RESEARCH ARTICLE

Phylogeny of *Discosia* and *Seimatosporium*, and introduction of *Adisciso* and *Immersidiscosia* genera nova

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Abstract   *Discosia* (teleomorph unknown) and *Seimatosporium* (teleomorph *Discostroma*) are saprobic or plant pathogenic, coelomycetous genera of so-called 'pestalotioid fungi' within the *Amphisphaeriaceae* (*Xylariales*). They share several morphological features and their generic circumscriptions appear unclear. We investigated the phylogenies of both genera on the basis of SSU, LSU and ITS nrDNA and β-tubulin gene sequences. *Discosia* was not monophyletic and was separated into two distinct lineages. *Discosia eucalypti* deviated from *Discosia* clade and was transferred to a new genus, *Immersidiscosia*, characterised by deeply immersed, pycnidioid conidiomata that are intraepidermal to subepidermal in origin, with a conidiomatal beak having periphyses. Subdividing *Discosia* into 'sections' was not considered phylogenetically significant at least for the three sections investigated (sect. *Discosia*, *Laurina*, and *Strobilina*). We recognised *Seimatosporium* s.l. as a monophyletic genus. An undescribed species belonging to *Discosia* with its associated teleomorph was collected on living leaves of *Symplocos prunifolia* from Yakushima Island, Japan. We have therefore established a new teleomorphic genus, *Adisciso*, for this new species, *A. yakushimense*. *Discostroma tricellulare* (anamorph: *Seimatosporium azaleae*), previously described from *Rhododendron* species, was transferred to *Adisciso* based on morphological and phylogenetic grounds. *Adisciso* is characterised by relatively small-sized ascomata without stromatic tissue, obclavate to broadly cylindrical asci with biseriate ascospores that have 2 transverse septa, and its *Discosia* anamorph. Based on these features, it can easily be distinguished from *Discostroma*, a similar genus within the *Amphisphaeriaceae*.

INTRODUCTION

During investigations into fungal diversity on Yakushima Island, south-eastern Japan (Tanaka & Hosoya 2006, 2008, Hosoya & Tanaka 2007a, b, Sato et al. 2008, Yonezawa et al. 2008, Tanaka et al. 2009), an interesting coelomycetous fungus associated with an unknown ascomycete was collected on living leaves of *Symplocos prunifolia* (*Symplocaceae*). The anamorphic fungus was identified provisionally as '*Discosia* sp. KT 1907' (Hosoya & Tanaka 2007a) based on its morphology, including disc-like applanate conidiomata and conidia with bipolar appendages. No teleomorph has yet been discovered for the more than 100 described taxa in *Discosia* (Nag Raj 1993), but cultural studies of '*Discosia* sp. KT 1907' revealed that a monoconidial isolate of the specimen could produce a teleomorphic state identical to the ascomycete found on the host plant. The teleomorph somewhat resembled some members of *Discostroma* (e.g., *D. tricellulare*) in the *Amphisphaeriaceae, Xylariales*. However, anamorphs of 28 species in *Discostroma* have thus far been accommodated in *Seimatosporium* (= *Sporocadus*) (Shoemaker & Müller 1964, Brockmann 1976, Paulus et al. 2006).

Discosia and *Seimatosporium* are saprobic or plant pathogenic coelomycetous genera of so-called 'pestalotioid fungi', and share several morphological features. In general, they have been distinguished primarily on the basis of their conidial mor-

phology. *Discosia* has almost hyaline conidia with bipolar appendages derived from the concave side of the conidium. *Seimatosporium* has conidia with pigmented median cells, with or without one apical appendage, and with or without one branched or unbranched basal appendage (Nag Raj 1993). These generic circumscriptions, however, appear to be unclear for some species with intermediate conidial morphology. *Seimatosporium grammitum*, for example, has been accepted as *Discosia* by Subramanian & Chandra-Reddy (1974) and Chandra-Reddy (1984), but later transferred to *Seimatosporium* on account of its pigmented conidia with or without apical appendages (Nag Raj 1991, Vanev 1992a). The delimitation of these genera has not been verified via molecular techniques either. Phylogenetic analyses of pestalotioid fungi based on LSU and internal transcribed spacer region (ITS1, 5.8S, ITS2) nrDNA revealed that *Discosia* is a sister taxon to the *Seimatosporium* group, and their relationship is supported by relatively high bootstrap values (Jeewon et al. 2002). However, the phylogenetic relatedness of these genera remained unclear, because only one strain of *Discosia* was included in their analyses (Jeewon et al. 2002). The heterogeneity of both genera is also problematic (Sutton 1980, Nag Raj 1993, Wu & Sutton 1996), and thus generic monophyly should be assessed based on molecular data.

Our aims were 1) to assess the generic monophyly of *Discosia* and *Seimatosporium* using sequence analyses of SSU, LSU and ITS nrDNA; 2) to evaluate the taxonomic significance of the morphological distinction between *Discosia* and *Seimatosporium*; 3) to establish the species validity of '*Discosia* sp. KT 1907' based on morphological comparison with other related species and β-tubulin (BT) gene analyses; and 4) to compare the teleomorph of '*Discosia* sp. KT 1907' with the morphologically similar ascomycetous genus, *Discostroma* (the teleomorph

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Table 1 Isolate data and GenBank accession numbers of *Discosia* and *Seimatosporium*.

Fungal species	Teleomorph	Original no.	Culture collection no.	Voucher specimen	Host	GenBank accession no.			
						SSU	LSU	ITS	BT
Discosia aquatica	Unknown	–	NBRC 32624	–	*Quercus fusiformis*	–	–	03262401[6]	–
Discosia artocreas	Unknown	–	NBRC 8975	NBRC H-11891	*Poa pratensis*	–	AB593705	AB594773	AB594172
	Unknown	–	NBRC 31640	–	Decayed leaves	–	–	03164001[6]	–
	Unknown	–	NBRC 31883	–	*Prunus mume*	–	–	03188301[6]	–
Discosia aff. *artocreas*	Unknown	KT 2118	MAFF 242776	HHUF 29924	*Betula ermanii*	AB593695	AB593704	AB594772	–
	Unknown	SH 290	MAFF 242785	HHUF 29927	*Hamamelis japonica*	AB593697	AB593711	AB594779	AB594177
	Unknown	–	MAFF 238070	–	*Fallopia japonica*	–	AB593720	AB594788	AB594186
Discosia aff. *brasiliensis*	Unknown	KT 2193	NBRC 104198	HHUF 29935	Decayed leaves	–	AB593706	AB594774	–
	Unknown	KT 2194	NBRC 104199	HHUF 29936	Decayed leaves	–	AB593707	AB594775	AB594173
	Unknown	KT 2190	MAFF 242780	HHUF 29932	Decayed leaves	–	AB593717	AB594785	AB594183
	Unknown	–	MAFF 237018	–	*Rosa rugosa*	–	AB593719	AB594787	AB594185
Discosia eucalypti[1]	Unknown	KT 2091	NBRC 104195	HHUF 29920	*Quercus myrsinifolia*	AB593703	AB593722	AB594790	–
	Unknown	KT 2115	NBRC 104196	HHUF 29922	*Quercus myrsinifolia*	AB593701	AB593723	AB594791	–
	Unknown	KT 2117	NBRC 104197	HHUF 29923	*Ardisia japonica*	AB593702	AB593724	AB594792	–
	Unknown	KT 2191	MAFF 242781	HHUF 29933	Decayed leaves	–	AB593725	AB594793	–
Discosia pini	Unknown	–	MAFF 410149	–	*Pinus densiflora*	–	AB593708	AB594776	AB594174
Discosia aff. *pleurochaeta*	Unknown	KT 2179	MAFF 242778	HHUF 29929	Decayed leaves	–	AB593709	AB594777	AB594175
	Unknown	KT 2188	MAFF 242779	HHUF 29930	Decayed leaves	–	AB593713	AB594781	AB594179
	Unknown	KT 2192	MAFF 242782	HHUF 29934	Decayed leaves	–	AB593714	AB594782	AB594180
Discosia sp. 1	Unknown	KT 2131	MAFF 242777	HHUF 29925	*Betula platyphylla* var. *japonica*	AB593696	AB593710	AB594778	AB594176
Discosia sp. 2	Unknown	KT 2109	MAFF 242775	HHUF 29921	Decayed leaves	AB593698	–	AB594780	AB594178
Discosia sp. 3	Unknown	SH 125	MAFF 242783	–	*Castanea crenata*	–	AB593715	AB594783	AB594181
	Unknown	SH 288	MAFF 242784	HHUF 29926	*Machilus thunbergii*	AB593699	AB593716	AB594784	AB594182
Discosia sp. 4	Unknown	–	MAFF 236709	–	*Parthenocissus tricuspidata*	–	AB593718	AB594786	AB594184
Discosia sp.	Ascomycete[2]	KT 1907	MAFF 242774 = NBRC 104194T	HHUF 29671	*Symplocos prunifolia*	AB593700	AB593721	AB594789	AB594187
Seimatosporium alzaleae[3]	*Discostroma tricellulare*	–	NBRC 32705	NBRC H-12205	*Rhododendron indicum*	–	AB593728	AB594796	AB594188
		–	NBRC 32706	NBRC H-12206	*Rhododendron macrosepalum*	–		03270601[6]	–
		–	NBRC 32707	NBRC H-12207	*Rhododendron obtusum*	–		03270701[6]	–
		–	NBRC 100406	–	*Rhododendron indicum*	–		10040601[6]	–
		–	NBRC 100407	–	*Rhododendron indicum*	–		10040701[6]	AB594189
		–	MAFF 237478	–	*Rhododendron indicum*	–	AB593730	AB594798	–
Se. botan	*Discostroma botan*	H 4619	NBRC 104200T	HHUF 27946	*Paeonia suffruticosa*	–	AB593731	AB594799	–
Se. dilophosporum[4]	*Discostroma stoneae*	–	NBRC 32690	–	–	–	AB593729	AB594797	–
Se. discosioides	Unknown	H 4621	NBRC 104201	–	*Punica granatum*	–	AB593732	AB594800	–
Se. elegans	*Discostroma elegans*	–	NBRC 32674	–	*Melaleuca ericifolia*	–	AB593733	AB594801	–
Se. foliicola	Unknown	–	NBRC 32676	–	*Juniperus phoenicea*	–	AB593734	AB594802	–
Se. glandigenum	Unknown	–	NBRC 32677	–	*Fagus sylvatica*	–	AB593735	AB594803	–
Se. hakeae	Unknown	–	NBRC 32678	–	*Pteridium aquilinum*	–	AB593736	AB594804	–
Se. hypericinum	Unknown	–	NBRC 32647	–	*Hypericum* sp.	–	AB593737	AB594805	–
Se. kriegerianum	*Discostroma callistemonis*	–	NBRC 32679	–	*Callistemon paludosus*	–	AB593739	03267901[6]	–
Se. lichenicola	*Discostroma fuscellum*	–	NBRC 32680	–	*Ribes* sp.	–	AB593726	AB594806	–
Se. lichenicola[4]	*Discostroma corticola*[5]	–	NBRC 32625	–	*Rosa canina*	–	AB593740	AB594794	–
Se. mariae	Unknown	–	NBRC 32681	–	*Correa reflexa*	–	AB593741	AB594807	–
Se. parasiticum[4]	Unknown	–	NBRC 32682	–	*Physocarpus amurensis*	–	AB593727	AB594808	–
Se. passerinii[4]	*Discostroma tostum*	–	NBRC 32626	–	–	–		AB594795	–

[1] Described in this study as *Immersidiscosia eucalypti*.
[2] Described in this study as *Adisciso yakushimense*.
[3] Described in this study as *Adisciso tricellulare*.
[4] Deposited in NBRC as ex-teleomorph strain.
[5] This species has been treated as a synonym of *Discostroma fuscellum* by Huhndorf (1992).
[6] Sequence ID of NBRC strains; data obtained from NBRC web site (http://www.nbrc.nite.go.jp/NBRC2/NBRCDispSearchServlet?lang=jp).

of *Seimatosporium*), in order to resolve their morphological differences.

