

A PHYLOGENY OF THE RICE TRIBE ORYZEAE (POACEAE) BASED ON *MATK* SEQUENCE DATA¹

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Phylogenetic relationships were inferred using nucleotide sequences of the chloroplast gene *matK* for 26 species representing 11 genera of the tribe Oryzeae and three outgroup species. The sequenced fragments varied from 1522 base pairs (bp) to 1534 bp in length with 15.4% variable and 7.9% phylogenetically informative sites when the outgroups were excluded. The aligned sequences were analyzed by maximum parsimony and neighbor-joining methods. Analyses of the sequence data indicated that species of Oryzeae form a strongly supported monophyletic group, concordant with previous morphological and anatomical evidence. The tribe Oryzeae can be divided into two monophyletic lineages, corresponding to the traditionally recognized subtribes Oryzinae and Zizaniinae. The first subtribe consists of *Oryza* and *Leersia*, while the subtribe Zizaniinae includes the remaining genera. The *matK* sequence data did not support the close affinities of the monoecious genera in Oryzeae, implying the possibility of multiple origins of the floral structures in the tribe. It is noteworthy that *Porteresia coarctata* is closely related to *Oryza* species, suggesting that it should be treated as a member in the genus *Oryza* rather than a separate monotypic genus.

Key words: chloroplast DNA; *matK*; molecular phylogeny; *Oryza*; Oryzeae.

The rice tribe, Oryzeae, consists of 12 genera and is distributed in tropical and temperate regions worldwide (Clayton and Renvoize, 1986; Vaughan, 1994). Species in the genus *Oryza* and in other genera closely related to *Oryza* have been extensively studied because of their agronomically useful traits in rice genetic improvement (wild *Oryza* species, *Porteresia*, and *Leersia*) (Tateoka, 1965; Flowers et al., 1990; Naredo, Vaughan, and Cruz, 1993; Vaughan, 1994; Katayama, 1995) or economic values as a well-known part of cuisine (*Zizania*) (Duvall et al., 1993; Kennard et al., 1999). However, the systematic treatments and phylogenetic relationships among genera in Oryzeae have not been well studied in detail although evidence shows that this tribe is a distinct and monophyletic lineage (Duistermaat, 1987; Zhang and Second, 1989; Kellogg and Watson, 1993). The taxonomic bifurcation of the monoecious vs. bisexual groups in the oryzoid grasses has been proposed and variously recognized at tribal or subtribal levels (Hitchcock, 1935; Stebbins and Crampton, 1961; Pyrah, 1969). Later investigations, however, indicated that the maintenance of monoecious genera (*Zizania*, *Zizaniopsis*, *Luziola*, and *Hydrochloa*) in the same group was in conflict with their characters such as anatomy, cytology, embryology, and cp-DNA restriction sites (Terrell and Robinson, 1974; Duvall et al., 1993). The three subtribes proposed by Terrell and Robinson (1974) were followed by subsequent treatments (Tsvetlev, 1983) but not supported by a recent molecular study (Du-

vall et al., 1993). In addition, the circumscription and taxonomic position of some genera in this tribe have been problematic and are still controversial. For instance, the mutually exclusive hypotheses that the genus *Zizania* is more closely related to the monoecious genera or to the bisexual genera have been in dispute for decades (Terrell and Robinson, 1974; Duvall et al., 1993). In particular, the taxonomic position of *Porteresia coarctata* that interests scientists for its unique salt tolerance has long been the subject of dispute (Tateoka, 1965; Vaughan, 1989; Flowers et al., 1990; Ge et al., 2001). *Porteresia* was established as a new genus by Tateoka (1965) including only a single species that had formerly been a member of *Oryza* (*Oryza coarctata*). This treatment has been widely accepted (Terrell and Robinson, 1974; Clayton and Renvoize, 1986; Tzvelev, 1989; Vaughan, 1994). However, some authors have retained this species in *Oryza* (Cope, 1982; Oka, 1988). Recent molecular data provided strong evidence that *P. coarctata* should be included in *Oryza* (Ge et al., 1999, 2001). A reasonable taxonomic treatment based on the phylogenetic relationships revealed by more powerful methods is urgently needed.

