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REVIEW

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Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping

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Abstract Currently, rDNA-ITS sequence analysis seems to be the most appropriate method for comprehensive classification of *Rhizoctonia* spp. Our previous review article was concerned with detailed analysis of multinucleate *Rhizoctonia* (MNR), and the current review complements the previous one with detailed analysis of binucleate *Rhizoctonia* (BNR) (teleomorphs: *Ceratobasidium* spp. and *Tulasnella* spp.) and uninucleate *Rhizoctonia* (UNR) (teleomorph: *C. bicorne*). Data of all the appropriate BNR and UNR accumulated in GenBank were analyzed together in neighbor-joining (NJ) trees supplemented with percent sequence similarity within and among the anastomosis groups (AGs) and subgroups. Generally, the clusters of the isolate sequences supported the genetic basis for the AG based on hyphal fusion anastomosis. Comprehensive interrelationships among all the currently available MNR, BNR, and UNR groups and subgroups in GenBank were subsequently analyzed in NJ and maximum-parsimony (MP) trees, showing the genetic relatedness among the different groups and indicating possible bridging groups between MNR, BNR, and UNR. The review also indicates serious inaccuracies in designation of sequences of some isolates deposited in GenBank. Several additional teleomorph genera with *Rhizoctonia* spp. anamorphs have also been reported in the literature. However, as they have not been intensively studied, there were no available data on their rDNA-ITS sequences that could be included in this review.

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Introduction

The first part of the review on the advancing identification and classification of *Rhizoctonia* spp. (Sharon et al. 2006) described, summarized, and compared the efficacy of the various biochemical, molecular, and the classical anastomosis grouping methods used for the identification and classification currently available for *Rhizoctonia* spp. The first part also included a more detailed multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis of the multinucleate (MNR) anastomosis groups (AGs) and subgroups of *R. solani*, *R. praticola*, and *R. circinata* (teleomorphs: *Thanatephorus cucumeris*, *T. praticola*, and *Waitea circinata*, respectively; Sharon et al. 2006).

Burpee et al. (1980) divided the binucleate *Rhizoctonia* (BNR) (*Ceratobasidium cornigerum* and related fungi) into 7 "*Ceratobasidium* Anastomosis Groups" (CAG-1 to CAG-7), whereas Ogoshi et al. (1983) divided the BNR into 17 anastomosis groups (AG-A to AG-Q). As the isolates of most of the different CAGs were the same as some of the BNR AGs designated in Japan (Ogoshi et al. 1983), they were subsequently incorporated into the AG system developed in Japan. CAG 2 to AG-A; CAG 1 to AG-D; CAGs-3 and CAG-6 (which belong to the same AG) to AG-E; and CAG 4 to AG-F. CAGs 5 and 7 were designated as the new AG-R and AG-S, respectively (Sneh et al. 1991). Since then, Ogoshi's classification for BNR AGs has been accepted and used accordingly.

Of the 21 BNR AGs of the *Ceratobasidium* teleomorph [not including fungi belonging to the *Tulasnella* teleomorph (*Epulorhiza* anamorph) and others] reported as AG-A to AG-S (Sneh et al. 1991) and AG-T, AG-U (Hyakumachi et al. 2005), only 16 AGs are currently known, as (1) AG-J was excluded from *Rhizoctonia* because its hyphae form clamp connections; (2) the representative isolates of AG-M were lost (Sneh et al. 1991), and no isolates of this AG

Table 1. *Rhizoctonia* spp. isolates that were inaccurately designated to anastomosis groups (AGs) in GenBank, followed by their possible AG designation according to their rDNA-internal transcribed spacer (ITS) sequence location in clusters of the neighbor-joining tree [Fig. 1, supported by the maximum-parsimony (MP) tree] and their % sequence similarity to the closest AGs

Isolate number	Accession number	Inaccurate AG[a]	% Similarity	To AG
SIR-2	AF354091	B(o)	94–100	A
CFM-1	AF446088	B(o)	86–90	A & K
TC1	AJ242902	N	94–99	A
76150	AJ242903	P	95–100	A
2Shi-1999	AB196659	T	94–100	A
Aic0400	AB196660	T	94–100	A
4Oit-800	AB196661	T	94–99	A
3Iba-1000	AB196662	T	94–99	A
1Fuk-600	AB196663	T	94–100	A
Rh-521	U19950	MNR	94–99	A
S5	AJ427400	S	99	B(o)
AGS	AB196656	S	99	B(o)
76146	AJ242901	L	99–100	C
Rh155	AJ242896	E	90–99	C
Bn-37	AF354082	R	94–99	F
AV-2	AJ242898	I	96–97	G
MWR-20	AB196664	U	98–100	P
MWR-24	AB196665	U	98–100	P
MWR-22	AB196666	U	98–100	P
BN31	AF354080	E	90–95	R
C4	AJ301900	E	90–96	R
C8	AJ302006	S	91–96	R
STC-43[b]	AB286935	N	only 61–72	BNR

[a] According to the rDNA-ITS sequence location in the tree
[b] Not *Rhizoctonia*

remained in any known culture collection; (3) AG-N is excluded from the BNR (see following text, and Tables 1, 3, 4, and Figs. 1, 3, 4, 5, 6); (4) AG-T was reported as a new BNR AG by Hyakumachi et al. (2005), but its isolates were subsequently found to anastomose with additional AG-A representatives (Hyakumachi, unpublished data) and were confirmed to belong to AG-A also by rDNA-ITS sequence analysis (see Table 1, Fig. 1); (5) AG-U was reported as a new BNR AG by Hyakumachi et al. (2005), but its isolates were subsequently found in the current analysis (see following text, and Tables 1, 3, and Figs. 1, 3, 4, 5, 6) to anastomose with additional AG-P isolates.

AG-B was divided into three subgroups (Ogoshi et al. 1983; Sneh et al. 1991). AG-D was initially divided into two subgroups (Toda et al. 1999) and subsequently into three subgroups (Hayakawa et al. 2006; see Fig. 1). AG-F was suggested to be divided into two subgroups (Sharon et al. 2007; Fig. 1), although additional research with more isolates is needed to support it. Results of future research on the identification of newly recovered isolates might be expected to indicate the existence of additional AGs and subgroups.

The classical hyphal fusion method for the identification and classification of *Rhizoctonia* spp. into anastomosis groups has been widely used; it is still valid and was genetically supported in recent years by the use of DNA-based molecular methods. However, for some *Rhizoctonia* spp. isolates, anastomosis reactions are actually insufficient for their accurate identification and classification into the appropriate AGs for the following reasons. Some isolates have lost their capability even to self-anastomose (Hyakumachi and Ui 1987). Carling et al. (2002) reported that some isolates of the MNR AG 2 had no anastomosis reaction (C0) with certain isolates of the same AG, whereas they did have up to clonal reaction (C3) with other isolates of the same subgroup. Similarly, some BNR isolates of certain AGs failed to anastomose with certain representative isolates of the same AG, although they did anastomose with other representative isolates of the same AG: both CAG-3 and CAG-6 belong to AG-E (Sneh et al. 1991), AG-A (Martin 2000), and isolates of various additional AGs (Naito, unpublished data). On the other hand, some isolates actually anastomose with representatives of more than one AG, as demonstrated by Ogoshi for isolates of BNR AG-C with AG-I isolates (which are suggested by him to be combined into AG-C), or MNR isolates of AG 2 (that includes the former separate group of AG BI) with AG 3, AG 6, AG 8, and AG 11 (Ogoshi et al. 1983; Sneh et al. 1991; Carling 1996; Carling et al. 2002).

Isolates of certain AGs have been investigated more intensively than others, because of their greater relative importance as plant pathogens or as mycorrhizal endophytes in orchids, such as *Epulorhiza* (synonym *Rhizoctonia*, teleomorph *Tulasnella*) spp. Therefore, rDNA-ITS data on more isolates of these AGs are currently available in GenBank and in publications than on the other existing groups of fungi in nature. The BNR AGs of which relatively more isolate sequences have been deposited in GenBank are AG-A, AG-B, AG-D, AG-G, AG-E, AG-F, AG-P, AG-R, and AG-I. There are only a few available sequences

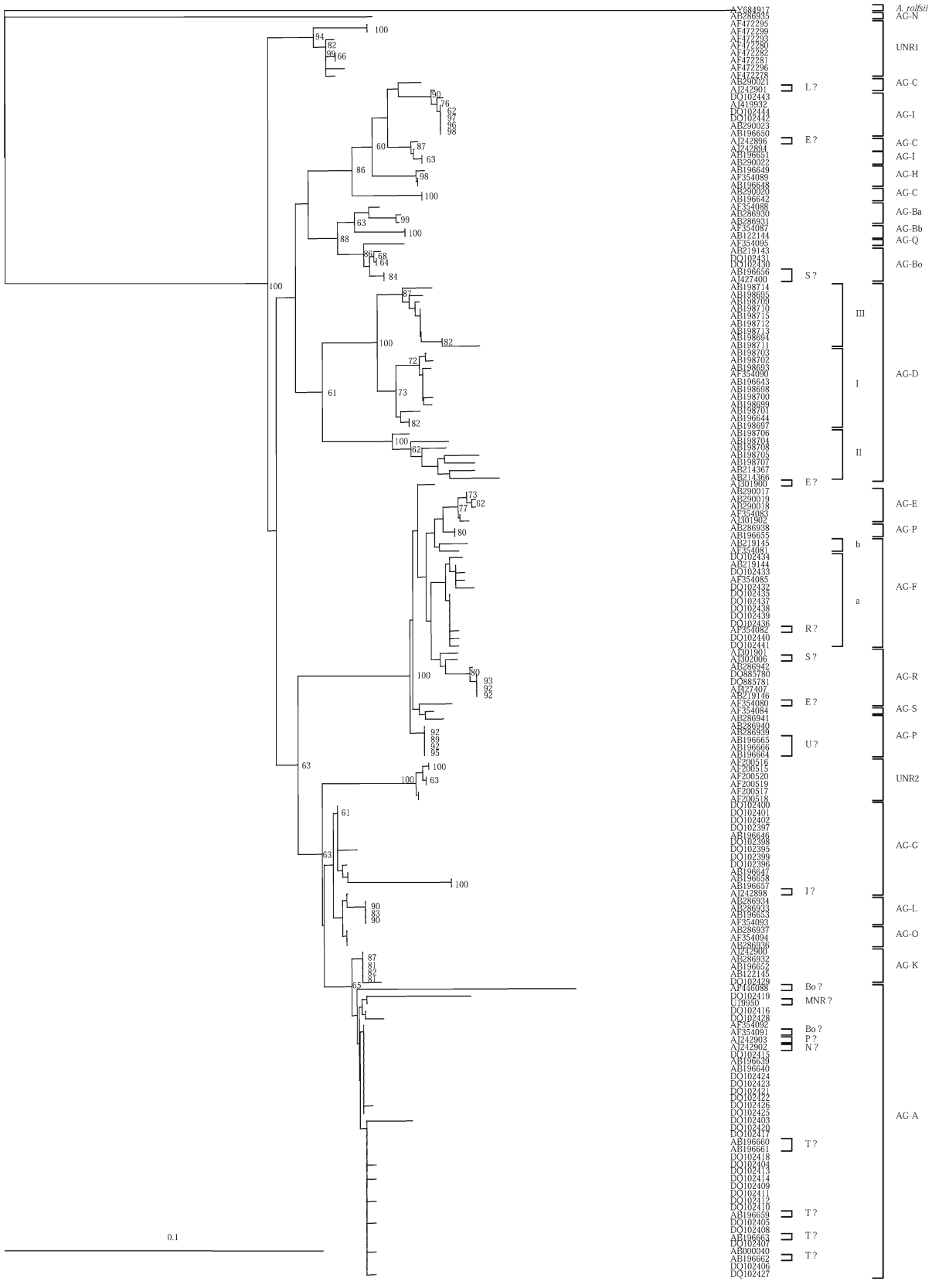

Fig. 1. A neighbor-joining tree of binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) anastomosis groups (AGs) isolates (represented by their DNA accession number) available in GenBank, clustered according to multiple alignment of rDNA-internal transcribed spacer (ITS) sequence analysis. The distances were determined according to Kimura's two-parameter model. Bootstraps of 111 trials are positioned alongside the branches with values over 60. Isolate AY684917 [*Athelia* (*Sclerotium*) *rolfsii*] was used as an outgroup. The AGs and subgroups for the clusters are indicated. *Bar* indicates 1 base change per 10 nucleotide positions

for isolates of AG-C, AG-H, AG-K, and AG-L, and sequences for only one isolate each of AG-N, AG-O, AG-Q, and AG-S.

Hyakumachi et al. (2005) reported on two new groups, AG-T and AG-U. However, the isolates of AG-T were subsequently identified to actually belong to AG-A (see Fig. 1), and isolates of AG-U have been subsequently found to anastomose with certain isolates of AG-P (Naito and Kuninaga, unpublished data). For the accuracy of classification purposes, using only one isolate to represent an AG or a subgroup for anastomosis testing or for other classification purposes such as rDNA-ITS sequence analysis is therefore insufficient and may lead to inaccurate conclusions (see Table 1, Fig. 1). The suggestion of the new AGs, T and -U (Hyakumachi et al. 2005), probably stemmed from the fact that some isolates did not anastomose with certain representative members of an AG but did anastomose with others of the same AG. Isolates of the restriction fragment length polymorphism (RFLP) group O of AG-A, which are genetically close within AG-A, anastomosed with certain isolates but not with other AG-A isolates (Martin 2000). Isolates of AG-U anastomosed at a high frequency with several isolates of AG-P but at low frequencies or not at all with other isolates of AG-P. Similarly, the other AG-P isolates had low fusion frequency with this group of AG-P, indicating that AG-P is a heterogeneous AG (Naito and Kuninaga, unpublished data). This result is also supported by the % rDNA-ITS sequence similarity among the isolates within AG-P. For some isolates it ranged higher, between 93% and 100%, but for others it was lower, from 90% to 94% (Sharon and Sneh, unpublished data). Isolates of AG-P were located in two separate clusters in the neighbor-joining (NJ) tree (Fig. 1), but in one cluster in the maximum-parsimony (MP) tree (not shown). The AG-U isolate sequences were located on the same cluster with some of the AG-P isolates, and the % sequence similarity range between the AG-U and the AG-P isolates was 88% to 96%. The similarity among the only three available AG-U isolates is very high (98%–100%), as expected for isolates obtained from one source (roses). These data indicate that AG-P is a heterogeneous group, which may include several subgroups, and the AG-U isolates together with some of the AG-P isolates are in one of the AG-P subgroups. Additional work with more isolates may support the existence of these AG-P subgroups.

