

Molecular phylogeny of two coelomycetous fungal genera with stellate conidia, *Prosthemium* and *Asterosporium*, on Fagales trees

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Abstract: *Prosthemium* (teleomorph *Pleomassaria*) and *Asterosporium* (teleomorph unknown) are coelomycetous genera with stellate conidia on Fagales trees. Their morphological resemblance suggests their close relationship, but phylogenetic relatedness remains unknown. They have been distinguished on the basis of either conidiomatal morphology (pycnidia in *Prosthemium* and acervuli in *Asterosporium*) or their differing conidial septation (euseptate in *Prosthemium* and distoseptate in *Asterosporium*). To reveal their phylogenetic affinities and clarify reliable distinguishing phenotypical characters, five species of *Prosthemium* and two species of *Asterosporium* were investigated using sequences of the small subunit, large subunit, and internal transcribed spacer region of nuclear ribosomal DNA and β -tubulin gene from 43 isolates of these species. The analyses revealed the following: (i) *Asterosporium* typified by *Asterosporium asterospermum* on *Fagus* is a member of the Sordariomycetes and is distinct from *Prosthemium* belonging to the Dothideomycetes; (ii) *Asterosporium betulinum* on *Betula* species should be excluded from *Asterosporium* and transferred to *Prosthemium* as *Prosthemium neobetulinum* nom. nov.; (iii) conidial septation does not seem to have a diagnostic value, whereas conidiomatal morphology is useful in distinguishing both genera; (iv) the number and length of conidial arms are useful criteria in distinguishing *Prosthemium* species. A new species, *Prosthemium intermedium* sp. nov., found on both *Betula ernanii* and *Betula maximowicziana*, is described and illustrated.

Key words: anamorphic fungi, Ascomycetes, Diaporthales, *Pleomassaria*, Pelosporales.

Résumé : Les *Prosthemium* (téléomorphe *Pleomassaria*) et *Asterosporium* (téléomorphe inconnu) constituent des genres de Coelomycètes possédant des conidies étoilées et venant sur des arbres appartenant aux Fagales. Leur ressemblance morphologique suggère une étroite relation, mais le lien phylogénétique demeure inconnu. On les a distingués soit sur la base de la morphologie des conidiomata (pycnidies chez les *Prosthemium* et acervules chez les *Asterosporium*), ou sur celle des différences de leurs septations (euseptée chez les *Prosthemium* et distoseptée chez les *Asterosporium*). Afin de révéler leurs affinités phylogénétiques et d'établir des caractères phénotypiques distinctifs, les auteurs ont examiné cinq espèces de *Prosthemium* et deux espèces d'*Asterosporium*, en utilisant les séquences petite sous-unité, grande sous-unité, l'espaceur interne transcrit du l'ADN ribosomal, et le gène de la β -tubuline, à partir de 43 isolats de ces espèces. Les analyses révèlent ce qui suit ; (i) l'*Asterosporium* typifié par l'*Asterosporium asterospermum* sur *Fagus* appartient aux Sordariomycètes et diffère des *Prosthemium* appartenant aux Dothidéomycètes ; (ii) l'*Asterosporium betulinum* sur *Betula* spp. doit être exclu des *Asterosporium* et transféré au genre *Prosthemium* comme *Prosthemium neobetulinum* nom. nov. ; (iii) la septation conidiale ne semble pas avoir de valeurs diagnostiques, alors que la morphologie conidiomatale demeure utile pour distinguer les deux genres ; (iv) le nombre et la longueur des bras conidiens se révèlent comme des caractères utiles pour distinguer les espèces de *Prosthemium*. Les auteurs décrivent et illustrent une nouvelle espèce, le *Prosthemium intermedium* sp. nov., venant sur les *Betula ernanii* et *Betula maximowicziana*.

Mots-clés : champignons anamorphes, Ascomycètes, Diaporthales, *Pleomassaria*, Pélosporales.

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Introduction

During our recent studies of coelomycetous fungi in Japan and Russia (Endo et al. 2008; Hatakeyama et al. 2008; Sato et al. 2008; Shabunin et al. 2008; Yonezawa and Tanaka

2008; Kamiyama et al. 2009), a number of anamorphic species with star-shaped conidia were encountered on twigs of Fagales trees such as *Alnus*, *Betula*, and *Fagus*. Morphological studies of these fungi suggested that they are members of either *Prosthemium* or *Asterosporium*. These genera share

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several morphological and ecological features, but their phylogenetic relatedness is unknown. *Prosthemium* species have *Pleomassaria* teleomorphs belonging to the Pleosporales (Dothideomycetes) (Sivanesan 1984; Hantula et al. 1998; Paavolainen et al. 2000; Tanaka et al. 2005), and their familial affiliations have been discussed by several authors (Liew et al. 2000; Lumbsch et al. 2000; Lumbsch and Lindemuth 2001; Schoch et al. 2006). In contrast, no teleomorph information or DNA sequence data exists to suggest the phylogenetic placement of *Asterosporium* species.

Traditionally, these two genera have been differentiated by their conidiomatal morphology, with pycnidia in *Prosthemium* and acervuli in *Asterosporium* (Saccardo 1884; Morgan-Jones and Kendrick 1972). Sutton (1980), however, regarded the conidiomata of *Prosthemium* as acervuli or eustromata and preferred to use conidial septation as the distinguishing feature between the two genera, where *Asterosporium* is distoseptate and *Prosthemium* is euseptate. These discrepancies in morphological circumscription were probably sources of taxonomic confusion. In fact, one species of *Asterosporium*, *Asterosporium orientale*, originally described from twigs of *Betula ermanii* in Russia (Mel'nik 1988), has been recently transferred to *Prosthemium* on the basis of its similarities to the type species of *Prosthemium* (*Prosthemium betulinum*) rather than the type of *Asterosporium*, *Asterosporium asterospermum* (Kamiyama et al. 2009).

Prosthemium species have been mainly reported as endophytes or phelloglyphs from twigs of *Betula* and *Alnus* in Betulaceae (Kowalski and Kehr 1992, 1996). Although seven taxa have been previously recognized in the genus (Saccardo 1884, 1895, 1899, 1906; Kowalski and Holdenrieder 1996; Tanaka et al. 2005; Kamiyama et al. 2009), the boundaries between these species are not necessarily clearly defined. For example, *Prosthemium stellare* has conidia with 10–14 radiating arms equally developed (Sivanesan 1984), but *Prosthemium orientale*, characterized typically by 4-armed conidia, also rarely produces up to 10-armed conidia (Kamiyama et al. 2009). *Prosthemium canba* was introduced as a distinct species primarily based on its largest conidial arm (Tanaka et al. 2005), but later some reduced conidia resembling those of *P. betulinum* were observed in culture (K. Tanaka, personal observation). Furthermore, an unnamed *Prosthemium* sp. showing conidial morphology intermediate between *P. orientale* and *P. canba*, was also found in our recent survey. To evaluate the monophyly of *Prosthemium* taxa that possess a continuum of morphological characters, a molecular phylogenetic analysis was much needed.

This study was undertaken with three objectives: (i) to infer the taxonomic placement of *Asterosporium* species and to clarify their phylogenetic affinities to *Prosthemium* species using molecular data from the small and large subunit nuclear ribosomal DNA (SSU and LSU nrDNA, respectively); (ii) to determine reliable morphological characters as taxonomic criteria for the separation of these genera; and (iii) to evaluate the species validity of each *Prosthemium* taxon, including an unidentified *Prosthemium* sp., based on sequence analyses of the internal transcribed spacer 5.8S nrDNA (ITS) and the β -tubulin gene (*BT*).

Materials and methods

Morphological studies and fungal isolates

Collections of *Prosthemium* and *Asterosporium* were made from woody plants such as *Alnus*, *Betula*, and *Fagus*, primarily in Japan and Russia. Voucher specimens were deposited in the herbaria of Hirosaki University (HHUF) and Komarov Botanical Institute (LE) (Table 1). Methods of morphological observation used are described by Tanaka et al. (2009). Single-spore cultures were obtained according to the methods of Shearer et al. (2004). To validate isolations, the induction of conidiomatal formation was encouraged by placing a small piece of mycelial culture on rice straw agar (Tanaka and Harada 2003). Fungal cultures newly obtained in this study were deposited at the Japan Collection of Microorganisms (JCM); the Ministry of Agriculture, Forestry, and Fisheries, Japan (MAFF); and the Centraalbureau voor Schimmelcultures (CBS).