The *matK* gene, located within the intron of the chloroplast gene *trnK*, has relatively high rates of substitution compared to other chloroplast genes and has been used effectively for phylogenetic studies at a variety of taxonomic levels (Johnson and Soltis, 1994; Olmstead and Palmer, 1994; Hilu and Liang, 1997; Sang, Crawford, and Stuessy, 1997) including *Oryza* and Poaceae (Liang and Hilu, 1996; Ge et al., 1999; Hilu, Alice, and Liang, 1999). In this paper, we report the results of phylogenetic analyses of chloroplast *matK* gene sequences for species of the tribe Oryzeae. Our objective was to reconstruct the phylogeny of the rice tribe and evaluate the previous circumscription of the groups at tribal and subtribal levels. We were particularly interested in revealing the phylogenetic relationship between the genus *Oryza* and other genera of Oryzeae. This information may facilitate the utilization of the genetic resource in wild rice germplasm and provide an impor-

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TABLE 1. Pairwise Kimura two-parameter distance matrix including 23 *Oryzae* species and three outgroups.

Species	1	2	3	4	5	6	7	8	9	10	11
1 <i>Oryza sativa</i>	—										
2 <i>Oryza punctata</i>	0.00993	—									
3 <i>Oryza officinalis</i>	0.00992	0.00926	—								
4 <i>Oryza malapuzhanensis</i>	0.00993	0.00000	0.00926	—							
5 <i>Oryza latifolia</i>	0.00992	0.00926	0.00395	0.00926	—						
6 <i>Oryza australiensis</i>	0.01393	0.01328	0.00926	0.01328	0.00926	—					
7 <i>Oryza brachyantha</i>	0.02613	0.02411	0.02004	0.02411	0.02138	0.02138	—				
8 <i>Oryza granulata</i>	0.02271	0.02206	0.01799	0.02206	0.01932	0.02068	0.01732	—			
9 <i>Oryza longiglumis</i>	0.02611	0.02478	0.02137	0.02478	0.02271	0.02408	0.02002	0.02068	—		
10 <i>Oryza schlechteri</i>	0.01260	0.01061	0.00926	0.01061	0.00926	0.00926	0.01530	0.01395	0.02001	—	
11 <i>Porteresia coarctata</i>	0.01461	0.01395	0.01126	0.01395	0.01126	0.01126	0.01866	0.01596	0.02204	0.00329	—
12 <i>Leersia perrieri</i>	0.02885	0.02817	0.02408	0.02817	0.02475	0.02408	0.02408	0.02611	0.02611	0.02270	0.02474
13 <i>Leersia hexandra</i>	0.02951	0.03021	0.02474	0.03021	0.02609	0.02473	0.02474	0.02677	0.02677	0.02337	0.02540
14 <i>Leersia tisseranti</i>	0.03227	0.03298	0.02749	0.03298	0.02884	0.02748	0.02749	0.02952	0.02952	0.02610	0.02814
15 <i>Rhynchoryza sublulata</i>	0.03989	0.04337	0.03848	0.04337	0.03985	0.03985	0.03850	0.03778	0.04196	0.03432	0.03639
16 <i>Luziola leiocarpa</i>	0.05388	0.05390	0.05035	0.05390	0.05174	0.05176	0.05180	0.05249	0.05318	0.04754	0.05035
17 <i>Chikusichloa aquatica</i>	0.03225	0.03295	0.02814	0.03295	0.02950	0.02950	0.03091	0.03020	0.03295	0.02541	0.02745
18 <i>Hygroryza aristata</i>	0.04541	0.04470	0.04192	0.04470	0.04330	0.04330	0.04543	0.04401	0.04751	0.04052	0.04191
19 <i>Zizaniopsis villanensis</i>	0.04407	0.04619	0.04127	0.04619	0.04264	0.04264	0.04270	0.04337	0.04759	0.03709	0.03779
20 <i>Potamophila parviflora</i>	0.03430	0.03500	0.02881	0.03500	0.03017	0.03223	0.03088	0.03018	0.03292	0.02676	0.02881
21 <i>Prospyrtchloa prehen-</i> <i>silis</i>	0.03362	0.03432	0.02814	0.03432	0.02950	0.03155	0.03020	0.02950	0.03224	0.02608	0.02813
22 <i>Zizania aquatica</i>	0.05269	0.05418	0.04911	0.05418	0.05053	0.04909	0.04913	0.05127	0.05563	0.04482	0.04623
23 <i>Zizania latifolia</i>	0.04546	0.04619	0.04121	0.04619	0.04259	0.04259	0.04261	0.04258	0.04758	0.03699	0.03840
24 <i>Ehrharta longifolia</i>	0.08976	0.08911	0.08154	0.08911	0.08300	0.08225	0.08534	0.08526	0.08688	0.08074	0.08297
25 <i>Phyllostachys aurea</i>	0.06281	0.06286	0.05774	0.06286	0.05990	0.05918	0.06141	0.06068	0.06581	0.05483	0.05698
26 <i>Chusquea coronalis</i>	0.06651	0.06582	0.06141	0.06582	0.06359	0.06215	0.06144	0.06285	0.06729	0.05775	0.05990

