Phylogenetic analysis of *Kengyilia* species based on nuclear ribosomal DNA internal transcribed spacer sequences

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Abstract

Phylogenetic analysis was conducted based on sequences of the internal transcribed spacer region (ITS) of nuclear ribosomal DNA in 17 species of *Kengyilia*, together with those of 18 species from *Pseudoroegneria*, *Agropyron*, *Roegneria* and *Douglasdeweya* by the maximum parsimony, maximum likelihood and neighbor-joining distance methods. The results indicate that species of *Kengyilia* had close affinities to species of *Douglasdeweya* and *Agropyron.* The species in *Kengyilia* was identified as two subgroups with regard to geographic distribution, indicating that species from the same distribution had a closer phylogenetic relationship. The genus *Kengyilia* was found as a ligament-group between *Roegneria* and *Agropyron*. The ITS sequence is a useful tool for studying the phylogeny of closely related species.

Additional key words: *Triticeae*, morphology, StYP genomes, cluster analysis, phylogenetic relationship.

Introduction

Kengyilia is a genus in the tribe *Triticeae* (family *Poaceae*) with *Kengyilia gobicola* Yen *et* J.L. Yang as the typical species (Yen and Yang 1990). Species of *Kengyilia* can be utilized as the potential contributor to genes of cold hardiness, drought resistance, and resistance to head scab for cereal crops in *Triticeae*. Twenty-six species and six varieties were involved in this genus (Cai and Zhi 1999). They are mainly distributed from the Pamir and Qinghai-Tibet plateau to the Karkorum, Altai, Tianshan, and Qilian mountain ranges at altitudes from 1100 to 5100 m (Yang *et al*. 1992).

Morphologically, species in *Kengyilia* is intermediate between species of *Roegneria* C. Koch and *Agropyron* Gaertn. They differ from *Roegneria* species by having the erect spike with densely placed spikelets, lemmas densely pilose or hirsute, short-awned, and distinguish from species of *Agropyron* by the flat glumes, lemmas with rounded back and not keeled from tip to bottom, and a terminal spikelet frequently presented (Yen and Yang 1990, Baum *et al*. 1995). Karyotype study and genome analysis on *Kengyilia* species showed that they are hexaploids (2n=6x=42) and contained the StYP genomes (Jensen 1990, 1996, Zhou 1994, Zhang *et al*. 2000). The StY genomes of *Kengyilia* come from *Roegneria* and the P genome is derived from *Agropyron* (Yen and Yang 1990, Yang *et al*. 1992). However, the taxonomic treatments of *Kengyilia* species have been unstable from time to time. Nevski (1934) and Tzvelev (1976) combined a number of these species into *Elytrigia* sect. *Hyalolepis*. Keng and Chen (1963) classified them as *Roegneria* sect. *Paragropyron*. Löve (1984) combined the species into *Elymus* sect. *Goulardia*. Chen *et al*. (1991) and Yang *et al*. (1992) transferred the species of *Roegneria* sect. *Paragropyron* to *Kengyilia*. Since then, the new species of *Kengyilia* will be constantly discovered and new combinations will be combined to this genus. Cai and Zhi (1999) systematically coordinated the species of *Kengyilia* and carried out the phylogenetic analysis according to the morphological characters (Cai 1999). However, the treatment of some species and the circumscription of the genus were different from previous scholar's opinions. Some grass researchers still includes

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Abbreviations: bp - base pair; CTAB - cetyltrimethylammonium bromide; ITS - internal transcribed spacer region; PCR - polymerase chain reaction.
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these species in *Elymus* (Dewey 1984, Jensen and Chen 1992, Lu 1994, Jensen 1996, Liu *et al*. 2006).

