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MOLECULAR PHYLOGENY OF ORYZEAE (POACEAE) BASED ON DNA SEQUENCES FROM CHLOROPLAST, MITOCHONDRIAL, AND NUCLEAR GENOMES[1]

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The phylogeny and evolutionary history of the rice tribe (Oryzeae) were explored using sequences of five DNA fragments (*matK, trnL, nad1, Adh2,* and *GPA1*) from chloroplast, mitochondrial, and nuclear genomes. Results indicate that (1) Oryzeae is monophyletic and falls into two main clades corresponding to the traditionally recognized subtribes; (2) previous recognition of three monotypic genera (*Hydrochloa*, *Porteresia*, and *Prosphytochloa*) is not justified; and (3) close affinities of the monoecious genera are not supported, suggesting the possibility of multiple origins of unisexual florets. Based on the magnitude of *matK* and *GPA1* sequence divergence, we suggest that *Oryza* and *Leersia* branched off from the remaining genera of Oryzeae ~20 million years ago (mya), and separated from each other ~14 mya. A divergence time of ~9 mya is obtained for the most basal split within *Oryza*. These estimates suggest that Oryzeae diverged during the Miocene, and thus imply that long-distance dispersal appears to be one of the important factors in the diversification of the tribe.

Key words: biogeography; divergence time; multiple sequences; Oryzeae; phylogeny.

The tribe Oryzeae (Poaceae), as conventionally delimited, includes approximately 12 genera and more than 70 species distributed throughout the tropical and temperate regions of the world (Clayton and Renvoize, 1986; Vaughan, 1994). As the largest tribe in the subfamily Ehrhartoideae, Oryzeae contains more than half of both genera and species of the subfamily (Watson and Dallwitz, 1999; GPWG, 2001). In this tribe, *Oryza* L. with approximately 23 species and *Leersia* Sw. with approximately 18 species are the two largest genera and distributed worldwide (Watson and Dallwitz, 1999; Terrell et al., 2001). Except for *Zizania* L., which is disjunctly distributed in eastern Asia and eastern North America, the remaining nine genera are distributed regionally or confined to a specific continent (Watson and Dallwitz, 1999). *Chikusichloa* Koidz and two monotypic genera (*Hygroryza* Nees and *Porteresia* Tateoka) are distributed in Asia. *Zizaniopsis* Doell ex Asch. and *Luziola* Juss., as well as the monotypic *Rhynchoryza* Baill., occur in North and South America, while monotypic *Potamophila* R. Br. is endemic to Australia and *Prosphytochloa* Schweick. to Africa.

In the genus *Oryza*, the Asian cultivated rice (*O. sativa*) is one of the world's most important crops and a primary food source for more than one-half of the world's population (Chandler and Wessler, 2001; Ge et al., 2001). Other members of Oryzeae have important economic value, such as the wild species of *Oryza* that contain agronomically useful traits for rice genetic improvement, *Zizania* species that are a well-known part of cuisine in China (*Z. latifolia*) and North America (*Z. palustris* L.), and *Leersia* species that are useful forage (Vaughan, 1994; Kennard et al., 1999). Importantly, rice is becoming an excellent research model due to its small genome size, efficient genetic transformation, and extensive colinearity with other grass species (Gale and Devos, 1998; Schmidt, 2000; Shimamoto and Kyozuka, 2002). In particular, the completion of the rice genome sequence provides tremendous value of rice as a model plant (Bennetzen, 2002; Rensink and Buell, 2004). Therefore, a clear picture of phylogenetic relationships of rice and its relatives will provide an important foundation for future breeding programs and for biological studies of rice and grasses in general (Kellogg, 1998; Ge et al., 2001; Rensink and Buell, 2004).

Because of the economical and theoretical importance of rice, classification and phylogeny of *Oryza* and its closely related genera have been studied through various approaches including morphology (Weatherwax, 1929; Launert, 1965; Terrell and Robinson, 1974; Kellogg and Watson, 1993; Terrell et al., 2001), cytology (Ramanujam, 1938; Li et al., 1963; Nayar, 1973), restriction site analysis (Zhang and Second, 1989; Duvall et al., 1993), and DNA sequencing (Ge et al., 2002; Hass et al., 2003). Although evidence showed that this tribe was a distinct and monophyletic group (Duistermaat, 1987; Zhang and Second, 1989; Kellogg and Watson, 1993; Ge et al., 2002), its subdivision and phylogenetic relationships among genera have been inconsistent among studies (Hitchcock, 1920; Hubbard, 1934, 1959; Hitchcock and Chase, 1951; Stebbins and Crampton, 1961; Pyrah, 1969; Terrell and Robinson, 1974; Duvall et al., 1993). Previous morphological investigations treated the oryzoid grasses as a taxonomic bifurcation of monoecious vs. bisexual groups and recognized the two groups variously at tribal (Oryzeae vs. Zizanieae) (Hitchcock, 1920; Hitchcock and Chase, 1951; Stebbins and Crampton, 1961) or subtribal (Oryzinae vs. Zizaniinae) (Hubbard, 1934, 1959; Pyrah, 1969) levels. Through morphological and

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anatomical investigations, Terrell and Robinson (1974) established a new subtribe Luziolinae and proposed a three-subtribe system, but this treatment was not supported by later studies of restriction sites and sequence data (Zhang and Second, 1989; Duvall et al., 1993; Ge et al., 2002). In addition, the delimitation and taxonomic position of some genera in this tribe have long been problematic and are still controversial mainly due to limited samples or insufficient character information used in previous studies. For example, the mutually exclusive hypotheses that *Zizania* is more closely related to the monocious genera or to the bisexual genera have been in dispute for decades (Terrell and Robinson, 1974; Duvall et al., 1993; Ge et al., 2002). Whether or not to place the monotypic genus *Hydrochloa* Beauv. in synonymy under *Luziola* and to treat the monotypic genus *Porteresia* as a member of *Oryza* are also not resolved with certainty (Terrell and Robinson, 1974; Duvall et al., 1993).

Based on sequences of the chloroplast *matK* gene, Ge et al. (2002) studied the phylogeny of Oryzeae. They verified that the tribe was a monophyletic group consisting of two strongly supported monophyletic groups corresponding to the two traditionally recognized subtribes, and they also suggested treating *Porteresia* as a member of *Oryza*. Although the *matK* work was so far the most comprehensive phylogenetic study of this tribe using sequence data in terms of species and generic inclusion, many of the questions just mentioned remain unsolved or the answers uncertain because of the relatively low resolution of the chloroplast gene. Growing examples in the recent literature have demonstrated that low-copy nuclear genes have great potential to compensate cpDNA and nrDNA for improvement of resolution and robustness of plant phylogenetic reconstruction (Soltis and Soltis, 1998; Doyle and Doyle, 1999; Sang, 2002; Grob et al., 2004). The alcohol dehydrogenase (*Adh*) gene is the most widely used low-copy nuclear gene, whereas the chloroplast *matK* gene and *trnL* intron as well as the mitochondrial *nad1* intron 2 are also used in phylogenetic studies (Soltis and Soltis, 1998; Ge et al., 1999, 2002; Gugerli et al., 2001; Sang, 2002; Hass et al., 2003). Because nuclear *GPA1* that encodes the G protein α subunit is present as a single copy in higher plants and is well characterized in function and structure (Seo et al., 1995; Fujisawa et al., 1999), we successfully used it to investigate reticulate evolution of tetraploids in *Oryza* (Bao and Ge, 2004). In the present study, we reconstruct the phylogeny of Oryzeae using sequences of five DNA fragments from three genomes, including two chloroplast (*matK* and *trnL* intron), one mitochondrial (*nad1* intron 2), and two nuclear (*Adh2* and *GPA1*) fragments. Our specific objectives are: (1) to evaluate the circumscription of the groups at tribal and subtribal levels and the subtribal classification systems of the Oryzeae, (2) to investigate generic relationships within the tribe with particular emphasis on the systematic positions of some genera, and (3) to gain insights into the origin and biogeographic history of the tribe from a molecular phylogenetic perspective.