The genes and gene expressions involved in the hyphal fusion process (anastomosis reactions) have not yet been recovered. The reason for low fusion frequencies or no fusion at all of isolates of the same AG may be a consequence of some minor mutations in one or more of these genes, rather than a result of a distinct genetic or evolutionary distance, whereas the rDNA-ITS sequence analysis and % sequence similarity may provide more substantial genetic relatedness and evolutionary phylogenetic information of these isolates within and among AGs.

One of the authors (S. Naito) continued to collect, test, and confirm anastomosis of BNR isolates to the correct AGs and maintain the culture collection previously established by A. Ogoshi at Hokkaido University, Sapporo, Japan. Its purpose is to serve as a reliable source for several standard and genuine representative isolates for each of the various BNR AGs and subgroups and avoid misidentification. It is an important initiative for *Rhizoctonia* researchers; this initiative requires continued volunteering and stronger international support. The representative isolates have been carefully tested for anastomosis reactions with a considerable number of the available isolates of the various BNR (*Ceratobasidium* spp.) AGs and subgroups, as well as for rDNA-ITS sequence analysis (Naito and Kuninaga, unpublished data). The representative tester isolates for each AG and subgroups and their accession numbers are listed in Table 2.

Table 2. List of binucleate *Rhizoctonia* (BNR *Ceratobasidium* spp.) representative isolates for anastomosis testing

AG	Number			Source		
	Isolate	Accession	MAFF	Host	Location	Collector
A	AH-1	AB196639	305267	Peanut	Chiba Jpn	Ogoshi
	C-538	AB196640	305271	Potato	Hokkaido Jpn	Oniki
	4Oit-800	AB196661	240158	Rose	Oita Jpn	Hyakumachi
	1Fuk-600	AB196663	240157	Rose	Fukuoka Jpn	Hyakumachi
	RU56-8	DQ102417		Soil	W. Virg. USA	Sneh
Ba	C-314	AB286931	240159	Soil	Fukuoka Jpn	Oniki
	C-460	AF354088	305277	Rice	Fukuoka Jpn	Oniki
	C-484	AB196641	305278	Rice	Miyagi Jpn	Oniki
	Scl-2	AB286930		Rice	Japan	Ogoshi
Bb	C-350	AB122144	240160	Rice	Fukuoka Jpn	Oniki
	C-455	AF354087	305284	Rice	Fukuoka Jpn	Oniki
B(o)	C-302	AB219143		Soil	Fukuoka Jpn	Oniki
	RU89-1	DQ102431		Soil	Conn. USA	Sneh
	RU18-1	DQ102430		Soil	W. Virg. USA	Sneh
C[a]	Ao-1-2	AB290020	305287	Soil	Aomori Jpn	Ogoshi
	OR706 (= 70B)	AJ242894		*Gymnadenia*	Japan	Tsutsui
	55D25	AB290021	240161	Sugar beet	Hokkaido Jpn	Uchino

Table 2. *Continued*

AG	Number			Source		
	Isolate	Accession	MAFF	Host	Location	Collector
D I	Ayu-WP-1	AB198703	240162	*Agrostis*	Kagawa Jpn	Tanaka
	BrG-WP-1	AB198693	240163	*Agrostis*	Yamanashi Jpn	Tanaka
	YC-SDS-1	AB198699	240164	*Zoysia*	Mie Jpn	Tanaka
	W-12	AB198697		Wheat	Hokkaido Jpn	Yanagida
D II	YG-EF-1	AB198704	240165	*Zoysia*	Yamagata Jpn	Tanaka
	MW-EF-1	AB198708		*Zoysia*	Yamaguchi Jpn	Tanaka
	Oak-EF-1	AB198707	240166	*Zoysia*	Okayama Jpn	Tanaka
	TAK-14KT	AB214367	240167	*Zoysia*	Shizuoka Jpn	Hayakawa
D III	KOU04-12FW	AB198713	240168	*Zoysia*	Shizuoka Jpn	Hayakawa
	KOU04-18FW1	AB198714		*Zoysia*	Shizuoka Jpn	Hayakawa
	KAG 9R	AB198709		*Zoysia*	Shizuoka Jpn	Hayakawa
E	TMA1-1(= TM1-1)	AB290017	240169	Soil	Hokkaido Jpn	Kuninaga
	Oc-1	AB290019	305299	*Oxalis*	Tokyo Jpn	Ogoshi
	BN74	AF354083		*Erigeron*	Florida USA	Burpee
	Lu-5	AB290018	305296	Flax	Hokkaido Jpn	Mitsui
Fa	Str10	DQ102434		Strawberry	Israel	Sneh
	Str36	DQ102435		Strawberry	Israel	Sneh
	PS-17	AB219144	305303	Pea	Tokushima Jpn	Ogoshi
Fb	AH-6	AB196645	240170	Peanut	Chiba Jpn	Ogoshi
	FKO2-28	AB219145	305302	Soil	Fukuoka Jpn	Ogoshi
	Bn38	AF354081		Soybean	Georgia USA	Burpee
G	AH-9	AB196646	305305	Peanut	Chiba Jpn	Ogoshi
	Gm1	DQ102395		Strawberry	Calif. USA	Martin
	Str15	DQ102396		Strawberry	Israel	Sneh
	Su-1	AB196647	305307	*Stellaria*	Tokyo Jpn	Ogoshi
	1Shi-1299	AB196657		Rose	Shizuoka Jpn	Hyakumachi
H	STC-9	AF354089		Soil	Tochigi Jpn	Ogoshi
	STC-10	AB196648	305310	Soil	Tochigi Jpn	Ogoshi
	STC-11	AB196649	305311	Soil	Tochigi Jpn	Ogoshi
I[a]	AV-2	AB196650	240173	*Artemisia*	Tokyo Jpn	Ogoshi
	FKO-6-7	AB290022	305313	Soil	Fukuoka Jpn	Ogoshi
	FKO-1-17	AB196651	305312	Soil	Fukuoka Jpn	Ogoshi
	55D21	AB290023	240174	Sugar beet	Hokkaido Jpn	Uchino
	Im2	DQ102444		Strawberry	Calif. USA	Martin
	Ibs1	DQ102442		?	?	Sneh
J[b]	Not *Rhizoctonia*					
K	SH-10	AB196652	305319	Soil	Hokkaido Jpn	Ogoshi
	AC-1	AB122145	240175	Onion	Hokkaido Jpn	Ogoshi
	56D17	AB286932		Sugar beet	Hokkaido Jpn	Uchino
L	FKO-2-11	AB286934	305321	Soil	Fukuoka Jpn	Ogoshi
	FKO-2-16	AB286933	305322	Soil	Fukuoka Jpn	Ogoshi
	FKO-2-26	AB196653	305324	Soil	Fukuoka Jpn	Ogoshi
M	Lost					
N	Not *Rhizoctonia*					
O	FKO-6-2	AF354094	305328	Soil	Fukuoka Jpn	Ogoshi
	FKO-2-10	AB286936	305329	Soil	Fukuoka Jpn	Ogoshi
	FKO-2-32	AB286937	305330	Soil	Fukuoka Jpn	Ogoshi
P[c]	C-578	AB196654	305335	Tea	Shizuoka Jpn	Takaya
	C-584	AB286938	305339	Soil	Ishikawa Jpn	Oniki
	X1-2-1	AB286940		*Betula*	Yunnan Ch	Yang
	Xb-3	AB286939		Ginger	Yunnan Ch	Yang
Q	C-620	AF354095	305911	*Cynodon*	Chiba Jpn	Oniki
R	Bn-37	AB219146		Cucumber	Georgia USA	Burpee
	X4-3	AB286942		Ginger	Yunnan Ch	Yang
	Xb-1-3	DQ885784		*Betula*	Yunnan Ch	Yang
S	S5	AB196656		Soil	Japan	
T[d]	= AG-A					
U[c]	MWR-20	AB196664	240176	Rose	Gifu Jpn	Hyakumachi
	MWR-22	AB196666	240177	Rose	Gifu Jpn	Hyakumachi
	MWR-24	AB196665	240178	Rose	Gifu Jpn	Hyakumachi

[a] Some isolates of AG-C anastomose with some isolates of AG-I (see text)
[b] Hyphae of AG-J have clamp connections
[c] Some isolates of AG-P anastomose with isolates of AG-U, indicating that AG-U may belongs to AG-P (see text)
[d] AG-T isolates belong to AG-A (see text)

Classification of the BNR AGs by the rDNA-ITS sequence analysis

Compared to the knowledge accumulated for MNR AGs reviewed by Sharon et al. (2006), fewer studies have examined the genetic diversity by rDNA-ITS sequence analyses of isolates within and among BNR AGs (Gonzalez et al. 2002; Otero et al. 2002; Ma et al. 2003; Hyakumachi et al. 2005; Sharon et al. 2007). It has been reported that although fewer BNR isolates have been investigated, their rDNA-ITS sequence homologies were lower than those of MNR isolates (Gonzalez et al. 2001). On the other hand, Sharon et al. (2007) reported that the percent rDNA-ITS sequence similarities calculated in a pairwise manner ranged between 93% and 100% for isolates within the various BNR AGs or subgroups and only from 89% to 95% for isolates among BNR AGs or subgroups in AGs -A, -B, -F, -G, -I, and -K. From the summary of a considerable number of currently available sequence data for BNR isolates in GenBank as they are presented in Fig. 1, it is evident that the rDNA-ITS sequences of BNR (*Ceratobasidium* spp.) AGs are well arranged within distinct clusters according to their AGs and subgroups. The % sequence similarity within AGs or subgroups ranged from 93% to 100% [except for some AGs, which further work may indicate that they may well include several subgroups, such as AG-C (87%–98%); AG-P (90%–100%); AG-R (91%–100%); AG-A (93%–100%)], and among the AGs or their subgroups it was 75% to 95% [except for lower values for AG-N (only 61%–72%), which its belonging to BNR (*Ceratobasidium*) is questionable (see following); or high upper values for AG-C with AG-I (86%–100%), which may belong to the same AG (see following); AG-G with AG-L or with AG-O (93%–97%); and AG-L with AG-O (97%–98%) (Table 3)].

rDNA-ITS sequences of BNR AGs, their subgroups and UNR clusters in the neighbor-joining (NJ) and maximum-parsimony (MP) trees

In the present review, the rDNA-ITS sequences of a considerable number of the isolates designated to BNR and UNR AGs currently available in GenBank were initially retrieved and analyzed with the ClustalW program (Thompson et al. 1994) from DDBJ (DNA Data Bank of Japan, Research Organization of Information and Systems, National Institute of Genetics, Shizuoka, Japan; http://www.ddbj.nig.ac.jp) for multiple alignment, and additionally manual multiple alignments were performed using the Genedoc program, version 2.5.000 (Nicholas et al. 1997). For each data set, sequences were aligned with the ClustalW program at DDBJ, using the default options for DNA analysis (blosum matrix, gap opening penalty 15, gap extension penalty 6.66, and gap separation distance 8) and later

Table 3. Pairwise percent similarity ranges of rDNA-ITS sequences of uninucleate (UNR) and binucleate (BNR) *Ceratobasidium* spp. AGs and subgroups

AG	A	B	Ba	Bb	Bo	C	D	D(I)	D(II)	D(III)	E	F	Fa	Fb
A	93–100													
B	76–87	82–100												
Ba	85–88	84–94	98–100											
Bb	76–80	82–86	84–86	97										
Bo	84–87	82–94	92–94	82–84	99–100									
C	80–88	76–87	82–86	76–79	84–87	87–98								
D	79–84	75–85	82–85	75–81	81–85	78–84	78–100							
D(I)	81–84	78–84	82–84	78–81	83–84	81–84	86–95	97–100						
D(II)	79–83	75–85	82–85	75–80	82–85	78–84	84–89	86–89	93–96					
D(III)	81–83	76–84	82–84	76–80	81–83	80–84	84–95	91–95	84–89	95–100				
E	80–89	77–85	83–85	77–80	84	80–85	78–82	80–82	78–82	79–82	93–99			
F	80–86	77–86	82–86	77–80	81–85	79–85	77–82	80–83	77–82	78–82	89–93	90–100		
Fa	80–86	77–86	82–86	77–80	81–84	79–85	77–82	80–83	77–82	78–82	89–95	90–92	94–100	
Fb	81–85	78–86	84–86	78–80	84–85	80–83	78–82	80–81	78–82	78–81	91–93	90–92	90–92	95
G	85–92	76–86	84–86	76–80	85–87	84–89	78–85	80–85	78–84	80–85	82–89	82–88	82–88	82–86
H	82–88	75–86	83–85	75–79	84–86	88–94	80–84	81–84	80–83	80–84	81–84	80–84	80–84	80–82
I	82–88	77–86	84–85	77–79	84–86	86–100	80–85	82–84	80–84	81–85	83–86	81–85	81–85	81–83
K	92–96	79–87	86–87	79–81	86	83–86	82–86	82–84	83–86	83–85	85–88	82–86	82–86	85–86
L	87–92	78–87	86–87	78–80	86	86–88	81–84	83–84	81–83	82–84	85–88	84–88	84–88	86–87
N	69–71	61–70	69	61–62	69–70	70–72	66–69	67–68	66–68	67–69	67–69	66–68	66–68	66–67
O	87–93	78–87	87	78–80	87	87–89	81–85	83–84	81–83	82–85	86–89	84–88	84–88	86–87
P	79–86	76–85	80–85	76–80	81–85	78–83	75–82	78–81	75–82	77–82	86–92	89–93	89–93	89–91
Q	80–82	83–91	88–89	83–85	90–91	81–82	79–83	81–82	80–83	79–82	80–82	80–82	80–82	81–82
R	78–88	76–85	80–85	76–80	79–84	75–84	74–82	78–81	74–81	77–82	85–94	86–93	86–93	87–92
S	82–85	78–89	82–84	78–89	83	80–82	78–82	81–82	78–80	80–81	89–92	91–93	91–93	92
T ?	94–100	78–87	86–87	78–80	86	84–88	80–84	81–84	80–83	81–83	84–88	82–86	82–86	84–85
U ?	82–88	76–84	83–84	76–77	83–84	81–85	78–81	79–80	78–81	78–81	89–91	88–91	88–91	88–90
UNR1	85–89	81–95	89–92	81–85	94–95	85–88	80–85	82–85	80–84	82–85	81–86	80–84	80–84	83–84
UNR2	83–87	78–85	83–85	78–79	83–84	81–84	79–84	81–84	79–84	80–84	83–84	82–86	82–86	84–86
S. rolfsii	62–64	62–64	62–64	63	63–64	63–64	60–64	62–64	60–63	60–62	63–64	62–65	64–65	62–65

adjusted manually by visual examination. The consequent alignments were used to create either an NJ tree (by the Tree and Bootstrap options in DDBJ), or MP trees (by the Seqboot, Dnapars, and Consense tools in the Phylip Package 3.6). Manual alignments have to be meticulously performed, even though there may be more than one reasonably correct alignment; this may be a source for differences in positions of isolates and clusters in trees obtained from these alignments. The anastomosis grouping may also provide the researcher with an assisting framework for choosing the most appropriate manual alignment. The sequences of ITS1 are more variable than ITS2 among fungal isolates. Some of the researchers prefer analyzing the ITS1 and ITS2 separately (Kuninaga et al. 1997; Kuninaga 2002), while others commonly use the ITS1 + 5.8S + ITS2 sequences. Using the ITS1 + 5.8S + ITS2 includes more information than either of them used separately, despite the fact that the general clades were not significantly changed. In the present review the ITS1 + 5.8S + ITS2 sequences were used for the analyses. Analyses of the 5.8S rDNA were used for relatedness, phylogeny, and evolution of fungi (Sugiyama 1998).

