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 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 oryzae 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 cpDNA restriction sites (Terrell and Robinson, 1974; Duvall et al., 1993). The three subtribes proposed by Terrell and Robinson (1974) were followed by subsequent treatments (Tsvel-ev, 1983) but not supported by a recent molecular study (Duvall 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; Tsvelev, 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 relationships 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-
Plant materials—In this study, 23 species included in the Oryzeae representing all 12 genera recognized by Clayton and Renvoize (1986) and were transferred from Oryza to Leersia, Zizania, and Potamophila (Vaughan, 1994). Ten Oryza species were included, representing all of the ten genome types, of the genera Oryza, Leersia, Zizania, Potamophila, and Rhynchoryza (Vaughan, 1994). Ten Oryza species were used including the cultivated O. sativa. Based on a recent comprehensive study of the subfamily classification of the grass family (GPWG, 2001), the subfamily Ehrhartioideae consists of three tribes: Ehrhartieae, Oryzeneae, and Phyllorachideae. Ehrhartieae is the most closely related tribe to Oryzeae. Therefore, we chose the genus Ehrhartia 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://ajb supp. 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 GenBank (http://aldbsupp. 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 RAND stepwise addition with 1000 replicates. The sequence data were also analyzed with a neighbor-joining (NJ) method using the Juke-Cantor and Kimura two-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 Oryzeae species, 111 (47.2%) were potentially phylogenetically
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 corollinais 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 Rhyynchoryza subsulata, 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 coarc-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 + Prophytochloa 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 + Rhyynchoryza + 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 coarcctata 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

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having one-flowered spikelets, which are compressed or te-
rete, 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 Oryzeae formed a monophyletic
group in all three trees they presented. The matK sequence
data from this study further strongly support the tribe Ory-
zeae as a monophyletic group.

The tribe Oryzeae is usually divided into two subtribes,
Oryzininae and Zizaniinae, on the basis of possession of bisex-
ual or unisexual flowers (Pyrah, 1969). Based on morpholog-
ical and anatomical studies of seven genera of Oryzeae, 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 Zizi-
nia. In their study on the phylogeny of North American ory-
zoid grasses using cpDNA restriction sites, Duvall et al.
(1993) obtained high support for the recognition of two monophyletic
groups corresponding to subtribes Zizaniinae and Oryzininae,
but only weak support for the recognition of subtribe Luziolin-
ae, as sister to the Zizaniinae. However, in the above studies,
important genera such as Chikusichloa, Hygroryza, and Pot-
amophila were not included. The present matK sequence data
strongly supported two monophyletic clades corresponding to
the two traditional herbaceous oryzoid subtribes, Oryzininae 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 Oryzininae (Figs. 1 and 2).
The pairwise comparisons of sequence divergence also support the
study of two subtribes in Oryzeae (Table 1).

As indicated by Duistermaat (1987), the three subtribes pro-
posed by Terrell and Robinson (1974) in the Oryzeae were
partly based on the presence of unisexual florets in the non-
Oryzininae, 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 (Rynchoryza, Hygroryza,
Prophytochloa, and Chikusichloa) and unisexual genera (Lu-
ziola, 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
cludes 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. tisserantti 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. tisserantti, 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 Revoieze (1986) that Leersia is linked to Chlorisichloa 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), Prophytochloa Schweickert (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, Prophytochloa 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 Prophytochloa 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 Oryzeae.

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 Oryzeae 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 Rhynchospora, 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 Reveloize, 1986; Duistermaat, 1987; Zhang and Second, 1989; Aggarwal 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 Oryzeae in terms of species and genera inclusion. Our phylogenetic analysis of the matK sequences of Oryzeae leads to the following conclusions: (1) the tribe Oryzeae 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 Zizianinae; (3) the hypothesis of close affinities of the monoeocious genera in Oryzeae 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.

LITERATURE CITED