tant basis for addressing many intriguing questions involving the biogeography and genome evolution in the tribe and the grass family.

MATERIALS AND METHODS

Plant materials—In this study, 23 species included in the *Oryzae* representing all 12 genera recognized by Clayton and Renvoize (1986) and Vaughan (1994) were used, except for *Maltebrunia*, which was placed in synonymy under the genus *Potamophila* by Duistermaat (1987). Apart from the genera *Oryza*, *Leersia*, and *Zizania*, one species from each of the eight genera were sampled, including five monotypic genera: *Hygroryza*, *Porteresia*, *Potamophila*, *Prospyrtchloa*, and *Rhynchoryza* (Vaughan, 1994). Ten *Oryza* species were included, representing all of the ten genome types, of which the AA genome species are the recently diverged group while the GG species are the most basal lineage in the genus (Ge et al., 1999). Three species of *Leersia* were used including two species (*L. perrieri* and *L. tisseranti*) that were transferred from *Oryza* to *Leersia* by Launert (1965). Two *Zizania* species were used including the cultivated *Z. latifolia*. Based on a recent comprehensive study of the subfamilial classification of the grass family (GPWG, 2001), the subfamily Ehrhartoideae consists of three tribes: Ehrharteae, *Oryzae*, and Phyllostachydeae. Ehrharteae is the most closely related tribe to *Oryzae*. Therefore, we chose the genus *Ehrharta* as the outgroup. Two additional species of the closely related subfamily Bambusoideae were also included in the phylogenetic analyses as outgroups. Seed and leaf samples were provided by the Genetic Resources Center of the International Rice Research Institute (IRRI) at Los Banos, Philippines. The scientific names, accession numbers, chromosome numbers, and origins of the species under study are listed in the *American Journal of Botany*'s supplementary data website (<http://ajbsupp.botany.org/v89/>).

DNA isolation, amplification, and sequencing—Total DNA was isolated from silica-gel dried leaves using the cetyltrimethyl ammonium bromide (CTAB) method as described by Ge et al. (1999). Four primers for amplifying and sequencing the *matK* coding region were designed based on the conservative regions between rice and maize and were specified in Ge et al. (1999).

The forward and reverse polymerase chain reaction (PCR) primers are located at the beginning and the end of the *matK* coding region, respectively. The PCR products of the *matK* gene were purified and sequenced directly on an ABI373 or an ABI377 automated DNA sequencer (Applied Biosystems, Foster City, California, USA).

