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The taxonomic status of the wild rice species *Oryza ridleyi* Hook. f. and *O. longiglumis* Jansen (Ser. *Ridleyanae* Sharma et Shastry) from Southeast Asia

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Abstract

The taxonomic status of the two species *O. ridleyi* and *O. longiglumis* in *Oryza* series *Ridleyanae* was resolved using morphological and molecular markers. Cluster analysis using a similarity matrix based on 12 qualitative characters separated the *O. ridleyi* from the *O. longiglumis* samples and the *O. ridleyi* samples from Papua New Guinea (PNG). *O. ridleyi* and *O. longiglumis* formed distinct groups upon cluster analysis based on 16 quantitative characters. Canonical discriminant analysis showed significant differences between the two species with anther and ligule length and spikelet and leaf dimensions as the most discriminating characters. Cluster analysis based on RAPD markers showed distinct clusters for *O. longiglumis* and *O. ridleyi* samples from different geographic origins. Hybridization studies revealed an F_1 sterility barrier in interspecific hybrids and those obtained from intraspecific crosses between *O. ridleyi* from Southeast Asia and PNG.

Introduction

In a proposed taxonomic treatment of the genus *Oryza*, Lu (1999) classified the 24 species in the genus in three sections, *Padia* (Zoll. et Mor.) Baill., *Brachyantha* B. R. Lu, and *Oryza* L. Two species, *O. ridleyi* Hook. f. and *O. longiglumis* Jansen, were classified in Sect. *Padia* series *Ridleyanae* Sharma et Shastry (= *O. ridleyi* complex of Vaughan 1989a). These two species are quite distinct from all other wild rice species in terms of the setaceous pair of sterile lemmas with a longitudinally punctulate outer lemma and palea surface that is hispid along the keel and margins Sharma and Shastry (1965). The sterile lemma length to the fertile lemma (spikelet) length ratio is conventionally used to differentiate the two species (Takeoka 1962, 1963; Chang 1976; Duistermaat 1987; Vaughan 1989a). The sterile lemma length is only about half the length of the spikelet in *O. ridleyi*, but almost as long as or longer than the spikelet in *O. longiglumis*. However, S.V.S. Shastry (as noted by Vaughan 1990) indicated the possible presence of intermediates for this character upon examining a herbarium sample that did not completely conform to the type of *O. longiglumis*. He suggested that *O. longiglumis* might be relegated as a subspecies of *O. ridleyi*. Vaughan (1991) also recognized that the extent to which the two species differ was not entirely clear because of variation in sterile lemma length.

O. ridleyi was first described by Hooker in 1897 based on specimens collected by H. N. Ridley from Pahang in the Malayan Peninsula (Hooker 1897). *O. ridleyi* occurs across Southeast Asia and Irian Jaya, Indonesia, and has also been found in Papua New Guinea (Figure 1). Jansen (1953) described *O. longiglumis* based on specimens from ''New Guinea, Western District''(= Papua New Guinea). Figure 1 shows that, compared with *O. ridleyi*, this species is narrowly distributed and known only from a few sites along the Koembe River in Irian Jaya (Vaughan 1989a) and the Western Province of Papua New

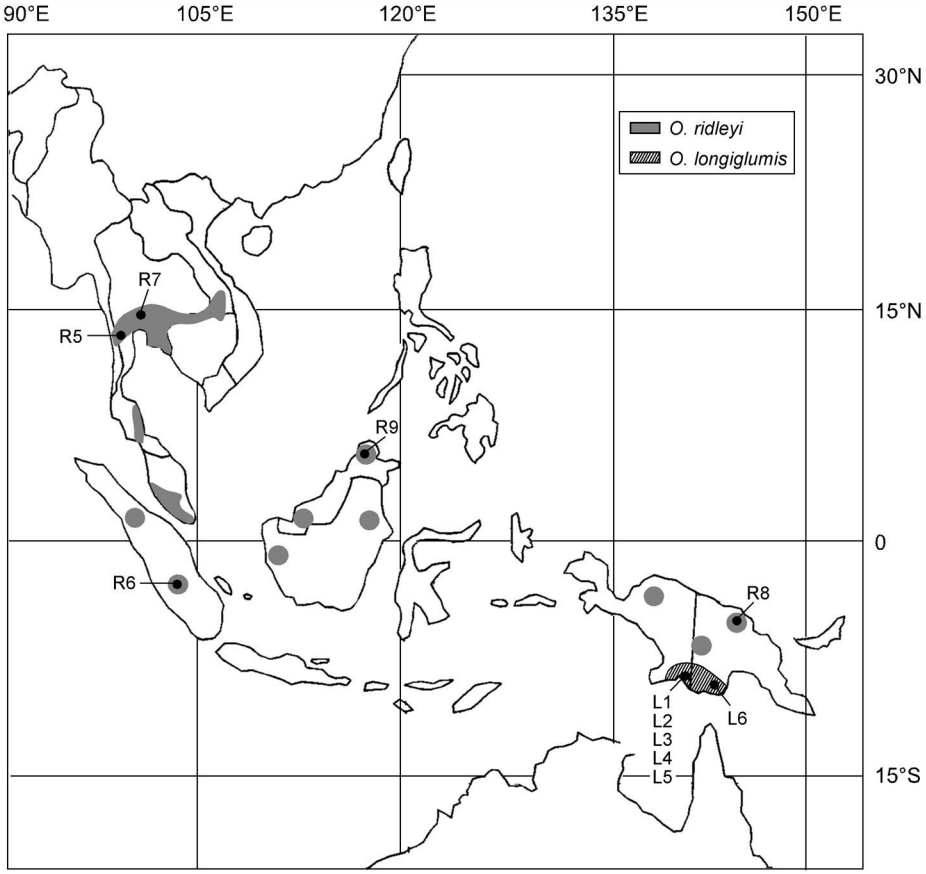

Figure 1. Distribution of *O. longiglumis* and *O. ridleyi* across Southeast Asia, Irian Jaya, and Papua New Guinea based on collections in the IRG and herbarium specimens. Accessions identified by codes are included in the study. R1, R2, R3, and R4 are not plotted because of unavailable latitude and longitude data.