DNA extraction and amplification

A total of 43 isolates, including six strains obtained from CBS and one herbarium specimen, were used for DNA extraction (Table 1). DNA from mycelia was extracted using the ISOPLANT Kit (Nippon Gene Co., Tokyo, Japan) according to the manufacturer's instructions. Partial SSU (17 isolates, ca. 1000–1300 bp of the 5' end) and LSU nrDNA (43 isolates, ca. 1250 bp of the 5' end) were determined to elucidate familial and generic positioning. The complete ITS region of nrDNA (ca. 500 bp), and exons 1–6 and the respective introns of the *BT* gene (ca. 600 bp) were sequenced (37 isolates) to confirm generic and species-level placements (Table 1). Four primer sets, NS1–NS4 (White et al. 1990), LR0R–LR7 (Rehner and Samuels 1994), ITS1–ITS4 (White et al. 1990), and T1–BT2B (Glass and Donaldson 1995; O'Donnell and Cigel'nik 1997), 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).

Sequence analysis

SSU and LSU sequences of *Prosthemium* and *Asterosporium* were aligned along with those of other related species obtained from GenBank. To clarify their validity at the species level, the alignments of ITS and *BT* sequences were also generated. A combined data set of ITS + *BT* was used for the analysis, because the phylogenetic resolution from each ITS and *BT* data set was relatively low. Preliminary multiple alignments of sequences were generated using MAFFT version 6 (Katoh and Toh 2008; mafft.cbrc.jp/alignment/software). Final alignments were manually adjusted using BioEdit version 7.08 (Hall 1999). Alignment gaps and ambiguous positions were excluded from the analysis. All alignments used in this study were deposited in TreeBASE (www.treebase.org). Maximum parsimony (MP) analyses were carried out using PAUP version 4.0b10 (Swofford 2003). MP analyses with the heuristic search option using the tree bisection reconstruction (TBR) algorithm with 1000 random sequence additions were performed to find the global optimum tree. All sites were treated as unordered and unweighted. Neighbor-joining (NJ) analyses based

on the Kimura two-parameter substitution model were carried out using MEGA version 4 (Tamura et al. 2007). Characters were weighted equally. Bootstrap support (BS) values for nodes were computed from 1000 replicates for both the MP and NJ analyses. Bayesian analyses were done using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). MrModeltest version 2.3 (Nylander 2004), in conjunction with PAUP version 4.0b10 (Swofford 2003), was used to select substitution models for Bayesian analyses. On the basis of the Akaike information criterion of MrModeltest version 2.3, a general time-reversible, invariant, γ -distributed (GTR+I+G) model was applied to the LSU data sets of *Prosthemium* and *Asterosporium*. In the ITS + *BT* data sets of *Prosthemium* species, the symmetrical invariant (SYM+I) and Hasewaga–Kishino–Yano invariant (HKY+I) models were applied to ITS and *BT* sequences, respectively. Two runs with 10 chains of Markov chain Monte Carlo (MCMC) iterations were performed for 4.5 million generations of the LSU data set of *Prosthemium*, 1.3 million generations of the LSU data set of *Asterosporium*, and 3 million generations of the ITS + *BT* data sets of *Prosthemium* species, keeping one tree every 100 generations. The runs were deemed to have converged if the mean standard deviation of split frequencies became less than 0.01. The first 3.5, 0.3, and 2 million generations of the LSU of *Prosthemium*, the LSU of *Asterosporium* and ITS + *BT* of *Prosthemium* species 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

Phylogenetic analyses

SSU phylogeny

An SSU alignment consisting of 18 sequences of *Prosthemium*/*Asterosporium* and 40 sequences retrieved from GenBank, after excluding insertions in *Lecanora hybocarpa* (DQ782883; 257–361) and *Geoglossum nigritum* (AY544694; 485–877), resulted in a 953 character data set with 325 (34.1%) variable sites. The NJ tree generated from this alignment showed that all *Prosthemium* species and one species of *Asterosporium*, *Asterosporium betulinum* (not *P. betulinum*), constituted a monophyletic clade belonging to the Pleosporales (Dothideomycetes). On the other hand, five strains of *A. asterospermum*, the type species of *Asterosporium*, grouped within the Diaporthales clade (Sordariomycetes) with 99% BS (data not shown but found in TreeBASE).

LSU phylogeny

The LSU sequences from *Prosthemium* species as well as two isolates of *A. betulinum* (suggested as a member of *Prosthemium* by SSU analysis) were aligned with representative members of the Pleosporales obtained from GenBank. *Lophium mytilinum* (DQ 678081; Mytiliniales) and *Rhytidhysterion rufulum* (GU397354; Hysteriales) were selected as the outgroup (Schoch et al. 2009). The final LSU alignment of 73 taxa resulted in a 1220 character data set, of which 282 characters (23.1%) were variable and 210 characters (17.2%) were parsimony informative. The 50% majority rule tree generated from 20 002 Bayesian trees is shown in

Fig. 1. All *Prosthemium* species, including *Pleomassaria siparia* (the teleomorph of *P. betulinum*) from GenBank, and two isolates of *A. betulinum*, formed a robust clade with high statistical support (1.00 PP, 96%–99% BS). These nested within the Melanommataceae along with an unidentified Pleosporales sp. (HC27033) (Fig. 1).

To clarify their familial placement in the Diaporthales, an LSU data set consisting of five isolates of *A. asterospermum* and 34 related taxa from GenBank was generated. The data set comprised 1086 aligned characters, including 210 variable positions (19.3%) and 185 parsimony-informative positions (17.0%). *Magnaporthe grisea* (AB026819) and *Gaeumannomyces graminis* (AF362556) in the Magnaporthaceae were used as the outgroup (Gryzenhout et al. 2006). The 50% majority rule tree generated from 20 002 Bayesian trees is shown in Fig. 2. Five isolates of *A. asterospermum* formed a strongly supported monophyletic group (0.99 PP and 100% BS) and grouped within the Diaporthales. The clade of *A. asterospermum* was sister to the main diaporthalean families, except for the Togniniaceae, and this relationship received strong support (1.00 PP, 98%–99% BS) (Fig. 2).

ITS + BT phylogeny

To clarify the species validity and boundaries of *Prosthemium* species, a combined alignment of ITS + *BT* from 37 taxa of *Prosthemium* species and *A. betulinum* was generated. A Pleosporales sp. (HC27033), closely related to *Prosthemium* (Fig. 1), was used for the outgroup. Out of 1014 characters, 177 (17.5%) and 83 (8.2%) were variable and parsimony-informative, respectively. An MP analysis resulted in three equally parsimonious trees with a length of 212 steps (consistency index = 0.9245, retention index = 0.9497) (Fig. 3). The trees obtained from NJ and Bayesian analyses had a topology identical to that of the MP tree. All species formed distinct monophyletic lineages with strong or moderate statistical support (>0.95 PP and 81%–100% BS) in all analyses, with the exception of the *Prosthemium* sp.; this was without PP support (<0.95). *Prosthemium stellare*, a parasite on *Alnus*, was sister to the clade consisting of species mainly occurring on *Betula*. *Asterosporium betulinum* was in the basal lineage of a large group containing *P. canba*, *P. orientale*, *P. betulinum*, and the *Prosthemium* sp. (Fig. 3).

Taxonomy

Conidiomata of *Prosthemium* and *Asterosporium* were sectioned with a freezing microtome and their morphology compared (Figs. 4–9). As expected, all conidiomata of *Prosthemium* species were globose to subglobose pycnidia with a circular ostiole (Figs. 6–9). The type species of *Asterosporium* (*A. asterospermum*) had typical flattened acervuli with a wide opening (Fig. 4), as was noted in several previous reports (Morgan-Jones and Kendrick 1972; Sutton 1980; Kobayashi and Kubono 1986; Prášil and Réblová 1995). Conidiomata of *A. betulinum* were pycnidial with a wide ostiole of more than 100 μ m diameter (Fig. 5). Conidial septation was considered euseptate in all species (Figs. 10–15). A distinct central cell connecting each conidial arm was found in all *Prosthemium* species as well as in *A. betulinum*, whereas the central cell of *A. asterospermum* was indistinct.

Table 1. Isolate data and GenBank accession Nos. of *Asterosporium* and *Prosthemia*.