Data analysis—Alignment of the *matK* sequences was unambiguous and can be done manually with the rice (*Oryza sativa*) sequence as the reference. The sequences reported here were deposited in GeneBank (<http://ajbsupp.botany.org/v89/>), and the sequences of *Zizania aquatica* and three outgroups were taken from published data (Hilu, Alice, and Liang, 1999). Phylogenetic analyses of the sequence data were conducted using the parsimony and distance methods as implemented in PAUP* 4.0 (Swofford, 1998). Maximum parsimony (MP) analyses were performed using heuristic search with MULPARS, tree-bisection-reconnection (TBR) branch swapping, and RANDOM stepwise addition with 1000 replicates. The sequence data were also analyzed with a neighbor-joining (NJ) method using the Juke-Cantor and Kimura two-parameter distance estimates (Kimura, 1980; Saitou and Nei, 1987). Topological robustness was assessed by bootstrap analysis with 1000 replicates using simple taxon addition (Felsenstein, 1985). Gaps were treated as missing data.

RESULTS

Variation in *matK*—The generated sequences were the coding region of the *matK* gene that corresponded to codons 68–1589 in *O. sativa*. The sequence sizes varied from 1522 base pairs (bp) to 1534 bp. Mean guanine + cytosine (G + C) content is 34.6% excluding the outgroups. The aligned sequences resulted in a final data matrix with 1540 bp with three 6-bp alignment gaps, which were inferred as insertions or deletions (indels). Of them, two indels were autapomorphies and the other one was potentially phylogenetically informative. Excluding polymorphisms introduced by gaps, 330 of the characters (21.9%) were variable in the data set. For the *Oryzae* species, 111 (47.2%) were potentially phylogenetically

TABLE 1. Extended.

12	13	14	15	16	17	18	19	20	21	22	23	24	25
—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.00461	—	—	—	—	—	—	—	—	—	—	—	—	—
0.01663	0.01595	—	—	—	—	—	—	—	—	—	—	—	—
0.04124	0.04191	0.04262	—	—	—	—	—	—	—	—	—	—	—
0.05458	0.05385	0.05529	0.03432	—	—	—	—	—	—	—	—	—	—
0.03361	0.03291	0.03567	0.01864	0.03156	—	—	—	—	—	—	—	—	—
0.04679	0.04747	0.04818	0.02950	0.03639	0.02404	—	—	—	—	—	—	—	—
0.04825	0.04892	0.04895	0.02271	0.03164	0.02408	0.03502	—	—	—	—	—	—	—
0.03360	0.03428	0.03566	0.01728	0.02950	0.01059	0.02200	0.02270	—	—	—	—	—	—
0.03291	0.03359	0.03497	0.01661	0.02882	0.00992	0.02133	0.02204	0.00197	—	—	—	—	—
0.05482	0.05551	0.05555	0.03426	0.04842	0.03075	0.04055	0.03922	0.02933	0.02865	—	—	—	—
0.04688	0.04612	0.04538	0.02716	0.04190	0.02305	0.03486	0.03282	0.02168	0.02099	0.01232	—	—	—
0.08829	0.08895	0.08976	0.07991	0.08753	0.07321	0.08368	0.08521	0.07249	0.07179	0.09436	0.08724	—	—
0.06356	0.06424	0.06426	0.05621	0.06497	0.05119	0.05765	0.06200	0.05043	0.04973	0.07087	0.06217	0.07481	—
0.06507	0.06574	0.06577	0.05766	0.06867	0.05405	0.06348	0.06346	0.05185	0.05115	0.07236	0.06435	0.08012	0.02171

informative out of the 235 variable nucleotides. For the gene as a whole, therefore, 7.3% of the nucleotides were potentially phylogenetically informative excluding the outgroups. The Kimura two-parameter distances are presented in Table 1. Pairwise divergence of sequences ranges from 4.97 to 9.44% between the outgroups and Oryzeae and from 0 to 5.56% within Oryzeae.