MATERIALS AND METHODS

Morphological studies

Specimens of *Discosia* and *Seimatosporium* were taken from leaves and twigs of various plants in Japan and were mainly deposited in the herbarium of Hirosaki University (HHUF). Morphological methods are described by Tanaka et al. (2009, 2010). Monoconidial cultures were obtained according to the methods of Shearer et al. (2004). Nineteen cultures were isolated from the collections and were deposited at the National Institute of Agrobiological Sciences (NIAS), Japan (MAFF), and the National Institute of Technology and Evaluation (NITE), Biological Resource Center (NBRC). Other strains were obtained from the culture collection of NIAS and NBRC (Table 1). To validate isolations, sporulation was promoted by placing a small piece of mycelial culture on rice straw agar (RSA; Tanaka & Harada 2003). Colony colours on potato-dextrose agar (PDA, Difco) are taken from Rayner (1970). Four specimens of *Discostroma massarinum* (the type species of the genus) preserved in the herbarium of the ETH, Zürich (ZT) were borrowed and examined for morphological comparison with the teleomorph of '*Discosia* sp. KT 1907.' Nomenclatural novelties were deposited in Myco-Bank (Crous et al. 2004).

Molecular analyses

A total of 45 isolates were used for DNA extraction (Table 1). DNA from mycelia was extracted using the ISOPLANT Kit (Nippon Gene, Tokyo) according to the manufacturer's instructions. Partial SSU (9 isolates, c. 1 000–1 300 bp of the 5' end) and LSU nrDNA (37 isolates, c. 1 250 bp of the 5' end) were analysed to elucidate familial and generic positioning. The complete ITS region of nrDNA (45 isolates, c. 500 bp), and exons 1–6 and the respective introns of the BT gene (18 isolates, c. 600 bp) were sequenced to confirm generic and species-level placements (Table 1). Four primer sets, NS1–NS4 (White et al. 1990), LR0R–LR7 (Rehner & Samuels 1994), ITS1–ITS4 (White et al. 1990), and T1–Bt2B (O'Donnell & Cigelnik 1997, Glass & Donaldson 1995, respectively) were used for the amplification of SSU, LSU, ITS, and BT, respectively. DNA was amplified and sequenced according to the methods described by Tanaka et al. (2009). Newly obtained sequences were deposited in GenBank (Table 1). These sequences were aligned manually along with published sequence data retrieved from GenBank, using Clustal W included in the program Molecular Evolutionary Genetic Analysis (MEGA) v4 (Tamura et al. 2007). The aligned dataset was subjected to two phylogenetic analyses involving maximum-parsimony (MP) and neighbour-joining (NJ) using MEGA 4. The MP tree was generated using the close-neighbour-interchange heuristic search (level 1), the initial tree by random addition sequence (100 replicates), and the 'use all site' option. The NJ tree was obtained using the Kimura 2-parameter model, a uniform rate among sites, and pairwise deletion of gaps. Confidence in topologies was assessed using a bootstrap (BS) test involving 1 000 replicates. Alignments used in this study were deposited in TreeBASE (http://www.treebase.org).

RESULTS

Phylogenetic analyses

An SSU alignment comprised of nine sequences of *Discosia* and 24 representative sequences within the *Xylariales* from GenBank, resulted in a 1 012 character dataset with 114 (11.3 %) variable sites. The NJ tree generated from this alignment confirmed that '*Discosia* sp. KT 1907' is close to other pestalotioid fungi and is a member of the *Amphisphaeriaceae* in *Xylariales* (data not shown but available from TreeBASE).

To clarify phylogenetic relatedness more precisely, our LSU sequences of *Discosia* and *Seimatosporium* were aligned with sequences of other pestalotioid fungi from GenBank. The dataset comprised 795 aligned characters, including 139 variable positions (17.5 %) and 87 parsimony-informative positions (10.9 %). A total of 237 equally MP trees with a length of 291 steps (consistency index (CI) = 0.5910, retention index (RI) = 0.8496) were generated and one of the MP trees is shown in Fig. 1. Twenty-one taxa of *Seimatosporium* formed a strongly supported monophyletic group (more than 97 % MP/NJ BS), while species of *Discosia* were divided into two distinct clades. Nineteen taxa of *Discosia*, including '*Discosia* sp. KT 1907' and *S. azaleae*, grouped as a sister clade to *Seimatosporium*, although the MP BS support of the clade (*Discosia* I) was relatively low (51–85 %). Four strains identified as *D. eucalypti* deviated from the clade of *Discosia* I, and were in a basal position to *Discosia* I, *Seimatosporium*, and the other pestalotioid genera (*Truncatella*, *Monochaetiopsis*, and *Bartalinia*).

A total of 52 ITS sequences from *Discosia* and *Seimatosporium* were aligned with outgroup taxa (*Pestalotiopsis* spp.). Out of 545 characters, 126 (23.1 %) were variable and 100 (18.3 %) were parsimony-informative. An MP analysis resulted in 252 equally most parsimonious trees with a length of 220 steps (CI = 0.6772, RI = 0.9147) (Fig. 2). The three clades found in the LSU tree (i.e., *Discosia* I, *Discosia* II, and *Seimatosporium*) were confirmed as monophyletic groups in the ITS tree, although the monophyly of *Seimatosporium* received low BS support in MP analysis (< 50 %). '*Discosia* sp. KT 1907' nested within the *Discosia* I, a clade with strong or moderate BS support (87–93 %), and was positioned as a sister taxon to *S. azaleae*. The robust clade of *Discosia* II (100 %) was sister to *Seimatosporium*, but this relationship was not well-supported (54–56 %).

To clarify species boundaries within *Discosia*, an alignment of BT sequences from 18 strains of *Discosia* was generated. Of the 505 characters, 96 (19.9 %) were variable, of which 63 (12.5 %) were parsimony informative. An MP analysis yielded 90 equally MP trees with a tree length of 138 steps (RI = 0.8333, CI = 0.8861) and an unrooted MP tree is shown in Fig. 3. '*Discosia* sp. KT 1907' clustered most closely with *S. azaleae*, and this relationship was strongly supported (98–99 %). In general, groups that were recognisable from their conidial morphologies received higher support, with the exception of '*Discosia* aff. *brasiliensis*'; this was not well-supported by the MP BS value (< 50 %).