Isolate No.	Culture collection No.	Herbarium specimen	Host ^d	Locality	GenBank acc. No. ^b				Notes ^c
					SSU	LSU	ITS	BT	
<i>Asterosporium asterospermum</i>									
KT 2095	JCM 16962	HHUF 30036	<i>F. crenata</i>	Japan	AB553634	AB553741	—	—	C
KT 2101	JCM 16963	HHUF 30037	<i>F. crenata</i>	Japan	AB553635	AB553742	—	—	C
KT 2125	MAFF 242300	HHUF 30038	<i>F. crenata</i>	Japan	AB553636	AB553743	—	—	C
KT 2138	MAFF 242299	HHUF 30039	<i>F. crenata</i>	Japan	AB553637	AB553744	—	—	C
—	CBS 112404	—	<i>F. sylvatica</i>	Italy	AB553638	AB553745	—	—	C
<i>Asterosporium betulinum</i> (= <i>Prosthemia neobetulinum</i>)									
VM 20081002	CBS 126960	LE230931 = HHUF 30040	<i>B. davurica</i>	Russia	AB553639	AB553746	AB554077	AB554114	C
—	CBS 121.51	—	<i>B. alleghaniensis</i>	Canada	AB553640	AB553747	AB554078	AB554115	C
<i>Prosthemia betulinum</i>									
VM 20040721	CBS 127468	LE 226142	<i>P. rhoifolia</i>	Russia	AB553644	AB553754	AB554085	AB554122	C
VM 20061225	CBS 126961	LE 255828	<i>P. rhoifolia</i>	Russia	—	AB553755	AB554086	AB554123	C
—	—	LE 212484	<i>B. raddeana</i>	Russia	AB553645	AB553756	—	—	—
VM 20070116R	CBS 126962	HHUF 29954	<i>B. raddeana</i>	Russia	—	AB553757	AB554087	AB554124	A
VM 20070116P	CBS 126963	HHUF 29953	<i>B. papyrifera</i>	Russia	—	AB553758	AB554088	AB554125	A
—	CBS 279.74	CBS H-258	<i>B. pendula</i>	Netherlands	(DQ678027)	AB553759	AB554089	AB554126	A
<i>Prosthemia canba</i>									
KT 2083-1	JCM 16966	HHUF 29948	<i>B. ermanii</i>	Japan	AB553646	AB553760	AB554090	AB554127	C
KT 2149-1	JCM 16967	HHUF 30046	<i>Betula</i> sp.	Japan	—	AB553761	AB554091	AB554128	C
KT 2157-1	JCM 16968	HHUF 30047	<i>B. ermanii</i>	Japan	—	AB553762	AB554092	AB554129	A
KT 2224	JCM 16969	HHUF 30048	<i>B. ermanii</i>	Japan	—	AB553763	AB554093	AB554130	A
KT 2229A	JCM 16970	HHUF 30049	<i>B. ermanii</i>	Japan	—	AB553764	AB554094	AB554131	A
KT 2229C	JCM 16971	HHUF 30050	<i>B. ermanii</i>	Japan	—	AB553765	AB554095	AB554132	C
KT 2230	MAFF 242297	HHUF 30051	<i>B. ermanii</i>	Japan	—	AB553766	AB554096	AB554133	C
KT 2231	MAFF 242296	HHUF 30052	<i>B. ermanii</i>	Japan	—	AB553767	AB554097	AB554134	C
MK 14	MAFF 242295	HHUF 30053	<i>B. ermanii</i>	Japan	—	AB553768	AB554098	AB554135	C
MK 28	MAFF 242294	HHUF 30054	<i>B. ermanii</i>	Japan	—	AB553769	AB554099	AB554136	C
MK 30	MAFF 242293	HHUF 30055	<i>B. ermanii</i>	Japan	—	AB553770	AB554100	AB554137	C
<i>Prosthemia orientale</i>									
KT 1669	JCM 12841 = MAFF 239509	HHUF 28524	<i>B. pendula</i>	Japan	AB553641	AB553748	AB554079	AB554116	A
KT 2088-1	JCM 16964	HHUF 29946	<i>B. platyphylla</i> var. <i>japonica</i>	Japan	—	AB553749	AB554080	AB554117	C
KT 2093-1	JCM 16965	HHUF 30041	<i>B. pendula</i>	Japan	AB553642	AB553750	AB554081	AB554118	C
KT 2103	MAFF 242298	HHUF 30042	<i>B. platyphylla</i> var. <i>japonica</i>	Japan	AB553643	AB553751	AB554082	AB554119	C
—	CBS 431.96	CBS H-7671	<i>B. pendula</i>	Poland	—	AB553752	AB554083	AB554120	C
—	CBS 114278	—	<i>S. caprea</i>	Sweden	—	AB553753	AB554084	AB554121	C
<i>Prosthemia stellare</i>									
VM 20050611	CBS 126964	HHUF 29951	<i>A. glutinosa</i>	Lithuania	AB553650	AB553781	AB554111	AB554148	A
—	CBS 558.70	CBS H-17355	<i>A. glutinosa</i>	Netherlands	—	AB553782	AB554112	AB554149	C
<i>Prosthemia</i> sp. (= <i>Prosthemia intermedium</i>)									
KT 2082-1	MAFF 242292	HHUF 29952	<i>B. ermanii</i>	Japan	AB553647	AB553771	AB554101	AB554138	C
KT 2096-1	MAFF 242291	HHUF 30057	<i>B. ermanii</i>	Japan	AB553648	AB553772	AB554102	AB554139	C
KT 2143	MAFF 242290	HHUF 30058	<i>B. maximowicziana</i>	Japan	AB553649	AB553773	AB554103	AB554140	C
KT 2158	MAFF 242289	HHUF 30059	<i>B. ermanii</i>	Japan	—	AB553774	AB554104	AB554141	C
KT 2159	JCM 16972	HHUF 30060	<i>B. ermanii</i>	Japan	—	AB553775	AB554105	AB554142	C
KT 2160	JCM 16973	HHUF 30061	<i>B. ermanii</i>	Japan	—	AB553776	AB554106	AB554143	C
KT 2225	JCM 16974	HHUF 30062	<i>B. ermanii</i>	Japan	—	AB553777	AB554107	AB554144	A
KT 2226	JCM 16975 = MAFF 242288	HHUF 30063	<i>B. ermanii</i>	Japan	—	AB553778	AB554108	AB554145	C
KT 2228	JCM 16976	HHUF 30064	<i>B. ermanii</i>	Japan	—	AB553779	AB554109	AB554146	C
MK2	JCM 16977	HHUF 30065	<i>B. ermanii</i>	Japan	—	AB553780	AB554110	AB554147	C
Pleosporales sp.									
HC27033	—	HHUF 27033	<i>F. crenata</i>	Japan	—	AB553783	AB554113	AB554150	A

^aGeneric names of host plants: *A.*, *Alnus*; *B.*, *Betula*; *F.*, *Fagus*; *P.*, *Pterocarya*; *S.*, *Salix*.^bGenBank accession No. in parentheses indicates a sequence obtained from a previous work (Schoch et al. 2006).^cOrigin of isolates: A, single ascospore; C, single conidium.

Morphologically, the *Prosthemium* sp. and *A. betulinum* were close to the generic type of *Prosthemium*. Our phylogenetic analyses revealed that these constitute a separate lineage within the genus. Therefore, these two taxa are described below as new *Prosthemium* species. Some morphological and phylogenetic characters of existing species in *Prosthemium* are also noted below.

Prosthemium intermedium Kaz. Tanaka & Melnik, sp. nov. (Figs. 16–29)

MYCOBANK ACCESSION NO.: MB 518629.

TELEOMORPH: *Pleomassaria* sp.

ETYMOLOGY: In reference to the intermediate morphology of conidia between *P. canba* and *P. orientale*.

Conidiomata pycnidioidea, 490–560 μm alta, 920–1180 μm diametro, unilocularia, immersa, sparsa vel gregaria, depresso-globosa vel globosa. Paries conidiomatis "textura angularis," 20–30(–45) μm crassus ad latus et basim. Conidiophora ad 300 μm longa, 2–4 μm lata, simplicia, septata, hyalina. Cellulae conidiogenae holoblasticae. Conidia stellata, ex 4 brachiis obclavatis composita, echinulata, brunnea, ad cellulam apicales pallidiora; brachium longissimum 57–73 μm \times 16.5–23 μm (mean = 63.4 μm \times 19.6 μm , n = 60), 4–5-euseptatum; brachium aliud (37.5–)40–55.5(–60) μm \times (11–)13–19.5 μm (mean = 46.3 μm \times 16.0 μm , n = 60), 3–4-euseptata.