Guinea (Vaughan 1991). However, the two species occupy similar habitats and are usually found in shaded areas along rivers and streams (Vaughan 1994). Both species are tetraploid with 48 chromosomes (Sitch et al. 1991), and have been assigned the same genome, HHJJ (Aggarwal et al. 1997).

We undertook the present study to determine whether *O. ridleyi* and *O. longiglumis* are just variants of a single species or distinct taxonomic species. The taxonomic relationship between the two species was based on morphological variation and RAPD markers, and on hybridization studies.

Materials and methods

Plant materials

The materials used in the study (all from the International Rice Genebank Collection at IRRI) included five accessions from Thailand, two from Malaysia, six from Indonesia, and two from Papua New Guinea (Table 1). Figure 1 shows the distribution of 11 of these accessions across Southeast Asia including Irian Jaya and Papua New Guinea. R1, R2, R3, and R4 were not plotted since no geographic data were available. We adopted the identity of each accession as *O. ridleyi* or *O. longiglumis* based on the collector's identification.

All accessions except for R6 (which was from an original collection) had been rejuvenated at IRRI. Seed dormancy was broken by soaking the seeds in 1000 ppm gibberellic acid for 48 hours. The seeds were air-dried for 15 min and germinated in Petri dishes lined with filter paper moistened with sterilized distilled water. The seedlings were transferred to culture solution (pH 5) (Yoshida et al. 1976) and allowed to grow in an indoor cabinet under controlled

Table 1. O. ridleyi and *O. longiglumis* accessions used in morphological, molecular, and hybridization studies.

Code	IRGC accession number	Species designation by the collector	Origin		
			Province	Country	Latitude/longitude
R1	100820	*O. ridleyi*	–	Thailand	Not known
R2	100821	*O. ridleyi*	–	Thailand	Not known
R3	100877	*O. ridleyi*	Bangkok	Thailand	Not known
R4	101453	*O. ridleyi*	–	Malaysia	Not known
R5	105366	*O. ridleyi*	Central Thailand	Thailand	13°40′N/100°20′E
R6	105973	*O. ridleyi*	South Sumatra	Indonesia	3°00′ S/104°00′E
R7	106028	*O. ridleyi*	Northeast Thailand	Thailand	14°20′ N/101°00′E
R8	106259	*O. ridleyi*	Madang	Papua New Guinea	4°05′ S/144°40′E
R9	106471	*O. ridleyi*	Sabah	Malaysia	5°10′ N/116°20′E
L1	100974	*O. longiglumis*	Irian Jaya	Indonesia	8°00′ S/140°20′E
L2	105146	*O. longiglumis*	Irian Jaya	Indonesia	8°00′ S/140°20′E
L3	105147	*O. longiglumis*	Irian Jaya	Indonesia	8°00′ S/140°20′E
L4	105148	*O. longiglumis*	Irian Jaya	Indonesia	8°00′ S/140°20′E
L5	105562	*O. longiglumis*	Irian jaya	Indonesia	8°00 S/140°20′E
L6	106525	*O. longiglumis*	Western Province	Papua New Guinea	9°12′ S/142°55′E

conditions (21 °C/29 °C, 70% RH, 12H/12H 1000 $\mu\varepsilon$ m^{-2} s^{-1} light intensity). The seedlings were transplanted singly to 30-cm plastic pots with highly organic soil and maintained under ambient conditions at about 30 °C in the International Rice Genebank (IRG) screenhouse under partial shade.

Morphological characterization

Each accession was represented by two to four plants and scored for 12 qualitative and 16 quantitative traits (leaf, culm, panicle, and spikelet) listed in Table 2. For continuously variable characters, five measurements were taken from each plant.

RAPD analysis

DNA was extracted following Virk et al. (1995). DNA concentration was estimated by comparison with different concentrations of λ DNA in 0.7% agarose after electrophoresis in 0.5x TBE buffer. Sixty-nine 10-base oligonucleotide primers from Operon Technologies Inc. were screened but only 13 were finally used for DNA amplification (Table 3). At least two amplifications were carried out in 25 μL reaction mixture containing 5 ng DNA, 0.4 μM primer, 200 μM of each dNTP, 2.5 mM magnesium chloride, 10x ammonium reaction buffer, and 1 unit Taq polymerase (BIOTAQ). The mixture was overlaid with mineral oil. The reaction was performed with the following temperature profile: 2 min at 94 °C; 2 cycles of 30 s at 94 °C, 1 min at 37 °C, and 2 min at 72 °C; 2 cycles of 30 s at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; 41 cycles of 30 s at 93 °C, 1 min at 35 °C, and 2 min at 72 °C; and 5 min at 72 °C. The amplification products were separated in 1.4% agarose gel using 0.5x TBE buffer and stained with ethidium bromide and visualized under UV light. Only reproducible bands were scored and analyzed.

Data analysis

Each plant was treated as an operational taxonomic unit (OTU). Separate analyses were carried out on qualitative and quantitative morphological characters. For continuously variable characters, the mean from 5 measurements per plant was used in the analysis. A similarity matrix and a dissimilarity matrix for 12 qualitative and 16 quantitative characters, respectively, were obtained using NTSYS-pc 2.02k version (Rohlf 1994). Cluster analyses were carried out on the similarity/dissimilarity matrices using the SAHN (sequential, agglomerative, hierarchical, and nested) option of NTSYS-pc with UPGMA as the clustering algorithm. Canonical discriminant analysis (CDA) was performed with the CANDISC procedure of (SAS Institute 1989).

RAPD band patterns scored as 1 = present or 0 = absent from individual plants were used to obtain a similarity matrix of Jaccard coefficients. Cluster analysis (UPGMA method) was carried out on the similarity matrix using NTSYS-pc. Statistical support of the branches was tested with a bootstrap analysis using the software program 'WinBoot' developed at IRRI (Yap and Nelson 1996) with 10,000 data resamples.

Table 2. Morphological characters scored for taxonomic analysis.