Taxonomy

'*Discosia* sp. KT 1907' grouped with other species of *Discosia* in a monophyletic clade, in both our LSU (Fig. 1) and our ITS nrDNA (Fig. 2) analyses. A new genus, *Adisciso*, is established for the clade containing the teleomorph of '*Discosia* sp. KT 1907'. No ascomatal state has previously been discovered for any *Discosia*-like species, and the genus is paraphyletic. *Discostroma tricellulare* (anamorph: *S. azaleae*) is transferred to the genus *Adisciso*. For morphological comparison between *Adisciso* and *Discostroma*, the type species of the latter genus (*D. massarinum*) is briefly described. The new genus *Immersidiscosia*, a segregate of *Discosia*, is proposed for *Discosia eucalypti*, as this is not only shown to deviate from the *Discosia* clade (Fig. 1, 2), but has a distinct conidiomatal morphology from species in the other *Discosia* clade.
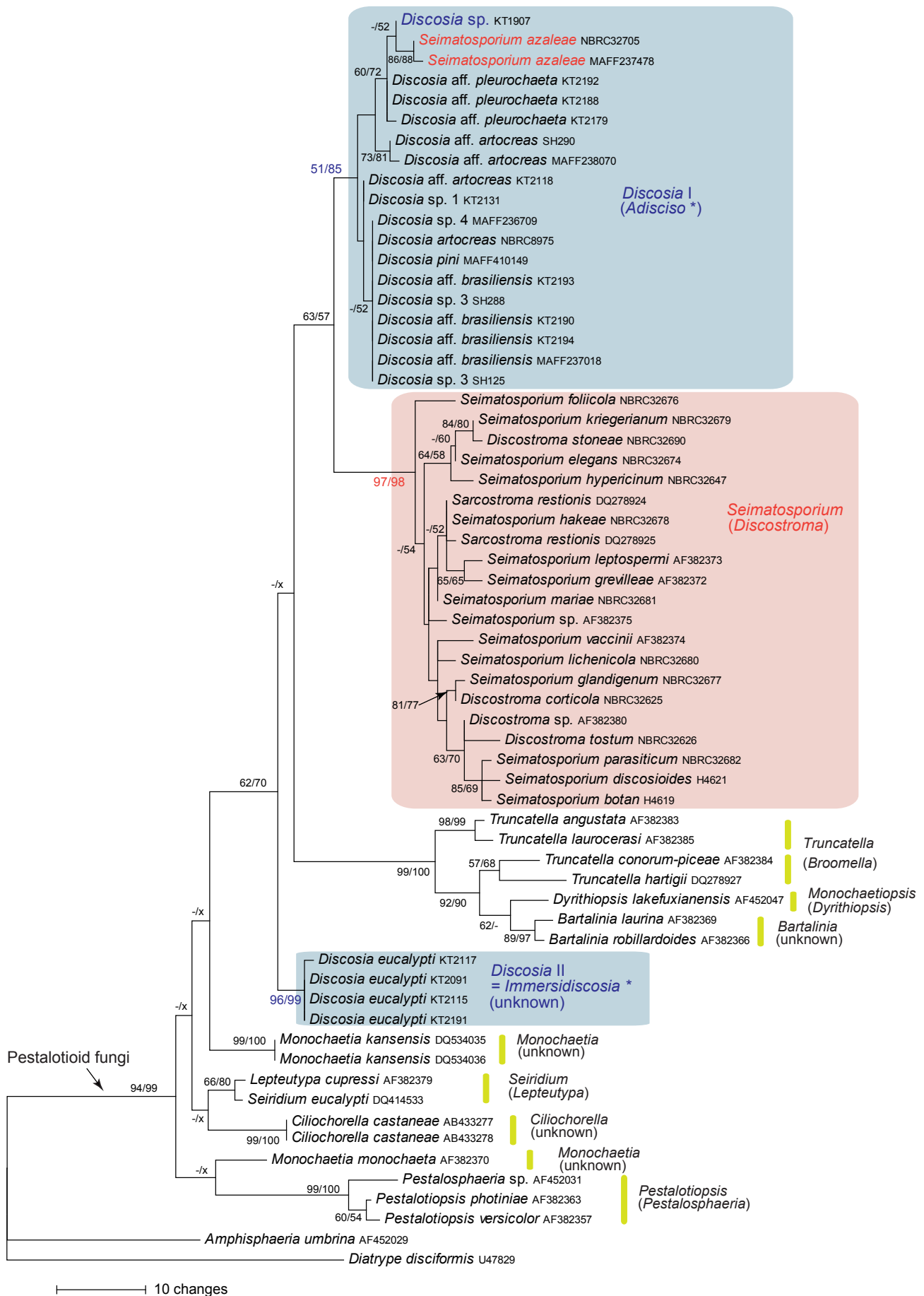
Fig. 1 One of 237 equally most parsimonious trees of *Discosia*, *Seimatosporium*, and related amphisphaeriaceous genera based on the LSU sequences (795 bp). The clades of *Discosia* and *Seimatosporium* are shown in blue and red backgrounds, respectively. Maximum parsimony (MP) and neighbour-joining (NJ) bootstrap (BS) values greater than 50 % are indicated at the nodes as MPBS/NJBS. A hyphen ('–') indicates values lower than 50 % BS and a node not present in an analysis is shown with 'x'. An original isolate designation is noted after the species name. Generic name in parentheses is teleomorphic name. An asterisk indicates a new name proposed in this study.
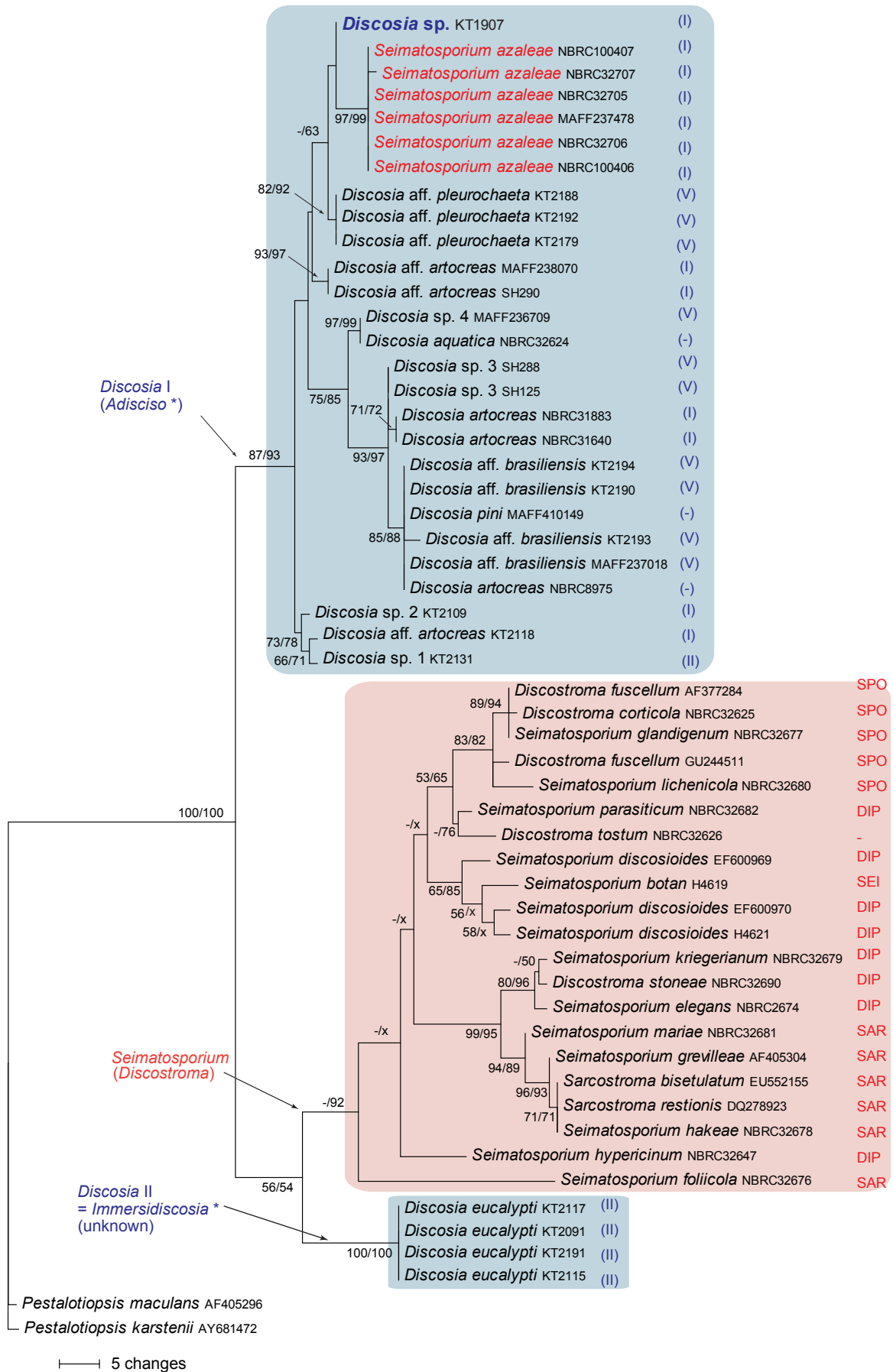
Fig. 2 One of 252 equally most parsimonious trees of *Discosia* and *Seimatosporium* based on the ITS sequences (545 bp). The clades of *Discosia* and *Seimatosporium* are shown in blue and red backgrounds, respectively. Maximum parsimony (MP) and neighbour-joining (NJ) bootstrap (BS) values greater than 50 % are indicated at the nodes as MPBS / NJBS. A hyphen ('–') indicates values lower than 50 % BS and a node not present in an analysis is shown with 'x'. An original isolate designation is noted after the species name. Generic name in parentheses is teleomorphic name. An asterisk indicates a new name proposed in this study. A Roman numeral in parentheses shows a section within *Discosia* (Vanev 1991; I = sect. *Discosia*, II = sect. *Laurina*, V = sect. *Strobilina*). Capital letters of *Seimatosporium* indicate a segregated genus from *Seimatosporium* (Nag Raj 1993; DIP = *Diploceras*, SAR = *Sarcostroma*, SEI = *Seimatosporium* s.str., SPO = *Sporocadus*).
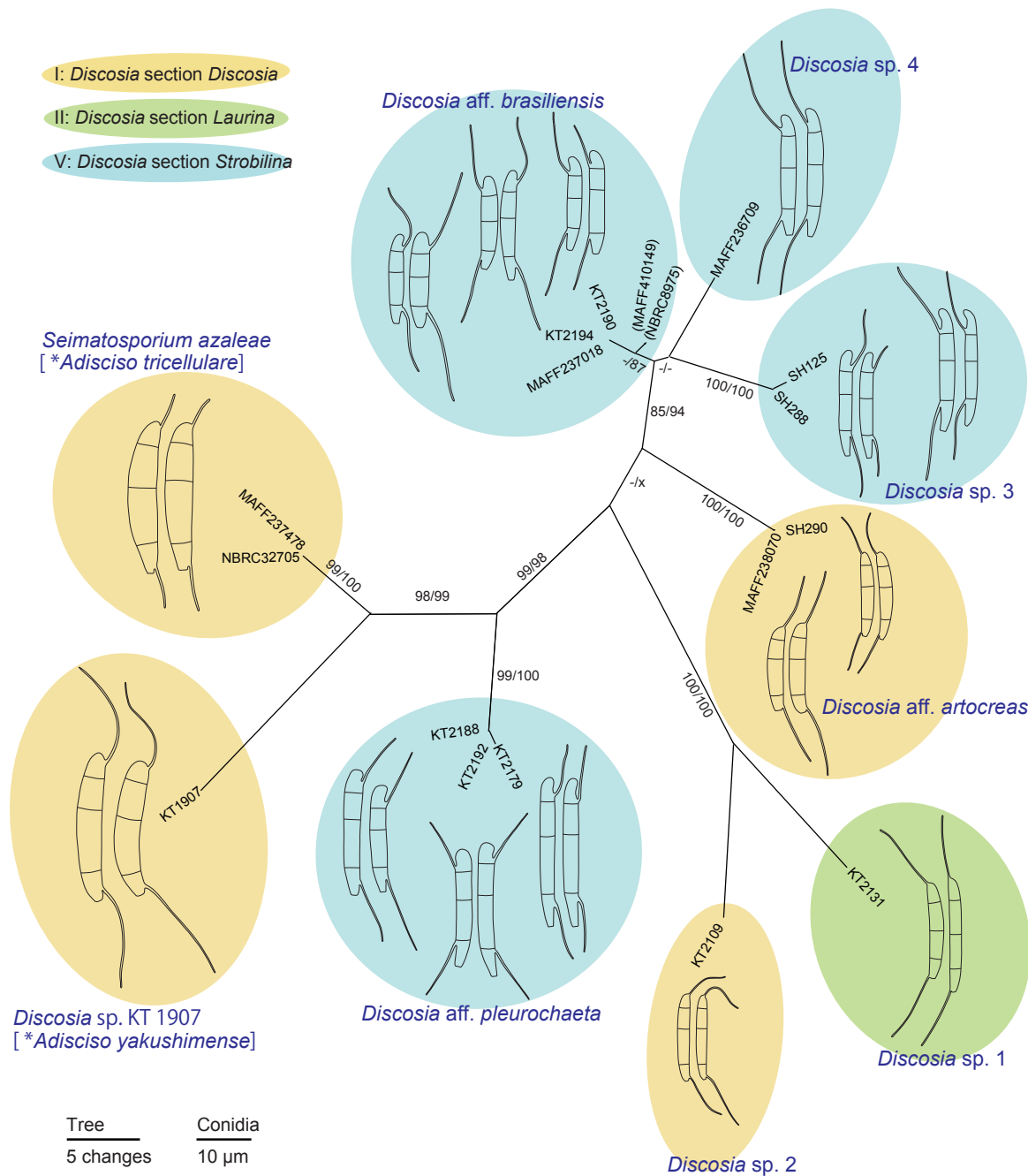
Fig. 3 One of 90 equally most parsimonious trees of *Discosia* based on the BT sequences (505 bp). Maximum parsimony (MP) and neighbour-joining (NJ) bootstrap (BS) values greater than 50 % are indicated at the nodes as MPBS/NJBS. A hyphen ('–') indicates values lower than 50 % BS and a node not present in an analysis is shown with 'x'. An original isolate designation is noted after the species name. An asterisk indicates a new name proposed in this study. Conidial morphology is shown at the side of each species (MAFF 410149 and NBRC 8975 are deposited as different species names, *D. pini* and *D. artocreas* respectively, but their conidial morphology could not be observed in culture media).