Multiple alignments containing incomplete rDNA-ITS sequences lacking a substantial portion of the start or the end may cause inaccuracies in the sequence analyses. Sequences of such isolates were omitted from the summarized isolate sequences of the present review. To get better standardization of the deposited rDNA-ITS sequences in GenBank, it is recommended to deposit only complete sequences, starting with the end of the conserved area of the 18S (primers ITS1-TCCGTAGGTGAACCTGCGG or ITS5-GGAAGTAAAAGTCGTAACAAGG) and ending at the beginning of the conserved area of the 28S region (primer ITS4-TCCTCCGCTTATTGATATGC). The rDNA-ITS size between these two primers may vary among groups of isolates. Currently, the rDNA-ITS sequences deposited in GenBank vary from the longer known ones, for the MNR AG 2-2IV (isolate BC10), about 678 bp, to shorter MNR sequences such as that of *W. circinata*, about 570 bp. The longer sequence for the BNR AG-Bb (isolate C-350 accession number AB122144) is about 679 bp, and the shorter BNR sequence of AG-L (isolate FKO-2-16 AB286933) is about 611 bp.

The MP analysis provides a number of maximum parsimonious trees, and the researcher can either choose the most appropriate tree according to past knowledge or create a consensus tree based on all the best parsimonious trees. As it was technically impossible to include all the available MNR, BNR, and UNR isolate sequences in one tree, partial NJ trees were initially prepared, calculated by the "NJ tree" option in DDBJ (found under the ClustalW), from the manual alignment performed earlier. The first tree (see Fig. 1) included the BNR (*Ceratobasidium* spp.) AGs and subgroups and UNR (*Ceratobasidium bicorne*) isolate sequences available and compatible (lengthwise) in GenBank. The second tree (see Fig. 2) included all the available and com-

G	H	I	K	L	N	O	P	Q	R	S	T ?	U ?	UNR1	UNR2
95–100														
83–89	97–99													
84–89	91–95	94–100												
87–90	84–86	84–86	100											
93–97	87–88	86–88	90	100										
68–70	69–71	69–72	69	70–71	–									
93–97	87–89	86–88	89–90	97–98	70	100								
79–86	78–82	78–84	83–86	82–87	66–69	83–88	90–100							
80–81	80–82	81–82	82	80–81	66	82	80–82	–	–					
77–87	76–83	78–84	81–88	81–87	65–68	82–90	86–91	80–81	91–100					
82–85	80–81	80–81	85–86	86	68	86	90–92	81	91–92					
88–91	87–88	85–88	94–96	91	70–71	92	82–86	81–82	80–87	84–85	99–100			
83–88	82–83	82–85	86	87–89	68	88–90	88–96	79	86–92	89	86–88	98–100		
85–88	85–88	84–88	85–87	88–89	70–71	88–89	79–85	88–89	78–84	81–82	87–89	82–85	94–100	
85–89	80–83	81–83	86–88	87–89	65–68	88–89	82–84	81	80–85	83	85–86	82–84	82–85	98–100
62–63	62–63	62–64	64	63	64	62	63–65	64	63–65	64	62–63	61	62–63	61

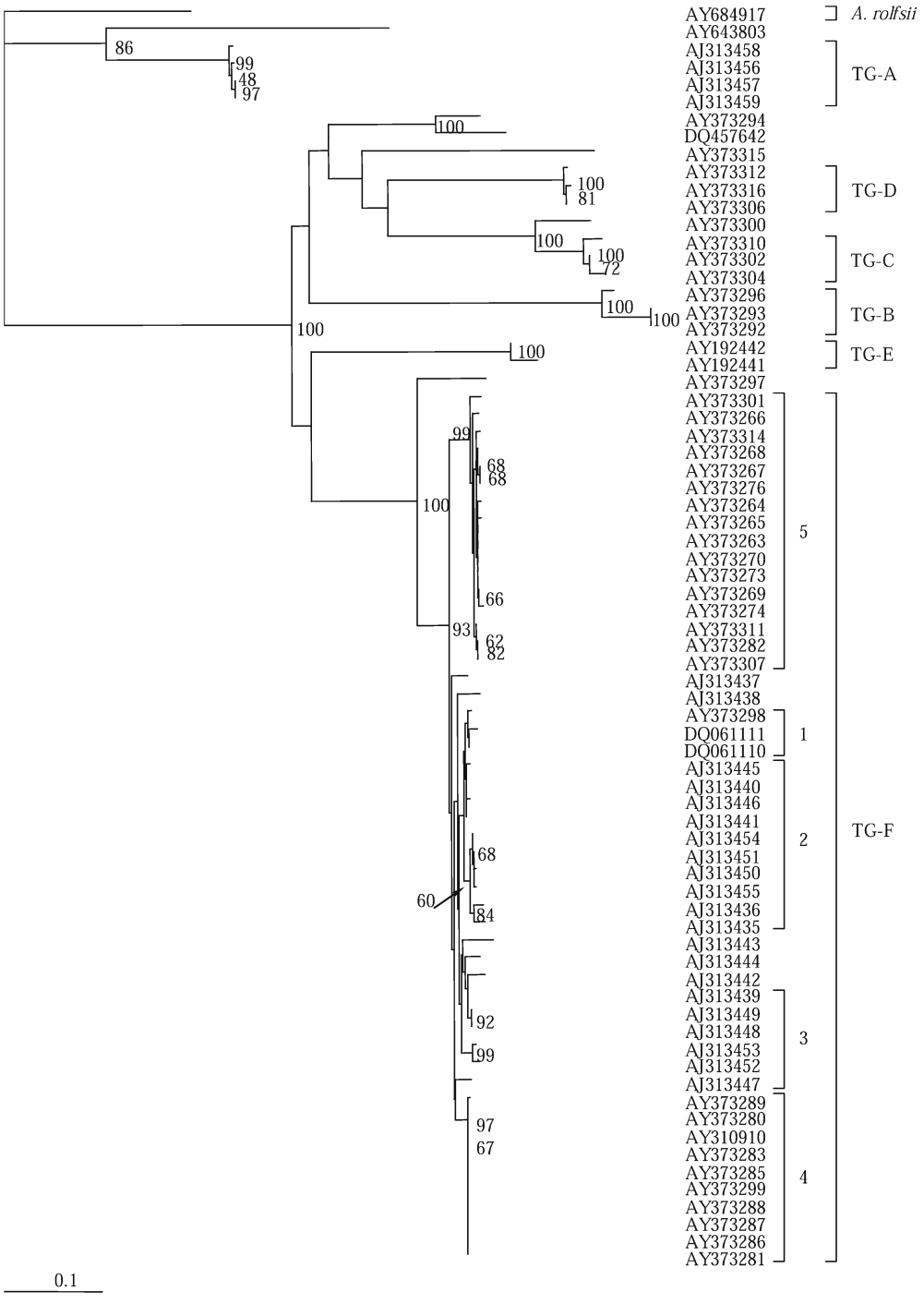

Fig. 2. A neighbor-joining tree composed of rDNA-ITS sequences of binucleate *Epulorhiza* spp. (syn. *Rhizoctonia* spp.; teleomorph *Tulasnella* spp.) isolates (represented by their DNA accession number) available in GenBank, clustered according to multiple alignment of rDNA-ITS sequence analysis. (See legend for Fig. 1)

patible BNR *Epulorhiza* spp. (teleomorph: *Tulasnella* spp.) isolate sequences in GenBank (sequence length was around 650 bp). The isolates of *Tulasnella* spp. have not been generally tested for anastomosis grouping, and therefore there was no realistic option to relate the clusters of isolate sequences to AGs, as was arranged for the investigated AGs of the other BNR and MNR groups. The third tree (see Fig. 3) included representative isolate sequences (distantly located sequences in each of the AG or subgroup clusters) of the UNR and BNR, including the *Tulasnella* ones. The fourth tree (see Fig. 4) included the representative isolate sequences (distantly located in each of the clusters) of the UNR and BNR, not including the *Tulasnella*. The fifth tree (see Fig. 5) included the representative isolate sequences of UNR, BNR, and MNR AGs. The sixth tree (see Fig. 6) included the representative isolate sequences of UNR, BNR, and MNR in one consensus MP tree, using the program Dnapars, followed by the Consense program, which generates a consensus tree from all the best possible trees. Bootstraps values were calculated with the seqboot program, using 100 replicates. Seqboot, Dnapars, and Consense were all from the Phylip Package 3.6 (Felsenstein

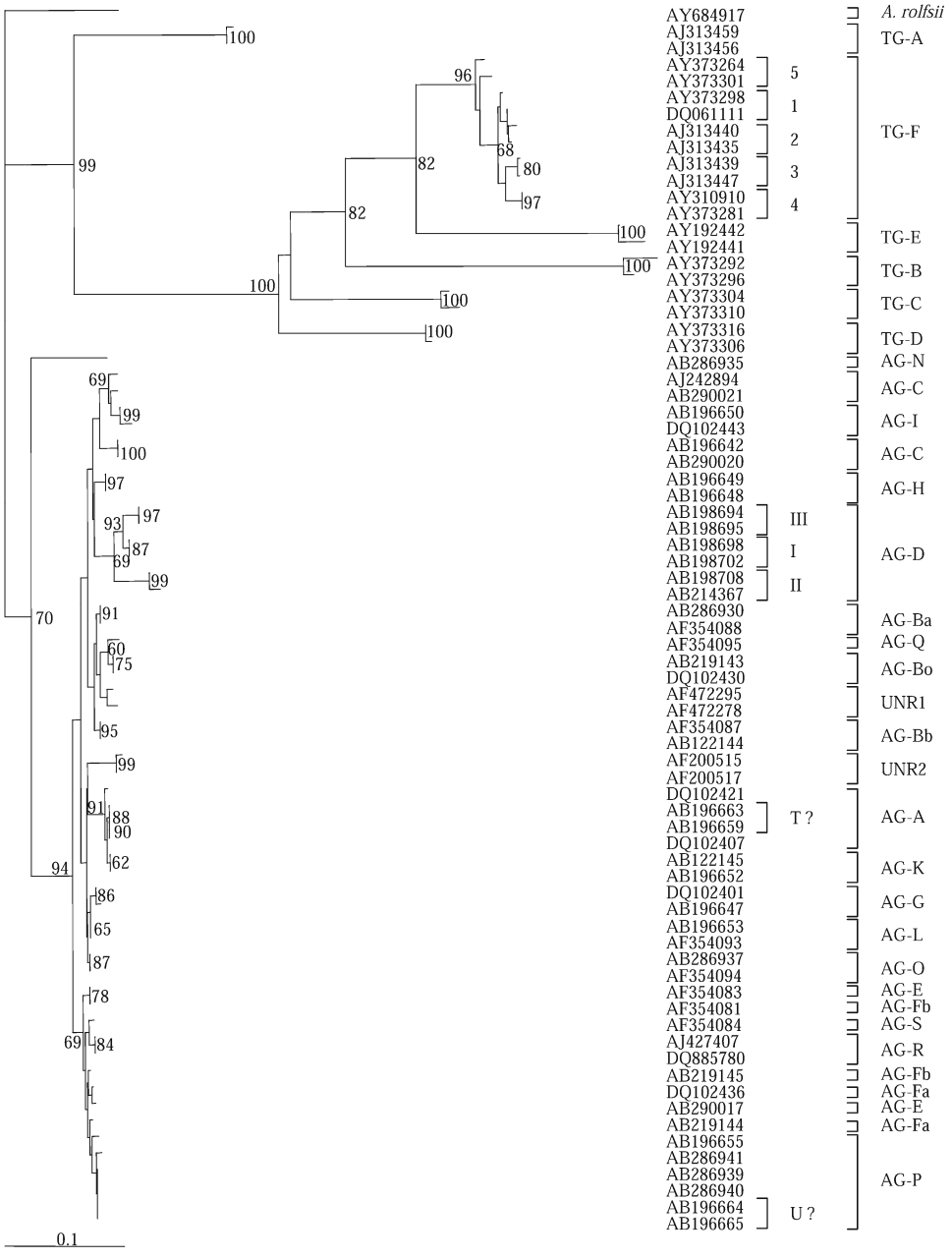

Fig. 3. A neighbor-joining tree of representative AGs isolates of uninucleate and binucleate *Rhizoctonia* spp. (including *Tulasnella*) isolates (represented by their DNA accession number) clustered according to multiple alignment of rDNA-ITS sequence analysis. (See legend for Fig. 1)

1989). All trees were viewed by TreeView version 1.6.6 (Page 1996). Pairwise percentages of sequence similarities of all the isolates within AGs and subgroups as well as among the AGs and subgroups [calculated with the MatGat program (Campanella et al. 2003)] are summarized in Table 3 for UNR + BNR (*Ceratobasidium*), in Table 4 for BNR *Tulasnella* spp., and in Table 5 for between all the *Ceratobasidium* spp. UNR + BNR and the MNR isolates. It was also important to include rDNA-ITS sequences of most of the isolates available in GenBank (summarized in Figs. 1 and 2) to obtain information on the extent rates of % sequence similarities within the AGs or subgroups, as well as to identify inaccuracies of isolate sequences and AG identification of certain isolate sequences deposited in GenBank.