Growth stage scored	Character	Code[a]
Vegetative (30 days after transplanting)	Basal leaf sheath color	
	Auricle hairiness	
	Ligule color	
	Collar color	
	Upper leaf surface pubescence	
	Lower leaf surface pubescence	
	Growth habit	
Reproductive (14 days after flowering)	Flag leaf width/flag leaf length	FWD/FLT
	Second leaf width/second leaf length	LWD/LLT
	Second leaf ligule length	LIGLT
	Culm length	CULT
	Culm diameter	CUDI
	Panicle type	
	Panicle length	PANLT
	Number of panicle branches	BRANCHES
	Number of spikelets/panicle	SPIKES
	Lemma color	
	Awn color	
	Stigma color	
	Anther length	ANLT
	Stigma length	STGLT
	Style length	STYLT
	Spikelet width/spikelet length	SPKWD/SPKLT
	Spikelet length/awn length	SPKLT/AWNLT
	Sterile lemma length/spikelet length	STLT/SPKLT
	Awn width	AWNWD
Reproductive (30 days after flowering)	Basal leaf ligule shape	
	Number of tillers per plant	TILLERS

[a]Only quantitative characters are indicated by codes.

Table 3. Primers used for the amplification of total DNA from individual samples from *O. ridleyi* and *O. longiglumis*.

Primer code	Sequence (5′ to 3′)	Number of bands scored	Polymorphic bands (%)
OPA-09	GGGTAACGCC	22	81.8
OPA-19	CAAACGTCGG	11	90.9
OPB-13	TTCCCCCGCT	9	100.0
OPC-03	GGGGGTCTTT	12	75.0
OPC-12	TGTCATCCCC	5	60.0
OPD-17	TTTCCCACGG	4	100.0
OPE-12	TTATCGCCCC	7	57.1
OPH-09	TGTAGCTGGG	8	62.5
OPJ-05	CTCCATGGGG	14	78.6
OPJ-14	CACCCGGATG	10	70.0
OPN-19	GTCCGTACTG	9	88.9
OPO-10	TCAGAGCGCC	7	100.0
OPQ-02	TCTGTCGGTC	15	93.3

Hybridization

Intraspecific and interspecific crosses were made in September to November 1996 and in April 1998. Each accession was represented by at least five plants. Emasculation and pollination were the same as described by Naredo et al. (1997). F_1 seeds were harvested at 10-12 days after pollination to avoid dehydration and/or deterioration of the young embryos. Seeds were germinated on MS medium and maintained in the IRG screenhouse. The pollen fertility of each hybrid plant was obtained from five individual spikelets based on the frequency of round, intact, and fully stained pollen grains after immersion in I_2-KI

solution for 3 min. Spikelet fertility was based on the ratio of filled grains to the total number of grains from five individually bagged panicles. Chromosome pairing was observed at late diakinesis or metaphase I in pollen mother cells (PMC).

Results

Qualitative morphological characters

The UPGMA dendrogram shows two clusters defined at a similarity level of 0.486 (Figure 2). The first (cluster 1) was composed mainly of samples representing *O. longiglumis* and the *O. ridleyi* (R8) samples from Papua New Guinea. The *O. ridleyi* samples and the *O. longiglumis* samples L2-1, L2-2, L5-3, and L1-3 formed cluster 2. Table 4 lists the characters that differentiate the two clusters.

Figure 2 shows that the four *O. longiglumis* samples in cluster 2 showed $< 20\%$ panicle fertility, which is comparable to that shown by artificial hybrids obtained from interspecific crosses (see Table 6). These and other highly sterile samples (L2-4, L5-2, R3-1, and R7-3) are putative hybrids obtained from seed multiplication under screenhouse conditions. In contrast, the other samples showed $> 30\%$ panicle fertility except for R9, which was derived from an accession originally collected from a highly sterile population in nature (Vaughan 1989b). To