Adisciso Kaz. Tanaka, Okane & Hosoya, *gen. nov.* — Myco-Bank MB519743

Anamorph. Discosia.

Foliicola. Ascomata dispersa, immersa, ad apicem erumpentia, globosa. Rostrum ascomatis papillatum, cum periphysibus. Paraphyses filiformes. Asci unitunicati, obclavati vel anguste cylindrici, octospori. Ascosporae obovatae vel ellipsoideae, hyalinae, septatae. Conidiomata stromatica, origine subcuticularia vel intraepidermalia. Rostrum conidiomatis obscurum, sine periphysibus. Conidia cylindrica, 3-septata, hyalina, utrinque appendiculata.

Type species. Adisciso yakushimense.

Etymology. An anagram of *Discosia.*

Foliicolous. Ascomata scattered, immersed, slightly erumpent at the beak, globose, ostiolate. *Beak* of ascomata papillate, with numerous periphyses. *Ascomatal wall* composed of sev-

eral layers of polygonal to rectangular, thin-walled cells. *Paraphyses* filiform, hyaline. *Asci* unitunicate, obclavate to broadly cylindrical, rounded and thick-walled at the apex, with subapical ring of J+ or J- in Melzer's reagent, with 8 biseriate ascospores. *Ascospores* obovoid to ellipsoid, transversally euseptate. *Conidiomata* stromatic, scattered to gregarious, subcuticular to intraepidermal in origin, appearing as almost superficial, applanate, black, depressed globose to lenticular in longitudinal sectional. *Beak* of conidiomata inconspicuous, without periphyses. *Conidiophores* mostly reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to lageniform, not branched, holoblastic, hyaline, smooth. *Conidia* fusiform to subcylindrical, slightly curved, euseptate, with unequal or equal cells, with bipolar appendages derived from the concave side of conidia.
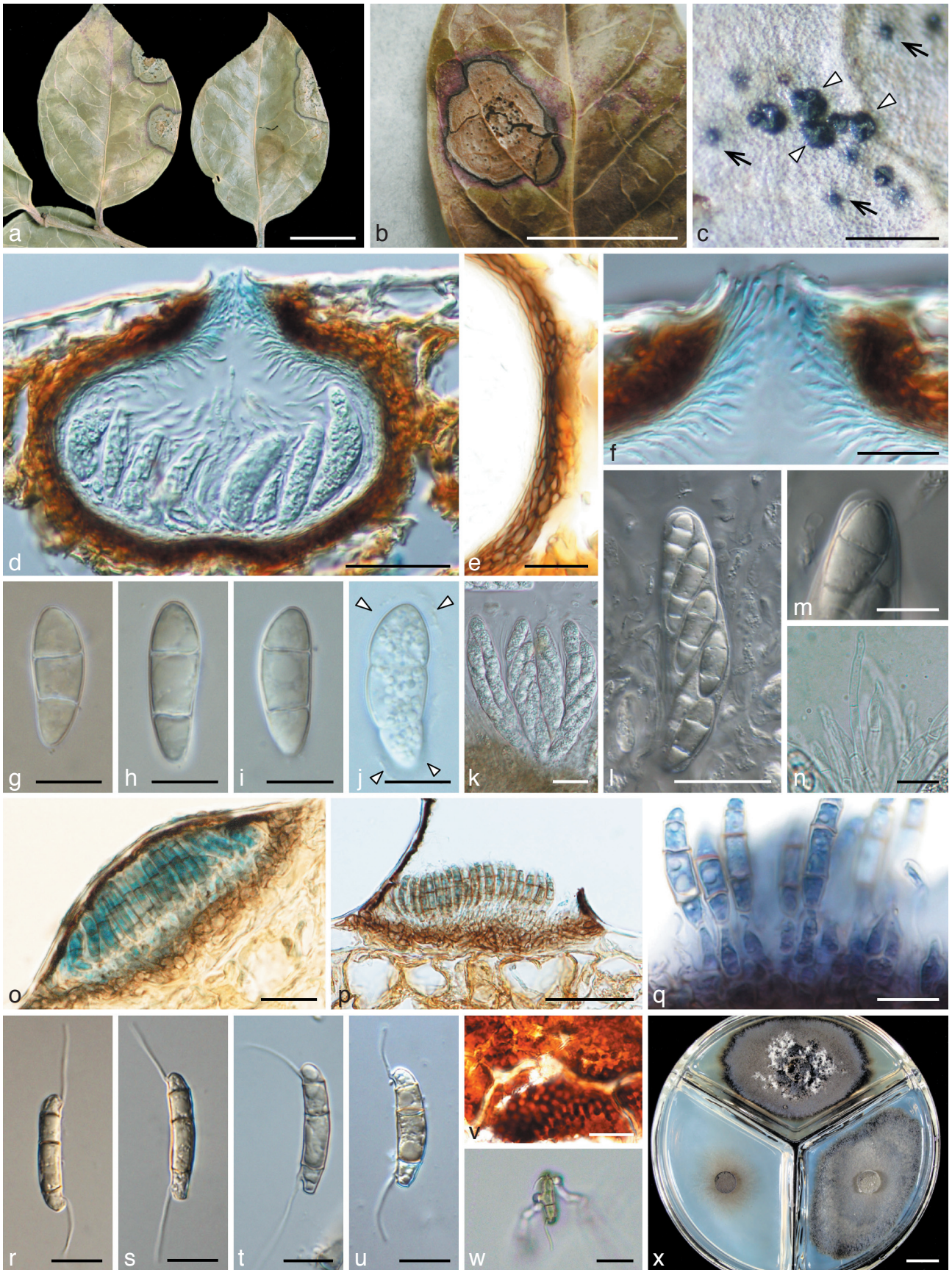
Fig. 4 *Adisciso yakushimense*. a, b. Leaf spots of *Symplocos prunifolia*; c. ascomata (black arrows) and conidiomata (white arrowheads) on host surface; d. ascoma in longitudinal section; e. ascomatal wall; f. ostiole with periphyses; g–j. ascospores (arrowheads indicate sheath of ascospore); k, l. asci; m. ascus apex; n. paraphyses; o. conidioma immersed under host epidermis; p. conidioma in longitudinal section; q. conidia and conidiogenous cells; r–u. conidia; v. conidiomatal wall of surface view; w. germinating conidium; x. colonies on PDA (upper), CMA (left), and MEA (right) after 30 d at 20 °C in the dark (a–i, l, m, o–w: HHUF 29671 (holotype); j, k, n, x: culture MAFF 242774 = NBRC 104194T). — Scale bars: a, b, x = 1 cm; c = 500 μm; d, p = 50 μm; e, f, k, l, n, o, w = 20 μm; g–j, m, q–v = 10 μm.

Adisciso yakushimense Kaz. Tanaka, Okane & Hosoya,
　sp. nov. — MycoBank MB519744; Fig. 4, 7a–f

　Anamorph. _Discosia_ sp.

Foliicola. Ascomata 110–150 µm alt, 150–210 µm diam, dispersa, immersa, ad apicem erumpentia, globosa. Rostrum ascomatis 25–32 µm alt, 42–60 µm diam, papillatum, cum periphysibus. Paraphyses filiformes. Asci 65–95(–102) × 15–23 µm, unitunicati, obclavati vel anguste cylindrici, octospori. Ascosporae 20.5–27.5 × 7–8.5 µm, obovatae vel ellipsoideae, hyalinae, septatae. Conidiomata 45–70 µm alt, 130–200 µm diam, origine subcuticularia vel intraepidermalia. Conidia 22–32.5 × 4–6 µm, subcylindrica, 3-septata; appendix apicalis 10–20 µm; appendix basalis 11–22 µm.

　Etymology. In reference to the location where the specimen was collected.

Foliicolous. _Ascomata_ 110–150 µm high, 150–210 µm diam, scattered, epiphyllous, immersed, slightly erumpent at the beak, globose, ostiolate. _Beak_ 25–32 µm high, 42–60 µm diam (ostiole 25–30 µm diam), papillate, with numerous periphyses. _Ascomatal wall_ 7.5–12.5 µm thick at sides and base, composed of 3–5 layers of polygonal to rectangular, pale brown to brown, thin-walled cells, 4–10 × 2–4 µm. _Paraphyses_ filiform, hyaline, 5–6 µm wide, with septa at 17–42 µm intervals. _Asci_ 65–95 (–102) × 15–23 µm (av. 82.6 × 20.2 µm, $n = 43$), L/W 3.3–4.7 (av. 4.1, $n = 43$) unitunicate, obclavate to broadly cylindrical, rounded and thick-walled at the apex, with subapical ring of J- in Melzer's reagent, with 8 biseriate ascospores. _Ascospores_ 20.5–27.5 × 7–8.5 µm (av. 23.8 × 7.8 µm, $n = 38$), L/W 2.6–3.6 (av. 3.0, $n = 38$), obovoid to ellipsoid, slightly curved, 2-septate, slightly constricted at the septa, slightly enlarged at the second cell from apex (length of the second cell 7–10.5 µm, av. 8.8 µm, $n = 38$), hyaline, smooth, with an entire sheath when fresh. _Conidiomata_ 45–70 µm high, 130–200 µm diam, stromatic, epiphyllous, scattered to gregarious, occasionally confluent, subcuticular to intraepidermal in origin, applanate, rounded to irregular in outline, glistening black, depressed globose to lenticular in longitudinal sectional, unilocular; basal stroma 7–15 µm thick, composed of 3–5 layers of pale brown, thin-walled, subglobose to rectangular cells, 2–7 µm diam; upper wall composed of dark brown to black, thick-walled cells. _Conidiophores_ reduced to conidiogenous cells. _Conidiogenous cells_ 4.5–10 × 2–3.5 µm (av. 8.1 × 2.8 µm, $n = 20$), ampulliform to lageniform, unbranched, holoblastic, hyaline, smooth. _Conidia_ 22–32.5 × 4–6 µm (av. 26.9 × 5.1 µm, $n = 83$), L/W 4.2–6.8 (av. 5.3, $n = 83$), subcylindrical, slightly curved, 3-septate, with unequal cells, smooth, with bipolar appendages; basal cell 4–7 µm long (av. 5.5 µm, $n = 83$), hyaline to pale brown, with a truncate base of 2.5–3 µm wide; 2 median cells together 12–21.5 µm long (av. 17.4 µm, $n = 83$) (second cell form the base 8–14 µm long (av. 10.9 µm, $n = 83$); third cell 4–9 µm long (av. 6.4 µm, $n = 83$)), pale brown; apical cell 3–5.5 µm long (av. 4.1 µm, $n = 83$), with a rounded apex, hyaline to pale brown; apical appendage 10–20 µm long (av. 14.6 µm, $n = 26$), inserted about 2–3 µm from the basal septum; basal appendage 11–22 µm long (av. 17.3, $n = 38$), inserted about 2–3 µm from the apical septum.