A considerable number of the AGs were frequently represented in publications, by sequences of only one or two isolates per group (Gonzalez et al. 2001, 2002; Otero et al. 2002). Sharon et al. (2007) have indicated that a relatively wide rage of percent similarity for rDNA-ITS sequences could be found for isolates within several BNR AGs. Therefore, statistically, the probability to obtain a wider range

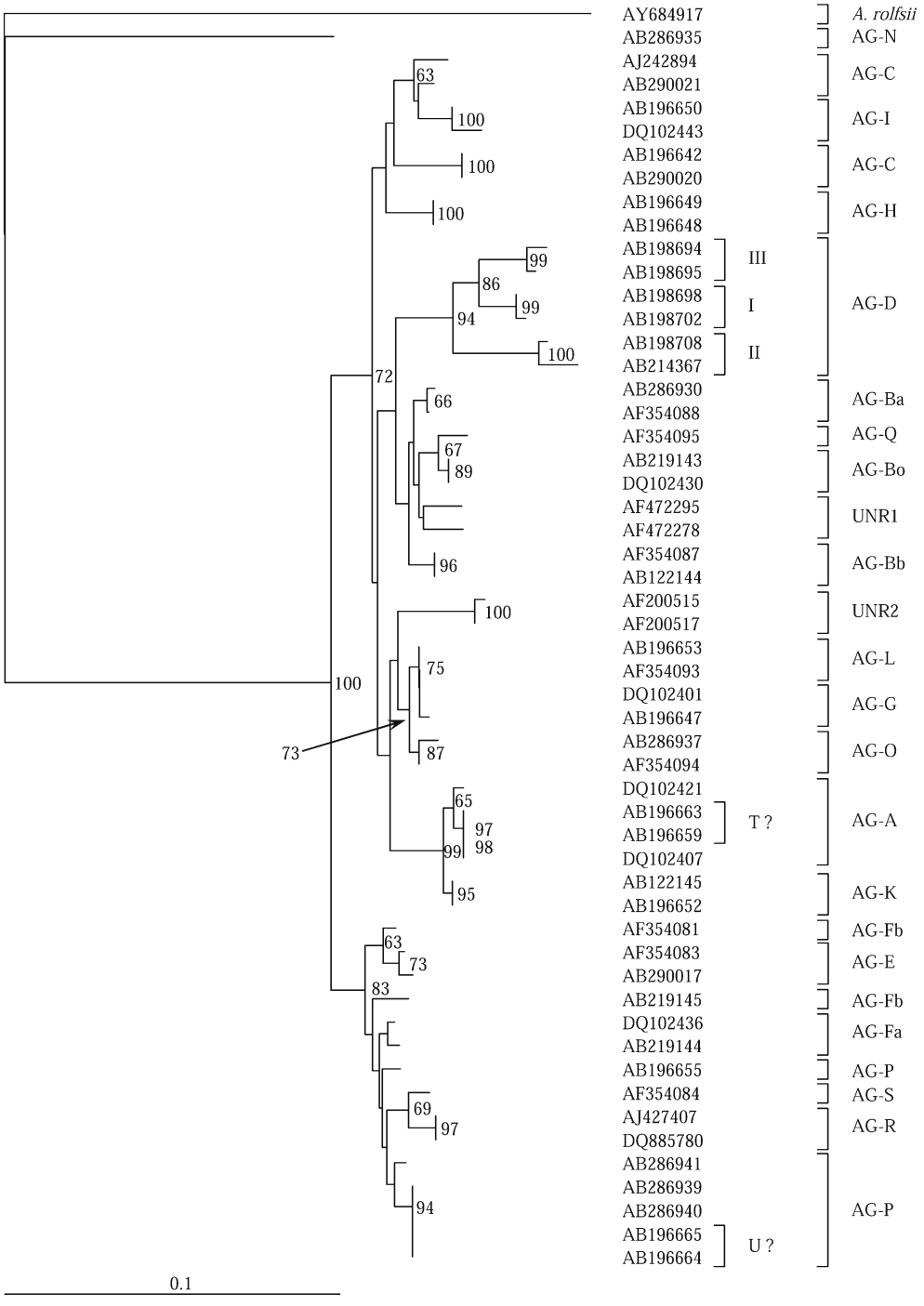

Fig. 4. A neighbor-joining tree of representative AGS isolates of uninucleate and binucleate *Rhizoctonia* spp. isolates (represented by their DNA accession number) clustered according to multiple alignment of rDNA-ITS sequence analysis. (See legend for Fig. 1)

and, thus, a more reliable representative result is higher when sequences of more isolates of the same AG or subgroup are analyzed; this is especially important for analyses of closely related isolates as within *Rhizoctonia* spp. It is rather unfortunate that for some BNR AGs a sequence of only one or few isolates are available, which may render the accuracy and location of these AGs in the tree insufficiently supported.

Although attempts to determine a threshold of percent similarity of rDNA-ITS sequences for differentiating isolates belonging to different AGs or subgroups were made, they could not be consistently obtained because in some instances there was an overlap between the percent sequence similarity ranges for isolates within an AG with the range among the different AGs. A relatively wide % similarity range within an AG may also indicate the possible existence of subgroups, which future research may support. Clusters locations of different AGs in trees based on rDNA-ITS sequence analysis are calculated by the relevant computer program used, yet there are additional differences among

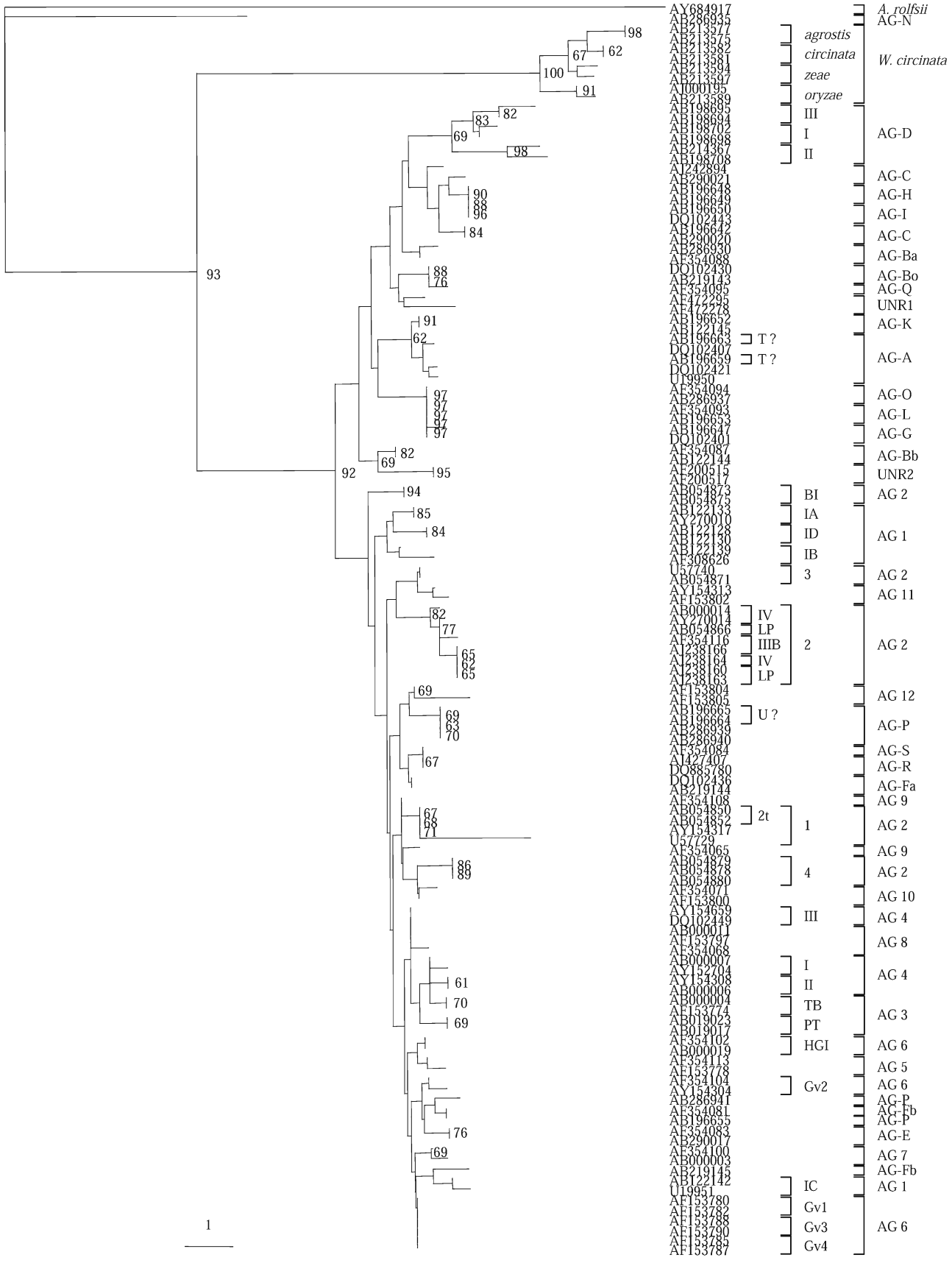

Fig. 5. A neighbor-joining tree of representative AGs isolates (represented by their DNA accession number) of all the uninucleate and binucleate AGs and subgroups (see Fig. 4) and multinucleate AGs and subgroups (Sharon et al. 2006) clustered according to multiple alignment of rDNA-ITS sequence analysis. (See legend for Fig. 1)