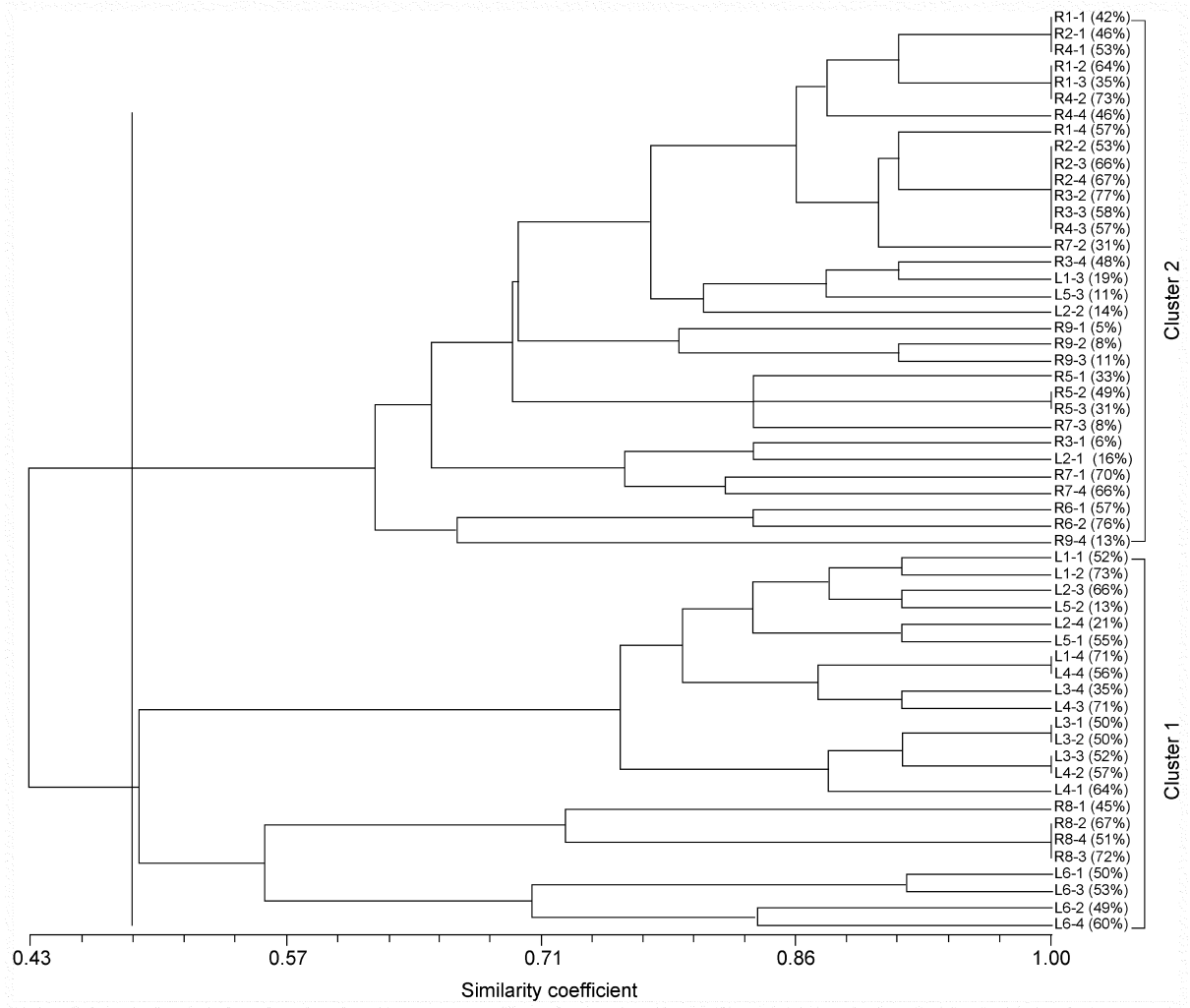

Figure 2. UPGMA dendrogram of *O. ridleyi* and *O. longiglumis* samples from cluster analysis based on a similarity matrix for 12 qualitative characters. Percentage values in parentheses correspond to panicle fertility of the sample.

Table 4. Differences in character states of samples in two clusters obtained from cluster analysis.

Character	Cluster 1 (mainly *O. longiglumis*)	Cluster 2 (mainly *O. ridleyi*)
Basal leaf sheath color	Green	Mainly purple
Ligule shape	Truncate	Acute
Auricle hairiness	Glabrous to hairy	Hairy to very hairy
Lower leaf surface pubescence	Slightly pubescent to pubescent margins	Glabrous to slightly pubescent margins
Panicle type	Compact or open	Mainly intermediate

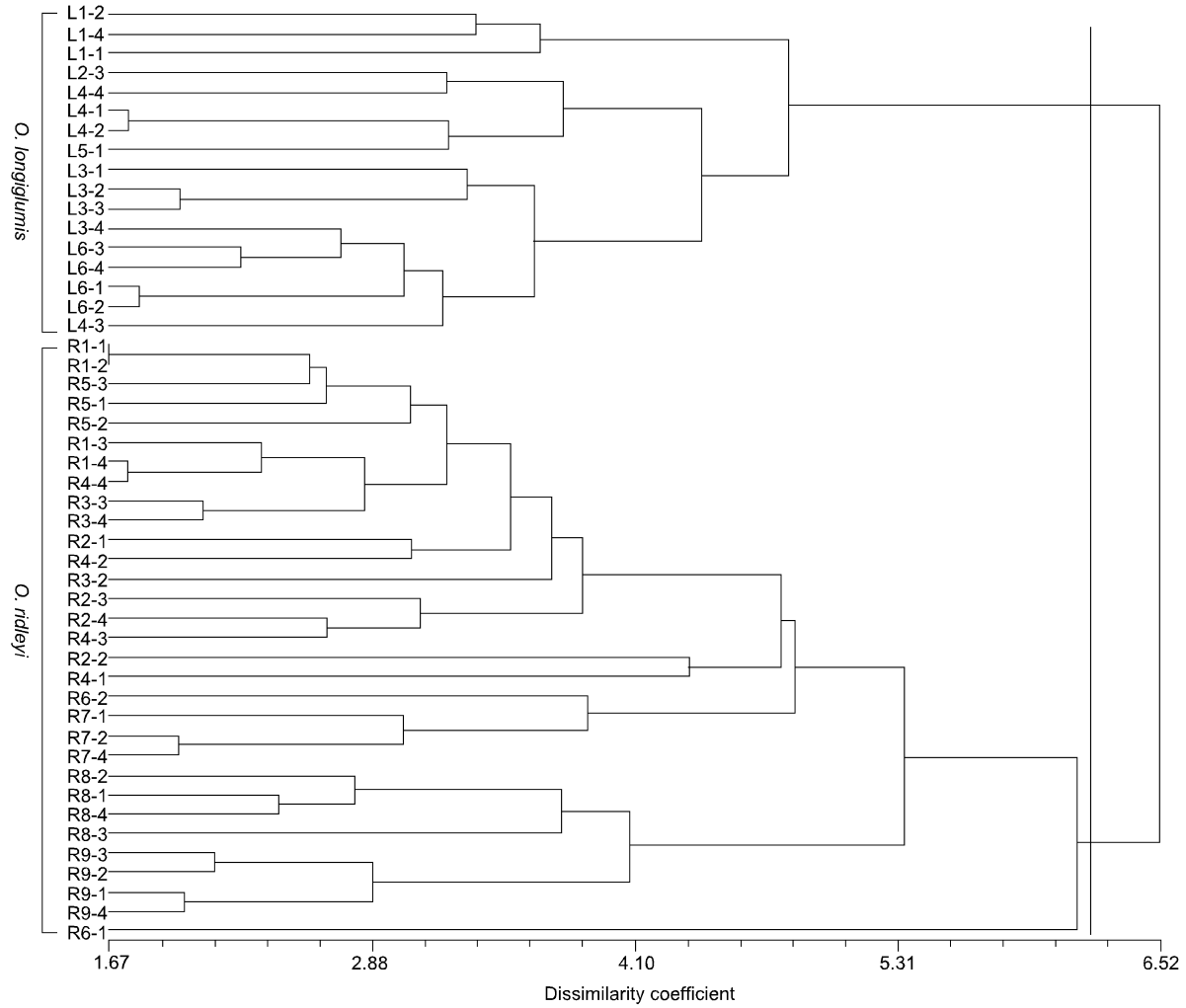

Figure 3. UPGMA dendrogram of *O. ridleyi* and *O. longiglumis* samples based on a dissimilarity matrix of Euclidian distances for 16 quantitative characters.

obtain an accurate measure of differences between the two species, we therefore excluded the putative hybrids from subsequent analyses.

Quantitative morphological characters

The UPGMA dendrogram obtained from cluster analysis carried out on the dissimilarity matrix for 16 quantitative characters shows that *O. longiglumis* and *O. ridleyi* formed distinct clusters at a dissimilarity level of 0.614 (Figure 3).