DESCRIPTION: Conidiomata pycnidial, 490–560 μm high, 920–1180 μm in diameter, unilocular, immersed, scattered to crowded, depressed globose to globose, ostiolate. Wall of textura angularis at sides and base, brown, almost uniformly 20–30(–45) μm thick, composed of 5–7 layers of flattened cells (1.5–3 μm thick), with brown sparse hyphae 3.5–5 μm wide; wall around the ostiole clypeus-like, 20–38 μm thick, composed of small black cells of 1–2 μm diameter. Conidiophores up to 300 μm long, 2–4 μm thick, unbranched, septate, hyaline, smooth. Conidiogenous cells holoblastic, integrated, determinate, hyaline, smooth, terminal. Conidia stellate, brown, minutely verrucose, mostly composed of a long arm and 3 equally developed arms, (72–)84–114 μm (mean = 99.2 μm , n = 60) between the apex of longest and the adjacent arms; longest arms 57–73 μm \times 16.5–23 μm (mean = 63.4 μm \times 19.6 μm , n = 60), L/W ratio 2.6–3.9 (mean = 3.3, n = 60), 4–5 euseptate, hyaline to pale brown at the terminal cell; other arms (37.5–)40–55.5(–60) μm \times (11–)13–19.5 μm (mean = 46.3 μm \times 16.0 μm , n = 60), L/W ratio 2.3–3.8 (mean = 2.9, n = 60), 3–4 euseptate, constricted and connected at the central cell; central cells hyaline to pale brown, isodiametric to somewhat round, 8–11.5(–13.5) μm in diameter.

TYPUS: Japan, Aomori, Mount Hakkoda (140°52.4'E, 40°42'N), on twigs of *B. ermanii*, 31 March 2007, KT 2226 (HHUF 30063 holotype designated here; monoconidial isolate from the holotype, JCM 16975 = MAFF 242288).

ADDITIONAL MATERIALS EXAMINED: Japan, Aomori, Mount Hakkoda (140°52.4'E, 40°42'N), on twigs of *B. ermanii*, 15 April 2006, KT 2082-1 (HHUF 29952; monoconidial isolate MAFF 242292); 25 May 2006, KT 2096-1 (HHUF 30057; monoconidial isolate MAFF 242291); 4 March 2007, KT 2158 (HHUF 30059; monoconidial isolate MAFF 242289); KT 2159 (HHUF 30060; monoconidial isolate JCM 16972);

KT 2160 (HHUF 30061; monoconidial isolate JCM 16973); 31 March 2007, KT 2225 (HHUF 30062; single ascospore isolate JCM 16974); KT 2228 (HHUF 30064; monoconidial isolate JCM 16976); Ohanabe mountain pass, 4 May 2008, MK2 (HHUF 30065; monoconidial isolate JCM 16977); Shirakami, Iwasaki-mura (140°03'15.7"E, 40°33'54.5"N), on twigs of *Betula maximowicziana*, 6 August 2006, KT 2143 (HHUF 30058; monoconidial isolate MAFF 242290).

NOTES: This fungus, found on *B. ermanii* and *B. maximowicziana*, is introduced as a new species in this study. It is characterized by conidia composed of 1 longer arm, with 3 other arms equally developed. The longest arm of *P. intermedium* (57–73 μm \times 16.5–23 μm) is similar to that of *P. canba* (63–88.5 μm \times 14–20 μm ; Tanaka et al. 2005), but differs in its L/W ratio (3.3 vs. 4.4). Moreover, all conidial arms are obviously unevenly developed in the latter species. The size range of other conidial arms of *P. intermedium* (37.5–60 μm \times 11–19.5 μm) overlaps with that of *P. orientale* (36–45 μm \times 10–14 μm ; Kamiyama et al. 2009), but *P. orientale*, by contrast, does not have 1 longer arm in its conidia. In addition to these distinguishing morphological features, *P. intermedium* was demonstrated to be a single species in the phylogenetic tree, with strong bootstrap support (96%–97%) in NJ and MP, although the PP value was less than 0.95 in Bayesian analysis (Fig. 3). The clade of *P. intermedium* resides as basal and sister to the clade that includes *P. canba*, *P. orientale*, and *P. betulinum* (Fig. 3).

Prosthemium neobetulinum Kaz. Tanaka & Melnik, nom. nov. (Figs. 5 and 11)

MYCOBANK ACCESSION NO.: MB 518630

\equiv *Asterosporium betulinum* Peck, Ann. Rep. N.Y. St. Mus. Nat. Hist. 33:26, 1883 (1880), non *P. betulinum* Kunze, Mykologische Hefte (Leipzig) 1:18, 1817.

TELEOMORPH: Unknown

DESCRIPTION: Conidiomata pycnidial, 200–280 μm high, 580–750 μm in diameter, unilocular, immersed, scattered to crowded, depressed globose, with a wide ostiole of more than 100 μm diameter. Conidia stellate, brown, composed of 4 equally developed arms, 30.5–55(–61) μm (mean = 40.4 μm , n = 50) between the widest points; arms 17–28 (–30) μm \times 8–9.5(–11) μm (mean = 21.7 μm \times 8.7 μm , n = 50), L/W ratio 1.9–3.2 (mean = 2.5, n = 50), 3–5 euseptate, connected and slightly constricted at the central pale brown cell of 5–6 μm in diameter.

MATERIALS EXAMINED: Canada, Ontario, Ottawa, near Richmond Hill, on twigs of *Betula alleghaniensis*, 11 June 1929, H.S. Jackson (BPI US0404970); 28 April 1933, H.S. Jackson (BPI US0404971, US0404972); Central Experimental Farm, 28 July 1953, R. Horner (BPI US0404967, US0404969), 2 June 1955, R. Horner (BPI US0404968), 1 November 1955, R. Horner (BPI US0404966). Monoconidial isolate CBS 121.51 (Ontario, Dorset, May 1949, B.W. Dance). France, on twigs of *Betula medwediewii*, 19 March 1957, P.X. Peltier (BPI US0404977 as *Asterosporium hoffmannii* Kunze). Russia, St. Petersburg, botanical garden of Komarov Botanical Institute, on twigs of *Betula davurica*,

Fig. 1. Majority rule consensus tree of *Prosthemium* species within the Pleosporales (Dothideomycetes) from Bayesian analysis based on LSU nrDNA sequences (1220 bp). Bayesian posterior probabilities (PP) above 0.90 and maximum parsimony (MP) and neighbor-joining (NJ) bootstrap values greater than 70% are indicated at the nodes as PP/MPBS/NJBS. A hyphen (“-”) indicates values lower than 0.90 (PP) or 70% bootstrap support (BS), and a node not present in an analysis is shown with “x”. A thick line is used for a clade with high statistical support (more than 1.00 PP and 90% BS). Either GenBank accession Nos. or the original isolate numbers are noted after the species names. The tree was rooted to *Lophium mytilinum* (Mytiliniales) and *Rhytidhysteron rufulum* (Hysteriales).