Table 4. Pairwise percent similarity of rDNA-ITS sequences of binucleate (BNR) *Tulasnella* spp. isolates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1. AY373267																																			
2. AY373276	100																																		
3. AY373268	100	100																																	
4. AY373270	99	99	99																																
5. AY373273	100	100	99	99																															
6. AY373282	99	99	99	99	99																														
7. AY373307	99	99	99	99	99	99																													
8. AY373311	99	99	99	99	99	99	99																												
9. AY373265	99	99	99	99	99	99	99	99																											
10. AY373269	99	99	99	99	99	98	99	99	99																										
11. AY373314	98	98	98	98	98	98	98	98	98	98																									
12. AY373274	97	97	97	97	98	97	97	97	97	97	97																								
13. AY373264	99	99	99	99	99	98	99	98	98	98	98	97																							
14. AY373263	98	98	98	98	98	98	98	98	98	98	97	96	98																						
15. AY373266	95	95	95	95	95	95	95	95	95	95	94	94	95	95																					
16. AY373301	95	96	95	95	95	95	95	95	95	95	95	94	95	96	95																				
17. AJ313446	90	90	89	89	90	90	90	90	90	90	90	88	90	90	90	90																			
18. AJ313441	89	89	89	89	89	89	89	89	90	89	90	89	90	90	89	89	98																		
19. AJ313440	89	89	89	89	89	89	89	89	89	89	90	88	90	90	89	89	98	100																	
20. AJ313445	89	89	89	89	89	89	89	89	89	89	90	88	90	89	89	88	97	99	99																
21. AJ313451	89	89	88	88	89	89	89	89	89	89	89	88	88	89	89	88	94	95	95	95															
22. AJ313450	88	88	88	88	88	89	88	88	89	89	88	87	88	88	88	88	94	94	95	94	100														
23. AJ313454	89	89	88	88	89	89	89	89	89	89	89	88	88	89	89	88	94	94	95	94	100	99													
24. AJ313455	89	89	88	88	89	89	89	89	89	89	89	88	88	89	88	88	94	94	95	94	100	99	99												
25. AJ313436	87	87	87	87	88	88	88	87	88	88	88	87	88	88	89	88	94	94	94	94	96	96	96	96											
26. AJ313435	87	87	87	87	88	88	88	87	88	88	88	87	88	88	89	88	94	94	95	94	97	96	96	96	98										
27. DQ061111	89	89	89	89	89	89	89	89	89	89	89	87	89	89	88	88	94	94	94	94	94	94	94	94	94	94									
28. DQ061110	89	89	89	89	90	90	90	89	90	90	90	88	90	90	89	89	95	95	95	95	95	95	95	95	95	95	99								
29. AY373298	89	89	89	89	89	89	89	89	90	90	88	90	90	90	90	90	95	95	95	95	95	95	95	95	95	95	97	99							
30. AJ313444	89	89	88	88	89	89	89	89	89	89	88	87	89	89	89	88	91	92	92	92	92	92	92	92	91	92	92	93	93						
31. AJ313442	88	88	88	88	88	88	88	88	88	88	88	86	88	89	88	88	91	92	92	91	90	90	90	90	90	91	92	92	94						
32. AJ313438	88	88	88	87	88	88	88	87	87	88	87	87	88	88	88	88	90	91	91	91	90	90	90	90	89	89	90	91	91	90	90				
33. AJ313443	88	88	88	87	87	88	88	87	88	88	87	86	87	87	87	86	89	89	89	89	88	87	88	87	87	87	88	89	88	89	88	88			
34. AJ313437	90	90	90	90	90	90	90	90	90	91	90	88	90	91	91	90	92	93	93	92	92	92	92	92	91	91	93	94	93	95	93	90	88		
35. AJ313449	90	90	90	89	90	89	90	89	90	90	89	88	90	90	90	90	92	92	92	92	92	92	92	92	92	92	94	95	94	94	94	91	89	95	
36. AJ313448	90	90	90	89	90	89	90	89	90	90	89	88	90	90	90	90	92	92	92	92	92	92	92	92	92	92	94	95	94	94	94	91	89	95	100
37. AJ313439	91	91	90	90	90	90	90	90	90	91	89	89	90	90	90	90	92	92	93	92	92	92	92	92	91	91	94	95	94	94	94	91	89	94	99
38. AJ313453	90	90	90	90	90	89	89	89	90	90	89	88	89	90	90	90	92	92	92	92	92	92	91	92	91	91	93	94	93	92	92	90	88	92	96
39. AJ313452	90	90	89	89	89	89	89	89	89	90	89	87	89	89	90	89	92	92	92	92	91	91	91	91	90	90	92	93	92	92	91	89	88	92	96
40. AJ313447	89	89	89	89	89	89	89	89	89	89	89	87	89	89	88	89	91	91	91	91	91	91	91	91	90	90	92	94	93	93	92	90	88	93	96
41. AY310910	89	89	89	88	89	88	89	89	89	89	88	87	88	89	88	88	90	90	90	90	89	89	89	89	89	89	90	92	91	92	90	89	87	91	95
42. AY373285	91	91	91	90	90	90	91	90	90	91	90	89	90	91	90	90	92	92	92	92	91	91	91	91	91	91	91	93	92	93	91	90	89	93	96
43. AY373299	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	91	92	92	92	91	91	91	91	90	91	91	92	92	93	91	90	89	93	96
44. AY373288	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	92	92	92	92	91	91	91	91	91	91	91	93	92	93	91	90	89	93	96
45. AY373287	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	92	92	92	92	91	91	91	91	91	91	91	93	92	93	91	90	89	93	96
46. AY373286	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	92	92	92	92	91	91	91	91	91	91	91	93	92	93	91	90	89	93	96
47. AY373281	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	92	92	92	92	91	91	91	91	91	91	91	93	92	93	91	90	89	93	96
48. AY373280	90	90	91	90	90	90	90	90	90	91	90	89	90	90	90	90	92	92	92	92	91	91	91	91	91	91	92	93	93	93	91	90	89	93	96
49. AY373289	90	90	90	89	90	89	90	90	90	90	89	88	89	90	89	90	91	92	92	91	91	90	90	90	90	90	91	92	92	93	91	90	89	93	96
50. AY373283	90	90	90	90	90	90	90	90	90	91	90	89	89	90	90	90	91	91	91	91	91	91	91	91	90	91	92	93	93	93	91	90	89	93	96
51. AY373297	76	76	76	76	76	77	76	76	76	76	76	76	76	76	76	76	78	79	79	79	77	77	77	77	76	76	77	78	78	78	78	80	77	79	79
52. AY192442	63	63	63	63	63	63	63	62	63	63	63	63	63	63	63	62	63	63	63	62	62	61	62	61	62	62	59	61	61	62	62	60	64	63	64
53. AY192441	64	64	63	63	64	63	63	63	63	64	64	64	64	64	64	64	63	63	63	62	62	63	63	62	61	62	63	64	62	61	62	61	63	63	63
54. AY373294	63	63	63	63	63	63	63	63	63	63	63	64	62	63	61	62	63	63	63	63	64	64	64	64	62	63	62	63	64	64	63	63	61	63	64
55. DQ457642	62	62	61	61	62	62	62	61	62	62	61	62	61	61	63	63	61	62	61	62	61	61	61	61	61	62	63	65	62	64	63	62	63	62	63
56. AY373316	63	63	63	62	63	63	64	63	63	63	63	64	62	63	62	63	64	64	64	65	63	63	63	64	62	62	62	61	63	63	64	63	64	62	64
57. AY373312	62	62	62	62	62	63	63	62	62	62	62	63	62	63	62	63	64	64	64	65	63	63	63	63	62	61	61	61	62	63	63	63	64	62	64
58. AY373306	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	65	65	65	65	63	63	62	63	63	62	61	61	61	63	65	63	64	62	63
59. AY373302	59	59	59	59	60	60	60	60	60	59	60	60	61	60	61	59	60	61	61	60	60	60	60	60	59	59	61	60	60	60	61	60	60	60	60
60. AY373304	58	58	59	58	58	58	58	59	58	58	59	59	59	59	59	58	60	60	60	60	60	59	59	59	60	60	61	61	60	61	61	60	60	61	60
61. AY373310	60	60	60	60	60	60	60	60	60	60	60	60	60	60	61	61	62	62	62	61	62	62	62	62	61	61	62	63	61	62	63	62	62	62	61
62. AY373300	61	62	61	60	61	61	61	62	61	60	62	61	61	61	62	62	61	62	62	62	62	62	62	62	62	62	62	62	61	63	63	61	60	61	63
63. AY373315	59	61	59	59	60	60	59	61	61	62	61	60	61	60	62	61	63	62	62	61	63	62	62	63	62	62	64	64	61	62	63	62	62	61	62
64. AY373293	55	55	55	54	55	55	55	55	55	55	55	53	54	53	54	54	53	53	53	53	54	54	54	54	53	52	53	53	53	54	53	54	53	54	54
65. AY373292	55	55	55	54	55	55	55	55	55	55	55	54	55	54	55	55	54	54	54	54	55	55	55	55	53	53	53	54	54	54	54	55	54	54	55
66. AY373296	54	54	54	54	53	54	55	54	54	54	55	53	55	54	53	55	53	52	52	52	53	53	52	53	52	52	53	52	53	52	53	54	53	52	54
67. AJ313457	57	58	58	57	58	58	58	58	58	57	57	57	58	57	57	57	56	57	57	57	57	56	57	57	56	57	58	58	57	57	57	57	57	57	58
68. AJ313459	57	58	57	57	57	58	58	57	58	58	58	57	58	57	57	57	57	57	57	58	57	57	57	57	57	57	57	57	57	56	58	57	58	57	58
69. AJ313458	57	57	56	56	58	57	57	57	57	57	57	58	57	57	57	58	57	58	57	58	58	58	58	58	56	57	58	58	57	57	58	56	58	58	57
70. AJ313456	57	58	57	57	58	57	57	57	58	57	57	58	58	57	57	57	57	57	57	58	57	57	57	57	56	57	57	56	56	57	58	56	58	57	58
71. AY643803	54	54	55	55	55	55	54	55	54	54	55	55	55	54	56	54	54	55	55	54	55	54	54	55	56	55	55	56	56	57	56	54	54	55	55
72. AY684917	53	54	53	53	54	54	54	54	53	54	53	53	54	53	53	53	54	54	54	54	54	54	53	54	54	55	54	54	54	54	55	53	53	53	54

range = 94–100%

36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71

range = 86–100%

99																																			
96	96				range = 94–100%																														
96	96	99																																	
96	96	94	94																																
95	95	94	94	95																															
96	96	95	94	95	98																														
96	96	95	94	95	98	100																													
96	96	95	94	95	98	100	100																												
96	96	95	94	95	98	100	100	100																											
96	96	95	94	95	98	100	100	100	100																										
96	96	95	94	95	98	100	100	100	100	100																									
96	97	95	94	95	98	100	100	100	100	100	100																								
96	96	94	94	95	98	99	99	100	100	100	100	99																							
96	96	95	94	95	98	100	100	100	100	100	100	100	99																						
79	80	79	79	80	80	79	79	79	79	79	79	78	78	79																					
64	64	62	62	63	64	64	64	64	64	64	64	64	65	65	62																				
63	64	63	63	64	64	64	64	64	64	64	64	64	64	63	61	96																			
64	63	62	63	64	64	63	63	63	63	63	63	63	62	63	65	59	58																		
63	63	62	62	63	65	64	64	64	64	64	64	64	64	63	67	60	61	86																	
64	64	63	63	62	60	62	61	62	62	62	62	62	61	61	63	61	60	61	61	range = 97–99%															
64	63	63	63	63	62	63	63	64	64	64	64	62	60	61	62	60	60	60	62	97															
63	63	63	63	63	61	62	62	62	62	62	62	64	62	64	62	61	60	61	61	98	99														
60	60	62	62	60	60	60	60	60	60	60	60	60	60	60	62	59	59	65	66	61	61	62	range = 92–97%												
60	60	61	61	60	59	60	60	60	60	60	60	60	60	60	62	59	57	65	65	61	61	62	97												
61	61	61	62	62	60	61	61	61	61	61	61	60	60	61	62	60	60	64	66	62	62	63	93	92											
63	62	62	63	63	60	61	61	61	61	61	61	61	61	61	61	60	59	69	68	62	62	61	84	85	86										
62	62	62	62	64	61	62	62	62	62	62	62	63	62	63	63	61	60	64	63	61	61	61	64	65	65	64									
54	53	53	52	53	53	53	53	54	54	54	54	54	54	53	51	52	52	52	53	54	54	54	51	51	52	52	49	range = 86–99%							
55	54	53	52	53	53	53	53	54	54	54	54	54	54	53	51	53	52	52	53	54	54	53	50	50	52	52	50	99							
54	54	52	52	52	52	53	53	53	53	53	53	52	52	52	49	52	52	51	52	53	53	53	52	51	51	49	50	86	87						
58	58	58	58	57	55	57	57	57	57	57	57	57	56	57	53	57	57	53	53	58	59	58	55	55	54	55	56	54	53	52	range = 98–99%				
58	58	58	58	57	56	57	57	57	57	57	57	57	57	57	54	58	58	54	54	58	59	58	57	56	55	55	56	55	53	52	98				
57	57	58	59	57	56	57	57	57	57	57	57	57	57	57	54	58	58	54	54	58	60	58	57	57	56	54	56	54	53	52	98	99			
58	58	58	58	57	55	56	56	57	57	57	57	56	56	56	54	58	59	54	53	58	58	58	57	58	56	55	56	54	52	52	98	99	99		
55	55	55	55	55	55	55	55	55	55	55	55	55	56	55	53	54	54	55	55	55	55	55	56	56	57	56	54	48	51	50	62	63	63	63	
54	54	55	55	54	54	53	53	53	53	53	53	54	54	54	54	53	55	53	53	54	53	53	54	54	55	53	54	54	47	47	48	59	59	59	65

Table 5. Pairwise percent similarity ranges of rDNA-ITS sequences of uninucleate (UNR), binucleate (BNR) *Ceratobasidium* spp., and multinucleate (MNR) *Thanatephorus* spp. and *Waitea circinata* ssp. AGs and subgroups

AG	A	B	Ba	Bb	Bo	C	D	D(I)	D(II)	D(III)	E	F	Fa	Fb
1	77–85	76–84	79–85	78–81	76–84	75–82	74–81	77–81	74–81	75–81	82–91	83–91	83–91	83–90
1 IA	80–85	78–84	81–84	78–81	81–83	77–81	77–80	78–80	77–80	77–80	86–90	87–91	88–91	87–89
1 IB	77–82	79–83	79–83	79–81	79–81	75–79	75–80	77–80	75–80	75–78	82–88	83–89	83–89	83–87
1 IC	80–84	78–81	81–85	78–81	81–84	78–82	75–81	79–81	75–81	76–81	86–91	87–91	87–91	87–90
1 ID	77–81	76–81	79–80	79–81	76–79	75–78	74–78	78	74–78	76–78	83–86	83–87	83–87	85
2	75–84	77–85	77–85	78–82	77–82	74–82	73–82	75–82	73–81	73–80	83–91	82–92	84–92	82–91
2–1	75–83	77–83	77–83	78–82	77–81	74–82	73–80	76–80	73–79	73–79	84–91	82–91	84–91	82–90
2–12t	77–81	81–83	81–83	81–82	81	77–81	76–80	79–80	76–78	77–78	88–91	88–90	88–90	88–89
2–2	75–80	78–82	78–81	78–82	78–80	74–78	74–78	75–78	74–78	74–77	83–87	84–87	84–87	85–87
2–2 IIIB	75–80	78–82	78–81	78–82	78–80	74–78	74–78	75–78	74–78	74–77	83–87	84–87	84–87	85–87
2–2 IV	75–79	78–82	78–80	81–82	79–80	74–78	74–77	76–77	74–77	75–77	84–87	85–87	85–87	85–87
2–2 LP	76–80	78–82	78–81	81–82	79–80	74–78	74–78	75–78	75–77	74–77	83–87	84–87	84–87	85–87
2–3	78–84	78–85	81–85	78–81	81–82	78–82	77–82	79–82	77–81	77–80	86–91	87–92	87–92	88–91
2–4	78–81	80–82	81–82	80–81	80	76–80	76–80	79–80	76–79	76–78	87–89	86–89	86–89	88–89
BI	73–79	77–81	78–79	79–81	77–79	73–76	74–78	76–78	73–77	74–76	81–85	82–85	82–84	83–85
3	78–82	79–83	80–83	79–81	79–83	77–81	75–81	78–81	75–80	75–79	86–89	87–90	87–90	87–90
3 TB	78–82	80–83	82–83	80–81	82–83	77–80	76–81	80–81	77–80	76–78	87–89	87–90	87–90	89–90
3 PT	78–82	79–82	80–82	79–81	79–81	77–81	75–80	78–80	75–79	75–79	86–89	87–90	87–90	87–89
4	77–82	77–82	79–82	77–81	78–82	74–80	74–80	76–80	74–80	75–80	85–91	86–90	86–90	86–90
4 HGI	77–82	77–82	79–82	77–80	79–82	75–80	74–80	77–80	74–77	76–80	86–91	86–90	87–81	86–90
4 HGII	78–81	78–81	80–81	78–80	80–81	74–79	76–80	79–80	76–80	77–79	87–91	87–90	88–90	87–89
4 HGIII	77–81	78–81	79–80	78–81	78–79	75–78	75–80	76–77	76–80	75–77	85–86	86–89	86–89	86–87
5	80–84	78–85	82–85	78–81	82–84	78–82	77–81	80–81	77–81	78–81	88–92	89–93	89–93	91–92
6	81–87	78–87	82–87	78–81	84–86	79–84	76–82	78–82	76–82	78–82	89–94	87–96	87–95	91–96
6 HGI	81–85	78–85	84–85	78–80	83–84	80–83	78–81	80–82	78–80	79–81	91–93	90–95	90–93	95
6 Gv1	81–86	7685	82–85	76–78	84–85	79–83	76–82	78–82	76–81	77–81	89–93	87–95	87–93	91–95
6 Gv2	82–86	79–86	84–86	79–80	84–85	80–83	78–82	80–82	78–82	79–82	92–94	90–96	90–93	95–96
6 Gv3	83–87	80–87	85–87	80–81	86	81–84	78–82	81–82	79–82	78–81	92–94	92–96	92–95	96
6 Gv4	82–86	79–87	85–87	79–81	85–86	81–84	79–82	81–82	79–82	79–82	91–94	91–96	91–94	96
7	81–85	78–86	83–86	78–80	82–84	79–84	78–82	80–82	78–82	78–81	90–96	90–94	90–94	91–93
8	81–86	78–87	85–87	78–80	85–86	80–85	80–83	81–83	80–81	80–82	91–94	90–95	90–93	92–95
9	77–82	79–84	81–84	79–83	80–83	78–82	76–81	78–81	76–79	76–78	86–91	88–91	88–91	88–90
10	79–83	7884	82–84	78–81	81–82	77–81	76–80	79–80	76–79	76–79	87–90	87–90	87–90	88–90
11	77–82	78–83	80–83	78–81	80–82	77–81	76–81	79–81	77–80	76–77	85–88	87–89	87–89	87–89
12	79–83	80–84	81–84	80–81	81–82	78–81	76–80	77–80	76–80	77–80	85–90	88–93	88–93	89–91
C. zeae	68–72	61–71	69–71	61–65	70–71	69–71	66–71	66–70	66–69	68–71	67–70	66–70	66–70	67–68
C. agrostis	69–73	64–72	70–72	64–67	71–72	69–72	67–71	69–71	67–70	69–71	67–71	67–69	67–69	68–69
C. circinata	69–73	63–69	68–69	63–65	68–69	69–72	66–71	67–70	66–69	68–71	67–70	66–70	66–70	68–70
C. oryzae	68–72	64–70	69–70	64–66	69–70	68–71	65–70	67–69	65–69	67–70	67–69	66–70	66–70	67–70

the isolates that are not expressed only by the % sequence similarity which are exhibited in the trees.