Molecular phylogenetic studies have successfully revealed the origin and evolutionary history of polyploids in plants and clarified the nature of different polyploids and the hybridization events involved in their formation (Soltis *et al*. 2003). The region of the internal transcribed spacers ITS1 and ITS2 of the 18S-26S nuclear ribosomal DNA has been established as a useful marker to decipher phylogenetic relationships and genomic relationship of plants at lower taxonomic levels (Baldwin *et al*. 1995, Hsiao *et al*. 1995). Some taxa in *Triticeae*, such as diploid species (Hsiao *et al*. 1995), *Aegilops* (Wang *et al*. 2000) and *Elymus* (Liu *et al*. 2006) have been successfully investigated for the phylogenetic relationship analysis based on ITS sequences.

The aims of the present study are: *a*) to evaluate the taxonomic status of *Kengyilia* and its related genera, *b*) to investigate the interspecific relationship in *Kengyilia*, and *c*) to reveal genome relationships of the StYP, StP, StY, St and P genomes.

Materials and methods

Plants: A total of 35 accessions, including seventeen *Kengyilia* (StYP), ten *Roegneria* (StY), four *Pseudoroegneria* (St, St_1St_2), two *Agropyron* (P) and two *Douglasdeweya* (StP) species were used in this study (Table 1). *Bromus tectorum* L. was used as outgroup. The accessions with PI numbers were kindly provided by American National Plant Germplasm System (Pullman, Washington, USA) and the others were collected from the field by Prof. Chi Yen, Jun-Liang Yang and Yong-Hong Zhou (Sichuan Agricultural University, Sichuan, China). The voucher species and the plant materials are deposited in Triticeae Research Institute, Sichuan Agricultural University, China (SAUTI).

DNA extraction, amplification and sequencing: Total DNA was extracted from fresh leaves of 10 - 15 individual plants using the CTAB method (Doyle and Doyle 1987). The entire ITS region of the nuclear ribosomal DNA was amplified by polymerase chain reaction (PCR) with primers
ITSL: 5'-TCGTAACAAGGTTTCCGTAGGTG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (Hsiao *et al*. 1995). The PCR amplification of ITS DNA was performed in a total reaction volumes of 0.05 cm^3 containing 1× ExTaq polymerase buffer, 1.5 mM of $MgCl_2$, 0.8 mM of dNTP mixture, 1.5 μM of each primer, 1.25 U of ExTaq polymerase (*TaKaRa*, Dalian, Liaoning, China) and 10 - 20 ng template DNA. PCR reactions were carried out in a *GeneAmp 9700* thermal cycler (*Applied Biosystems*, Foster City, USA). The thermal cycling profile consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 1 min at 94 °C for denaturing, 1 min at 52 °C for annealing, 1 min at 72 °C for extension and final extension step of 8 min at 72 °C. The PCR products were purified using a gel extraction kit *ENZA*^{TM} (*Omega*, Georgia, USA) and ligated into a pMD18-T vector according to the manufacturer's instruction. Transformation, plating and isolation of plasmids were performed as described in Liu *et al*. (2006). Purified plasmid DNA was digested with *Eco*RI and *Hind*III. For each accession, 3 - 5 cloned PCR products were sequenced in *TaKaRa* biotechnology company (Dalian, Liaoning, China).

Phylogenetic analysis: The ITS sequences were aligned with *Clustal X* (Thompson *et al*. 1999) and refined manually. The boundaries of ITS region (ITS1 - 5.8S - ITS2) were determined according to the available sequences in GenBank (Hsiao *et al*. 1995). Phylogenetic analysis was carried out using the maximum parsimony cladistic method and maximum likelihood method with *PAUP 4.0b10* (Swofford 2003). All characters were equally weighted and the gaps were treated as missing data. Parsimony analyses were performed by heuristic search with tree bisection-reconnection (TBR) branch swapping, MULPARS option, ACCTRAN optimization, and 100 random addition replicates. The nonparametric bootstrap technique was used to assess the reliability of individual branches in the phylogenetic trees with 1000 replicates of random addition sequences. The aligned sequences were also analyzed with the Neighbor-Joining program of *PAUP*, and carried out with 1000 bootstrap replicates.