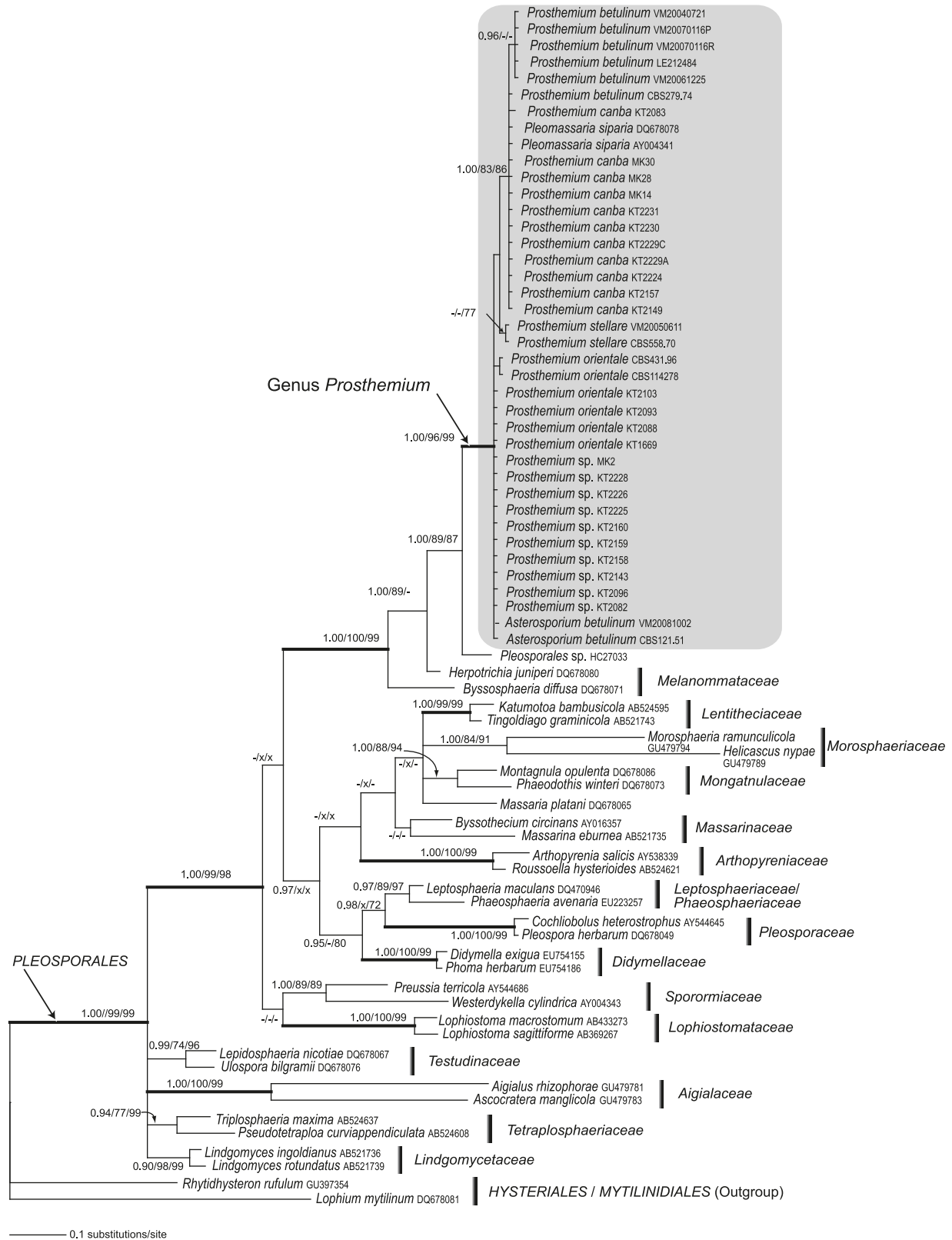
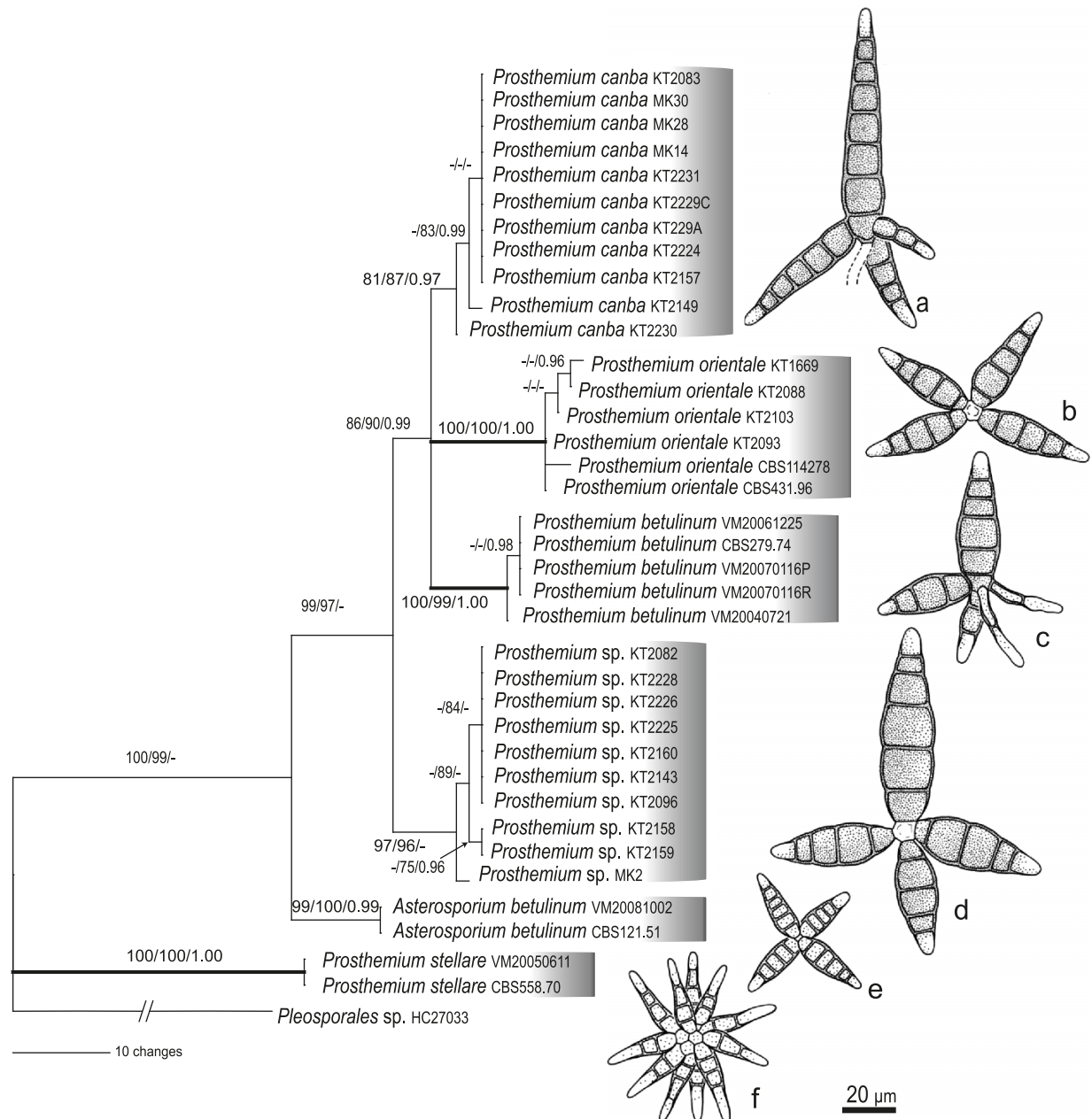


Fig. 3. One of the three most parsimonious trees of *Prosthemium* species based on a combined data set of ITS (507 bp) and *BT* (507 bp). Length = 212 steps, consistency index = 0.9245, retention index = 0.9497. Maximum parsimony (MP) and neighbor-joining (NJ) bootstrap values greater than 70% and Bayesian posterior probabilities (PP) above 0.90 are indicated at the nodes as MPBS/NJBS/PP. A hyphen (“-”) indicates values lower than 70% bootstrap support (BS) or 0.90 (PP). A thick line is used for a clade with high statistical support (more than 90% BS and 1.00 PP). An original isolate number is noted after the species name. Conidial morphology is shown at the side of each species. (a) *Prosthemium canba* from HHUF 27340, redrawn from Tanaka et al. (2005). (b) *Prosthemium orientale* from paratype (ZT) of *Prosthemium asterosporum*, redrawn from Kamiyama et al. (2009). (c) *Prosthemium betulinum* from LE 255828. (d) *Prosthemium* sp. (= *Prosthemium intermedium*) from HHUF 30063. (e) *Asterosporium betulinum* (= *Prosthemium neobetulinum*) from BPI US0404967. (f) *Prosthemium stellare* from CBS 126964.