Phylogenetic analyses—Parsimony analysis with gaps coded as missing data yielded three equally most parsimonious trees, each 476 steps long with a consistency index (CI) of 0.790 and a retention index (RI) of 0.819. The topology of the ingroup (all Oryzeae species) was exactly same when either *Ehrharta longifolia* or two species of subfamily Bambusoideae (*Chusquea coronalis* and *Phyllostachys aurea*) were specified as the outgroups. The strict consensus of the three equally most parsimonious (MP) trees is shown in Fig. 1. The neighbor-joining (NJ) tree shows essentially the same topology except for one clade consisting of *Rhynchoryza subulata*, *Luziola leiocarpa*, and *Zizaniopsis villanensis*, which obtains weak support (52% bootstrap) on the NJ tree but not on the MP tree (Figs. 1 and 2). In addition, maximum likelihood (ML) analyses were conducted on the data set, which produced exactly the same topology as that by MP analysis (not shown).

Both parsimony and distance methods show that the species of Oryzeae form a clade with 100% bootstrap support, indicating monophyly of the tribe Oryzeae (Figs. 1 and 2). In addition, the genera of the tribe fall into two main clades with very strong bootstrap support (100% and 96% on both MP and NJ trees). The first clade includes three genera, *Leersia*, *Oryza*, and *Porteresia*, with *Leersia* as the basal lineage. In this clade, three *Leersia* species form a highly supported group (100% bootstrap). The *Oryza* species and *Porteresia* *coar-*

tata, however, form a weakly supported group (61% and 63% bootstraps on the MP and NJ trees, respectively). The second clade consists of the remaining eight genera with *Chikusichloa* as the basal lineage followed by a weakly supported group, including the *Potamophila* + *Prosphytochloa* subclade (99% and 100% bootstraps on the MP and NJ trees, respectively), *Zizania* subclade (100% bootstraps on both MP and NJ trees), and *Luziola* + *Zizaniopsis* + *Rhynchoryza* + *Hygroryza* subclade (Figs. 1 and 2). Compared with the mean sequence divergence within each clade (1.96% and 2.65%, respectively), the mean pairwise sequence divergence between the two clades (4.12%) is high, suggesting that significant divergence has occurred between the two clades.

It is noteworthy that *Porteresia coarctata* lies deeply in the subclade (95% bootstraps on both the MP and NJ trees) that includes most *Oryza* species and forms a lineage with *O. schlechteri* with high bootstrap support (87% and 99% bootstraps on the MP and NJ trees, respectively) (Figs. 1 and 2).

DISCUSSION

Circumscription and subdivision of the tribe Oryzeae—Since it was proposed by Dumortier (1823), the tribe Oryzeae has been characterized as an entity inconsistently comprising 7–16 genera (Pyrah, 1969), and its circumscription also varied greatly over time (for review, see Duistermaat, 1987). After Tateoka (1963) presented strong arguments for excluding members of the Ehrharteae, the Oryzeae has been questioned less as a monophyletic lineage (Kellogg and Watson, 1993), although its recognized genera varied from 10 to 13 (Tateoka, 1963; Tzvelev, 1989). As described by Pyrah (1969) and Clayton and Renvoize (1986), the tribe Oryzeae is morphologically characterized primarily on the basis of