Results

The lengths of the entire ITS sequences in this study varied from 592 to 603 bp. The ITS1 region ranged from 217 to 221 bp, while the ITS2 region ranged from 214 to 220 bp. The length of 5.8S subunit was uniformly 164 bp in all accessions. The average G + C content of entire ITS sequences was 62.27 %.

The alignment matrix of ITS1, 5.8S and ITS2 sequences consisted of 603 characters with 75 variable sites, and of which 38 positions were parsimony informative. Nucleotide substitution and deletion appeared to be the main source of variability. Maximum parsimony analysis of the aligned sequences yielded 486 maximally parsimonious trees. Each of these trees required 146 evolutionary steps and had a consistency index (CI) of 0.8151 and a relation index (RI) of 0.8412.

In the strict consensus tree (Fig. 1) four major groups

Table 1. The species of *Kengyilia* and its related genera used in this study. YYB - J.L. Yang, Yen *et* Baum; YZY - J.L. Yang, Y.H. Zhou *et* Yen; * - data from published sequences in the GenBank (http:// www.ncbi.nlm.nih.gov).

Species	Ploidity	Genome	Voucher	Locality	GenBank No.
Kengyilia gobicola Yen *et* J.L. Yang	6x	StYP	Y9503	Taxkorgan,Xinjiang, China	EF015600
Kengyilia mutica (Keng *et* S.L. Chen)	6x	StYP	Y2873	Geermu,Qinghai, China	EF011707
Kengyilia hirsuta (Keng *et* S.L. Chen) YYB	6x	StYP	PI504457	Qinhai Lake, Qinghai, China	EF011700
Kengyilia kokonorica (Keng *et* S.L. Chen) YYB	6x	StYP	Y2880	Gonghe,Qinghai, China	EF011706
Kengyiia batalinii (Krassn) J.L. YYB	6x	StYP	PI565002	Kazakhstan	EF011693
Kengyilia rigidula (Keng *et* S.L. Chen) YYB	6x	StYP	W622130	Xiahe, Gansu, China	EF011701
Kengyilia grandiglumis (Keng *et* S.L. Chen) YYB	6x	StYP	Y2857	Haiyan, Qinghai, China	EF011699
Kengyilia tahelacana YYB	6x	StYP	Y0852	Wensu,Xinjiang, China	EF011698
Kengyilia zhaosuensis YYB	6x	StYP	Y2633	Zhaosu, Xinjiang, China	EF011704
Kengyilia melanthera (Keng) YYB	6x	StYP	Y2891	Maduo, Qinghai, China	EF011692
Kengyilia melanthera var. *tahopaica* (Keng) S.L. Chen	6x	StYP	Y2885	Xinghai, Qinghai, China	EF011696
Kengyilia nana YYB	6x	StYP	Y9505	Taxkorgan, Xinjiang, China	EF011695
Kengyilia kaschgarica (D.F. Cui) L.B. Cai	6x	StYP	Y9506	Taxkorgan, Xinjiang, China	EF011705
Kengyilia alatavica (Drobov) YYB	6x	StYP	PI565001	Kazakhstan	EF011694
Kengyilia stenachyra (Keng *et* S.L. Chen) YYB	6x	StYP	W622128	Xiahe, Gansu, China	EF011702
Kengyilia thoroldiana (Oliver) YYB	6x	StYP	PI531686	Geermu, Qinghai, China	EF011703
Kengyilia longiglumis (Keng *et* S.L. Chen) YYB	6x	StYP	ZY3119	Xiahe, Gansu, China	EF011697
Douglasdeweya deweyi YYB	4x	StP	PI531756	Russia	EF014250
Douglasdeweya wangyii YYB	4x	StP	PI401330	Iran	EF014251
Roegneria grandis Keng	4x	StY	H3879	Lingtong, Shanxi, China	AY740829*
Roegneria pendulina Nevski	4x	StY	Y1412	Wenchuan, Sichuan, China	AY740849*
Roegneria ciliaris (Trin.) Nevski	4x	StY	H7000	Beijing, China	AY740831*
Roegneria tibetica (Melderis) H.L. Yang	4x	StY	H8366	Gongbogyamda, Tibet, China	AY740864*
Roegneria fedtschenkoi (Tzvelev) J.L. Yang *et* Yen	4x	StY	H4114	NWFP, Pakistan	AY740840*
Roegneria glaberrima Keng *et* S.L. Chen	4x	StY	Y2042	Jeminay, Xinjiang, China	AY740845*
Roegneria abolinii C. Koch	4x	StY	H3306	Alma-Ata, Kazakshtan	AY740899*
Roegneria semicostata (Sted.) Kitagawa	4x	StY	H4101	Hazara, Pakistan	AY740803*
Roegneria shandongensis (Salomon) J.L. YZY	4x	StY	H3202	Wuhan, Hubei, China	AY740817*
Roegneria caucasica C. Koch	4x	StY	H3207	Dilidjian, America	AY740808*
Pseudoroegneria elytrigioides (Yen *et* J.L. Yang) B.R. Lu	4x	St_1St_2	Z2005	Changdu, Tibet, China	AY740798*
Pseudoroegneria spicata (Pursh) A. Löve	2x	St	PI547161	Oregon, America	AY740793*
Pseudoroegneria strigosa (Bieb.) A. Löve	2x	St	PI499637	Urumqi, Xinjiang, China	AY740795*
Pseudoroegneria libanotica (Hackel) A. Löve	2x	St	PI228389	Iran	AY740794*
Agropyrom cristatum (L.) Gaertn.	2x	P	H10154	Altai, Xinjiang, China	AY740892*
Agropyron mongolicum Keng	2x	P	--	--	L36481*