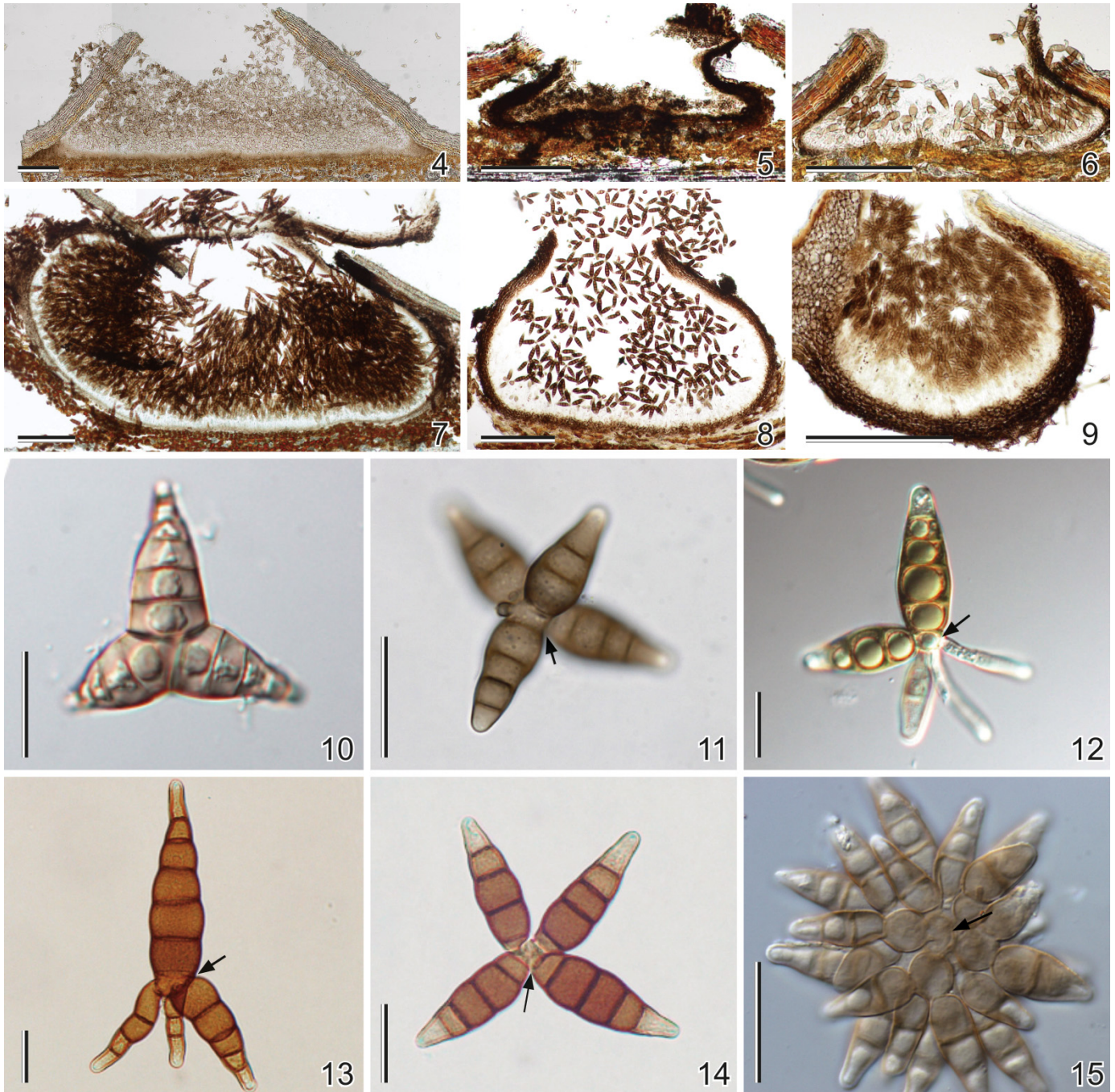
Prosthemium canba Kaz. Tanaka, Y. Harada & M.E. Barr, *Mycoscience* 46: 253, 2005 (Figs. 7 and 13)

TELEOMORPH: *Pleomassaria* sp.

NOTES: This species was originally described from *B. ermanii* solely based on morphological features such as conidia with their longer arm (73.2 μm × 16.9 μm), and an additional 2 or 3 arms unequally developed (Tanaka et al. 2005). In cul-

ture, abnormal, reduced conidia recalling those of *P. betulinum* were sometimes observed. However, in our phylogeny, *P. canba* was distinct from *P. betulinum* (Fig. 3). Although the monophyly of *P. canba* was weakly supported in all analyses using ITS or *BT* sequences (less than 70% BS), it received moderate support in the ITS + *BT* tree (81%–87% BS and 0.97 PP; Fig. 3).

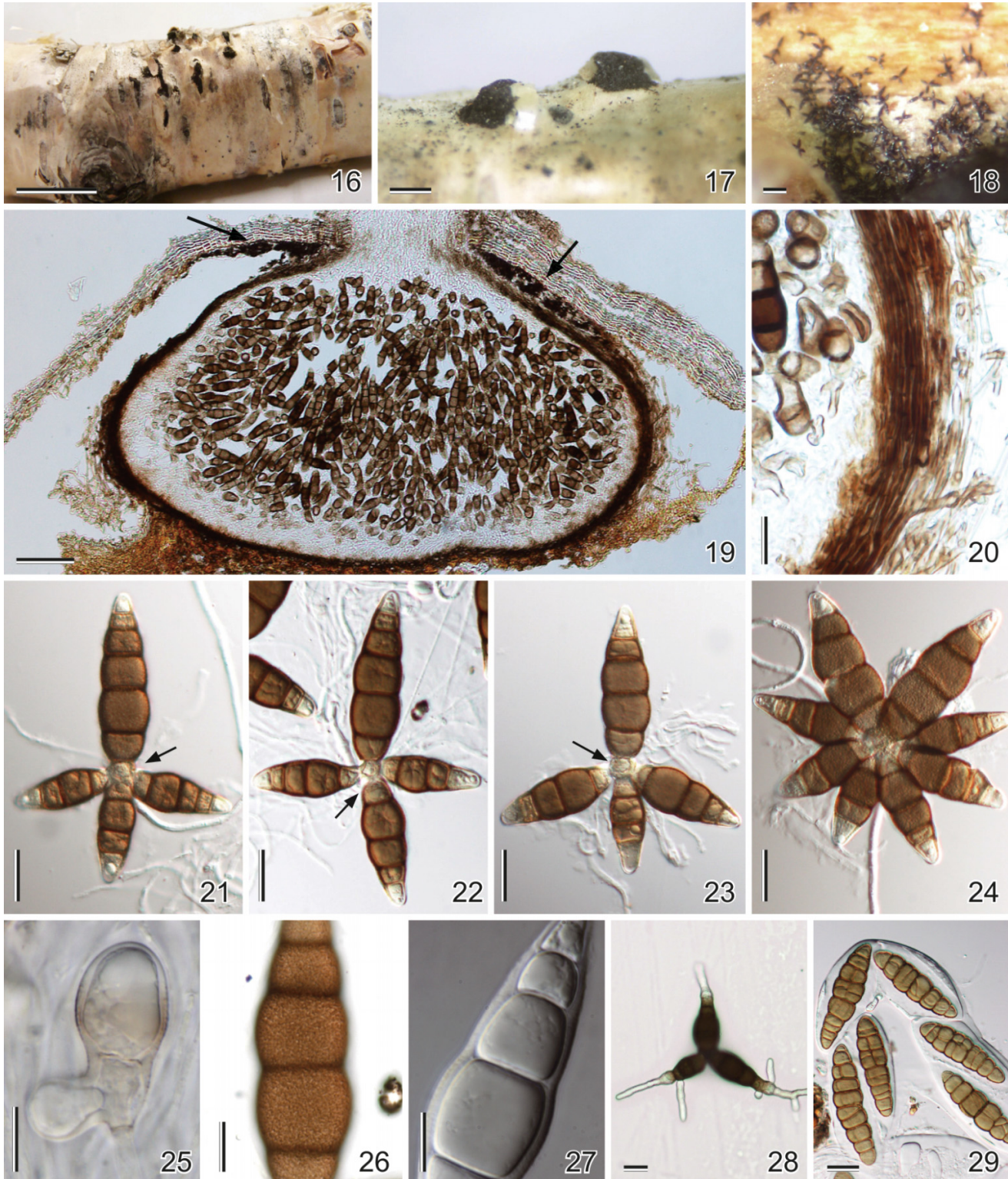
Figs. 4–15. Conidiomata (Figs. 4–9) and conidia (Figs. 10–15) of *Asterosporium* and *Prosthemium*. Fig. 4. Longitudinal section of an acervulus of *Asterosporium asterospermum* on *Fagus sylvatica* (from BPI US0404965). Fig. 5. Longitudinal section of a pycnidium of *Asterosporium betulinum* (= *Prosthemium neobetulinum*) on *Betula davurica* (from HHUF 30040). Fig. 6. Longitudinal section of a pycnidium of *Prosthemium betulinum* on *Betula raddeana* (from LE 212484). Fig. 7. Longitudinal section of a pycnidium of *Prosthemium canba* on *Betula ermanii* (from HHUF 27340). Fig. 8. Longitudinal section of a pycnidium of *Prosthemium orientale* on *B. ermanii* (from LE 73863). Fig. 9. Longitudinal section of a pycnidium of *Prosthemium stellare* on *Alnus glutinosa* (from HHUF 29951). Fig. 10. Conidium of *A. asterospermum* (from BPI US0404965). Fig. 11. Conidium of *A. betulinum* (= *P. neobetulinum*; from HHUF 30040). Fig. 12. Conidium of *P. betulinum* (from LE 255828). Fig. 13. Conidium of *P. canba* (from HHUF 29948). Fig. 14. Conidium of *P. orientale* (from LE 73863). Fig. 15. Conidium of *P. stellare* (from HHUF 29951). Arrows in Figs. 11–15 indicate the central connecting cell of conidia. Scale bars = 200 µm (Figs. 4–9); 20 µm (Figs. 10–15).