Canonical discriminant analysis was performed with the same variables used in cluster analysis to analyze the distinctiveness of *O. ridleyi* from *O.*

longiglumis. The multivariate test for differences between the two species was highly significant at the 0.0001 level. Figure 4 shows that the first canonical component (Can 1) effectively discriminated between the two species. This component was most highly correlated with anther length (ANLT) and ligule length (LIGLT) and the ratios of sterile lemma length to spikelet length (STLT/SPKLT), spikelet width to spikelet length (SPKWD/SPKLT), spikelet length to awn length (SPKLT/AWNLT), flag leaf width to flag leaf length (FWD/FLT), and second leaf width to second leaf length (LWD/LLT). The means of these variables shown in Table 5 reflect the differences between *O. longiglumis* and *O. ridleyi*. Figure 5,6 show the spikelet and ligule attributes of the two species.

RAPD analysis

The number of bands scored per primer varied from four (OPD-17) to 22 (OPA-9). Band polymorphism ranged from 57.1% (OPE-12) to 100% (OPB-13, OPD-17, and OPO-10) as shown in Table 3.

The UPGMA dendrogram obtained after cluster analysis shows four clusters at a similarity level of 0.684 (Figure 7). *O. longiglumis* from PNG and Indonesia and *O. ridleyi* from mainland Southeast Asia formed distinct clusters. *O. ridleyi* samples

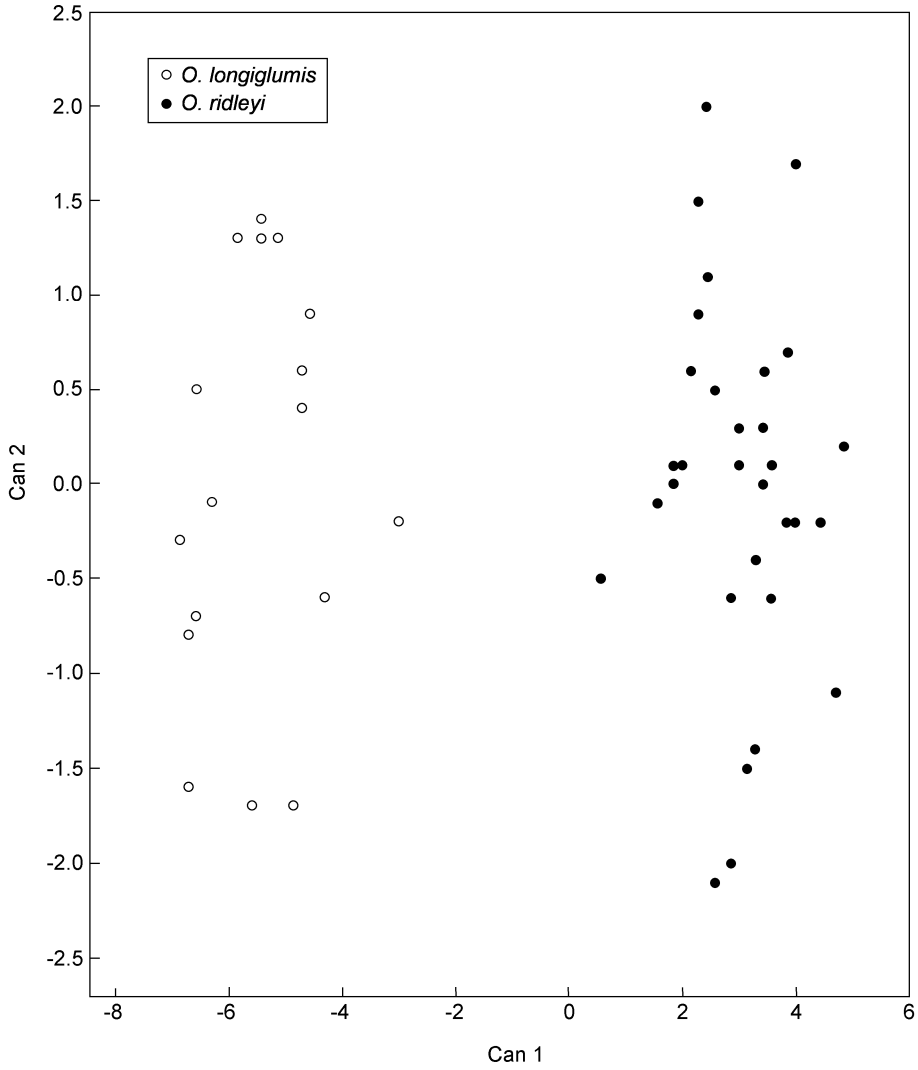

Figure 4. Plot of *O. ridleyi* and *O. longiglumis* along the first (Can 1) and second (Can 2) canonical components obtained from canonical discriminant analysis.

Table 5. Comparison between *O. longiglumis* and *O. ridleyi* based on the variables most highly correlated with the first canonical component obtained from canonical discriminant analysis.

Variable	Mean (95% C.I.)	
	O. longiglumis	*O. ridleyi*
FWD/FLT	0.064 (0.061, 0.067)	0.083 (0.079, 0.086)
LWD/LLT	0.055 (0.052, 0.058)	0.069 (0.066, 0.071)
LIGLT (mm)	1.218 (0.968, 1.468)	2.732 (2.470, 2.994)
ANLT (mm)	2.121 (2.041, 2.201)	2.988 (2.811, 3.164)
SPKWD/SPKLT	0.271 (0.261, 0.282)	0.207 (0.200, 0.214)
SPKLT/AWNLT	0.716 (0.666, 0.767)	1.406 (1.299, 1.513)
STLT/GLT	0.866 (0.827, 0.905)	0.580 (0.549, 0.611)

representing R9 from Sabah/Malaysia and R6 from South Sumatra/Indonesia and R8 from Papua New Guinea formed clusters distinct from the other *O. ridleyi* samples. The UPGMA dendrogram from NTSYS and the consensus tree from 10,000 replicates showed the same topology. The bootstrap P values (100%) shown at the corresponding node for each of the four clusters are highly significant. Figure 8 shows differences in RAPD band patterns among samples representative of each cluster.

Hybridization

More than 9,800 pollinations were made but less than 1% hybrids were obtained from both intraspecific and interspecific combinations. Table 6 shows that the percent hybrids obtained from crosses that produced hybrids ranged from 0.75% (L6 × L3) to 24.62% (R5 × L3).