Culture characteristics — Colonies on PDA, 49–51 mm diam after 25 d at 20 °C in the dark, Dull Green (69) to Greenish Glaucous (90), with entire margin; reverse similar, no pigment produced. On RSA both ascomata and conidiomata, almost identical with those on the natural specimen, were produced, but the ascospores produced were slightly larger, 20.5–30 × 7.5–10 µm (av. 25.6 × 8.7 µm, $n = 30$).

　Specimen examined. Japan, Kagoshima, Yakushima Island, Okenoguchi, near Nakasegawa, 30°16'39"N, 130°37'09"E, on living leaves of _Symplocos prunifolia_, 21 Oct. 2005, _K. Tanaka & T. Hosoya_, KT 1907 (HHUF 29671 holotype, TNS-F-12443 isotype, monoconidial isolate MAFF 242774 = NBRC 104194T).

Notes — The new species, _A. yakushimense_ found on _Symplocos prunifolia_ (_Symplocaceae_), is morphologically and phylogenetically close to _A. tricellulare_ (= _Discostroma tricellulare_) on _Rhododendron_ spp. (_R. indicum_, _R. macrosepalum_, and _R. obtusum_; _Ericaceae_). _Adisciso yakushimense_, however, differs from the latter in having asci with a J- apical ring, slightly longer ascospores (23.8 µm vs 18.4 µm on average), and shorter conidia (26.9 µm vs 30.9 µm on average) with longer appendages (10–22 µm vs 4.5–11 µm). Sequences of these two species were highly similar in the LSU (793/795 = 99.7 %) and ITS (541/545 = 99.3 %) nrDNA regions, but BT sequences differed at 23 positions.
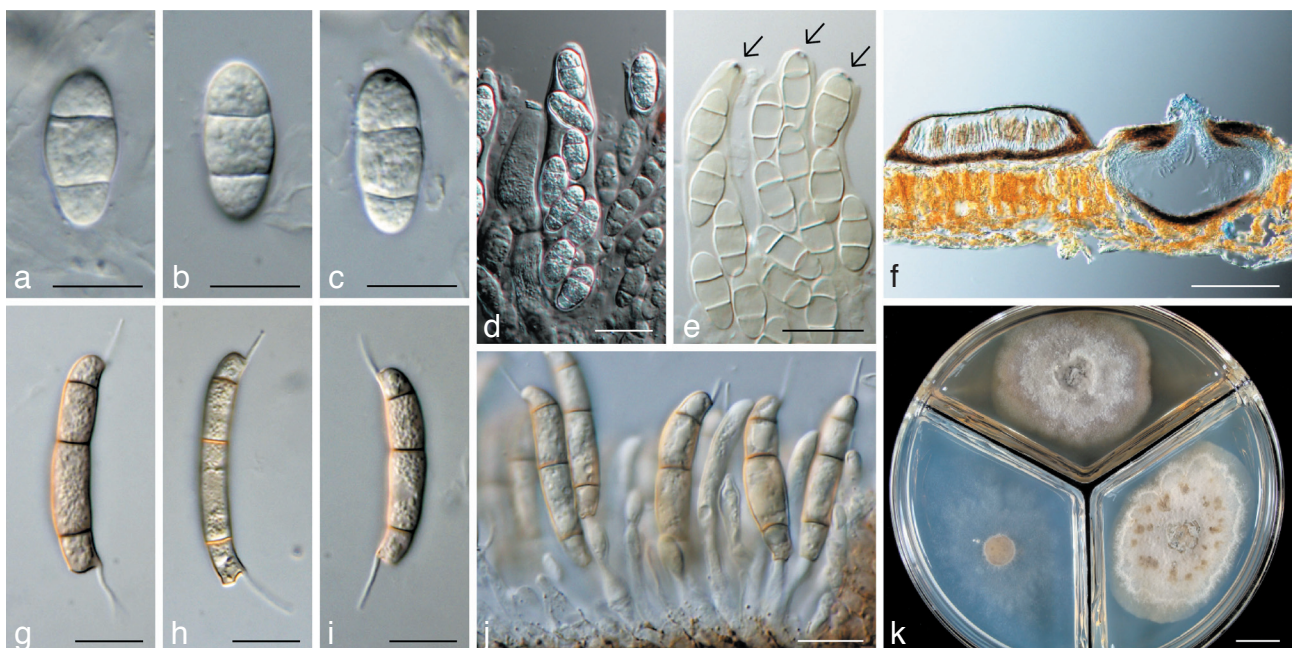

Fig. 5 _Adisciso tricellulare._ a–c. Ascospores; d. ascus; e. apex of asci with J+ rings (in Melzer's reagent); f. longitudinal section of conidioma (left) and ascoma (right); g–i. conidia; j. conidiogenous cells; k. colonies on PDA (upper), CMA (left), and MEA (right) after 30 d at 20 °C in the dark (a–j: NBRC H-12205 (holotype of _Discostroma tricellulare_); k: NBRC 32705). — Scale bars: a–c, g–j = 10 µm; d, e = 20 µm; f = 100 µm; k = 1 cm.

Adisciso tricellulare (Okane, Nakagiri & Tad. Ito) Kaz. Tanaka, Okane & Hosoya, *comb. nov.* — MycoBank MB519745; Fig. 5, 7g, h

Anamorph. Discosia sp. (*Seimatosporium azaleae* Okane, Nakagiri & Tad. Ito, Canad. J. Bot. 74: 1339. 1996).

Basionym. Discostroma tricellulare Okane, Nakagiri & Tad. Ito, Canad. J. Bot. 74: 1339. 1996.

Foliicolous. Ascomata immersed, globose. Asci 92–103 × 21.5–23 µm, L/W 4.1–4.7, with J+ subapical ring (0.5–1 µm high, 3–4 µm wide) in Melzer's reagent. *Ascospores* 17–20.5 × 7–8.5 µm (av. 18.4 × 7.8 µm, *n* = 20), with enlarged median cell (length 6–8 µm). *Conidiomata* subcuticular to intraepidermal in origin, applanate. *Conidia* 25.5–35.5 × 4–6 µm (av. 30.9 × 5.3 µm, *n* = 30) µm, L/W 4.9–7.4 (av. 5.9, *n* = 30), subcylindrical; basal cell 4.5–7.5 µm long (av. 5.7 µm, *n* = 30); 2 median cells together 18–22.5 µm long (av. 21.4 µm, *n* = 30) (second cell from the base 10.5–15 µm long (av.12.9 µm, *n* = 30); third cell 7–11 µm long (av. 8.6 µm, *n* = 30)); apical cell 3.5–5 µm long (av. 4.3 µm, *n* = 30); apical appendage 4.5–7.5 µm long (av. 5.9 µm, *n* = 30); basal appendage 5–11 µm long (av. 8.1 µm, *n* = 30).

Culture characteristics — Colonies on PDA, 25–28 mm diam after 25 d at 20 °C in the dark, Smoke Grey (105), with entire margin; reverse Grey Olivaceous (107) to Greenish Glaucous (90), no pigment produced.

Specimen examined. JAPAN, dried culture specimen on leaves of *Rhododendron indicum* (NBRC H-12205 (= IFO H-12205), holotype of *Discostroma tricellulare*) induced from single ascospore isolate NBRC 32705.

Notes — For further detailed description see Okane et al. (1996). Morphological features, such as small ascomata without stromatic tissue, clavate asci with biseriate ascospores, applanate conidiomata, and subcylindrical conidia with two subpolar appendages, indicate that this species should be transferred to *Adisciso*. All phylogenetic analyses in this study support this generic placement (Fig. 1–3).

Discostroma massarinum (Sacc.) Arx, Genera of Fungi Sporulating in Pure Culture, Ed 2: 131. 1974 — Fig. 6, 7i–k

Anamorph. Seimatosporium salicinum (Corda) Nag Raj, Coelomycetous Anamorphs with Appendage-bearing Conidia: 833. 1993 (= *S. ribis-alpini* (Fautrey) Shoemaker & E. Müll., Canad. J. Bot. 42: 403. 1964).

Basionym. Metasphaeria massarina Sacc., Atti Ist. Venet. Sci. Venetia 6, 2: 456. 1884.