Prosthemium orientale (Melnik) Kamiyama, Kaz. Tanaka & Melnik, *Mycoscience* 50: 438, 2009 (Figs. 8 and 14)
 TELEOMORPH: *Pleomassaria siparia* (Berk. & Broome) Sacc. type B (Paavolainen et al. 2000).

NOTES: Morphologically, this species is similar to *P. betulinum*, but differs in that the conidia have 4 equally developed arms (Kamiyama et al. 2009). This fungus was previously known as *A. orientale* (Mel'nik 1988; Mel'nik et al. 2001)

Figs. 16–29. *Prosthemium intermedium*. Figs. 16 and 17. Conidiomata on host surface. Fig. 18. Exuded conidia from conidioma. Fig. 19. Pycnidium in longitudinal section (arrows indicate clypeus-like structure around ostiole). Fig. 20. Wall of pycnidium with brown hyphae. Figs. 21–23. Conidia with 4 arms (arrows indicate central connecting cell of conidia). Fig. 24. Abnormal conidium with 8 arms. Fig. 25. Developing conidium. Fig. 26. Surface of conidium with verrucose ornamentation. Fig. 27. Euseptate conidium bleached with 5% sodium hypochlorite solution. Fig. 28. Germinating conidium. Fig. 29. Ascus and ascospores. Figs. 16–28. From HHUF 30063 (holotype); Fig. 29 From HHUF 30062. Scale bars = 1 cm (Fig. 16); 1 mm (Fig. 17); 100 μ m (Figs. 18 and 19); 10 μ m (Figs. 20 and 25–27); 20 μ m (Figs. 21–24, 28, and 29).



or *Prosthemium asterosporum* (Kowalski and Holdenrieder 1996; Barengo et al. 2000; Paavolainen et al. 2001) in Europe. It formed a robust clade (100% BS and 1.00 PP) in our analyses, based on six isolates, including an ex-type (CBS 431.96) of *P. asterosporum* (Fig. 3).

Prosthemium stellare Riess, Bot. Ztg. 11: 130, 1853 (Figs. 9 and 15)

TELEOMORPH: *Pleomassaria holoschista* (Berk. & Broome) Sacc.

NOTES: This quite distinctive species has relatively fewer pig-

mented conidia with several radiating arms. Conidial arms range from 6 to 20 in number, but in most cases (ca. 85%) there are 9–14. This species occurs on *Alnus*, and both the teleomorph (*Pleomassaria holoschista*) and anamorph are widely known from Europe (Shoemaker and LeClair 1975; Sutton 1980; Kirk and Spooner 1984; Sivanesan 1984; Eriksson 2009), but have not yet been reported from America (Barr 1982). We used two isolates (from Lithuania and the Netherlands), and this fully supported monophyletic clade was the most basal lineage (Fig. 3).

Key to the species of *Prosthemium*

- | | | |
|----|--|------------------------|
| 1a | Conidia mostly 9–14 armed, occurring on <i>Alnus</i> | <i>P. stellare</i> |
| 1b | Conidia mostly 4–5 armed, occurring on <i>Betula</i> | 2 |
| 2a | Conidia almost less than 60 µm diameter (between widest points of conidial arms) | <i>P. neobetulinum</i> |
| 2b | Conidia more than 60 µm diameter | 3 |
| 3a | Conidia with 4 equally developed arms | <i>P. orientale</i> |
| 3b | Conidia with unequally developed arms | 4 |
| 4a | Longer conidial arm is less than 55 µm long | <i>P. betulinum</i> |
| 4b | Longer conidial arm is more than 55 µm long | 5 |
| 5a | Conidia with a longer arm (L/W ca. 4.4) and 2 or 3 unequally developed arms | <i>P. canba</i> |
| 5b | Conidia with a longer arm (L/W ca. 3.3) and 3 equally developed arms | <i>P. intermedium</i> |

Discussion

Phylogenetic placements of *Prosthemium* and *Asterosporium*

The type species of *Prosthemium* (*P. betulinum*; Kunze 1817) has been known to possess the pleosporalean ascomatal state of *Pleomassaria* (Tonolo 1956), a genus previously placed in the Pleomassariaceae (type *Pleomassaria siparia*; Barr 1982; Tanaka et al. 2005; Kirk et al. 2008). Sequence data from several genes (e.g., SSU, LSU, *RPB2*, *TEF1*) from one strain of *Pleomassaria siparia* (CBS 279.74) have been used for phylogenetic analyses within the Dothideomycetes (Liew et al. 2000; Lumbsch et al. 2000; Lumbsch and Lindemuth 2001; Schoch et al. 2006). In our analysis of LSU nrDNA data, all *Prosthemium* species formed a strongly supported monophyletic clade within the Pleosporales (Dothideomycetes) and nested within the Melanommataceae (Fig. 1). This familial placement of *Prosthemium* species is in agreement with the recent reassessment of the family by Mugambi and Huhndorf (2009) based on the LSU and *TEF1* gene. In this study, *Pleomassaria siparia* was basal to other members of Melanommataceae but had relatively low bootstrap support (Mugambi and Huhndorf 2009). Similar topology was obtained in the phylogeny based on the SSU, LSU, *RPB2*, and *TEF1* provided by Zhang et al. (2009), who synonymized the Pleomassariaceae with the Melanommataceae. On morphological grounds, however, *Pleomassaria* species possessing *Prosthemium* anamorphs are rather unique within the Melanommataceae because of their large-sized ascospores, and star-shaped conidia (Barr 1982; Tanaka et al. 2005). Further investigation based on additional molecular data using additional *Pleo-*

massaria taxa will be needed to resolve phylogenetic relationships within the Melanommataceae and to evaluate the validity of the Pleomassariaceae as a family for *Pleomassaria* species with *Prosthemium* anamorphs.

In contrast, there was no prior information about the ordinal or familial affiliations of *Asterosporium*. It has been suspected that the type species of the genus (*A. asterospermum*; Kunze 1819; Hughes 1958) has a teleomorph belonging to the Massariaceae (Wehmeyer 1926) or to *Asteromassaria macrospora* (Spooner and Kirk 1982), but no teleomorph has been found. In our study, SSU and LSU sequences from seven strains of two *Asterosporium* taxa were analyzed to clarify their affinities. The results clearly reveal that *Asterosporium* is polyphyletic. One species of *Asterosporium* on *Betula*, *A. betulinum*, is in a different clade from the type of *Asterosporium* and groups with *Prosthemium* with high statistical support (1.00 PP and more than 96% BS; Fig. 1). Therefore, *A. betulinum* is transferred to *Prosthemium* and given a new name, *P. neobetulinum*, to avoid creating a later homonym of *P. betulinum*. While, *A. asterospermum* is located in a clade within the Diaporthales (Sordariomycetes) (Fig. 2). A BLAST search using the LSU sequence of *A. asterospermum* suggested that the species is close to members of the Diaporthaceae, in particular *Diaporthe pustulata* (AF408358), *Diaporthe padi* (AF408354), and *Diaporthe perijuncta* (AF408356). However, in the phylogenetic analyses, *A. asterospermum* was found as basal to the core members of the Diaporthales, except for the Togniniaceae clade (Fig. 2). The familial placement of *A. asterospermum* remains unknown. It seems likely that, *A. asterospermum* represents a new lineage which does not belong in any existing families of the Diaporthales. In contrast to *A. asterospermum*, anamorphic members of this or-

der are generally known to have non- or one-septate conidia, produced from phialidic or annellidic conidiogenous cells (Rossman et al. 2007).