MATERIALS AND METHODS

Plant materials and DNA isolation—Twenty-four species were used in the present study, representing all 12 genera of the tribe Oryzeae (Clayton and Renvoize, 1986; Vaughan, 1994), except for *Maltebrunia* that was placed in synonymy under the genus *Potamophila* by Duistermaat (1987). Nine *Oryza* species representing all six diploid genomes and four *Leersia* species including two diploids and two tetraploids were sampled in this study. Two species from each of *Luziola* and *Zizania* and one species from each of the remaining seven genera, including five monotypic genera (*Hygroryza*, *Porteresia*, *Potamophila*, *Prosphytochloa*, *Rhynchoryza*), were sampled. Based on a recent comprehensive study of the subfamilial classification of the grass family (GPWG, 2001), the subfamily Ehrhartoideae includes three tribes: Ehrharteae, Oryzeae, and Phyllorachideae, and the tribe Ehrharteae is the most closely related tribe to Oryzeae. Therefore, we chose one species of the genus *Ehrharta* Thunb. as an outgroup. One additional species of the closely related subfamily Bambusoideae was also included in the phylogenetic analysis as an outgroup. The details of the sampled species with their scientific names, accession numbers or vouchers, origins, chromosome numbers, and GenBank accession numbers are listed in the Appendix. Total DNA was isolated from silica-gel dried or fresh leaves using the cetyltrimethylammonium bromide (CTAB) method as described previous by Ge et al. (1999).

Amplification, cloning, and sequencing—To amplify the chloroplast *matK* and *trnL* intron, mitochondrial *nad1* intron 2, and nuclear *Adh2* and *GPA1*, the polymerase chain reactions (PCR) were carried out in a volume of 25 μL, containing 5–50 ng of genomic DNA, 5.0 pmol of each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$ and 0.65 units of *Taq* DNA polymerase. Amplifications were performed in a Peltier thermal cycler (PTC-200, PerkinElmer Corporation, Wellesley, USA) .

The primers for amplifying and sequencing *matK* followed those in Ge et al. (1999). Most sequences of *matK* were used in Ge et al. (2002), but additional sequences from *Leersia oryzoides*, *Luziola fluitans*, and *Ehrharta erecta* were generated in this study. The chloroplast *trnL* intron was amplified and sequenced with primers trn-L1 and trn-L2. The two primers, located on the 5′ exon and 3 exon of the *trnL* gene, respectively, were designed based on sequences of *Oryza sativa* (GenBank accession X15901), *Triticum aestivum* L. (AF148757), and *Zea mays* L. (X86563). The entire intron of mitochondrial *nad1* intron 2 and a small part of the 3′ exon was amplified and sequenced using the primers of Demesure et al. (1995). The PCR cycling parameters for the three loci were similar, with an initial 4 min at 70°C, followed by two cycles of 1 min at 94°C, 20 s at 52°C, and 1.5 min at 72°C; after that, 35 cycles of 20 s at 94°C, 20 s at 52°C, and 1.5 min at 72°C were conducted, with a final extension time of 10 min at 72°C.

The nuclear *Adh2* gene was amplified and sequenced with primers described in Ge et al. (1999). Two PCR primers (AdhF1 and AdhR1) are located in exon 2 and exon 8, respectively, and thus the sequenced *Adh2* region includes six introns and five exons (Ge et al., 1999). Sequences of all *Oryza* species and *Zizaniopsis villanensis* were used in Ge et al. (1999). The nuclear *GPA1* was amplified and sequenced with primers GPA1-FF and GPA1-14R (Bao and Ge, 2004). These two primers were designed based on *GPA1* sequences of *Oryza sativa* (L35844) and *Zea mays* (AF055471). The region amplified by the two primers spanned from exon 9 to exon 14, thus including four exons and five introns (Bao and Ge, 2004). The temperature profile for thermocycling to amplify the two nuclear genes was an initial 4 min at 70°C, followed by two cycles of 1 min at 94°C, 20 s at 54°C, and 1.5 min at 72°C; After that, 35 cycles of 20 s at 94°C, 20 s at 54°C, and 1.5 min at 72°C, with a final extension time of 10 min at 72°C. All the amplifying and internal sequencing primers for these fragments are listed in Table 1.

All PCR products were eclectrophoresed on and excised from 1.5% agarose gel. After purification using a DNA Purification kit (Amersham Pharmacia Biotech, Piscataway, USA) , the products of cytoplasmic genes (*matK*, *trnL* intron and *nad1* intron 2) were sequenced directly. Because the *Adh* gene was conducted using universal primers, amplified products contained both *Adh1* and *Adh2* sequences. Therefore, we used the pGEM-T Vector (Promega Corporation, Madison, USA) to clone the amplified sequences and sequenced individual clones for both diploids and tetraploids. For diploid species, we sequenced at least three clones until *Adh2* sequences were identified. For tetraploid species, 12–20 clones were screened by sequencing with one primer in an attempt to identify two homeologous loci and then were fully sequenced for both stands. The nuclear *GPA1* of diploid species were amplified and sequenced directly. When unable to yield a clean sequence for diploid species by direct sequencing, we cloned and sequenced them with a similar method used for the *Adh2* gene. The *GPA1* of tetraploid species were cloned and sequenced by the same strategy used for the *Adh2* tetraploids mentioned.

TABLE 1. The genes and primers used in this study. (The primers underlined are internal sequencing primers).

Locus	Primer	Direction	Sequence 5′-3′	Reference
Cytoplasmic				
matK	matKF1	Forward	TAATTAAGAGGATTCACCAG	Ge et al. (1999)
	matKR1	Reverse	ATGCAACACCCTGTTCTGAC	Ge et al. (1999)
	matKF2	Forward	ATTGCCTTTCCTTGATATCG	Ge et al. (1999)
	matKR2	Reverse	ACTACTCGAATTGGAATAG	Ge et al. (1999)
	matK-AF	Forward	CTATATCCACTTATCTTTCAGGAGT	Kato et al. (1998)
	matK-8R	Reverse	AAAGTTCTAGCACAAGAAAGTCGA	Kato et al. (1998)
trnL	trn-L1	Forward	TCGGTAGACGCTACGGAC	This paper
	trn-L2	Reverse	GGATAGAGGGACTTGAACC	This paper
nad1	nad1 exonB	Forward	GCATTACGATCTGCAGCTCA	Demesure et al. (1995)
	nad1 exonC	Reverse	GGAGCTCGATTAGTTTCTGC	Demesure et al. (1995)
	nad1 exB-FF	Forward	GGATATACACCAGGGCAAC	This paper
Nuclear				
Adh2	AdhF1	Forward	CACACCGACGTCTACTTCTG	Ge et al. (1999)
	Adh2-F1	Forward	TGCATGTGCTCCTCTATGG	This paper
	AdhR1	Reverse	ATCATGGCGTTGATGTTGCC	Ge et al. (1999)
	Adh2RR	Reverse	CCACCGTTGGTCATCTCAAT	Ge et al. (1999)
	Adh1R2	Reverse	ACTCACAGCAAGGCCTACAGC	Ge et al. (1999)
	Adh2R2	Reverse	ACAGCAAGGCCAACAGCTCC	Ge et al. (1999)
	AdhR3	Reverse	GTCACACCCTCTCCAACACTCT	Ge et al. (1999)
GPA1	GPA1-FF	Forward	GCAAGAGTACGGACAAATGGTG	Bao and Ge (2004)
	GPA1–14R	Reverse	GCTTGCTGCTCTGGAAGTAG	Bao and Ge (2004)
	GPA1–10F	Forward	GAGAGGTATATAGGTTGTATG	This paper
	GPA1–12FF	Forward	GACATATTCGAGAGGAAAATAC	This paper
	GPA1–10RR	Reverse	GGCAGCACAAAAGATTAC	This paper
	GPA1–13R	Reverse	GTCTTTAAACCACTCGCACAC	This paper

Clones with distinct inserts were fully sequenced for both strands and included in the phylogenetic analysis. Sequencing was done on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, California, USA) or a Megabase 1000 automatic DNA sequencer (Amersham Biosciences, Buckinghamshire, UK) .