Lignicolous. Ascomata 300–530 µm high, 320–600 µm diam, perithecial, single or 2–6 grouped under blackened clypeus, immersed to erumpent, globose to depressed globose, ostiolate. *Beak* 70–160 µm high, 100–130 µm diam, short cylindrical, with hyaline periphyses. *Ascomatal wall* 20–38 µm thick at sides, composed of 5–8 layers of thin-walled hyaline to pale brown polygonal cells (5–20 × 3.5–5 µm), with or without brown, dense hyphae at the side of ascomata; around the beak composed of thick-walled brown cells (2.5–8.5 × 2.5–5 µm). *Paraphyses* filiform, hyaline, 5 µm wide at the base, septate. *Asci* 138–193 × 10–13 µm (av. 167.3 × 11.0 µm, *n* = 30), L/W 13.5–17.0 (av. 15.2, *n* = 30), unitunicate, cylindrical, with subapical ring of J+ in Melzer's reagent, with 8 uniseriate ascospores. *Ascospores*
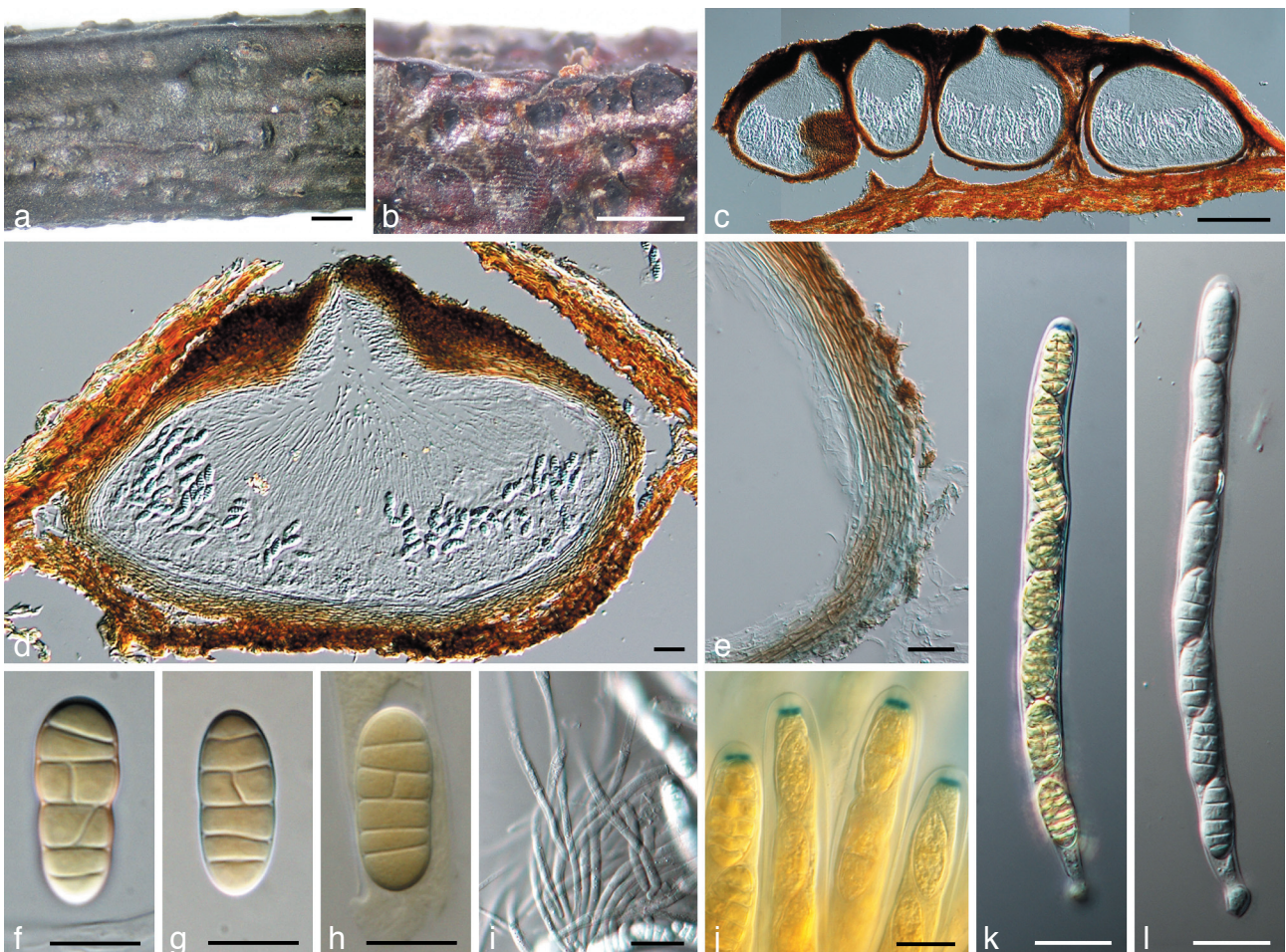

Fig. 6 *Discostroma massarinum*. a, b. Ascomata on host surface; c. grouped ascomata in longitudinal section; d. single ascoma in longitudinal section; e. ascomatal wall; f–h. ascospores (in Melzer's reagent); i. paraphyses; j. apex of asci with J+ rings (in Melzer's reagent); k, l. asci (k in Melzer's reagent) (a, f, g, i, l: ZT Petrak April 1934; b, d, e, j, k: ZT Brockmann 12 March 1974; c, h: ZT Müller 15 July 1956). — Scale bars: a, b = 1 mm; c = 200 µm; d = 50 µm; e, i, k, l = 20 µm; f–h, j = 10 µm.
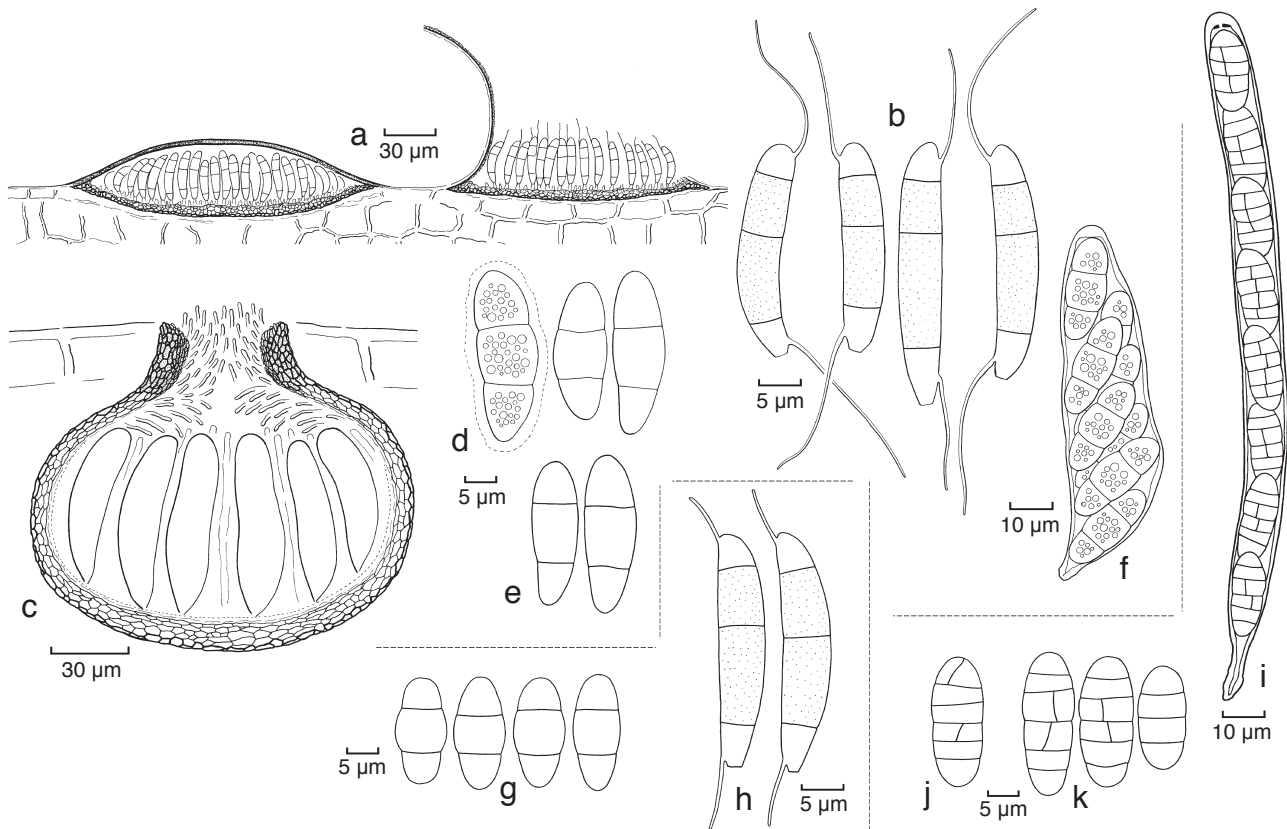
Fig. 7 Line drawings of pestalotioid fungi. a–f. *Adisciso yakushimense*. a. Conidioma in longitudinal section; b. conidia; c. ascoma in longitudinal section; d, e. ascospores; f. ascus. — g, h. *Adisciso tricellulare*. g. Ascospores; h. conidia. — i–k. *Discostroma massarinum*. i. Ascus; j, k. ascospores (a–c, e: HHUF 29671 holotype; d, f: culture MAFF 242774 = NBRC 104194T; g, h: IFO-H-12205 holotype of *Discostroma tricellulare*; i, k: ZT Petrak April 1934; j: ZT Brockmann 12 March 1974).

16–25 × 7.5–10 μm (av. 20.8 × 8.7 μm, n = 35), L/W 2.1–3.0 (av. 2.4, n = 35), ellipsoid, with 3–5(–7) transversal septa and 1 vertical septum, hyaline, smooth, without sheath.

Specimens examined. Czech Republic, Weisskirchen, Mähren, on twigs of *Ribes rubrum*, Apr. 1934, *F. Petrak* (ZT). – Switzerland, Chaclavouot, Val Tuors (Kanton Graubünden), on twigs of *R. petraeum*, 15 July 1955, *E. Müller* (ZT); Tramelan (Kanton Bern), on twigs of *R. rubrum*, 18 Aug. 1974, *I. Brockmann* (ZT); Zürich, Zollikon, on twigs of *R. rubrum*, 15 June 1986, *E. Müller* (ZT).

Notes — The holotype of *Metasphaeria massarina* deposited in NEU was not located. Therefore, for morphological comparison of *Discostroma* (type: *D. massarinum*) and *Adisciso*, we examined the above specimens; most were examined by Brockmann (1976) who also examined the type. The anamorph of this species has been reported as *Seimatosporium salicinum* (Shoemaker & Müller 1964, as *S. ribis-alpini*), but Nag Raj (1993) has questioned the existence of such a relationship.

Immersidiscosia Kaz. Tanaka, Okane & Hosoya, *gen. nov.* — MycoBank MB519746

Teleomorph. Unknown.

Foliicola. Conidiomata pycnidioidea, in sectione subglobosa vel lenticularia, immersa, origine intraepidermalia vel subepidermalia. Rostrum breve, cum periphysibus. Conidiophora cylindrica, ramosa. Cellulae conidiogenae cylindricae, holoblasticae, hyalinae. Conidia cylindrica, 3-septata, hyalina, utrinque appendiculata.

Type species. *Immersidiscosia eucalypti*.

Etymology. From the Latin *immersus,* meaning 'immersed' and generic name, *Discosia*.

Foliicolous. *Conidiomata* pycnidial, subglobose to sometimes lenticular in longitudinal section, immersed, intraepidermal to subepidermal in origin, scattered, unilocular, ostiolate. *Beak*

of conidiomata short, composed of thick-walled black small cells, with periphyses. *Conidiophores* cylindrical, branched. *Conidiogenous cells* cylindrical, holoblastic, hyaline, smooth. *Conidia* cylindrical, 3-septate, hyaline, with an appendage at both ends.

Immersidiscosia eucalypti (Pat.) Kaz. Tanaka, Okane & Hosoya, *comb. nov.* — MycoBank MB519747; Fig. 8

Basionym. *Cryptostictis eucalypti* Pat., Cat. Pl. Cell. Tunisie: 123. 1897.