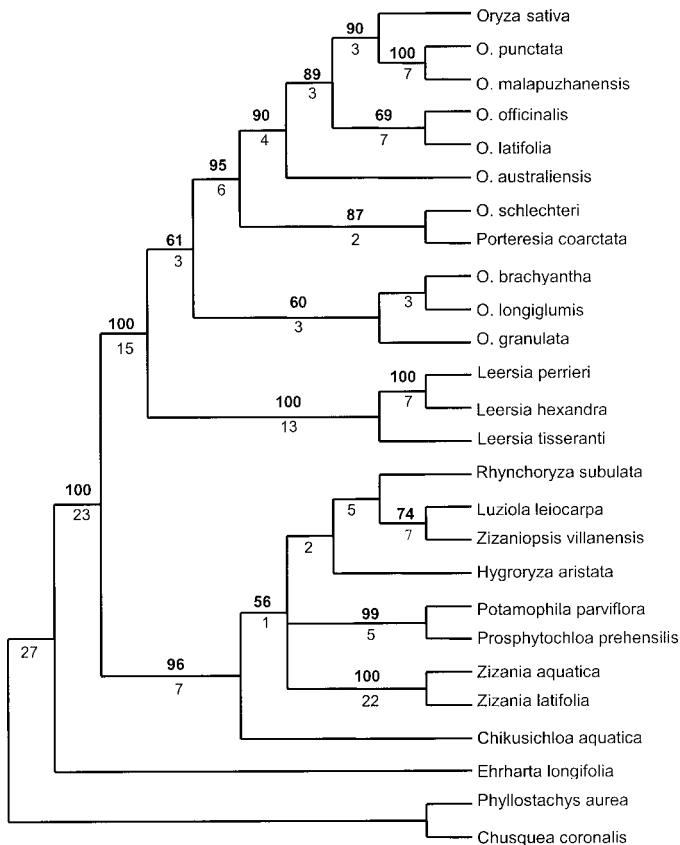


Fig. 1. Strict consensus of three equally most parsimonious trees of 23 Oryzaceae species and three outgroups (*Ehrharta longifolia*, *Chusquea coronalis*, and *Phyllostachys aurea*) generated from sequences of *matK* gene of chloroplast DNA. Tree length = 476, consistency index = 0.790, retention index = 0.819. Numbers above branches represent bootstrap values above 50%, and those below branches are nucleotide substitutions.

having one-flowered spikelets, which are compressed or terete, with a lemma and palea, and two well-developed bracts (degenerated flowers or sterile lemmas). The two tiny lobes below, sometimes referred to as cupules or glumes, appear to be expanded apices of the pedicels (Terrell, Peterson, and Wergin, 2001). Based on a large data set of morphological and anatomical characters, Kellogg and Watson (1993) conducted a phylogenetic analysis of the Bambusoid clade and found that seven genera of Oryzaceae formed a monophyletic group in all three trees they presented. The *matK* sequence data from this study further strongly support the tribe Oryzaceae as a monophyletic group.

The tribe Oryzaceae is usually divided into two subtribes, Oryzinae and Zizaniinae, on the basis of possession of bisexual or unisexual flowers (Pyrah, 1969). Based on morphological and anatomical studies of seven genera of Oryzaceae, Terrell and Robinson (1974) concluded that the genera with unisexual flowers represented two distinct phyletic lines and proposed a new subtribe Luziolinae (*Zizaniopsis* and *Luziola* sensu lato [s.l.]) by reducing the subtribe Zizaniinae to one genus *Zizania*. In their study on the phylogeny of North American oryzoid grasses using cpDNA restriction sites, Duvall et al. (1993) obtained high support for the recognition of two monophyletic groups corresponding to subtribes Zizaniinae and Oryzinae, but only weak support for the recognition of subtribe Luziolin-