Data analysis—Sequences were aligned using ClustalX version 1.81 (Thompson et al., 1997) and refined manually. The possibility of sequence saturation was examined using DAMBE version 4.2.13 (Xia and Xie, 2001). A plot of the number of transitions and transversions vs. divergence offers a visual display of substitution saturation, with an asymptotic relationship indicating the presence of saturation (Xia and Xie, 2001). Phylogenetic analyses of the sequence data were performed using the parsimony and Bayesian Markov chain Monte Carlo (MCMC) methods. Maximum parsimony (MP) analyses were conducted using PAUP version 4.0b10 (Swofford, 2001). All characters were equally weighted, gaps were treated as missing, and character states were treated as unordered. Heuristic search was performed with MULPARS option, tree-bisection-reconnection (TBR) branch swapping, and RANDOM stepwise addition with 1000 replicates. Topological robustness was assessed by bootstrap analysis with 1000 replicates using simple taxon addition (Felsenstein, 1985). An appropriate nucleotide substitution model was determined using Modeltest version 3.5 (Posada and Crandall, 1998) for each data set. The models were chosen according to the hierarchical likelihood ratio test (LRT) and then used for subsequent Bayesian analysis. Bayesian inference (BI) was conducted using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001). One cold and three incrementally heated MCMC chains were run for 10^6 generations, with trees sampled every 10 generation, using the random tree as its starting point and a temperature parameter value of 0.2 (the default setting of MrBayes). For each data set, MCMC runs were repeated twice as a safeguard against spurious results. The first 10^4 trees were discarded as burn-in, and the remaining trees were used to construct Bayesian trees. Examination of the log-likelihoods and the observed consistency between runs suggested that the burn-in periods were sufficiently long enough for chains to have become stationary.

The partition homogeneity test, as implemented in PAUP version 4.0b10 (Swofford, 2001), was used to evaluate the topological congruence between gene trees (Farris et al., 1995). Replicates were analyzed using the parsimony criterion, and branch swapping using TBR was performed and one tree held at each step during the stepwise addition. The partition homogeneity test was performed on either cytoplasmic (chloroplast plus mitochondrial) or nuclear loci, repectively. The results were used to determine whether each combined data set had significant incongruence. Following Cunningham (1997), we took a P value of 0.01 as a significance criterion for this test.

For a rough estimate of the divergence time of Oryzeae and its major lineages, a simple molecular clock assumption was employed, and the clock calibration was carried out based on the assumption that maize and rice diverged 50 million years ago [mya] (Stebbins, 1981; Gaut, 2002). Divergence times (T) of the Oryzeae and its major lineages were calculated by dividing the the estimated numbers of substitutions per site between homologous sequences (K) by twice the rate of nucleotide substitution (r), namely, using the formula $T = K/(2)\ (r)$. Relative rate tests were implemented by Hyphy (Kosakovsky Pond et al., 2005) and RRTree (Robinson-Rechavi and Huchon, 2000) and used to assess heterogeneity among different lineages. Rates of nucleotide substitution for individual genes were estimated by the Nei-Gojobori model with the Jukes-Cantor and Kimura two-parameter corrections as implemented in MEGA version 2.1 (Kumar et al., 2001).

RESULTS

Amplification, sequencing, and sequence characteristics—Amplifications for three cytoplasmic loci from all the species included in this study were successful except for the *matK* sequence of *E. erecta* for which no amplified product was obtained by using primers matKF1 and matKR1. Therefore, we used another pair of primers (matK-AF and matK-8R) (Kato et al., 1998) to amplify *matK* from *E. erecta* (Table 1). The resulting fragment was about 1.2 kilobases (kb), shorter than those of other species (1.5 kb). The sequence length of *matK* ranged from 1502 to 1562 bp (1143 bp for *E. erecta*) with an aligned length of 1574 bp. The characteristics of the

TABLE 2. Characteristics of chloroplast, mitochondrial, nuclear DNA data sets, and the combined data sets (excluding outgroups).

Sequence	Aligned length	Number of variable sites (%)	Number of informative sites (%)	GC (%)	Nucleotide substitution model	Gamma shape parameter
Cytoplasmic						
matK	1574	241 (15.3)	124 (7.9)	34.4	HKY + G	0.3614
trnL	609	87 (14.3)	46 (7.6)	34.9	HKY + G	0.3263
nad1	1485	61 (4.1)	33 (2.2)	54.1	HKY + I + G	0.9990
matK + *trnL* + *nad1*	3687	389 (10.6)	203 (5.5)	42.5	HKY + I + G	0.9550
Nuclear						
Adh2	903	360 (39.9)	233 (25.8)	42.6	TrNef + G	0.4517
GPA1	708	322 (45.5)	168 (23.7)	38.8	TrN + G	0.9440
Adh2 + *GPA1**	1611	654 (40.6%)	392 (24.3)	44.7	TrN + G	0.5816

* *Hygroryza aristata* was excluded from the combined data set.

three cytoplasmic loci are shown in Table 2. As expected, the mitochondrial *nad1* intron 2 is less variable and informative than the chloroplast genes. The mean GC content of mitochondrial *nad1* intron 2 (54.2%) is higher than those of chloroplast fragments (34.4% for *matK* and 34.9% for *trnL*). Overall, 3.6 kb of cytoplasmic DNA sequences are determined for all the species involved.

Using the universal primers (AdhF1 and AdhR1) of the *Adh* gene, we obtained a strong single product of about 1.7 kb for all the species. *Adh2* sequences were obtained from all the species except for *Potamophila parviflora* in which no *Adh2* sequence was found, although 24 clones were screened. By designing an *Adh2*-specific forward primer (Adh2-F1, Table 1) in conjunction with Adh2RR, an *Adh2*-specific reverse primer (Ge et al., 1999), we amplified successfully an ~1.1-kb *Adh2* fragment from *P. parviflora*. For multiple clones of diploid species, we obtained almost identical sequences but occasionally found several base pair differences among clones. However, initial phylogenetic analysis indicated that *Adh2* sequences of different clones from the same diploid species formed a monophyletic group, and thus we only retained one clone in our final analyses because exclusion of these minor types of clones have no impact on relationships among species. For tetraploid species, two distinct types of sequences were identified at the *Adh2* locus for the majority of tetraploid species except for two *Leersia* species (*L. oryzoides* and *L. hexandra*), in which only one type of sequence was sampled. The detection of only one *Adh2* sequence for the tetraploid *L. oryzoides* and *L. hexandra* is either indication of autoploidy or due to screening an insufficient number of clones. An exhaustive search for the other copy was not carried out, because it does not seem to be critical for understanding the phylogenetic relationships of Oryzeae in this study. In contrast, we got two distinct types of sequences from *L. perrieri*, which was previously identified as a diploid species ($2n = 24$) (Katayama, 1995). This possibly results from duplication in the *Adh2* or a wrongly recognized number of chromosomes for this species. The sequenced segments vary from 1558 to 1891 bp in length. Some parts of intron regions of the *Adh2* gene were difficult to align and thus were removed from the phylogenetic analysis. After excluding the ambiguous regions, the final alignment is 903 bp in length (including 594 bp of exons), with 360 sites (39.9%) variable and 233 sites (25.8%) informative.