≡ *Discosia eucalypti* (Pat.) Nag Raj, Coelomycetous Anamorphs with Appendage-bearing Conidia: 308. 1993.

Foliicolous. *Conidiomata* 200–370 μm high, 320–480 μm diam, pycnidial, subglobose to sometimes lenticular in section view, amphigenous, immersed (later erumpent at the ostiole), intraepidermal to subepidermal in origin, scattered, unilocular, ostiolate. *Beak* of conidiomata short, 50–115 μm long, 75–150 μm diam, composed of thick-walled, small black cells, with hyaline periphyses. *Wall* of conidiomata 15–25(–50) μm thick at the sides, composed of polygonal brown cells. *Conidiophores* up to 45 μm long, cylindrical, branched. *Conidiogenous cells* 5–20 μm long, 1.5–2 μm wide at the base, cylindrical, holoblastic, hyaline, smooth. *Conidia* 15–19.5 × 2.5–3 μm (av. 17.5 × 2.9 μm, n = 30), L/W 5.3–6.6 (av. 6.0, n = 30), cylindrical, 3-septate, hyaline, with an appendage at both ends; basal cell 2.5–3.1(–3.8) μm long (av. 2.9 μm, n = 30), obconic, truncate at the base; 2 median cells 10–13.8 μm long (av. 11.9 μm, n = 30), cylindrical (second cell from the base 5–7 μm long (av. 6.1 μm, n = 30), third cell 5–6.8 μm long (av. 5.8 μm, n = 30)); apical cell 2.5–3 μm long (av. 2.8 μm, n = 30). *Appendage* single, cellular, unbranched, filiform; apical appendage 8–15 μm long (av. 12.4 μm, n = 30); basal appendage 10–15 μm long (av. 12.1 μm, n = 30), eccentric.
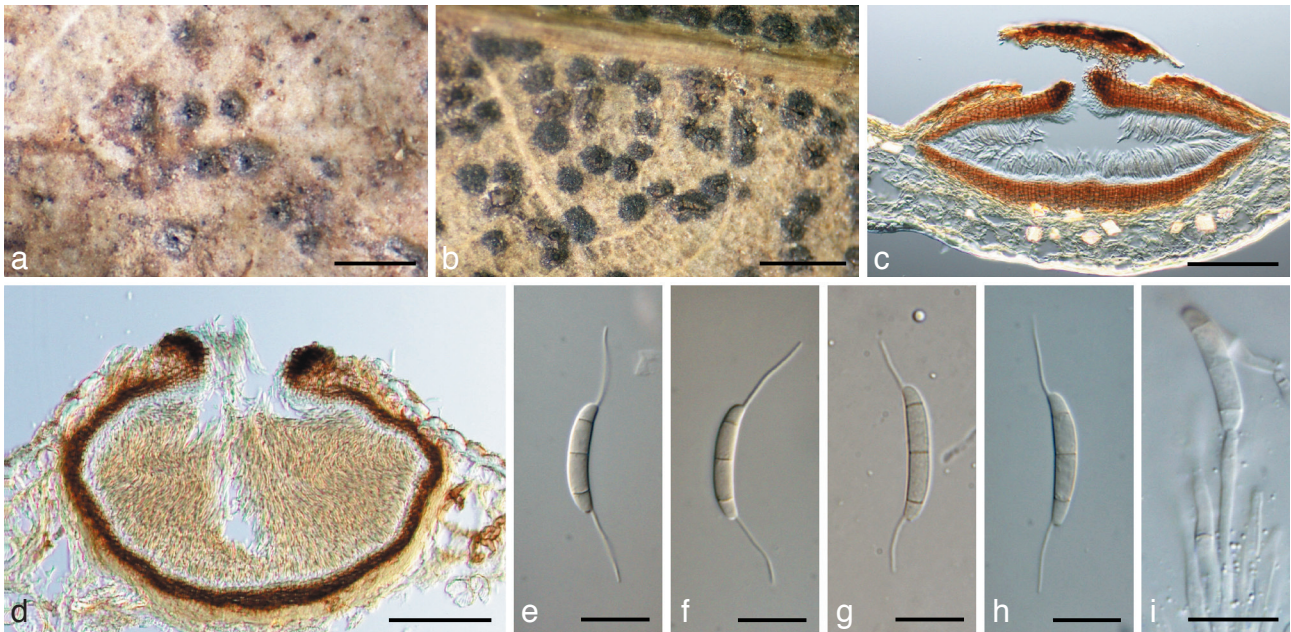
Fig. 8 *Immersidiscosia eucalypti*. a, b. Conidioma on host surface; c, d. longitudinal section of conidiomata; e–h. conidia; i. conidiogenous cells (a, d–f, i: FH holotype of *Cryptostictis eucalypti*; b: HHUF 29922; c, g: HHUF 29923; h: FH 1032). — Scale bars: a, b = 1 mm; c, d = 100 µm; e–i = 10 µm.

Specimens examined. FRANCE, Anduze (Gard), on dead leaves of *Laurus nobilis*, Oct. 1878, J. Therry (FH, Roumeguère Fungi Gallici exsiccati 1032, as *Discosia laurina* nob. in Herb, *D. artocreas* Fr. forma *laurina* Sacc. in litt.). – JAPAN, Aomori, Hirosaki, Nishigaoka, on dead leaves of *Quercus myrsinifolia*, 17 May 2006, *Y. Harada*, KT 2091 (HHUF 29920, monoconidial isolate NBRC 104195); 16 July 2006, *Y. Harada*, KT 2115 (HHUF 29922, monoconidial isolate NBRC 104196); on dead leaves of *Ardisia japonica*, 18 July 2006, *Y. Harada*, KT 2117 (HHUF 29923, monoconidial isolate NBRC 104197); Kagoshima, Yakushima Island, Nagata, on unknown dead leaves, 16 Mar. 2007, *K. Tanaka & H. Yonezawa*, KT 2191 (HHUF 29933, monoconidial isolate MAFF 242781). – TUNISIA, Souk-el-Arba, on dead leaves of *Eucalyptus* sp. 31 Oct. 1892 (FH, holotype of *Cryptostictis eucalypti*).

Notes — The main differences between *Immersidiscosia* and *Discosia* lie in the morphology of the conidiomata. The conidiomata of *I. eucalypti* are pycnidioid, deeply immersed (intraepidermal to subepidermal in origin), and have a developed beak (or opening) with hyaline periphyses, unlike representative species of *Discosia* as will be discussed later. The above description of *I. eucalypti* is based on the type. Compared with the mostly subglobose conidiomata found in the type (Fig. 8d), conidiomata from other materials (including those isolates used for phylogenetic analyses) were lenticular in longitudinal section (Fig. 8c). They are regarded as conspecific here, since all other details were identical.

DISCUSSION

Morphological delimitation of *Discosia* and *Seimatosporium*

Two coelomycetous genera, *Discosia* and *Seimatosporium*, have been distinguished on the basis of conidiomatal morphology, i.e., stromatic in *Discosia* and acervular to cupulate in *Seimatosporium* (Hawksworth et al. 1983), although these morphological features are variable or overlapping between the two genera, as illustrated by Nag Raj (1993). Sutton (1980) considered that they could be separated on the basis of conidial ontogeny (monoblastic in *Discosia* and annellidic in *Seimatosporium*), but their conidiogenous cells are now regarded as identical, and annellidic (Nag Raj 1993, Kirk et al. 2008). In the key to coelomycetous genera with appendage-bearing conidia, Nag Raj (1993) differentiated these genera based on conidial morphology. Namely, in *Seimatosporium*, two different types

of conidia could be found within a collection: i) conidia having apical and basal appendages and with central cells darker than end cells; and ii) conidia without either or without apical appendages and with a basal cell paler than the cells above. These different types of conidia were found to be produced according to differences in temperature (Hatakeyama & Harada 2004). *Discosia* has a single conidial type, that is almost hyaline to pale brown, and with polar/subpolar appendages inserted in the median part of the end cells (Nag Raj 1993). In addition to their morphological similarities, *Discosia* and *Seimatosporium* are phylogenetically related (Jeewon et al. 2002). Based on LSU and ITS analyses, Jeewon et al. (2002) revealed that *Discosia* is a sister taxon to the *Seimatosporium* group, with relationships supported by relatively high BS values (88–97 %). Although these authors suggested that *Discosia* could possibly be a synonym of *Seimatosporium*, they tentatively retained *Discosia* as a separate genus, because only one strain of *Discosia* was used in their analyses (Jeewon et al. 2002). Our analyses, based on LSU and ITS sequences, and using more than 20 taxa within each genus, confirmed that *Discosia* and *Seimatosporium* could be recognised as distinct genera, with the exception of *S. azaleae*, as this did not cluster with the *Seimatosporium* clade (Fig. 1, 2). It appears, therefore, that the minute morphological differences in conidiomata and conidia previously used for delineation of these genera may have taxonomic relevance. Our discovery of a teleomorph (*Adisciso*) for *Discosia* species could provide additional morphological evidence to discriminate *Discosia* from *Seimatosporium* (teleomorph: *Discostroma*).

Monophyly of *Discosia*

Our phylogenetic analyses of LSU and ITS sequences unexpectedly revealed that *Discosia* species are not monophyletic; they formed two separate lineages among pestalotioid fungi (Fig. 1, 2). All *Discosia* species used in our analyses, with the exception of *D. eucalypti*, clustered together in the *Discosia* I clade. On the other hand, *D. eucalypti* formed a distinct clade (*Discosia* II) supported by strong BS values (96–100 %, Fig. 1, 2). Unfortunately, an isolate from the type species of this genus (*D. strobilina*) was unavailable, and it is therefore difficult to judge from our results which clade is the type lineage of *Discosia*. Morphologically, however, we consider that the species

within the *Discosia* I clade may have phylogenetic affinity with the generic type. Species in *Discosia* I, as well as *D. strobilina*, have applanate conidiomata of subcuticular to intraepidermal origin (and appearing almost superficial), with an inconspicuous beak that lacks periphyses (Fig. 4o, p, 5f; in Subramanian & Chandra-Reddy 1974: fig. 1, in Nag Raj 1993: fig. 42-12). In contrast, *D. eucalypti* in *Discosia* II has deeply immersed (but later erumpent at the beak), pycnidioid conidiomata of intraepidermal to subepidermal origin, with a developed beak and periphyses (Fig. 8c, d). We propose, therefore, a new anamorphic genus, *Immersidiscosia*, for this member of the *Discosia* II clade (*D. eucalypti*), as a segregate from *Discosia* sensu Nag Raj (1993). To date, more than 100 names including intraspecific taxa have been recorded in *Discosia* according to the Index Fungorum (http://www.indexfungorum.org). Information on the conidiomatal anatomy of most species is lacking, since taxonomic revision of *Discosia* has been conducted based mainly on conidial morphology (Subramanian & Chandra-Reddy 1974, Chandra-Reddy 1984, Vanev 1991). Morphological re-examination of their conidiomatal features, as well as sequence analysis of LSU or ITS, will be necessary to validate the generic placement of species previously described as *Discosia*. According to the detailed description and illustration provided by Nag Raj (1994), *D. eucalypticola* might have affinity with *Immersidiscosia eucalypti*, because this species also has immersed, pycnidioid conidiomata with a conspicuous papillate beak.