Isolates of AG-K are genetically closely related to AG-A isolates. The isolate sequences of AG-K are located in an adjacent cluster to the AG-A cluster (see Figs. 1, 3, 4–6). However in our previous study (Sharon et al. 2007) and in some of the trees (not shown) the isolate sequences of AG-K were actually located within the AG-A cluster. The % sequence similarity between these two AGs ranged from 92% to 96%. Compared to the range of subgroups [within AG 4, 88%–97% (Sharon et al. 2006); or within AG-B, 82%–94%)], AG-K sequences are within the range of a subgroup of AG-A (the range within AG-A was 93%–100%, while within AG-K it was 100%). The lack of anastomosis between isolates of these AGs may indicate that they are distinctly separate AGs (may be only the result of minor mutations in genes regulating the hyphal fusion), although genetically closely related.

The percentages of rDNA-ITS sequence similarity ranges within some AGs are rather wide: AG-B (82%–100%), AG-D (84%–100%) (Table 3), AG 2 (81%–100%), AG 4 (88%–97%), AG 6 (90%–100%), and AG 1 (91%–100%) (Sharon et al. 2006). It stems from the existence of distinct subgroups that have already been recognized, in which the % sequence similarity ranges within their subgroups are narrower: AG-Ba (98%–100%), AG-Bb (97%), AG-B (o) (99%–100%), AG-DI (97%–100%), AG-DII (93%–96%), AG-DIII (95%–100%) (Table 3), AG 1-IA (96%–100%), AG 1-IC (96%–100%), AG 4 HG-I (94%–100%), AG 4 HG-II (98%–100%), and AG 4 HG-III (99%–100%) (Sharon et al. 2006). Future research using more isolate sequences may change the data obtained for the currently available isolates and indicate the existence of subgroups within the AGs, in which the % similarity range among their isolates is relatively wide, such as AG-C (87%–98%), AG-P (90%–100%), and AG-F (90%–100%).

The AG-F isolates are located in two distinctly separate clusters in the trees (see Figs. 1, 3–6). As anastomosis frequency between representative isolates of these two subclusters was only <30% (Kuninaga, unpublished data), and % sequence similarity range between isolates of the subclusters was relatively low (90%–92%), opposed to the

G	H	I	K	L	N	O	P	Q	R	S	T ?	U ?	UNI1	UNI2
76–84	75–81	76–82	80–85	79–85	64–67	79–85	83–89	79–83	84–90	79–83	79–84	83–90	78–84	79–85
80–84	78–80	79–81	83–85	83–85	65–66	83–85	87–89	81–83	87–90	80–83	82–84	87–89	80–82	81–83
76–81	75–78	76–79	80–82	79–82	64–66	79–82	83–89	80–82	84–88	79–81	79–81	83–86	78–81	79–82
80–84	77–81	79–82	82–84	82–85	65–67	83–85	86–89	81–83	85–90	81–83	81–84	86–90	81–84	83–85
77–80	76–77	77–78	80–81	79–80	64	80	83–87	79	85–86	79	80–81	84	78–79	79
75–84	75–80	75–81	78–85	78–84	62–67	78–84	81–88	78–83	84–89	77–82	77–83	81–87	75–83	77–82
75–82	75–80	76–81	78–83	78–83	62–66	78–83	81–88	78–82	84–89	77–81	77–82	81–86	75–82	78–82
78–81	78–79	80	82	81	65	81	85–88	81	88	80	80	85	79–81	81
76–80	75–78	75–78	78–80	78–80	63–65	78–80	83–86	79–80	84–87	78–80	77–79	81–84	76–79	77–79
76–80	75–77	75–78	79–80	78–80	63–65	78–80	83–86	79–80	85–87	78–80	77–79	81–83	76–79	77–79
77–80	75–77	76–78	78–79	79–80	63–64	78–79	83–86	79–80	84–86	79	77–78	81–84	76–78	77–78
76–80	75–78	76–78	78–80	79–80	63–64	79–80	83–86	79–80	84–87	78–80	77–79	81–83	76–79	77–78
79–84	77–80	78–81	81–85	81–84	66–67	82–84	85–88	81–83	86–89	80–82	81–83	84–87	80–83	80–82
78–80	77–78	78–80	81	81	65	81	85–87	82	87	79–80	80–81	84	79–80	81
74–78	74–77	75–76	77–80	78–79	61–62	78–79	80–84	79–80	82–84	77–79	76–78	79–80	76–78	77–78
77–82	77–80	78–80	81–82	81–82	63–66	81–82	84–88	81–83	86–88	79–82	79–82	84–85	78–81	79–82
79–82	78–80	79–80	82	82	66	82	85–88	83	88	82	81–82	85	81	81–82
77–81	77–80	78–80	81–82	81	63–65	81–82	84–88	81–82	86–87	79–80	79–82	84–85	78–81	79–80
77–83	75–80	77–80	80–82	80–83	63–67	81–83	85–89	79–82	85–90	79–81	79–81	84–87	78–82	79–82
77–83	77–80	77–80	80–82	81–83	66–67	81–83	86–89	80–82	86–90	79–81	80–81	84–87	78–82	80–82
78–81	76–79	78–79	81–82	81–82	65–66	81–82	86–88	81	86–89	80	80–81	84–85	80–81	80–82
78–81	75–79	77–78	80–81	80–81	63–64	81–82	85–87	79–80	85–88	79	79–80	84	78–79	79–80
81–85	79–82	81–82	84–85	83–85	65–68	84–85	88–89	82–83	87–90	82–83	82–83	86–88	81–83	82–84
81–87	80–83	81–84	84–86	85–87	66–69	86–88	86–92	80–83	84–93	83–85	84–86	88–92	82–85	84–88
83–87	80–82	81–83	86	86	67–69	86	89–91	81	88–92	83	84	88–89	82–84	84–85
81–87	80–83	81–83	84–86	85–87	67–68	86–87	86–91	80–82	84–92	84	84–86	89–92	82–84	82–85
83–87	81–83	82–83	86	87	68	87	89–90	82	88–92	84	85–86	89	83–85	85–86
84–87	81–83	83–84	87	87–88	66	88	90–92	83	88–93	85	85–86	90	84–85	86–87
83–86	81–83	82–83	86	87	67–68	87	90–91	82	88–92	84–85	84–85	89–90	83–84	85–86
82–86	80–82	81–83	84–86	85–86	68–69	85–86	89–91	82	87–92	82–83	83–84	91–89	82–84	82–84
83–87	81–83	83–85	85–86	86–87	69	86–87	88–91	81	86–91	85	84–85	89–91	83–85	83–85
78–83	78–81	79–82	81–82	81–83	63–65	81–84	86–88	81–84	86–89	80–82	80–82	85–86	79–82	80–83
78–82	78–80	79–81	81–82	82–83	64–65	82–83	85–87	82–83	87–88	80–82	81–82	84–86	79–81	80–81
79–83	77–79	79–80	82–83	81–82	65	81–82	84–88	80–82	85–87	79–81	80–82	83–84	79–82	79–82
80–84	77–80	78–80	82–84	83–85	67–68	83–85	87–93	80–82	88–91	80–82	81–82	85–88	80–82	81–82
70–72	68–71	68–71	70–71	70–72	70	70–71	66–68	67	66–69	70–72	70–71	68–69	69–71	67–69
71–73	69–72	70–72	71–72	71–72	69–70	72	66–68	67–68	66–70	71–72	71–72	67–69	71–72	69–71
71–73	69–72	70–72	71–72	72–73	70	72–73	65–66	66–67	66–69	69	71–72	66–69	68–70	67–71
69–72	69–71	69–71	70–72	70–71	68–69	70	64–67	65	64–69	69–70	70	67–69	68–70	68–69

higher % similarity within the subclusters (94%–100%) (see Table 3), it may be suggested to designate these groups of isolates as representatives of two AG-F subgroups, namely, AG-Fa and AG-Fb, respectively. More research is needed to substantiate this suggestion.

Ogoshi has already indicated that some anastomosis was evident between isolates of AG-C and AG-I [and he had already suggested that the isolates of these two AGs should belong to AG-C (Sneh et al. 1991)]. In the tree of Fig. 1, the AG-I isolates are in two clusters among three AG-C clusters (the AG-H cluster is also among them). A detailed anastomosis testing of a series of AG-C and AG-I isolates (Naito, unpublished data) indicated that isolates within and between these two designated AGs anastomosed in various fusion frequencies with each other, from high to very low fusion frequencies, or even no fusion at all. The % sequence similarity range among isolates within AG-I was 94%–100%, while that within AG-C was 87%–98%. The % sequence similarity between AG-I and AG-C was 86%–100%, indicating that [similarly to AG-P and AG-U (see earlier)] AG-C may be a variable group, which may include more than one subgroup that also includes the AG-I designated isolates. Future research with more isolate sequences and validation of anastomosis testing of both AGs may be required to support the designations of these two AGs into subgroups of one AG. AGs -C, -H, and -I are closely related. The % sequence similarity ranges between -C and -I is 86%–100%, between -H and -I is 91%–95%, and between -C and -H is 88%–94%.

It was also suggested to rename AG-B(o) as AG-Bc (Ogoshi and Kuninaga, personal communication). The “o” of AG-B(o) means “others” and was designated to indicate that, in contrast to AG-Ba and AG-Bb, which include isolates of similar cultural appearance, the AG-B(o) may include more variable isolates and is therefore likely to include more than one subgroup. Currently, there are only a few isolate sequences of the genuine AG-B(o) in GenBank. These isolates are genetically closely related (99%–100% sequence similarity) although they have been isolated from different locations (Sneh and Ichielevich-Auster 1998). Additional molecular work with many more isolates is needed to verify whether this subgroup should be divided

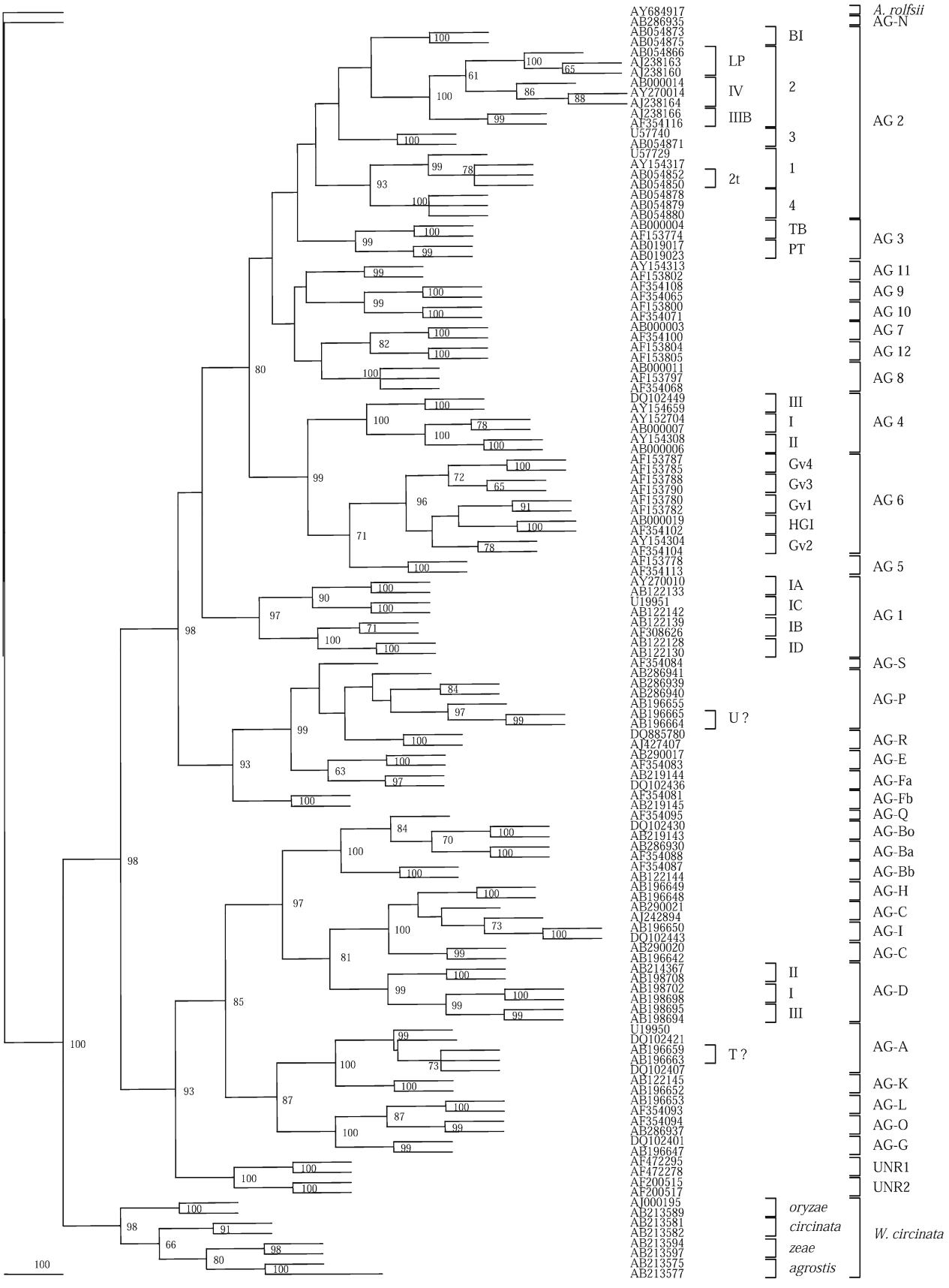

Fig. 6. A consensus tree assembling 1205 most parsimonious trees composed of rDNA-ITS sequences of representative AGs isolates (represented by their DNA accession number) of all the uninucleate and binucleate AGs and subgroups (see Fig. 4) and multinucleate AGs and subgroups (Sharon et al. 2006). Bootstrap of 113 trials are positioned alongside the branches where values exceed 60. Isolate AY684917 [*Athelia* (*Sclerotium*) *rolfsii*] was used as an outgroup. The AGs and subgroups for the clusters are indicated. *Bar* indicates one change