A single product of c. 1 kb in size for the *GPA1* gene was amplified in all species except for *Hygroryza aristata* and *E. erecta*, in which a single product of c.1.5 kb and 2.8 kb, respectively, was amplified. Therefore, we designed four additional primers (two forward and two reverse primers as listed in Table 1) to obtain the complete sequences of the two species. After comparing their sequences with those of other species, we found an ~630-bp fragment inserted into intron 13 of *GPA1* in *H. aristata* and three large segments inserted into intron 9 (~700 bp), intron 10 (~550 bp), and intron 13 (~620 bp), respectively, in *E. erecta*. Consequently, the sequence lengths for *H. aristata* and *E. erecta* are 1531 bp and 2860 bp, respectively, whereas the sequences for the remaining Oryzeae species vary from 871 to 990 bp. For the other outgroup, *Phyllostachys aurea*, a 280-bp fragment inserted into intron 10 was found, and the final sequence length of *P. aurea* is 1202 bp. Amplification products of *GPA1* for all diploid species were sequenced directly except for four species (*Luziola leiocarpa*, *Zizania latifolia*, *Zizaniopsis villanensis*, and *E. erecta*) in which unreadable sequences were found, suggestive of some extent of heterogeneity. Therefore, products from the four species, along with those from the tetraploid species, were subsequently cloned and sequenced using a similar strategy to that mentioned earlier. For each tetraploid, we identified two different types of sequences at the *GPA1* locus. Similar to the *Adh2* gene, we also got two different types of *GPA1* sequence for the diploid *L. perrieri*. After excluding the ambiguous regions, the final alignment of *GPA1* sequences is 708 bp long (including 369 bp of exons), of which 322 sites (45.5%) are variable and 168 sites (23.7%) are informative.

As shown in Table 2, cytoplasmic DNA is much less variable than the nuclear genes. Of the two nuclear genes, *Adh2* has higher parsimony information than *GPA1*, but the percentage of variable sites of *GPA1* is higher than that of *Adh2*. Because the *Adh2* and *GPA1* sequences had more than 30% variable sites (excluding the outgroups), we examined possible saturation of the two data sets. A good linear relationship was found for both *Adh2* and *GPA1* (not shown), indicating that the two data sets are not saturated in Oryzeae. Each of the sampled sequences and their most appropriate models are also presented in Table 2.

Phylogenetic analysis of the cytoplasmic genes—Phylogenetic analyses of three individual data sets from cytoplasmic genomes (*matK*, *trnL* intron, and *nad1* intron 2) provide approximately the same topology, in which the genera of the tribe fall into two main clades, but resolution was not sufficient to resolve relationships within clades (not shown). In the par-

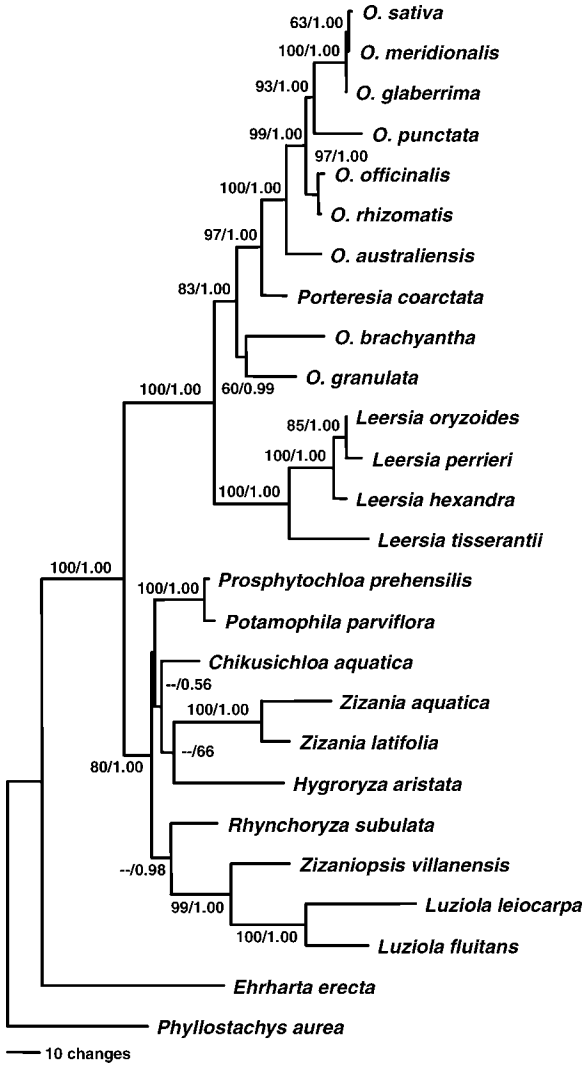

Fig. 1. A single most parsimonious tree (MPT) based on the combined data set of chloroplast *matK* and *trnL* intron and mitochondrial *nad*1 intron 2 (tree length = 654, consistency index = 0.806, retention index = 0.852). Bayesian phylogenetic analysis generates a similar topology. Numbers near branches are bootstrap percentages followed by Bayesian posterior probabilities.

tition homogeneity test, there was no significant incongruence among the three data sets ($P = 0.921$). Therefore, we combined the three fragments with a total length of 3687 bp. The combined data set generates one most parsimonious tree (654 steps, consistency index [CI] = 0.806, retention index [RI] = 0.852) (Fig. 1). The MP analysis shows that the Oryzeae species form a clade with 100% bootstrap support (BS) and can be divided into two highly supported clades (100% and 80% BS, respectively). The first clade includes three genera, *Leersia*, *Oryza*, and *Porteresia*. In this clade, four *Leersia* species form a highly supported group (100% BS) with the diploid species *L. tisserantii* at the basal position. The species of *Oryza* and the monotypic genus *Porteresia* form a strongly supported group (83% BS). The second clade consists of the remaining eight genera. Although the resolution in this clade is slightly lower, there are still several clades with strong support, including the *Prosphytochloa*/*Potamophila* clade (100% BS), two *Zizania* species (100% BS), and the *Zizaniopsis*/*Luziola* clade (99% BS).

The Bayesian analysis of the combined data set under the HKY + I + G model (Hasegawa-Kishino-Yano; I, G correction; Hasegawa et al., 1985) generates a similar topology, with only a few differences in bootstrap support and Bayesian posterior probability (PP) for some clades (Fig. 1). For example, the sister relationships between *Hygroryza* and *Zizania* (0.66 PP), between *Chikusichloa* and *Hygroryza*/*Zizania* (0.56 PP), and between *Rhynchoryza* and *Luziola*/*Zizaniopsis* (0.98 PP) in the Bayesian tree are not supported in the parsimony tree.

In addition, we conducted different rooting strategies using either *Phyllostachys aurea* or *E. erecta* or both as outgroups. In all analyses, ingroup topologies were not affected by outgroup selection, and the alternative outgroup was not nested within the ingroup, providing robust support for the monophyly of the tribe Oryzeae. For all analyses discussed here, both outgroups were used to root trees.

