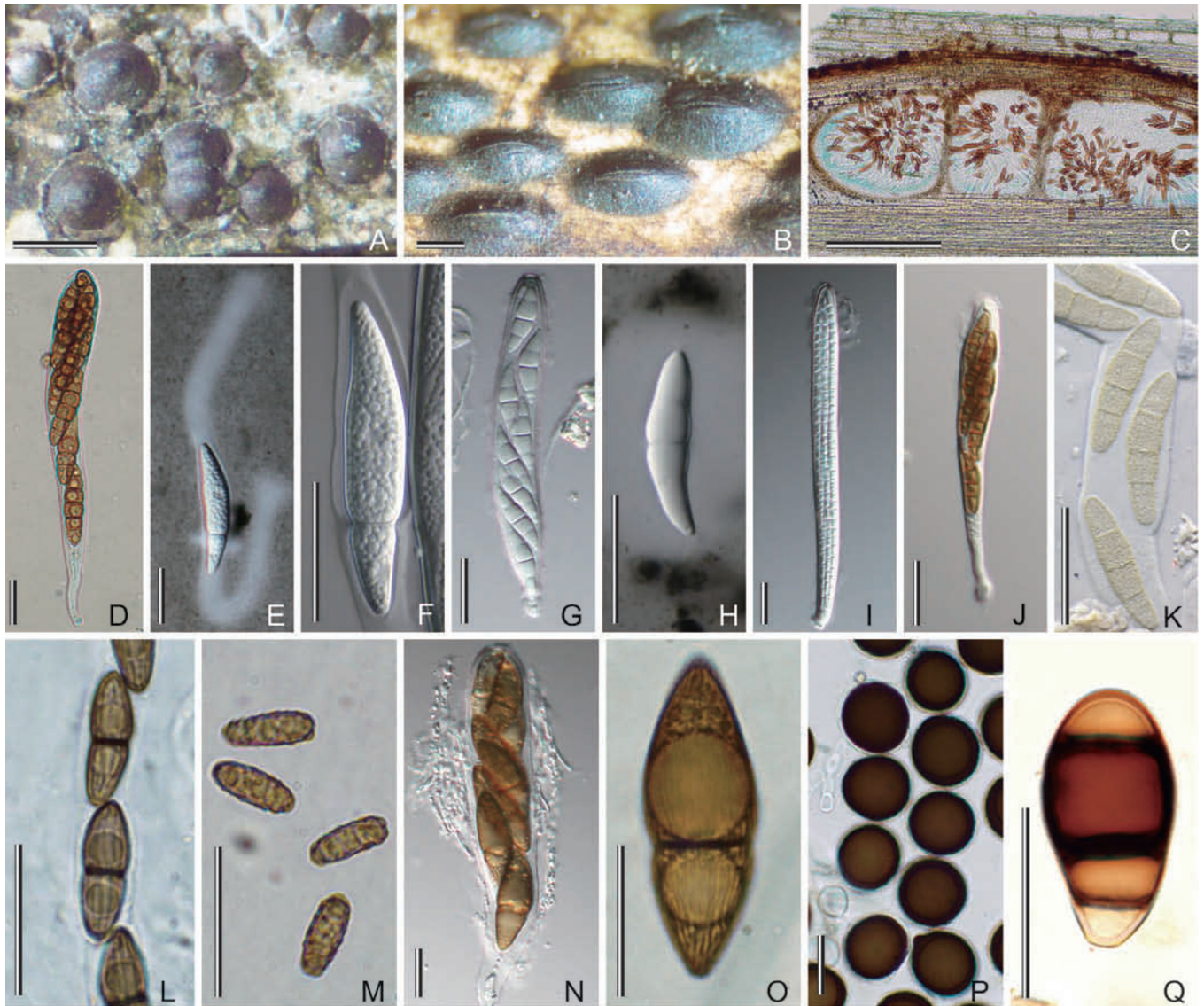
Fig. 19. Selected bambusicolous fungi; A. *Astrosphaeriella stellata* (HHUF 28494); B. *Astrosphaeriella aggregata* (HHUF 28232); C–D. *Kalmusia scabrispora* (HHUF 28608); E–F. *Katumotoa bambusicola* (culture KT 1517a); G–H. *Massarina arundinariae* (HHUF 30014); I. *Ophiosphaerella sasicola* (HHUF 29443); J. *Phaeosphaeria brevispora* (HHUF 30016); K. *Phaeosphaeria* sp. (HHUF 30017); L–M. *Roussoella hysterioides* (L from HHUF 29217; M from culture KT 1651); N–P. *Roussoellopsis tosaensis* (N–O from HHUF 29234; P from culture KT 1659); Q. *Versicolorisporium triseptatum* (HHUF 28815); A–B. Ascomata on host surface; C. Ascomata in longitudinal section; D, G, I–J, N. Asci; E–F, H, K–L, O. Ascospores; M, P–Q. Conidia. Scale bars: A–B = 500 μm; C = 200 μm; D–Q = 20 μm.