The hybrids from the *O. ridleyi* intraspecific cross R4 × R5 showed a mean pollen stainability of 88.1% and panicle fertility of 50.9%. However, hybrids from reciprocal crosses between R5 and R8 showed less than 3.0% pollen and panicle fertilities. *O. longiglumis* intraspecific crosses showed a mean pollen stainability that ranged from 71.1% to 81.4% and panicle fertility from 51.4% to 66.4%.

Most of the interspecific hybrids were highly sterile. The cross R8 × L6 produced the most sterile

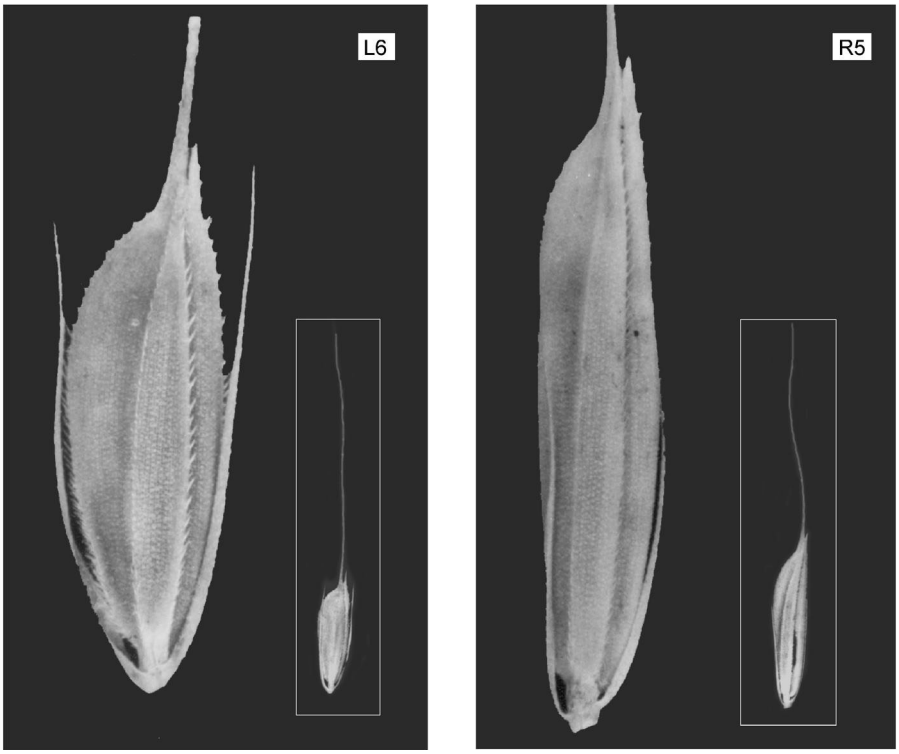

Figure 5. Spikelet attributes of representative samples of *O. longiglumis* and *O. ridleyi*. The *O. longiglumis* sample, L6, shows a roundish spikelet with a pair of sterile lemmas almost as long as the spikelet and an awn longer than the lemma. The *O. ridleyi* sample, R5, shows a long and narrow spikelet with a pair of sterile lemmas half its length and an awn shorter than or as long as the spikelet.

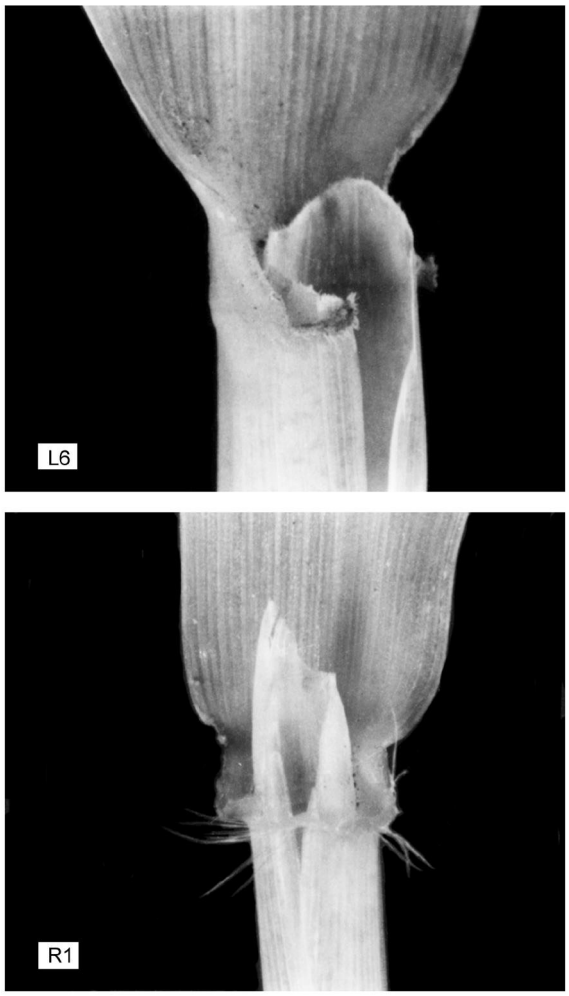

Figure 6. Ligule length in *O. longiglumis* and *O. ridleyi*. L6, representing *O. longiglumis*, shows a short, almost truncate ligule while the *O. ridleyi* sample, R1, shows a longer, almost acute ligule. Note, too, the glabrous pair of auricles in L6 compared with the hairy pair in R1.

hybrids with only 1.4% pollen stainability and 0% panicle fertility. The crosses L6 × R2 and L6 × R3, however, produced hybrids with 20.4% and 26.3% panicle fertility although the pollen stainability was only 13.9% and 7%, respectively.

Table 6 shows that chromosome pairing at metaphase I was normal in all the hybrids, indicated by high bivalent formation and chiasma frequency (Figure 9). Bivalent frequency was highest (23.83/PMC) in the L6 × R3 hybrids and lowest (22.13/PMC) in the L6 × R4 hybrids. The L6 × R5 hybrids showed the lowest chiasma frequency (47.70/PMC) because of the relatively high occurrence (0.30/PMC) of rod bivalents.