Phylogenetic analysis of the nuclear genes—Phylogenetic trees generated by individual data sets are essentially similar (not shown). Two and 18 most parsimonious trees were obtained for the *Adh2* (911 steps, CI = 0.630, RI = 0.727) and the *GPA1* (639 steps, CI = 0.714, RI = 0.789) data sets, respectively. The strict consensus of both gene sequences support the monophyly of the Oryzeae species, and two main clades within the tribe were recovered. The first main clade includes three genera, *Leersia*, *Oryza*, *Porteresia* (98% BS in *Adh2* and 88% BS in *GPA1*). Four genera (*Zizania*, *Rhynchoryza*, *Zizaniopsis*, and *Luziola*) form a second main clade with varying degrees of bootstrap support. A major exception is the placement of the genus *Hygroryza*. This monotypic genus is sister to the *Leersia*/*Oryza*/*Porteresia* clade in the *Adh2* tree but grouped with the *Zizaniopsis*/*Luziola*+*Zizania*/*Rhynchoryza* clade in the *GPA1* tree (not shown but see Fig. 3). The Bayesian method generates similar topologies under the models of TrNef + G (Equal-frequency Tamura-Nei; G correction; Tamura and Nei, 1993) (*Adh2*) and TrN + G (Tamura-Nei; G correction; Tamura and Nei, 1993) (*GPA1*), respectively.

The partition homogeneity test shows that there is marginally significant incongruence between the *Adh2* and *GPA1* data sets ($P = 0.028$) with the position of *Hygroryza aristata* between the *Adh2* and *GPA1* trees being apparently inconsistent. Therefore, we excluded the *H. aristata* sequences from the combined data set. Phylogenetic analysis of the combined data set recovers two equally most parsimonious trees (1529 steps, CI = 0.661, RI = 0.751), and their strict consensus tree is shown in Fig. 2. The strict consensus tree of the combined nuclear data set provides higher resolution than the individual nuclear and the combined cytoplasmic data sets. The monophyly of Oryzeae is strongly supported (100% BS), and the ingroup forms two clades with high support (100% and 82% BS, respectively). The first clade consists of the genera *Leersia*, *Oryza*, and *Porteresia*, and the second clade includes two subclades with strong support (99% and 99% BS, respectively). The first subclade consists of *Potamophila*, *Prosphytochloa*, and *Chikusichloa* (99% BS). The second subclade includes two sister groups: *Zizania*/*Rhynchoryza* (100% BS) and *Zizaniopsis*/*Luziola* (99% BS). The Bayesian phylogenetic analysis under the TrN + G model obtains a similar topology except for higher statistical support (bootstrap value and posterior probability) for several clades (Fig. 3). Significantly, the *Oryza* + *Porteresia* species are monophyletic with 0.67 sup-

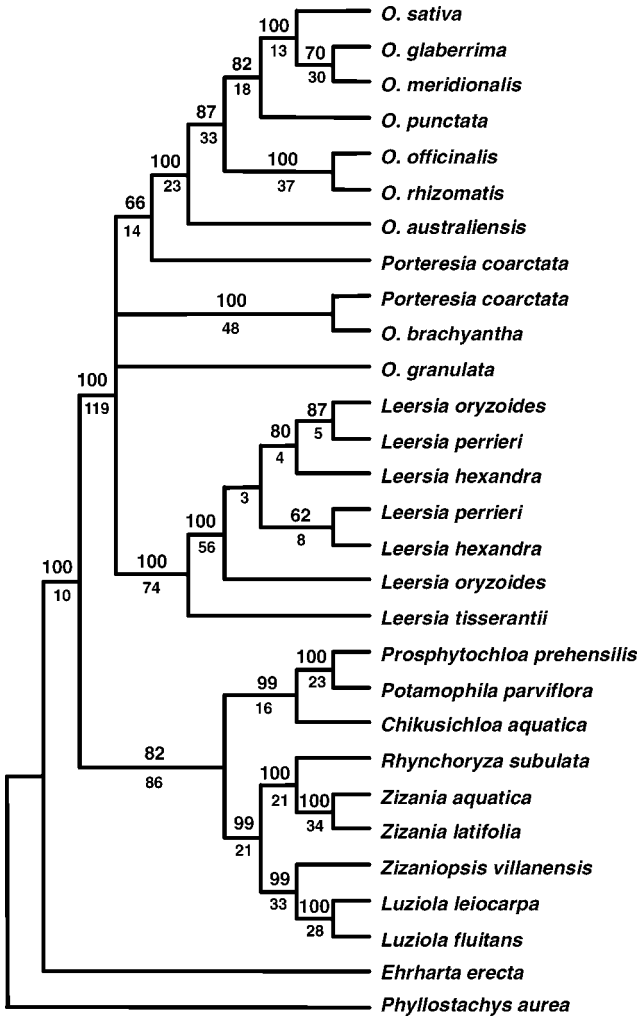

Fig. 2. The strict consensus of two equally most parsimonious trees (MPT) from the combined *Adh2* and *GPA1* data set after excluding *Hygroryza aristata* (tree length = 1529, consistency index = 0.661, and retention index = 0.751). Bayesian phylogenetic analysis generates a similar topology. Numbers above branches indicate bootstrap values above 50% and those below branches indicate nucleotide substitutions.

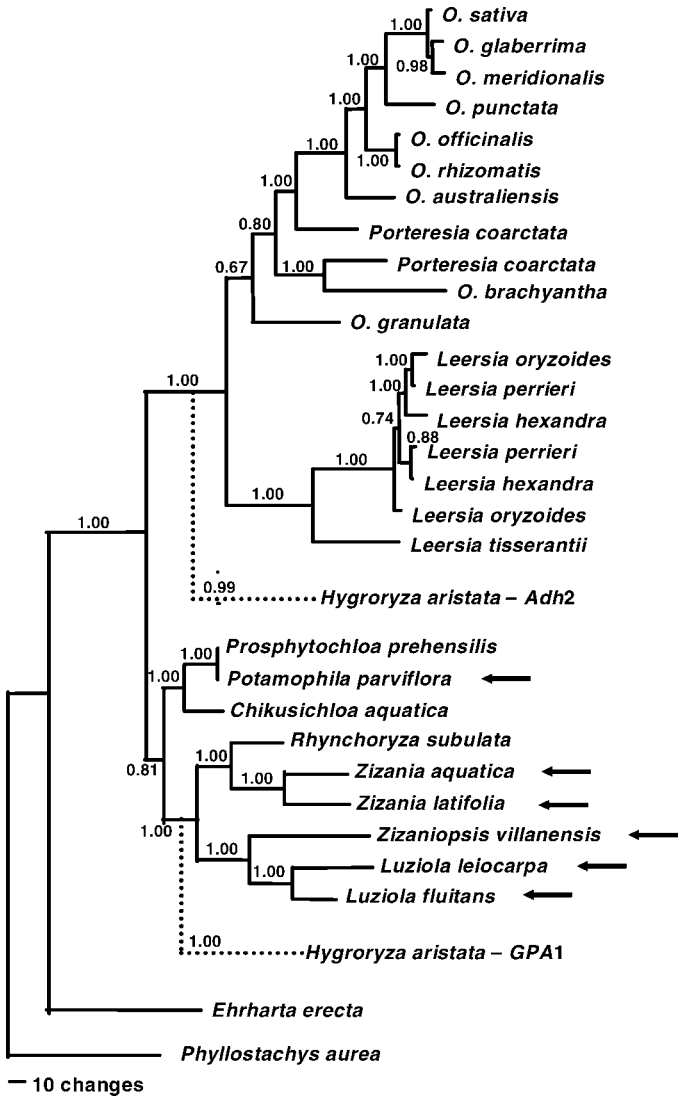

Fig. 3. Phylogeny of Oryzeae obtained from the combined *Adh2* and *GPA1* sequences by Bayesian inference under the TrN + G model after *Hygroryza aristata* was excluded. Numbers above branches are Bayesian posterior probabilities. The dashed lines indicate the position of *Hygroryza aristata* on the *Adh2* and *GPA1* trees, respectively. Arrows indicate the species with unisexual florets.

port of Bayesian posterior probability. The positions of *Hygroryza aristata* on the separate *Adh2* and *GPA1* concensus trees and species with unisexual flowers are shown in Fig. 3.