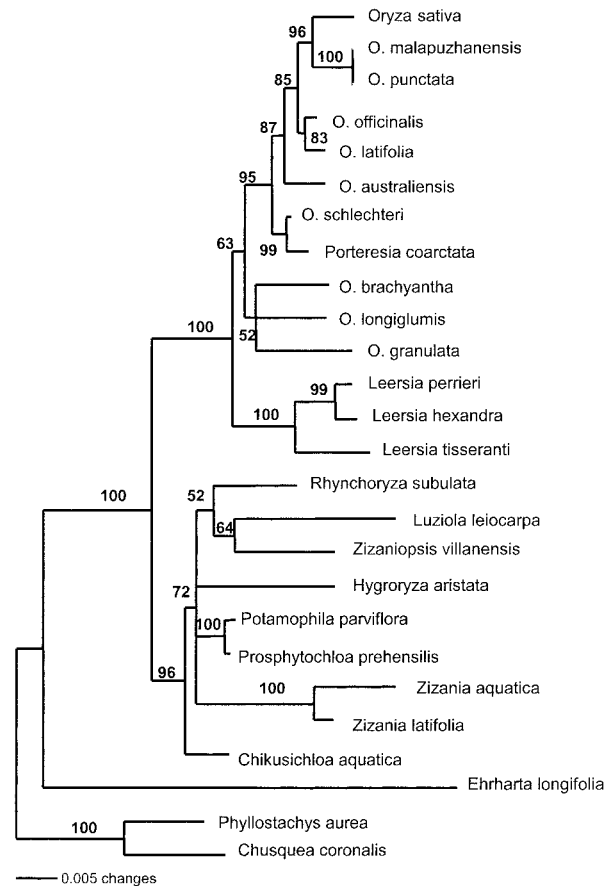


Fig. 2. Neighbor-joining phylogram based on Kimura two-parameter distance model. Numbers on the branches are bootstrap values from 1000 replicates. Branch lengths are proportional to distance.

ae, as sister to the Zizaniinae. However, in the above studies, important genera such as *Chikusichloa*, *Hygroryza*, and *Potamophila* were not included. The present *matK* sequence data strongly supported two monophyletic clades corresponding to the two traditional herbaceous oryzoid subtribes, Oryzinae and Zizaniinae. However, the subtribe Luziolinae sensu Terrell and Robinson (1974) was not supported because *Zizaniopsis* and *Luziola* were embedded in a strongly supported lineage (96% bootstrap on both the MP and NJ trees) that also included six other genera, excluding subtribe Oryzinae (Figs. 1 and 2). The pairwise comparisons of sequence divergence also support the treatment of two subtribes in Oryzaceae (Table 1).

As indicated by Duistermaat (1987), the three subtribes proposed by Terrell and Robinson (1974) in the Oryzaceae were partly based on the presence of unisexual florets in the non-Oryzinae, and they were apparently not aware of the fact that both uni- and bisexual florets existed in the spikelets of the Australian species *Potamophila parviflora*. It is clear from Figs. 1 and 2 that bisexual genera (*Rhynchoryza*, *Hygroryza*, *Prosphytochloa*, and *Chikusichloa*) and unisexual genera (*Luziola*, *Zizaniopsis*, and *Zizania*) are mingled with each other in the Zizaniinae clade. Therefore, the structure of the spikelets is more likely to be of multiple origins and its homology is questionable.

Delimitation and relationships of genera in the tribe—The three congeneric groups of species are each monophyletic

clades on the *matK* tree (Figs. 1 and 2). The monophyletic group of *Oryza* species reflected on the *matK* tree is weakly supported (61% and 63% bootstraps on the MP and NJ trees, respectively) but the relationships among species are in accordance with the previous multiple gene study (Ge et al., 1999). In comparison, however, three *Leersia* species, including *L. tiserantii* and *L. perrieri*, which were in the past classified in the genus *Oryza*, formed a monophyletic group with 100% bootstrap support. Therefore, the treatment by Launert (1965), in which three *Oryza* species (*O. tiserantii*, *O. angustifolia*, and *O. perrieri*) were transferred to the genus *Leersia*, has been justified (Zhang and Second, 1989) and gained strong support by *matK* sequence data from this study. Also, *matK* sequence data demonstrated that *Oryza* and *Leersia* are the most closely related genera in the tribe, as evidenced by previous investigations (Terrell and Robinson, 1974; Zhang and Second, 1989; Duvall et al., 1993). Therefore, the assertion by Clayton and Renvoize (1986) that *Leersia* is linked to *Chikusichloa* by the species with shortly stipitate florets is not supported by our *matK* data. The presumed link, shortly stipitate florets, has either been retained as a symplesiomorphy in these taxa or has arisen independently.