Discussion

The results of the present study provide strong support for retaining *O. ridleyi* and *O. longiglumis* as separate taxonomic species. In addition to the sterile lemma length to spikelet length ratio that is conventionally used to differentiate these species, other spikelet and leaf characters discriminate the two species, too. *O. ridleyi* has a longer and more acute basal leaf ligule shape and a more hairy pair of auricles than *O. longiglumis*, which shows a more or less truncate ligule with a pair of almost glabrous auricles. The spikelet in *O. ridleyi* is generally long and narrow, almost as long as or even longer than the awn, and with a pair of sterile lemmas half the length of the spikelet. The *O. longiglumis* spikelet is generally roundish and shorter than the awn with a pair of sterile lemmas almost as long as or even longer than the spikelet. The anther length in *O. ridleyi* at about 3 mm is longer than that in *O. longiglumis*, which is about 2 mm. In general, *O. longiglumis* has narrower but longer leaves than *O. ridleyi*.

The spikelet differences between the two species prove that for wild relatives not closely related to *O. sativa* the spikelet does provide appropriate taxonomic key characters (Vaughan 1989a). The results also support the original descriptions of *O. ridleyi* by Hooker (1897) and *O. longiglumis* by Jansen (1953) that emphasized spikelet and leaf characters and are adequate to reflect the differences between the two species. The revision of Hooker's description of *O. ridleyi* by Roschevicz (1931) to include a species identified as *O. stenothyrsus* as a synonym for *O. ridleyi* should, however, be reconsidered. *O. stenothyrsus* was described by K. Schumann and K. Lauterbach in 1905 (cited by Prodoehl 1922) based on a collection from Papua New Guinea. A population, IRGC 106259, was collected by Vaughan (1990) from the lower Ramu Valley where the population described as *O. stenothyrsus* was found. Results of the present study show that, although its spikelet attributes indicate *O. ridleyi*, its ligule was short and truncate, a characteristic of *O. stenothyrsus* as cited by Prodoehl (1922). This accession also showed distinct RAPD patterns and was isolated by an F_1 sterility barrier from samples designated as *O. ridleyi* and *O. longiglumis*. A more comprehensive biosystematic study involving more populations should be conducted to resolve the taxonomic status of this population.

RAPD analysis revealed that sufficient divergence at the molecular level has occurred among popula-

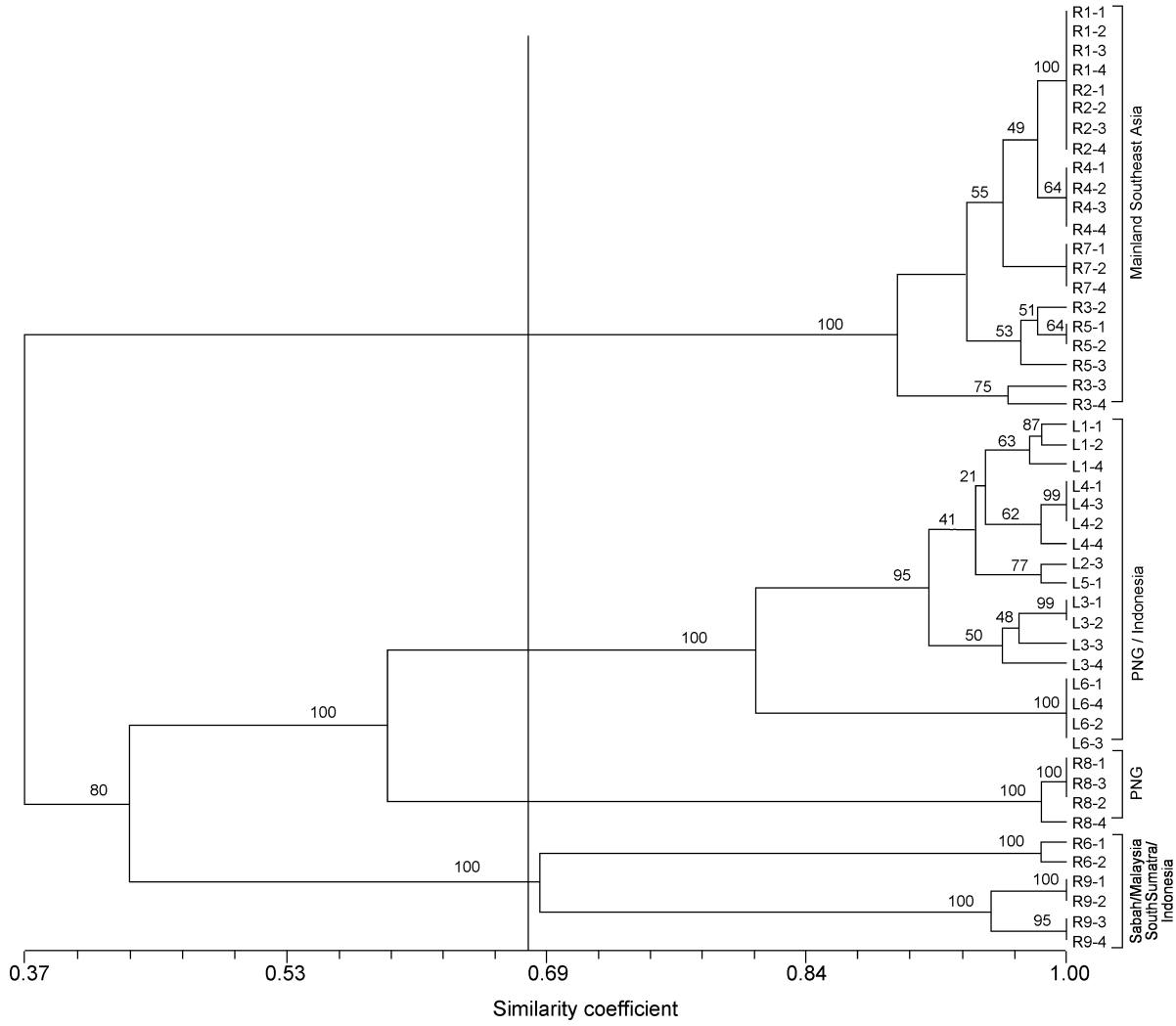

Figure 7. UPGMA dendrogram of 48 individual plant samples of Ser. *Ridleyanae* obtained upon cluster analysis based on a matrix of Jaccard coefficients for RAPD markers. The bootstrap P values (%) shown at the corresponding node for each cluster indicate the confidence limits for the grouping of the samples.