DISCUSSION

Monophyly and subdivision of the tribe Oryzeae—Since the tribe Oryzeae was proposed by Dumortier (1823), many genera have been added into Oryzeae and many of them were mistakenly treated. *Pharus* L. and *Leptaspis* R. Br. (including *Scrotochloa* Judziewicz) had been placed in Oryzeae by some researchers but later were treated as the separate tribe (Phareae) of Bambusoideae (Duistermaat, 1987). Similarly, genera that were unreasonably treated as members of Oryzeae include *Ehrharta* Thunb., *Microlaena* R. Br., *Tetrarrhena* R. Br., *Snowdenia* C. E. Hubb., *Achlaena* Griseb., and *Reynaudia* Kunth (Duistermaat, 1987). In addition, some authors (Avdulov, 1931; Gardner, 1952) had combined the tribes Oryzeae and Ehrharteae into a large rice tribe, namely Oryzeae sensu lato. Based on the study of anatomy and histology of leaf blades, Tateoka (1963) suggested that the two tribes should be treated separately. Since then, the recognized genera in Oryzeae have varied from 10 to 13 (Tateoka, 1963; Tzvelev, 1989), and the classification of 12 genera by Clayton and Renvoize (1986) and Vaughan (1994) has been accepted by most researchers (Zhang and Second, 1989; Duvall et al., 1993; Kellogg and Watson, 1993; Ge et al., 2002). From a morphological point of view, monophyly of the tribe Oryzeae is supported by the following synapomorphies: one-flowered spikelets, which are compressed or terete, with a lemma and palea, and two well-developed bracts (degenerated flowers or sterile lemmas) (Pyrah, 1969; Clayton and Renvoize, 1986); the two tiny lobes below, sometimes referred to as cupules or glumes, appear to be expanded apices of the pedicels (Terrell et al., 2001). Additionally, morphological and molecular studies also

TABLE 3. Main subdivision systems of the tribe Oryzeae and the proposed system in this study.

Hitchcock (1920) Hitchcock & Chase (1951)	Hubbard (1934, 1959)	Terrell (1974)	This study
Tribe Oryzeae	Subtribe Oryzinae	Subtribe Oryzinae	Subtribe Oryzinae
Leersia	*Leersia*	*Leersia*	*Leersia*
Oryza	*Oryza*	*Oryza*	*Oryza (Porteresia)*
	Hygroryza	*Porteresia*	
Tribe Zizanieae	Subtribe Zizaniinae	Subtribe Zizaniinae	Subtribe Zizaniinae
Zizania	*Zizania*	*Zizania*	*Zizania*
		Subtribe Luziolinae	
Luziola	*Luziola*	*Luziola*	*Luziola*
Zizaniopsis	*Zizaniopsis*	*Zizaniopsis*	*Zizaniopsis*
Hydrochloa	*Hydrochloa*		*Hygroryza*
			Chikusichloa
			Rhynchoryza
			Potamophila

support the distinctiveness and monophyly of Oryzeae (Duistermaat, 1987; Zhang and Second, 1989; Kellogg and Watson, 1993; Ge et al., 2002). Nevertheless, because most previous researchers (Pyrah, 1969; Clayton and Renvoize, 1986; Duistermaat, 1987; Zhang and Second, 1989; Kellogg and Watson, 1993) sampled limited species without some important genera included in their studies, the monophyly of Oryzeae was not determined with certainty. In this study, the analyses of both cytoplasmic and nuclear (either individual or combined) sequence data (Figs. 1–3) strongly support the monophyly of the tribe Oryzeae.

The subdivision of Oryzeae has varied among different researchers (Hitchcock, 1920; Hubbard, 1934, 1959; Hitchcock and Chase, 1951; Stebbins and Crampton, 1961; Pyrah, 1969; Terrell and Robinson, 1974), and three main subdivision systems of Oryzeae are summaried in Table 3. Whether they were recognized as two groups at tribal (Oryzeae vs. Zizanieae) (Hitchcock, 1920; Hitchcock and Chase, 1951; Stebbins and Crampton, 1961) or subtribal (Oryzinae vs. Zizaniinae) (Hubbard, 1934, 1959; Pyrah, 1969) levels or treated as three subtribes (Terrell and Robinson, 1974), they were all based on the monoecious vs. bisexual spikelet bifurcation. Based on cytoplasmic and nuclear sequence data, the present phylogenetic analyses of all genera in this tribe support recognition of two clades although the systematic position of *Hygroryza* remains ambiguous. The third subtribe Luziolinae proposed by Terrell and Robinson (1974) is not justified because in the cytoplasmic and nuclear phylogenetic trees the *Zizaniopsis*/*Luziola* clade forms a clade with the *Rhynchoryza*/*Zizania* clade, which is sister to the *Potamophila*/*Prosphytochloa*/*Chikusichloa* clade (Figs. 1 and 2).

Two clades supported by the present molecular data correspond to two traditionally recognized subtribes (Figs. 1 and 3; Table 3). The first subtribe is Oryzinae including three genera, i.e., *Leersia*, *Oryza*, and *Porteresia*. The second is Zizaniinae consisting of the other seven genera (*Potamophila*, *Prosphytochloa*, *Chikusichloa*, *Zizania*, *Rhynchoryza*, *Zizaniopsis*, and *Luziola*). Therefore, a subdivision system of this tribe based on the present study is presented in Table 3. It is worthwhile mentioning that the two subtribes accepted in this study (Table 3) are not essentially similar to those previously recognized based on the monoecious vs. bisexual classsification (Hubbard, 1934, 1959; Pyrah, 1969; and see Table 3) in which many genera were not included. It is apparent from Fig. 3 that bisexual genera (*Rhynchoryza*, *Prosphytochloa*, and *Chikusichloa*) and unisexual genera (*Luziola*, *Zizaniopsis*, and *Zizania*) are mingled with one another in the subtribe Zizaniinae. Beyond this, both unisexual and bisexual florets have been found in the spikelets of the Australian species *Potamophila parviflora* (Fig. 3). Consequently, the structure of unisexual florets is most likely to be of multiple origins and the bisexual structure is apparently the ancestral state.

Delimitation and phylogenetic relationships of genera in Oryzeae—Within Oryzeae, the monophyly of some genera is supported by the present multiple gene analyses. Four *Leersia* species, including *L. tisserantii* and *L. perrieri* that were previously classified in *Oryza* (Tateoka, 1963), form a highly supported monophyletic group, indicating that the transfer of *L. tisserantii* and *L. perrieri* from *Oryza* to *Leersia* by Launert (1965) is justified (Zhang and Second, 1989; Ge et al., 2002). *Luziola fluitans* has been treated as the monotypic genus *Hydrochloa* and later considered a member of *Luziola* based on growth habitat, chromosome number, and simple racemose staminate inflorescences (Pohl and Davidse, 1971). The work of Terrell and Robinson (1974) also indicated that *Hydrochloa* could not be differentiated satisfactorily from *Luziola* as they appeared to differ mainly by the reduced inflorescence in *Hydrochloa*. In the present analysis, two *Luziola* species including *Luziola fluitans* form a monophyletic group with high statistical (100% BS and 1.00 PP) support, suggesting that *Hydrochloa* be retained in *Luziola*. Similarly, previous researchers (Hubbard, 1967; Clayton, 1970) divided the genus *Potamophila* R. Br. into three genera, including two monotypic genera (*Prosphytochloa* and *Potamophila*) and *Maltebrunia* Kunth. This treatment was questioned by Duistermaat (1987) because these genera did not have fundamental differences in the structure of spikelets. In the present study, the close relationship between *Potamophila* and *Prosphytochloa* is highly supported in all the data sets (100 BS and 1.00 PP) (Figs. 1–3), and the sequence divergence between the two genera is much less than those between species of many other genera in the tribe (data not shown). Therefore, the present molecular data is in general accordance with the opinion that *Prosphytochloa* is within the generic limits of *Potamophila* (Duistermaat, 1987; Vaughan, 1994).