into additional subgroups (AG-Bc, AG-Bd, etc.). However, AG-B(o) is more closely related to AG-Ba (92%–94%) than to AG-Bb (82%–84%). The sequence similarity between AG-Ba to AG-Bb is lower (84%–86%). AG-B(o) is also close to UNR1 (94%–95%) and to a lesser extent to AG-Q (90%–91%). The UNR1 and AG-Q clusters are located among the clusters of the AG-B subgroups (see also Figs. 1, 3–6). Some nonpathogenic *Rhizoctonia* isolates, which colonize plant roots and protect them against infection by pathogenic *Rhizoctonia*, belong to AG-B(o) (Sneh and Ichielevich-Auster 1998; Sharon et al. 2007). It is interesting to note that UNR1 isolates, which are reported as mycorrhizal with orchid roots, are genetically closer to AG-B(o) than to the pathogenic UNR2 isolates (94%–95% compared to 83%–84%, respectively) (Table 3, Figs. 3–5). AG-S is closely related to AG-F (91%–93% sequence similarity), AG-P (91%–92%), and AG-R (91%–92%).

The rDNA-ITS sequence of the AG-N representative isolate STC43 is located at a great distance from all the other BNR AGs in the tree (see Figs. 1, 3–6). Furthermore, its sequence similarity range to the other BNR AGs is only 61%–72%, which is close to that of the outgroup isolate of *Athelia* (*Sclerotium*) *rolfsii*, which is a different genus (61%–65%). Also, the teleomorph of the AG-N isolate is unknown (Sneh et al. 1991), which may indicate that if this isolate is the valid representative of AG-N, then this AG may also be considered to be excluded from BNR (*Ceratobasidium*).

Groups of closely related BNR AGs

The locations of BNR isolate sequences in the clusters consistently supported the validity of the anastomosis groups, confirming that anastomosis grouping of BNR is also solidly based on genetic relatedness. During the analyses of the large number of data into NJ trees, it was evident that adding or subtracting a number of isolate sequences and/or exchanging the isolate sequence of the outgroup sometimes cause changes in the relative position and order of certain isolate sequences, clusters, or subclusters in the trees (see also differences between Figs. 1 and 3–5). Thus, the clusters of various AGs were also changing their relative locations along the tree. In some cases, it also caused a splitting of the isolate sequences of certain AGs to two distant locations, such as AG-A and AG-B (Fig. 1), AG-E and AG-F (Fig. 3), and AG-B and AG-P (Fig. 5). Clusters locations of the different MNR AGs in the MP tree (Sharon et al. 2006) were changed in the tree that included also the BNR + UNR AGs clusters (see Fig. 6). The positions of the isolate sequences and AGs in the clusters of the MP trees were kept more stable in response to such additions or deletions than in the NJ trees (see also Fig. 5 compared to Fig. 6), although the NJ analyses trees were frequently supported by corresponding MP analyses trees.

In some publications, attempts have been made to include several closely related AGs that are located in major clusters into groups of AGs. The rDNA-ITS sequence analyses of *Ceratobasidium* species were thus grouped into groups of AGs from 1 to 9 (Gonzalez et al. 2002) and the BNR and MNR AGs from 1 to 12 groups of AGs (Gonzalez et al. 2001). As the location order of the AGs may differ in various trees, it may be inappropriate to designate the groups of AGs with a sequential number order, because the order for the same groups will be different in various publications. From the gathered information summarized in the present review, the clusters of the BNR AGs are located in the NJ tree in five such groups of AG (see Figs. 1, 4):

AGs -A and -K are located in one group
AGs -G, -L, and -O are located in another group
AGs -E, -F, -P (and -U), -R, and -S are located in another group
AG-D (including its three subgroups) is located as a separate group
AGs -B (including its three subgroups) and -Q are in another group
AGs -C, -H, and -I are in another group

One of the two uninucleate *Rhizoctonia* clusters, UNR2 [the pine seedling pathogenic isolate sequences (Hietala 1995; Hietala and Sen 1996; Hietala et al. 1994, 2001)], was located near the AGs -A, -K, -G, -L, and -O groups, while the other cluster of UNR1 [the orchid endophytes (Otero et al. 2002)] was located outside of all the others (see Fig. 1). However, addition or subtraction of isolates from the alignment caused a change in the locations of these two UNR groups as well as others in the other trees (Figs. 1, 3–6). The relatively low % sequence similarity range between these two UNR clusters (82%–85%) indicates that these two clusters represent isolates of distinctly separate groups. The UNR2 isolates pathogenic to pine were identified as *Ceratobasidium bicorne*, while attempts to generate the teleomorph of the orchid endophytic isolates failed (Otero et al. 2002). Consequently, additional work is needed to substantiate the teleomorph taxonomic status of the UNR1 group. It would be important to clarify whether UNR1 is also *C. bicorne* or of a different teleomorph species. Differences in percent sequence similarity ranges among the groups may provide more important information on the closer or more distant genetic relatedness among the different AGs and complement the information obtained form the relative locations of the clusters in the trees.

The clusters of *Tulasnella* spp. groups (designated as TG-A to TG-F) are located on a separate cluster, which is considerably distant from all the other *Rhizoctonia* spp. groups (Fig. 3), as is also expressed in the low % sequence similarity range between *Tulasnella* spp. and the rest of the *Rhizoctonia* spp. (45%–62% with the other BNR AGs and 47%–60% with the UNR groups). As the locations of the different groups of AGs change in different trees, attempts to establish a genetically related justified group order may be more accurate if additional sequence analysis data will be considered. Differences in percent sequence similarity ranges among the groups may be one of these approaches taking into consideration that there is an overlap in % sequence similarity analysis among some groups.

Isolate sequences that were inaccurately designated in GenBank and in publications

Sequences deposited in GenBank are sometimes inaccurately designated to certain isolates or AGs. Such errors and using GenBank rDNA-ITS sequences for analyses of only one or two isolates per group may sometimes be misleading and consequently lead to wrong conclusions, as have already been widely reported in some past publications. Including rDNA-ITS sequences of most of the currently available isolates in GenBank in the analyses for the present review enabled tracing such inaccuracies and errors of certain deposited isolate sequences in GenBank. It is clearly evident from the location of some isolates marked within or among the clusters in Fig. 1 that they have been inaccurately designated in GenBank. For some of them it is clear to which AG their sequences may belong, while others still remain questionable, and future research may clarify with greater certainty to which AG they actually do belong. Some of the following examples have already been indicated by Sharon et al. (2007) and summarized in Table 1 (see also Fig. 1).

The rDNA-ITS sequence of isolate BN-37(gb) was deposited in GenBank by Gonzalez et al. (2002) as a representative of AG-R (synonym CAG-5), while its sequence was clearly located within the AG-F cluster. Moreover, the accurate ITS sequence of the original isolate BN-37 (AG-R, performed by Kuninaga) is located in a separate location in the tree for AG-R.

The isolate SIR2 was distributed among researchers as the AG-B(o) representative (Sneh et al. 1991) but the rDNA-ITS sequence deposited in GenBank for this isolate is of an unknown AG-A isolate (see Fig. 1). In the phylogenetic tree of Gonzalez et al. (2001) this isolate was also located on the same cluster of an AG-A isolate, but the authors did not comment in their paper on its unclear location in this tree. Isolate CFM1 (Kasiamdari et al. 2002) is similarly inaccurately designated as belonging to AG-B(o). The error undoubtedly occurred because of the distribution by mistake of an unknown AG-A isolate from Ogoshi's culture collection among *Rhizoctonia* researchers instead of the original SIR2 isolate (Ogoshi, personal communication). This AG-A isolate was used as the AG-B(o) representative for anastomosis with new and unknown BNR isolates. The rDNA-ITS sequence of isolate C-302 [a correct representative isolate for AG-B(o)] was clustered with two nonpathogenic isolates, RU89-1 and RU18-1 (DQ102430 and DQ102431, respectively), which anastomosed with this isolate and had a high sequence similarity (98%) to its sequence and were closely located in the tree, belong to the same AG. Isolate RU89-1 was already previously identified as an AG-B(o) isolate (Sneh and Ichielevich-Auster 1998).

Isolate AV2 is the representative isolate for AG-I (Sneh et al. 1991), and its sequence is also located with the other AG-I isolates in the same cluster (Fig. 1; Sharon et al. 2007). In GenBank, there are three accession numbers for the AV2 isolate. The following two of these, AJ419932 (Gronberg et al. 2003) and AB196650 (Hyakumachi et al. 2005), are identical and of the correct AV2 isolate. However, the other sequence, AJ242898 for AV2, designated also as belonging to AG-I (Gonzalez et al. 2002), is distinctly different. This sequence was also used in another study as an AG-I isolate, but it clustered with two AG-L isolates (Hyakumachi et al. 2005). The % sequence similarity for this unknown isolate with the AG-L isolates is only 95%, whereas it has a 96%–97% similarity to AG-G (Sharon et al. 2007), indicating that it may actually belongs to AG-G. Isolates S5 and AGS (AJ427400 and AB196656, respectively; Gonzales et al. 2001, 2002) were designated in GenBank as AG-S isolates. The locations of these two isolates in the NJ tree (Fig. 1) is with the AG-B(o) cluster, and their % sequence similarity with those of AG-B(o) isolate sequences is 99%; this indicates that these isolates actually belong to AG-B(o).

The nonpathogenic isolate Rh521 was inaccurately identified previously as an AG 4 (MNR) isolate (Ichielevich-Auster et al. 1985; Sneh and Ichielevich-Auster 1998) and, subsequently, as a BNR isolate of an undefined AG (Salazar et al. 2000; Gonzalez et al. 2001, 2002). Isolate RU56-8 was inaccurately identified as an AG-P isolate (Sneh and Ichielevich-Auster 1998). Both isolates were accurately identified as AG-A isolates via hyphal fusion and rDNA-ITS sequence analysis by Sharon et al. (2007; see also Fig. 1).

Hyakumachi et al. (2005) reported on two new BNR AGs according to their phylogenetic tree based on the rDNA-ITS sequence analysis: AG-T (the rose cut flower root and stem rot pathogen, AG-CUT) and AG-U (the miniature rose pathogen, AG-Min). As already described, the isolates of AG-T (which were actually located in the same cluster of AG-A in their original publication) were confirmed to belong to AG-A, indicating that these isolates were inaccurately designated as AG-T in GenBank, and the AG-U isolates may actually belong to the heterogeneous AG-P.

rDNA-ITS sequence analysis of endophytic mycorrhizal BNR (teleomorph *Tulasnella* spp.) and UNR (teleomorph *Ceratobasidium bicorne* and possibly additional *Ceratobasidium* spp.)

The involvement of endophytic *Rhizoctonia* spp. (including UNR, BNR, and MNR isolates) in mycorrhizal associations with orchids as well as of many other fungal species is widely documented (Warcup and Talbot 1971; Currah et al. 1987; Andersen and Stalpers 1994; Andersen and Rasmussen 1996; Zelmer et al. 1996; Currah et al. 1997; Carling et al. 1999; Sen et al. 1999; Otero et al. 2002; Rasmussen 2002; Ma et al. 2003; McCormick et al. 2004). In addition to the beneficial endophytic *Rhizoctonia* spp. in orchids, there are also beneficial nonpathogenic *Rhizoctonia* spp. that belong to several BNR (Sneh et al. 1986; Harris et al. 1994; Sneh 1996, 1998) and MNR AGs (Bandy and Tavantzis 1990) which colonize a wide variety of crop plants and are

involved in protection against plant pathogens and in plant growth promotion.

Moore (1987, 1996) and Sneh et al. (1991) suggested renaming the "form genus" anamorph *Rhizoctonia* to (1) *Moniliopsis* for the MNR teleomorph *Thanatephorus* [but this genus was proposed for conservation as *Rhizoctonia* by Stalpers and Andersen (1996)]; (2) *Chrysorhiza* for the MNR teleomorph *Waitea*; (3) *Ceratorhiza* for the BNR teleomorph *Ceratobasidium*; (4) *Epulorhiza* for the BNR teleomorph of *Tulasnella*; and (5) *Opadorhiza* for the BNR teleomorph of *Sebacina*. Although the new nomenclature for the "form genus" *Rhizoctonia* was not generally used by researchers working in recent years with *Rhizoctonia* spp. as plant pathogens, it has been more commonly used by the researchers working on orchid endophytic *Rhizoctonia* spp. belonging to *Tulasnella* spp. and *Ceratobasidium* spp. (Andersen and Rasmussen 1996; Currah et al. 1997; Otero et al. 2002; Ma et al. 2003; McCormick et al. 2004). *Sebacina* spp. is also an orchid endophyte (Warcup 1988; Sneh et al. 1991; Andersen and Rasmussen 1996; Bougoure et al. 2005).