tions in the series *Ridleyanae*. Geographic differentiation is reflected by the divergence of *O. ridleyi* accessions from South Sumatra/Indonesia and Sabah/Malaysia from those collected from mainland Southeast Asia, and from the *O. longiglumis* accessions from Irian Jaya and Papua New Guinea. Using AFLP markers, Aggarwal et al. (1999) similarly reported sufficient divergence between accessions designated as *O. longiglumis* from Indonesia and *O. ridleyi* from Thailand and Indonesia. The two species, however, share the same genome HHJJ as shown by total genomic DNA hybridization (Aggarwal et al. 1997). This explains the normal pairing and high chiasma frequency observed at meiosis of hybrids. Nevertheless, reproductive isolation between the two species is indicated by low pollen stainability and low spikelet fertility of F_1 hybrids derived from interspecific crosses. In this case, a reduction in fertility in the interspecific hybrids because of gamete deficiency might be the result of minor structural changes or cryptic changes in the genomes (Solbrig 1968). Moreover, the reproductive barrier is reinforced by the geographic isolation of the species. Spontaneous hybridization is possible, however, since we have observed

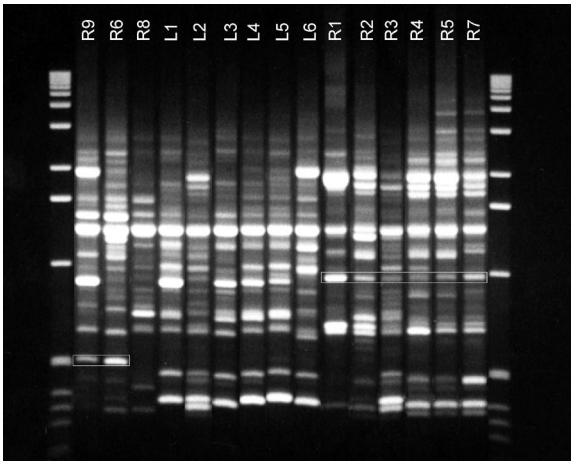

Figure 8. RAPD band patterns generated by primer OPQ-02 in samples representing accessions in the Ser. *Ridleyanae*. Lanes 1 and 17 -1 Kb DNA ladder markers; lanes 2-4, *O. ridleyi* samples from Sabah/Malaysia, South Sumatra/Indonesia, and PNG, respectively; lanes 5-10, *O. longiglumis*; lanes 11-16, *O. ridleyi* from mainland Southeast Asia. Unique alleles (boxed bands) were shown by *O. ridleyi* samples in lanes 2-3 and 11-16.

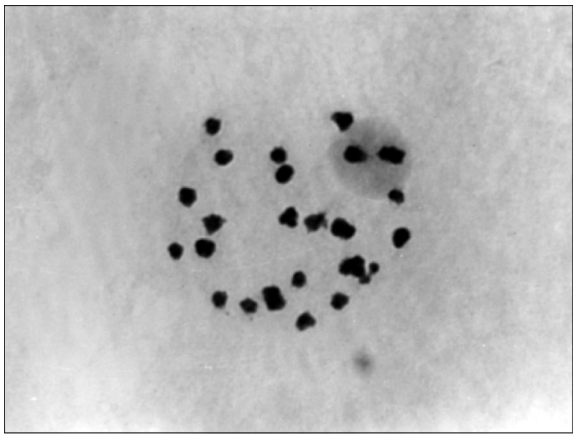

Figure 9. Meiotic configuration showing normal bivalent formation in the hybrid from the cross L6 × L2.

Table 6. Pollen stainability, panicle fertility, and meiotic pairing of hybrids obtained from crosses of *O. ridleyi* and *O. longiglumis*.

Combination/cross[a]	No. of spikelets pollinated	Hybrids obtained (%)[b]	Mean pollen stainability (%)	Mean panicle fertility (%)	No. of PMCs observed	Bivalent frequency/PMC		Chiasma frequency
						Total	Rod	
O. ridleyi × *O. ridleyi*								
R4 × R5	237	1.26	88.1	50.9	33	23.61	0.03	47.91
R5 × R8	–[c]	–	1.0	1.6	28	23.60	0	47.86
R8 × R5	–	–	2.7	0.2	–	–	–	–
O. longiglumis × *O. longiglumis*								
L3 × L6	711	0.98	81.4	60.0	–	–	–	–
L6 × L3	134	0.75	80.6	66.4	–	–	–	–
L6 × L4	204	4.41	71.1	51.4	158	23.63	0.06	47.87
O. ridleyi × *O. longiglumis*								
R5 × L3	65	24.62	10.1	3.9	51	23.54	0.06	47.87
R5 × L6	94	1.06	4.8	0.6	–	–	–	–
R8 × L6	–	–	1.4	0	80	23.70	0.02	47.92
O. longiglumis × *O. ridleyi*								
L3 × R7	125	7.20	11.9	4.6	69	23.47	0.09	47.90
L6 × R2	59	6.78	13.9	20.4	62	23.45	0.05	47.95
L6 × R3	136	3.68	7.0	26.3	68	23.83	0	48.00
L6 × R4	79	5.06	5.6	15.0	116	22.13	0.07	47.79
L6 × R5	204	2.94	9.5	4.4	74	23.56	0.30	47.70
L6 × R8	–	–	10.7	–	13	23.60	0	47.80

[a]Only crosses that produced hybrids are included in the table.
[b]% hybrids = (no. of hybrids obtained/no. of spikelets pollinated) × 100.
[c]Not observed.

this during seed multiplication of the two species in the IRG screenhouse. This is exemplified by some plants that we initially included for morphological analysis, but subsequently excluded because they were highly sterile and had characters intermediate between the two species. This occurrence must be

examined more closely because of its implications for the *ex situ* conservation of the two species.

The results of this study confirm the status of *O. ridleyi* and *O. longiglumis* as distinct taxonomic species. Several morphological characters differentiate the two species and molecular data largely support this morphological differentiation. The presence of an F_1 sterility barrier also supports *O. ridleyi* and *O. longiglumis* as distinct biological species. The elucidation of the taxonomy and genetic diversity of species in the Ser. *Ridleyanae* can greatly improve their conservation and increase their value in rice research and improvement.

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