In their taxonomic reassessment of *Discosia* species, Subramanian & Chandra-Reddy (1974) divided the genus into four sections on the basis of conidial characters. Similarly, Vanev (1991) expanded this concept and proposed six sections within the genus based on conidial morphology, viz., section I *Discosia*, having 3-septate conidia with the second cell (C_2) longer than the third cell (C_3) from the base, and with appendages originating from end cells; section II *Laurina*, having 3-septate conidia with two median cells of equal length ($C_2 = C_3$), and with appendages originating from end cells; section III *Clypeata*, having 3-septate conidia with C_2 shorter than C_3, and with appendages originating from end cells; section IV *Libertina*, having 3-septate conidia with two median cells of equal length ($C_2 = C_3$), and with appendages originating from the median part of the end cells; section V *Strobilina*, having 3-septate conidia with C_2 longer than C_3, and with appendages originating from the median part of the end cells; and section VI *Poikilomera*, having 4-septate conidia. Species in three of these sections, *Discosia*, *Laurina*, and *Strobilina*, were included in our phylogenetic analyses (Fig. 2, 3). Species in section II (*Laurina*) were distributed among the two *Discosia* clades as *Discosia* sp. 1 and *I. eucalypti* (Fig. 2), and species in sections I (*Discosia*) and V (*Strobilina*) did not constitute a natural grouping (Fig. 2, 3). Our results indicate that the subdivision proposed by Vanev (1991) is not phylogenetically significant, at least in the three sections examined.

Monophyly of Seimatosporium

Considerable controversy surrounds the generic concept of *Seimatosporium* as reviewed by Nag Raj (1993: 51–56). Sutton (1980) recognised the heterogeneity of this genus, but he provisionally provided a broad generic concept for *Seimatosporium*. In contrast, Nag Raj (1993) rearranged species in *Seimatosporium* s.l. into five related genera based on conidial morphology, including conidial shape, pigmentation of median cells, thickness of septa, and presence or absence of appendages. We adhered to *Seimatosporium* s.l. for our examined taxa, although species in all five segregate genera accepted by Nag Raj (1993) were included in our phylogenetic analyses (Fig. 1, 2). These are *Seimatosporium* s.str. (e.g., *Se. botan*),

Sporocadus (e.g., *Se. lichenicola*, type of the genus), *Sarcostroma* (e.g., *Se. foliicola*, type of the genus), *Diploceras* (e.g., *Se. hypericinum*, type of the genus), and *Vermisporium* (e.g., *Se. leptospermi*). With exception of *Se. azaleae*, which nested within *Discosia* I clade and is consequently transferred to *Adisciso*, all species in *Seimatosporium* and its teleomorph *Discostroma* clustered together as a monophyletic group in LSU (97–98 % BS; Fig. 1) and ITS (92 % BS in NJ but < 50 % BS in MP; Fig. 2) analyses. These results clearly indicate that species within *Seimatosporium* s.l. (Sutton 1980) have a close evolutionary relationship and constitute a single natural lineage, but it would be premature to reject the concepts of *Seimatosporium* s.str. and the other four genera circumscribed by Nag Raj (1993). The presence of several subgroups among *Seimatosporium* s.l., with relatively high BS support (Fig. 2), such as the clade including *Se. kriegerianum* to *Se. elegans* (80–96 % BS; *Diploceras* sensu Nag Raj 1993), or the clade including *Se. mariae* to *Se. hakeae* (89–94 % BS; *Sarcostroma* sensu Nag Raj 1993), may suggest that more well-defined, natural genera exist within the broadly-defined *Seimatosporium*, although these two groups deviate from their generic types, *Se. hypericinum* (*Diploceras*) and *Se. foliicola* (*Sarcostroma*). A similar suggestion on the subdivision of *Seimatosporium* is noted by Lee et al. (2006) based on molecular research. The morphological diversity of the teleomorphs reported as *Discostroma* should be considered grounds for subdivision of *Seimatosporium* s.l. Swart (1979) established *Discostromopsis* (type: *D. callistemonis* = anamorph: *Se. kriegerianum*) as the teleomorphic genus for *Se. kriegerianum*, *Se. dilophosporum*, *Se. elegans* (all within *Diploceras*), and *Se. leptospermi* (*Vermisporium*), on account of its ascus with a J- apical ring (vs J+ in *Discostroma*) and irregularly biseriate ascospores (uniseriate in *Discostroma*). *Discostromopsis* is now regarded as a synonym of *Discostroma* (Sivanesan 1983, Sivanesan & Shivas 2002, Paulus et al. 2006), but further consideration of the holomorph may provide reliable criteria for accurate taxonomic decisions regarding *Seimatosporium* s.l. In addition, more taxon sampling, particularly of the type of *Seimatosporium* (*Se. rosae* = teleomorph: *Discostroma rosae*) as well as the type of *Discostroma* (*D. massarinum* = anamorph: *Se. salicinum*), unfortunately not available for our analyses, will be required for reassessment of the *Seimatosporium* phylogeny.

Morphological differences between Adisciso and Discostroma

Despite there being over 100 *Discosia* taxa described, there has been no confirmed information regarding a possible teleomorph connection. A *Paradidymella* state (*P. tosta*) was noted as a teleomorph of *Discosia passerinii* (Müller & Corbaz 1956), but this species was later transferred to *Discostroma* (anamorph: *Seimatosporium*) on the basis of ascomata with well-developed clypeus and cylindrical asci with uniseriate ascospores (Brockmann 1976). We believe, therefore, that a new genus, *Adisciso*, is appropriate to accommodate *A. yakushimense* and *A. tricellulare* with *Discosia* anamorphs that may eventually be revealed to accommodate *Discosia* s.str.

Among the genera belonging to the *Amphisphaeriaceae* (Kang et al. 1999), *Adisciso* is similar to *Ellurema* in having subglobose, immersed, relatively small ascomata with a thinner peridial wall, and subclavate to clavate asci with biseriate ascospores. However, the monotypic genus *Ellurema*, based on *E. indica*, has hyaline to brown ascospores with verruculose ornamentation, and produces the *Hyalotiopsis* conidial state in culture (Punithalingam 1969, Nag Raj & Kendrick 1985). In addition, *Ellurema* is found to be phylogenetically related to *Lepteutypa* (*Seiridium* anamorph), based on ITS analysis (Kang et al. 1998). Another amphisphaeriaceous genus that might be

confused with *Adisciso* is *Paracainiella* with a 'Pestalotia-like' anamorph, but the latter genus is quite different as it has cylindrical to oblong asci and pigmented ascospores with a median septum (Vassilieva 1983).

In terms of overall morphology, the most similar genus to *Adisciso* is *Discostroma* typified by *D. massarinum* (von Arx 1974). *Discostroma* is circumscribed by a somewhat broad generic concept and currently includes 28 species (Paulus et al. 2006). We thus examined several reliable specimens of *D. massarinum* on twigs of *Ribes* spp., to clarify morphological differences between *Discostroma* and *Adisciso*. Our observations clearly revealed that these two taxa are not congeneric. *Adisciso* is foliicolous and has relatively small sized (150–210 μm diam), single ascomata without stromatic tissue or dense peripheral hyphae, obclavate to broadly cylindrical asci (L/W 3.3–4.7), and biseriate ascospores with 2 transverse septa (Fig. 4d–n). In contrast, the type species of *Discostroma* is lignicolous and has large sized (300–600 μm diam), 1–6 grouped ascomata under a black prominent clypeus, with or without stromatic tissue, and slender cylindrical asci (L/W 13.5–17.0) with uniseriate muriform ascospores (Fig. 6c–l). These characteristic features of *Discostroma* have been illustrated in detail by Berlese (1900: t. CXLII-2), Müller & Loeffler (1957: f. 1, 2), and Brockmann (1976: f. 5, 6). In addition to the morphological differences between holomorphs, evidence from molecular analyses supports the establishment of the new genus, *Adisciso* (Fig. 1, 2).

Two species of *Adisciso*, *A. yakushimense* and *A. tricellulare*, having ascal apices that are J- and J+ respectively, constituted a monophyletic clade. This indicates that the staining reaction of the ascal apex in Melzer's reagent is not an important criterion for the delimitation of genera within the *Amphisphaeriaceae*. This character, however, has often been used for generic circumscription within this family (Shoemaker 1963, Müller & von Arx 1973, Kang et al. 1999, Ono & Kobayashi 2003).

Future studies

Discosia species have been known as endophytes (Okane et al. 1998) or saprobes on various vascular plants (Subramanian & Chandra-Reddy 1974), but there are also parasitic members of this genus, forming leaf spots such as *A. yakushimense* (Vanev 1992c, Wołczańska et al. 2004). Since establishment of the genus *Discosia* by Libert in 1837 (lectotype: *D. strobilina*; Subramanian & Chandra-Reddy 1974, Vanev 1992b), more than 100 taxa have been described within it (http://www.indexfungorum.org) and taxonomic reassessments of many of these species have been carried out by several authors (e.g., Subramanian & Chandra-Reddy 1974, Chandra-Reddy 1984, Vanev 1991, Nag Raj 1993). Nevertheless, identification at species level is still difficult within this genus. Among the 25 strains of *Discosia* that we examined, most could not be fully identified to species level, and we thus tentatively labelled them 'aff. + epithet' or 'sp.' The conidial morphologies of *Discosia* sp. 3 and sp. 4 were extremely similar to those of *D.* aff. *brasiliensis* and *D.* aff. *pleurochaeta*, but they could be clearly separated as distinct lineages in our BT phylogeny (Fig. 3). This indicates that minute morphological differences of conidia, e.g., dimension of conidial body and length of appendages, may correlate with phylogenetic relations of these taxa as inferred from molecular data, but in practise have little meaning for reliable morphological identification. Vanev (1991) noted that among more than 2 500 specimens he examined almost 1/3 of the specimens were deposited as *D. artocreas* in herbaria. Certainly, this species is a well-known cosmopolitan saprobe occurring on dead leaves of more than 99 plant species within 67 genera (Farr & Rossman 2011), but the identity of this taxon remains doubtful. Most probably, *Discosia* encompasses more diverse species; hence more a precise taxonomic revision based on type specimens and reevaluation of morphological characters based on molecular data will be required.

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