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). *Roussoella* 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 *Roussoella* 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 *Roussoellopsis* and *Arthopyrenia salicis* (Fig. 16). This result might suggest that *Roussoella* belongs to *Arthopyreniaceae*, but this relationship is not

fully resolved because of the morphological differences between both taxa. Many of the characters found in *Arthopyreniaceae*, *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 *Roussoella*. Our results further suggest that *Roussoella* is not a monophyletic genus, but additional evidence would be necessary before taxonomic revisions of the genus can be proposed.

Roussoellopsis (Fig. 19N–P): Ascomata of this genus are extremely similar to those found in *Roussoella*, but *Roussoellopsis* species have clavate asci and large-sized (*ca.* 28–66 × 10–17 μm) fusiform ascospores strongly constricted at the submedian septum (Fig. 19N–O; Hino 1961, Hino & Katumoto 1965). All three species in *Roussoellopsis* have been considered to belong to *Astrosphaeriella* or *Roussoella* on the basis of their original descriptions (Aptroot 1995b). However, two isolates of *Roussoellopsis* appeared in the basal lineage of the main families in *Pleosporales* and far away from *Astrosphaeriella* clade in this

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 *Melanconiopsis* 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 *Rousoella* having a *Cytoplea* state (Fig. 19M; Hyde *et al.* 1996) indicate that they are not congeneric.

Versicolorisporium (Fig. 19Q): 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 *Roussoella-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 1978, Arambarri *et al.* 1987, Rifai *et al.* 1988, Révay 1993, Matsushima & Matsushima 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. setifera* (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. ellisii*.

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 1969, Descals & Moralejo 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* (Kodsueb *et al.* 2007), *Helminthosporium* (Oliver *et al.* 2000), *Saccharicola* (Eriksson & Hawksworth 2003). On the other hand, *Lophiostoma* characterised by the slit-like ostiole of ascomata 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 *Massarinaceae* 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 hysterioides* in Fig. 16. *Tetraplosphaeria* having *Tetraploa* anamorphs *s. str.* formed a single clade with four other genera (*Triplosphaeria*, *Polyplosphaeria*, *Pseudotetraploa* and *Quadricrura*) 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 based on 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* (Kodsueb *et al.* 2006) and for *Chaetosphaeriaceae* in *Sordariomycetes* (Réblová

2000, 2006, Réblová & Seifert 2007). However, the usefulness of anamorphic morphologies for species identification might be limited as in the case of *Tetraploa aristata s. l.* Similarly, *Triplosphaeria* species have relatively few morphological differences in their anamorphs, but significant differences in their teleomorphs, especially in their ascospores.

Relationshiphs between genera in *Tetraplosphaeri-aceae*

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. *Triplosphaeria* species produce conidia with pseudosepta similar to those of *Pseudotetraploa* but with a reduced number of conidial columns and setose appendages. Most species in *Triplosphaeria* 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 *Polyplosphaeria* 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, Worobiec *et al.* 2009). The oldest record of *Tetraploa*-like fossil from Devonian deposits has been reported as an acritarch genus *Frasnacritetrus* (Taugourdeau 1968), but this is regarded as a contamination by a recent *Tetraploa* (Worobiec *et al.* 2009). On account of the presence of *Tetraploa* fossils from the late Miocene accompanied by pollen grains of a bamboo (*Graminidites 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 (Worobiec *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 *Piricaudilium* (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 appendaged 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 & Bärlocher 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, *Arundinariinae* (*Pleioblastus* 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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