Whether the genus *Zizania* is more closely related to monoecious or to bisexual genera and its relationship with *Zizaniopsis* has not been resolved (Terrell and Robinson, 1974; Zhang and Second, 1989; Duvall et al., 1993; Terrell et al.,

2001; Ge et al., 2002). *Zizaniopsis* and *Zizania* are similar to one another in many characteristics including the lodicules, stamens, and pistil as well as the monoecious spikelets, and therefore *Zizaniopsis* was included under *Zizania* prior to 1871 (Weatherwax, 1929). But subsequent morphological studies showed that the fruits of *Zizania* and *Zizaniopsis* were conspicuously different and suggested that the two genera belonged to separate genera or even separate tribes of grasses (Martin, 1946; Terrell and Robinson, 1974). Recent molecular studies also indicated that the two genera separated from each other (Zhang and Second, 1989; Duvall et al., 1993; Ge et al., 2002). Our study of cytoplasmic and nuclear sequence data demonstrates that *Zizaniopsis* is more closely related to *Luziola* than to *Zizania* (Figs. 1 and 3). In addition, that *Porteresia* is nested within the clade of *Oryza* species further supports our previous statement that *P. coarctata* had a high affinity with *Oryza* species and should be treated as a member of *Oryza* rather than an independent monotypic genus (Ge et al., 2002).

It is noteworthy that the monotypic genus *Hygroryza* forms a clade with the Oryzinae clade on the *Adh2* tree but clusters with the Zizaniinae clade on the *GPA1* tree (Fig. 3). The combined cytoplasmic phylogeny (Fig. 1) presents a topology approximately similar to that of *GPA1*. The incongruence among phylogenies may often be due to many different causes such as gene choice and insufficient data, convergent evolution, rapid diversification, hybridization and introgression, and lineage sorting as well as rate heterogeneity among taxa or among sites of the sequences used (for reviews, see Johnson and Soltis, 1998; Wendel and Doyle, 1998). Because the sequences we used represent multiple loci from three genomes and include the nuclear genes that evolve rapidly with their substitution rates distributed evenly over sites (not shown), insufficient data and rate heterogeneity among sites are not likely to be reasons for the incongruence. As indicated by many authors, lineage sorting or phylogenetic sorting is one of the important factors causing phylogenetic incongruence if a polymorphism transcends one or more organismal divergence events (Wendel and Doyle, 1998). However, lineage sorting can be ruled out as a factor for discrepancy in the position of *Hygroryza* because it usually occurs at lower taxonomic ranks except in some exceptional cases (Rivers et al., 1993; Wendel and Doyle, 1998). Hybridization and subsequent polyploidization, which may result in reticulate evolution, would also be excluded for the cause of the incongruence because *Hygroryza* is a diploid ($2n = 24$) endemic to southeast Asia, and only one type of sequence was found for both *Adh2* and *GPA1* genes. Consequently, the ambiguous position of *Hygroryza* is probably the result of introgression and rapid speciation. Accumulating evidence shows that interspecific hybridization and introgression, though sometimes ''cryptic,'' are exceptionally widespread and are powerful and creative forces in evolution in plants (Wendel and Doyle, 1998; Wendel and Cronn, 2003). Whether this incongruence reflects ''real'' underlying biological phenomena or just ''spurious'' insufficient data or some other unknown artifact is currently unclear and needs to be further investigated.

Implications for the origin and divergence of Oryzeae—Because the rice tribe is a diverse and geographically widespread group with many economically important species, its time and place of origin and subsequent divergence have been subjects of considerable discussion (Clayton, 1975; Chang, 1985; Second, 1985; Duistermaat, 1987). In the Poaceae, macrofossils provide greater taxonomic resolution than pollen because there are more diagnostic characters that facilitate broad comparisons in phylogenetic studies (Jacobs et al., 1999). The oldest Oryzeae macrofossils have been reported from the end of the Eocene by Litke (1968) who discovered fossilized bits of leaf epidermis in coal deposits of eastern Germany. Other reported macrofossils identified as Oryzeae include silicified anthoecia (fertile lemmas and paleas) found in Nebraska (North America) in the Miocene age (Thomasson, 1980) and spikelets found in an excavation of Miocene age in Germany (Heer, 1855). The former was described as *Archaeoleersia nebraskensis* Thomasson, which has features in common with those of the living *Leersia ligularis* Trin., and the latter as *O. exasperata* (A. Braun) Heer., which closely resembles *O. meyeriana* (Heer, 1855). Therefore, the fossil reports suggest that Oryzeae had already differentiated by the end of the Eocene (36 to ~40 mya).

Molecular tools provide an alternative source of information with which to estimate divergence times. Based on isozyme electrophoresis data, Second (1985) estimated the divergence time of *Oryza* to be of the Miocene epoch (~15 mya) and the African A-genome species separated from the Asian A-genome species c. 7 mya. Although the divergence time of Poaceae has been estimated by molecular approaches (Bremer, 2002; Gaut, 2002), no attempt has been made to date the various events in Oryzeae using sequence data. Despite a number of limitations to the use of a molecular clock on sequence data, it is still useful for estimating divergence time if calibration can be made with confidence (Gaut, 2002; Wendel and Cronn, 2003).

To test the molecular clock hypothesis in Oryzeae, we performed relative rate tests for all the fragments used in this study. Results show that no rate heterogeneity exists at synonymous sites of *matK* sequences and at nonsynonymous sites of *GPA1* sequences whereas either saturation or rate heterogeneity among lineages are found for other data sets. Therefore, the data sets of *matK* synonymous sites and *GPA1* nonsynonymous sites are used for molecular dating. Another important aspect of divergence time estimation is clock calibration. Because the sequence divergence rates of these two loci either varied greatly among plant groups (*matK*) (Koch et al., 2001) or are unknown (*GPA1*), we estimated the absolute substitution rates of *matK* synonymous sites and *GPA1* nonsynonymous sites based on the assumption that maize and rice diverged 50 mya (Stebbins, 1981; Gaut, 2002). The estimated substitution rates for *matK* synonymous sites and *GPA1* nonsynonymous sites are 1.96×10^{-9} and 1.02×10^{-9} substitutions per site per year, respectively. Using the molecular clocks of *matK* synonymous sites and *GPA1* nonsynonymous sites, we calculated approximate divergence times for various lineages within the tribe and within *Oryza*. Based on *matK* synonymous sites, the mean sequence divergence between the two subtribes (the *Oryza–Leersia* clade vs. the clade including the remaining genera) is 8.1%, which translates into a divergence of 20.5 mya, close to the 22.1 mya estimate based on *GPA1* nonsynonymous sites. Interestingly, both *matK* and *GPA1* estimates indicate that *Oryza* and *Leersia* separated from each other c. 14.2 mya. Within *Oryza*, we obtain the divergence time of 10.2 mya (*matK*) and 8.8 mya (*GPA1*), respectively, for the most basal split in the genus (*O. granulata* vs. the remaining species). These estimations suggest that the tribe Oryzeae diversified during the Miocene epoch, in good agree-

ment with fossil evidence (Heer, 1855; Litke, 1968; Thomasson, 1980). Therefore, the previously estimated divergence time among *Oryza* species of about 15 mya based on isozyme electrophoresis data (Second, 1985) is probably inflated, and the assumption that the rice genus originated before the fragmentation of Gondwanaland (Chang, 1985) is not justified.