Of the 31 *Tulasnella* species identified (Stalpers and Andersen 1996), the following mycorrhitic species have been used so far in molecular identification studies (unfortunately, usually only one isolate per species): *T. albida*, *T. allantospora, T. assymetrica, T. bifrons, T. cruciata, T. danica, T. deliquenscens* (= *calospora*), *T. eichleriana, T. irregularis, T. pruinosa, T. tomaculum*, and *T. violea* (McCormick et al. 2004). The identification of the following anamorph species of *Epulorhiza* was based on morphological and cultural characteristics such as shape and dimension of monilioid cells: *E. albertaensis* and *E. anaticula* (Currah et al. 1990), *E. calendulina* (Zelmer et al. 1996), *E. inquilina* (Currah et al. 1997), and *E. repens* (Moore 1987). *E. repens* is the anamorph of *Tulasnella deliquescens*. It was previously considered to be *T. calospora* as a consequence of misidentification by Warcup and Talbot (1967) (Roberts 1994; Andersen and Rasmussen 1996; Moore 1996).

Ogoshi designated initially two anastomosis groups in *R. repens*: Rr1 and Rr2 (Sneh et al. 1991). However, subsequent work on the identification and classification of *Tulasnella* spp., as well as most of the other endophytic *Rhizoctonia* spp., did not include any hyphal fusion tests for anastomosis groups [as was basically performed for the other MNR (*Thanatephorus* and *Waitea*) and BNR (*Ceratobasidium*) isolates (Carling 1996; Sharon et al. 2006)], but only with molecular methods, such as the rDNA-ITS or mtLSU sequence analyses (Otero et al. 2002; Ma et al. 2003; McCormick et al. 2004). Furthermore, currently, the comparative genetic relatedness of this group with the other known *Rhizoctonia* AGs can be evaluated only by sequence analyses of isolates available in GenBank. This section of the review is therefore focused on the classification of isolates belonging to *Tulasnella* spp. by rDNA-ITS sequence analysis.

Molecular identification of orchid endophytes was carried out using RFLP of polymerase chain reaction (PCR)-amplified rDNA-ITS regions and mitochondrial large subunit (mtLSU) and rDNA-ITS sequence analysis methods (Otero et al. 2002; Ma et al. 2003; McCormick et al. 2004; Bougoure et al. 2005). The information in each of these publications provided limited and scattered separate cluster information on the isolates investigated, with very little integration of the information in a more comprehensive manner on the subject. Consequently, the relatively few reports could not provide an overview that might have included information on the others. As the rDNA-ITS sequence analysis method is currently the most appropriate one for comprehensive identification and classification of *Rhizoctonia* spp. (Sharon et al. 2006), attempts were made to summarize the GenBank data generated by this method in the present section. As only a few studies dealt with the rDNA-ITS sequence analysis of *Epulorhiza*, these data relate only to a small proportion of the 31 designated species of *Tulasnella.* A considerable proportion of the sequences were not identified to belong to any significant group. Therefore, the summary presented in this section should be considered only as an introduction to the identification of *Tulasnella* spp.

Quite a few of the groups that are considerably genetically distant from each other (according to their % rDNA-ITS sequence similarity, Table 4) are represented by a sequence of only a few isolates. Therefore, the probability of inaccurate designation of the isolates and their sequences may be relatively high. More research work with many more isolates of the same groups is needed, and is expected to support or modify the current summarized data, yielding more reliable identifications on new groups and subgroups of *Tulasnella* spp.

The results of the rDNA-ITS sequence analysis and percent sequence similarity of *Tulasnella* spp. isolates available in GenBank are summarized in Fig. 2 and Table 4. Some of the isolate sequences available in GenBank could not be included in this analysis because a critical section of the ITS region was missing in their sequences. Five major clusters of "*Tulasnella* groups" (TG-A to TG-F) are located in the NJ tree (Fig. 2). The percent sequence similarity ranges among these TGs are relatively very low (51%–65%) compared with the range (75%–95%) among the other BNR + UNR AGs (Table 3) and (78%–94%) among the *Thanatephorus* MNR AGs. The range between the *Waitea circinata* vars. and the *Thanatephorus* AGs was also relatively low (63%–70%) (Sharon et al. 2006). The low % sequence similarity range among all the *Tulasnella* groups compared to that from between them to the outgroup *Athelia rolfsii* (47%–59%) indicates that these groups are relatively genetically distant from each other. In addition to the major clusters some single isolates (accession numbers AY643803, AY373294, AY373315, AY373327, AY373300, DQ457642) are distantly related genetically, to the isolates of these in the clusters, as indicated by their % sequence similarity to all the other isolates (in the range of 49%–65% and one isolate, somewhat higher range, 54%–79%). These low similarity ranges may indicate that each of these isolates is likely to be a representative of a different and distinctly distant group.

Cluster TG-F includes the greatest number of TGs isolate sequences currently available in GenBank. The % sequence similarity range among the five subclusters of TG-F (TG-F1 to TG-F5) is 89%–93% (which is considerably

higher than the distances for the isolates in the previous paragraph). The range within these subclusters is higher (94%–100%), which is within the range of known MNR and BNR anastomosis groups or subgroups. In addition, the % sequence similarity range for some of the single isolates (accession numbers AJ313437, AJ313438, AJ313443, AJ313444) within TG-F (but are not included in any of these subgroups) with the other isolates is 88% to 94% (which is in the range of different subgroups); this may indicate that these isolates may represent additional subgroups as may be expected to be supported by future research data.

rDNA-ITS sequences of single isolates from several *Tulasnella* species in GenBank originated from the research of McCormick et al. (2004), who reported on a wide variety of endophytic fungi according to their various orchid hosts. The sequences of *T. tomaculum* (accession number AY373296), *T. violea* (AY373292), and *T. eichleriana* (AY373293) are located in the cluster of TG-B. However, the very close sequence similarity between the sequences of the isolates designated as *T. violea* and *T. eichleriana* (99%) indicate that the sequences of these two isolates are undoubtedly of the same species. The possibilities for this discrepancy may be (1) that one of the sequences is not of its designated species, (2) that the identification of one of the isolates was inaccurate, or (3) that the two species may be genetically the same species (the least likely). This finding indicates again that studies based only on one isolate per group are less reliable and might lead to errors. *Tulasnella albida* (AY373294) and *T. pruinosa* (DQ457642) are distinct but relatively close to each other (86% sequence similarity) and are separate from the major clusters. *Tulasnella deliquescens*, which is the identified teleomorph of *E. repens* (Moore 1996; Stalpers and Andersen 1996), belongs to cluster TG-F (but is separate from its five subclusters). The sequence of isolate AY643803 is located on a distant cluster from the other isolate sequences of *Tulasnella* spp. (Fig. 2). It has a very low sequence similarity (48%–66%) to all the other available sequences of *Tulasnella* spp. However, it has the highest closer similarity (86%) to AGs -C, -H, and -I, which is within the similarity range among the *Ceratobasidium* spp. BNR AGs. These data may suggest that this isolate could have been inaccurately designated as a *Tulasnella* isolate. The % sequence similarity range of the *Tulasnella* with the other *Ceratobasidium* BNR and UNR isolates is 45%–62%, with *Thanatephorus* MNR isolates is 45%–63%, and with *Waitea* MNR isolates is 45%–61% (data not presented in a table).

Ma et al. (2003) reported on separation of their *Epulorhiza* isolates designated according to their orchid hosts by rDNA-ITS analysis into two major groups. "Group 1," which was designated according to the morphological and cultural characteristics as *E. repens*, was separated into four clusters. Their sequences correspond with some subgroups of TG-F (see Fig. 2). Isolate sequences of clusters 1 and 2 correspond with TG-F4, those of cluster 3 correspond with TG-F3, and those of two isolates from cluster 4 correspond with TG-F3. Those of the other two isolates of cluster 4 are separate from the major subclusters of TG-F. The isolate sequences of "group 2" that were designated as *E. calendulina* (but the identification based on morphological and cultural characteristics may not be sufficiently supported; Ma, personal communication) correspond with TG-A (see Fig. 2). Isolate AY643803 was designated as *E. repens* (Bougoure et al. 2005) but, as it is very distant from all the other isolates (51%–63% similarity) and close to the outgroup (see Table 4), this may indicate that more research is needed to support the identification of anamorph species of *Epulorhiza*.

Otero et al. (2002) reported on separation of their *Epulorhiza* isolates designated according to their orchid hosts by rDNA-ITS analysis into four clusters of uninucleate (UNR) and binucleate (BNR) isolate sequences in addition to clusters of other BNR and MNR isolates. One of the clusters was located close to BNR AG-Q, while the other (two isolates) was close to AG-H. One of the other two isolates was located together with the AG-A cluster. In Fig. 2, the cluster of these endophytic UNR isolate sequences is positioned in a distinctly separate location in the NJ tree from the UNR *Ceratobasidium bicorne* isolate sequences reported by Hietala et al. (2001) as pine seedling pathogens. In certain isolates, some young hyphal cells were both uninucleate and binucleate (Otero et al. 2002).

A combined rDNA-ITS sequence analysis of MNR, BNR, and UNR groups

When best-fitting manual alignment was performed, subgroups were well organized within their AGs in NJ trees when analyzed separately for BNR + UNR (*Ceratobasidium*) isolate sequences (Figs. 1 and 3) or in the MP tree for MNR (*Thanatephorus* and *Waitea*) isolate sequences (Sharon et al. 2006), but the subgroups of AG 2 and AG 6 were separated in the NJ tree for the same multiple alignment for MNR. However, in trees where the MNR isolate sequences were analyzed together with BNR + UNR, some subgroups and also some isolate sequences of the same AG or even subgroup were split to distant locations in the NJ tree (Fig. 5) but were better organized in the MP tree (Fig. 6).

In the NJ tree (see Fig. 5), every addition or subtraction of isolate sequences may cause significant changes in the locations of the clusters, and sometimes isolate sequences of the same AG are split to two or more locations. In the MP tree (Fig. 6), the major clusters are generally more stable. Therefore, the data obtained are flexible to a certain extent and should be considered as such and not definite.

In the NJ tree (Fig. 5), the major clusters of the BNR (*Ceratobasidium*) are located at the upper part of the tree whereas the MNR (*Thanatephorus*) clusters are located at the lower part of the tree. However, some BNR clusters are located in between the MNR AGs. Clusters of some of the AG 2 subgroups are split in several locations on the tree. The AG-P isolate sequences are split in several clusters. One part of AG-P and AGs-S, -R, and -Fa clusters appear

between AG 2–1 and AG 2–2 and AG 12. The other AG-P cluster together with AGs -Fb and -E are between the separated AG 6 subgroups, AG 1–1C and AG 7. The cluster of the *Waitea circinata* MNR is located in the upper part of the tree on a separate cluster.

In the MP tree (Fig. 6), the major clusters of MNR AGs are located at the upper part of the tree while the BNR AGs are located at the lower part of the tree. The BNR AGs are divided into two major clusters. The first cluster includes AGs -G, -O, -L, -K, -A, -B, -D, -C, -I, -H, -B, and -Q. The clusters of UNR1 and UNR2 are located in this major cluster. The second cluster includes AGs -F, -E, -R, -P, and -S. This cluster is located in the major cluster of the MNR AGs. The cluster of the *Waitea circinata* MNR is located in the lower part of the tree on a separate cluster and had a very low sequence similarity with all the other MNR, BNR and UNR groups (only 61%–72%).

The % sequence similarity data (see Table 5) and the locations of these BNR + UNR AG clusters close to certain MNR AG clusters may indicate closer genetic relatedness and possible evolutionary bridges between certain BNR AGs to certain MNR AGs. Yokoyama and Ogoshi (1988) reported on hyphal fusion between an MNR isolate of AG 6 and a BNR isolate of AG-F. The percent sequence similarity range between AG-6 and AG-F is 87%–96%. Sequence similarity of 96% is within the range of isolate sequences that belong to the same AG or subgroup. AG-F is also close to AGs 7 and 8 (90%–95%), to AG 12 (89%–91%), to AG-2 (82%–92%), to AG 1–1C (87%–91%), and to AGs 3 and 4 (87%–90%). AG 6 is also closer to AGs -E (89%–94%), -R (84%–93%), -S (84%–93%), and -P (88%–92% with isolates designated as "U" and 86%–92% with the rest). AG 4 is also relatively close to these BNR AGs (85%–91%); AGs 1, 5, 7, 8, 9, 10, and 12 have also relatively close sequence similarities to these BNR AGs (82%–94%) (additional close relationships among MNR and BNR AGs are detailed in Table 5), compared to low sequence similarity between some BNR AGs (75%–83%) and between some MNR AGs (61%–88%).

Similarly to our previous suggestion for MNR isolate sequences (Sharon et al. 2006), it may be suggested to use, from the data summarized and analyzed in Fig. 4 and Table 3, the alignment of the BNR + UNR isolates of Fig. 4 for the identification of unknown BNR isolates into AGs and subgroups, by manually adding the new sequences to the existing alignment. As the access number of the full alignments in the TreeBase (temporary Study Access No. is SN3335) is not available yet for the public, the full alignments will be sent by e-mail upon request to the e-mail address of the corresponding author. Caution should be taken when new alignments are submitted to a computer program analysis, in which the original manual alignment will be disregarded; they should always be subjected to manual correction of the inaccuracies appearing in the alignment. The sequences should first be pairwise analyzed for percent sequence similarity with the rest of the BNR isolates, and then additional manual alignment should be made to the closest isolates before preparing the tree. Even so, the new alignment might result in changes in the locations of isolates and subgroups or AGs along the tree, compared to the presented complete BNR tree (see Fig. 1). If the sequence of the unknown isolate is within the range of 95%–100% similarity and its location is within a cluster of a certain AG or a subgroup, it indicates that the isolate is likely to belong to this AG or subgroup. This procedure may facilitate the laboratory work required for precise identification of unknown BNR isolates or verify previous identification of isolates.

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