The genus *Potamophila* R. Br. has been divided into three genera, i.e., *Potamophila* sensu stricto (s.s.) (only *P. parviflora*), *Prospytochloa* Schweickerdt (only *P. rehensilis*), and *Maltebrunia* Kunth (five species) by some authors (Hubbard, 1967; Clayton, 1970). However, Duistermaat (1987) indicated that they should not be separated because there was no fundamental difference in the structure of the spikelets, and, therefore, *Prospytochloa* and *Maltebrunia* were considered within the generic limits of *Potamophila* (Vaughan, 1994). The result, based on our *matK* phylogeny, is congruent with this consideration because *Prospytochloa rehensilis* and *Potamophila parviflora* formed a strongly supported group (99% and 100% bootstraps on the MP and NJ trees, respectively), although *Maltebrunia* species were not included in the present study. Further studies will require more extensive sampling, particularly of *Maltebrunia*, *Leersia*, and *Luziola*, and the sequencing of rapidly evolving nuclear DNA fragments in order to resolve the circumscription and relationships of the genera in Oryzaceae.

The systematic position of *Porteresia*—*Porteresia coarctata* was once recognized as *Oryza coarctata*, but later treated as a monotypic genus based on certain morphological distinctions (Tateoka, 1965; Vaughan, 1989). Based on sequence analysis of two nuclear genes (*Adh1* and *Adh2*) and a chloroplast gene (*matK*), Ge et al. (1999) found that *P. coarctata* was nested within the *Oryza* clade on both *Adh* and *matK* phylogenetic trees and suggested that *P. coarctata* should be an *Oryza* species. Zhang and Second (1989) reported their preliminary study on the phylogeny of the tribe Oryzaceae based on restriction fragment data of chloroplast DNA. In their average-linkage dendrogram, *P. coarctata* was within the *Oryza* group and clustered tightly with *O. meyeriana* followed by other *Oryza* species, while seven other genera formed two distinct groups (Zhang and Second, 1989). In recent amplified fragment length polymorphisms (AFLP) and intersimple sequence repeats (ISSR) studies on phylogenetic relationships among *Oryza* species, Aggarwal et al. (1999) and Joshi et al. (2000) have also documented the similar affinities between *P. coarctata* and *Oryza* species but they failed to give explanations. In contrast, the monotypic genus

Rhynchoryza, which was also classified previously in the genus *Oryza* (*Oryza subulata*), was apparently distinguished from *Oryza* on both the present *matK* tree and on evidence from previous morphological and molecular studies (Clayton and Renvoize, 1986; Duistermaat, 1987; Zhang and Second, 1989; Aggarwal et al., 1999; Ge et al., 1999). It is justified, therefore, to retain *Porteresia coarctata* in the genus *Oryza* (*O. coarctata*), although further studies on its distinct morphology are needed.

In conclusion, the present work is so far the most comprehensive phylogenetic study using molecular sequence data on the tribe Oryzaceae in terms of species and genera inclusion. Our phylogenetic analysis of the *matK* sequences of Oryzaceae leads to the following conclusions: (1) the tribe Oryzaceae is a monophyletic group, concordant with previous morphological and anatomical studies; (2) the tribe consists of two strongly supported monophyletic lineages that correspond to the two traditionally recognized subtribes, Oryzinae and Zizaniinae; (3) the hypothesis of close affinities of the monoecious genera in Oryzaceae was not supported by the *matK* sequence data, suggesting the possibility of multiple origins of the floral structures in the tribe; and (4) *Porteresia coarctata* has a high affinity with *Oryza* species and should be treated as a member of the genus *Oryza* rather than an independent monotypic genus.

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