Although molecular dating suffers from various errors including substitution rate heterogeneity among lineages, uncertainties of clock calibration, and other potential sources for errors (Gaut, 2002; Wendel and Cronn, 2003), the estimates in the present study provide a rough timeframe for understanding the evolutionary tempos and biogeographic history of this important group. To put the divergence of the rice tribe and rice genus into a temporal framework raises an interesting question regarding the biogeographic patterns in Oryzeae because both the tribe and particular genera, such as *Oryza* and *Leersia*, have achieved pantropical distribution. These distribution patterns imply that transoceanic dispersal or long-distance dispersal would contribute to the evolution and divergence of the Oryzeae given the fact that the two genera (*Oryza* and *Leersia*) branched from the remaining genera c. 20 mya and split from each other c. 14 mya. Long-distance dispersal events were also suggested for other recently diverged lineages such as *Gossypium* (Wendel and Cronn, 2003). It is worthwhile to note that the closely related genera *Chikusichloa*, *Potamophila*, and *Prosphytochloa* (Fig. 3) that are distributed in eastern Asia, Australia, and Africa, respectively, are currently isolated geographically from one another by thousands of kilometers of open ocean. This similarly implicates oceanic dispersal as a major factor in their evolution and divergence and suggests that this clade is likely to originate in Asia because *Chikusichola* is at the basal position (Fig. 3). Compared with the *Oryza*/*Leersia* clade, low sequence divergence was found among the genera in the clade corresponding to the subtribe Zizaniinae (not shown), which can be translated into phylogenetically short interior branches (Fig. 3) and may be one of the main reasons for the low resolution within this clade. This phenomenon may reflect rapid divergence or radiation during the early divergence of the tribe. It is also evident that the dispersal from America to Asia occurred in the *Rhynchoryza*/*Zizania*/*Zizaniopsis*/*Luziola* clade because *Zizania* is disjunctively distributed in eastern Asia and eastern North America while all the other genera are exclusively distributed in North and South America. Taken together, the rice tribe, in particular the worldwide genera *Oryza* and *Leersia*, is a successful group, as indicated by its extensive geographic ranges. Further studies will require a multidisciplinary approach and more extensive sampling to clarify their evolutionary history and biogeographic patterns.

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APPENDIX. Species of Oryzeae and outgroups used in this study.

Taxon (genome type of *Oryza* species); voucher or accession number; origin; chromosome number 2*n*; GenBank accession numbers: *MatK*; *trnL*; *nad1*; *Adh2*; *GPA1*.

O. sativa L. (AA); 30416[c]; Philippines; 24; AF148650[a]; AY792515; AY507930[a]; AF148602[a]; AY792541. ***O. glaberrima*** Steud. (AA); 104042[c]; Chad; 24; AF148654[a]; AY792516; AY792569; AF148606[a]; AY792542. ***O.***

meridionalis Ng (AA); 101147[c]; Australia; 24; AF148657[a]; AY792517; AY792570; AF148609[a]; AY792543. ***O. punctata*** Kotschy ex Steud. (BB); 104017[c]; Cameroon; 24; AF148661[a]; AY792518; AY507931[a]; AF148611[a]; AY299684[a]. ***O. officinalis*** Wall ex Watt (CC); 105085[c]; Philippines; 24; AF148658[a]; AY792519; AY507932[a]; AF148613[a]; AY188586[a]. ***O. rhizomatis*** Vaughan (CC); 105448[c]; Sri Lanka; 24; AF148660[a]; AY792520; AY792571; AF148614[a]; AY188587[a]. ***O. australiensis*** Domin (EE); 101144[c]; Australia; 24; AF148667[a]; AY792521; AY507937[a]; AF148623[a]; AY188596[a]. ***O. brachyantha*** A. Chev. et Roehr. (FF); 105151[c]; Sierra Leone; 24; AF148670[a]; AY792522; AY507934[a]; AF148632[a]; AY792546. ***O. granulata*** Nees et Arn. ex Watt. (GG); 2422; China; 24; AF148674[a]; AY792523; AY507938[a]; AF148631[a]; AY188597[a]. ***Porteresia coarctata*** Tateoka[b] (HHKK); 104502[c]; Bangladesh; 48; AF148669[a]; AY792524; AY507935[a]; AF148627[a], AF148628[a]; AY792544, AY792545. ***Leersia oryzoides*** (L.) SW. (—); GS0203; China; 48; AY792566; AY792525; AY792572; AY792577; AY792547, AY792548. ***Leersia perrieri*** (A. Camus.) Launert (—); 105164[c]; Madagascar; 24; AF148677[a]; AY792526; AY792573; AY792578, AY792579; AY792549, AY792550. ***Leersia hexandra*** Sw. (—); 105252[c]; Philippines; 48; AF489909[a]; AY792527; AY507940[a]; AY792580; AY792551, AY792552. ***Leersia tisserantii*** (A. Chev.) Launert (—); 105610[c]; Cameroon; 24; AF489910[a]; AY792528; AY507939[a]; AY792581; AY792553. ***Prosphytochloa prehensis*** (Nees) Schweick[b] (—); unknown[c]; S. Africa; 24; AF489916[a]; AY792529; AY507943[a]; AY792582; AY792554. ***Potamophila parviflora*** R. Br.[b] (—); 85424[c]; Australia; 24; AF489914[a]; AY792530; AY507944[a]; AY792583; AY792555. ***Chikusichloa aquatica*** Koidz. (—); 106186[c]; Japan; 24; AF489912[a]; AY792531; AY507948[a]; AY792584; AY792556. ***Rhynchoryza subulata*** (Nees) Baillon[b] (—); 100913[c]; Argentina; 24; AF148675[a]; AY792532; AY507945[a]; AY792585; AY792557. ***Zizania aquatica*** L. (—); *J. Alexander* 200301; MA, USA; 30; AF164393[a]; AY792533; AY792574; AY792586; AY792558. ***Zizania latifolia*** (Griseb.) Turcz. ex Stapf (—); GS0202; China; 34; AY092064[a]; AY792534; AY507941[a]; AY792587; AY792559. ***Zizaniopsis villanensis*** Quarin; 85425[c] (—); Argentina; 24; AF489917[a]; AY792535; AY507942[a]; AF148635[a]; AY792560. ***Luziola leiocarpa*** Lindm. (—); 82043[c]; Argentina; 24; AF489911[a]; AY792536; AY507946[a]; AY792588; AY792561. ***Luziola fluitans*** (Michx.) E. E. Terrell & H. Robinson (—); ^ 8434; USA; 24; AY792567; AY792537; AY792575; AY792589; AY792562. ***Hygroryza aristata*** (Retz.) Nees[b] (—); 105460[c]; Sri Lanka; 24; AF489913[a]; AY792538; AY507947[a]; AY792590; AY792563. ***Ehrharta erecta*** Lam.; *B. Bartholomeal* 9130; USA; 48; AY792568; AY792539; AY792576; AY792591; AY792564. ***Phyllostachys aurea*** Riviére & C. Riviére (—); GS0204; China; 48; AF1643901[a]; AY792540; AY507949[a]; AY792592; AY792565.

[a] used in our previous studies or downloaded from GenBank.

[b] monotypic genera.

[c] provided by the Genetic Resources Center of the International Rice Research Institute (IRRI) at Los Banos, Philippines and other accessions are obtained by the authors.