Morphological delimitation of *Prosthemium* and *Asterosporium*

Traditionally, *Prosthemium* and *Asterosporium* have been distinguished on the basis of conidiomatal morphology and have been placed in artificial anamorphic groups known as the Sphaeroideae and the Melanconieae, respectively (Saccardo 1884; Lindau 1922; Clements and Shear 1931). The delimitation of these genera was accepted and their conidiomata regarded as pycnidia in *Prosthemium* and as acervuli in *Asterosporium* (Morgan-Jones and Kendrick 1972; Kendrick and Nag Raj 1979). However, Sutton (1973, 1980) interpreted the conidiomata of *Prosthemium* as acervular to eustromatic, without an ostiole but dehiscing by the irregular rupture of the overlying tissues. In a dichotomous key to genera of acervular fungi, Sutton (1973) used the presence (in *Prosthemium*) or absence (in *Asterosporium*) of a distinct basal cell connecting each conidial arm to distinguish these genera. Later, Sutton (1980) adopted conidial septation as a key character distinguishing the euseptate *Prosthemium* from the distoseptate *Asterosporium*.

We examined the morphological characters previously used for circumscribing *Prosthemium* and *Asterosporium*. In longitudinal section the conidiomata of *A. asterospermum* were obviously acervuli with flattened bases (Fig. 4), as illustrated by several authors (Morgan-Jones and Kendrick 1972; Sutton 1980; Kobayashi and Kubono 1986; Prášil and Réblová 1995). All species in the *Prosthemium* lineage illustrated in our LSU tree (Fig. 1), including *A. betulinum* (= *P. neobetulinum*), had globose to subglobose pycnidia with circular ostioles (Figs. 5–9). In contrast to Sutton's (1980) idea, our result clearly reveals that conidiomatal morphology has taxonomic significance for the delimitation of these genera. The pycnidia of *A. betulinum* (= *P. neobetulinum*; Fig. 5) had a tendency to become somewhat incomplete because of the collapse of the upper wall layer surrounding the ostiole. These might be misinterpreted as acervuli, as illustrated by Sutton (1980).

We do not believe that conidial septation has diagnostic value, as we consider all species treated here to have euseptate conidia, at least at the light microscope level. A similar opinion has been noted for these fungi by Kowalski and Holdenrieder (1996). However, the presence or absence of a distinct basal cell connecting the conidial arms might have taxonomic significance in distinguishing between *Prosthemium* and *Asterosporium*. All species in *Prosthemium* have conidia with a central cell connecting several conidial arms (Figs. 11–15), but this was not found in *A. asterospermum* (Fig. 10). This result supports Sutton's (1973) circumscription of both genera, although later he did not use this character (Sutton 1980).

It appears that these genera can also be distinguished by host differences. Species of *Asterosporium* are restricted to *Fagus* (Fagaceae). With some exceptions, *Prosthemium* species are associated with *Alnus* and *Betula* (Betulaceae). *Prosthemium orientale* usually occurs on twigs of *Betula*. It has been recorded occasionally from other plant leaves such as those of *Carex* and *Salix*, but these examples are consid-

ered to result from conidia washed off their natural host by rainwater; there were none of the highly characteristic pycnidia on these substrates (Kamiyama et al. 2009). In our study, *P. betulinum*, a common fungus on *Betula* in Europe (Hantula et al. 1998; Paavolainen et al. 2000), was found on twigs of *Pterocarya rhoifolia* (Juglandaceae) (Table 1), and formation of typical pycnidia was confirmed on this substrate. Whether *P. betulinum* occurs on *Pterocarya* as a natural (not occasional) host remains unknown, because the specimens were collected from *Pterocarya* neighboring *Betula* trees in a botanical garden; more collecting surveys are needed to confirm the host range.

To summarize the diagnostic features of these genera, *Prosthemium* is characterized by pycnidial conidiomata with circular or widely open ostioles, conidia with a central cell connecting several arms, and a host usually within the Betulaceae. *Asterosporium* is characterized by acervular conidiomata with flattened bases, conidia without an obvious central cell, and a *Fagus* host.

Reconsideration of *Prosthemium* species

Our LSU tree of *Prosthemium* strongly confirmed the monophyly of the genus (Fig. 1). However, species boundaries within the genus could not be clarified because of the low sequence variability. Similar results were obtained from the ITS analyses, and the BS values for each node were relatively low (data not shown). The analyses of the *BT* gene showed most species as distinct monophyletic lineages with higher bootstrap support, but *P. canba* received low support (less than 70% NJ and MPBS). Consequently, we analyzed the combined data set of ITS and *BT* sequences to determine species boundaries within the genus. The ITS + *BT* tree revealed six significantly supported monophyletic species within *Prosthemium* (Fig. 3). These were divided into two groups correlated with their host and conidial morphology. The most basal clade thus consisted of *P. stellare* (mostly 9- to 14-armed conidia and an *Alnus* host), and the remaining clade consisted of other species, mostly with 4- to 5-armed conidia and a *Betula* host. Among the clade of *Betula* parasites, species recognized by both number and length of conidial arms were shown to be separate phylogenetically. Therefore, these morphological characters are considered to be reliable taxonomic indicators in *Prosthemium*, although they are to some degree overlapping among these species.

The challenge for holomorphic names

All *Prosthemium* species except for *P. neobetulinum* form teleomorphs belonging to *Pleomassaria* in nature, but we have used their anamorphic names because the application of the holomorphic names was somewhat complicated, particularly among pathogens of *Betula*. *Prosthemium stellare* on *Alnus* has the distinct teleomorph *Pleomassaria holo-schista*. This can be separated from other species in *Pleomassaria* by its relatively small ascospores (35–48 $\mu\text{m} \times$ 11–13 μm ; Sivanesan 1984). *Prosthemium betulinum* and *Prosthemium orientale* both have teleomorphs within the *Pleomassaria siparia* species complex that cannot be distinguished by their ascospore morphologies (Paavolainen et al. 2000). Consequently, the teleomorphs have been provisionally referred to as *Pleomassaria siparia* type A (anamorph *P. orientale*) and type B (anamorph *P. betulinum*) without

formal nomenclature (Paavolainen et al. 2000; Tanaka et al. 2005). A similar situation was observed between *P. canba* and *P. intermedium*. We obtained several isolates of these species from their teleomorphs (Table 1, Fig. 29), but we did not establish their holomorphic identity, mainly because of a lack of plentiful specimens of the teleomorph. However, even if we could obtain teleomorphic specimens in good condition, it may remain difficult to distinguish the teleomorphs of *P. canba* and *P. intermedium*. The ascospores of these species tend to be somewhat larger (ca. 61–89 $\mu\text{m} \times 14$ –22 μm) than those of *P. betulinum* and *P. orientale* (the *Pleomassaria siparia* complex; 51–74 $\mu\text{m} \times 15$ –26 μm ; Hantula et al. 1998), but those of *P. canba* and *P. intermedium* are almost identical. In addition to their teleomorphic similarity, *P. canba* and *P. intermedium* occur sympatrically on the same host (*B. ermanii*), and frequently even on the same twigs. Thus, we cannot decide whether a *Pleomassaria* species on a specimen was formed from a *P. canba* or a *P. intermedium* anamorph or even that a *Pleomassaria* species on a specimen represents a single species, unless we observe anamorphic states from each *Pleomassaria* fungus on the specimen. To characterize teleomorphs, it would be effective to observe ascomatal states induced by different mating pairs in culture. Apparently, all *Prosthemium* species used in this study are heterothallic, and their abilities to form ascomata in culture are unknown. Mating experiments have been successful within other dothideomycetous genera such as *Didymella* (Chilvers et al. 2009), *Leptosphaeria* (Shoemaker and Brun 2001), and *Mycosphaerella* (Mondal et al. 2004). Results from mating experiments could provide some taxonomic implications for the differentiation of teleomorphs among *Prosthemium* species.

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