were formed. The group I comprised *Pseudoroegneria* (St_1St_2 and St) and *Roegneria* species from the central Asia and its adjacent areas. The group II consisted of *Pseudoroegneria* (St) and *Roegneria* (StY) species mainly distributed in China except for *Pseudoroegneria spicata* and *P. libanotica*. *P. spicata* comes from America, while *P. libanotica* is from Iran. The group III consisted of *Roegneria tibetica* and *R. shandongensis*. The group IV included species of *Kengyilia, Douglasdeweya* and *Agropyron* with StYP, StP and P genomes, respectively. In group IV, three subgroups (I, II and III) were recognized with corresponding to their different distributions. *Kengyilia longiglumis* alone formed subgroup I, which comes from Gansu, China. The subgroup II included the species distributed in Xinjiang of China (70 % bootstrap support), such as *K. kaschgarica*, *K. tahelacana*, *K. nana*, *K. zhaosuensis* and *K. gobicola* except *K. alatavica* and *K. batalinii*, together with species of *Agropyron* and *Douglasdeweya*. The subgroup III consisted of the *Kengyilia* species from Qinghai and the adjacent areas in China (63 % bootstrap support). They are *K. hirsuta*, *K. thoroldiana*, *K. mutica*, *K. stenachyra*, *K. rigidula*, *K. melanthera*, *K. melathera* var. *tahopaica* and *K. kokonorica*. No obvious Y genome specific group was detected in the phylogenetic tree because of the absence of diploid species with the Y genome. The similar topologies with different bootstrap values were generated by Maximum Likelihood and Neighbor-Joining analyses.

Discussion

Morphological and cytogenetic studies have greatly improved our understanding of the origin and relationship between species of *Kengyilia* and its relatives. However, the taxonomic status, precise treatment and circum-

scription, and the interspecific relationships in *Kengyilia* have been in dispute (Yang *et al*. 1992, Baum *et al*. 1995, Cai 1998, Cai and Zhi 1999, Zhou *et al*. 2000). Analysis of the ITS sequences of *Kengyilia* species and their related genera will provide a valuable source of evidence for understanding their phylogenetic relationships and polyploidization events in the speciation process.

The *Kengyilia* species show intermediacy between

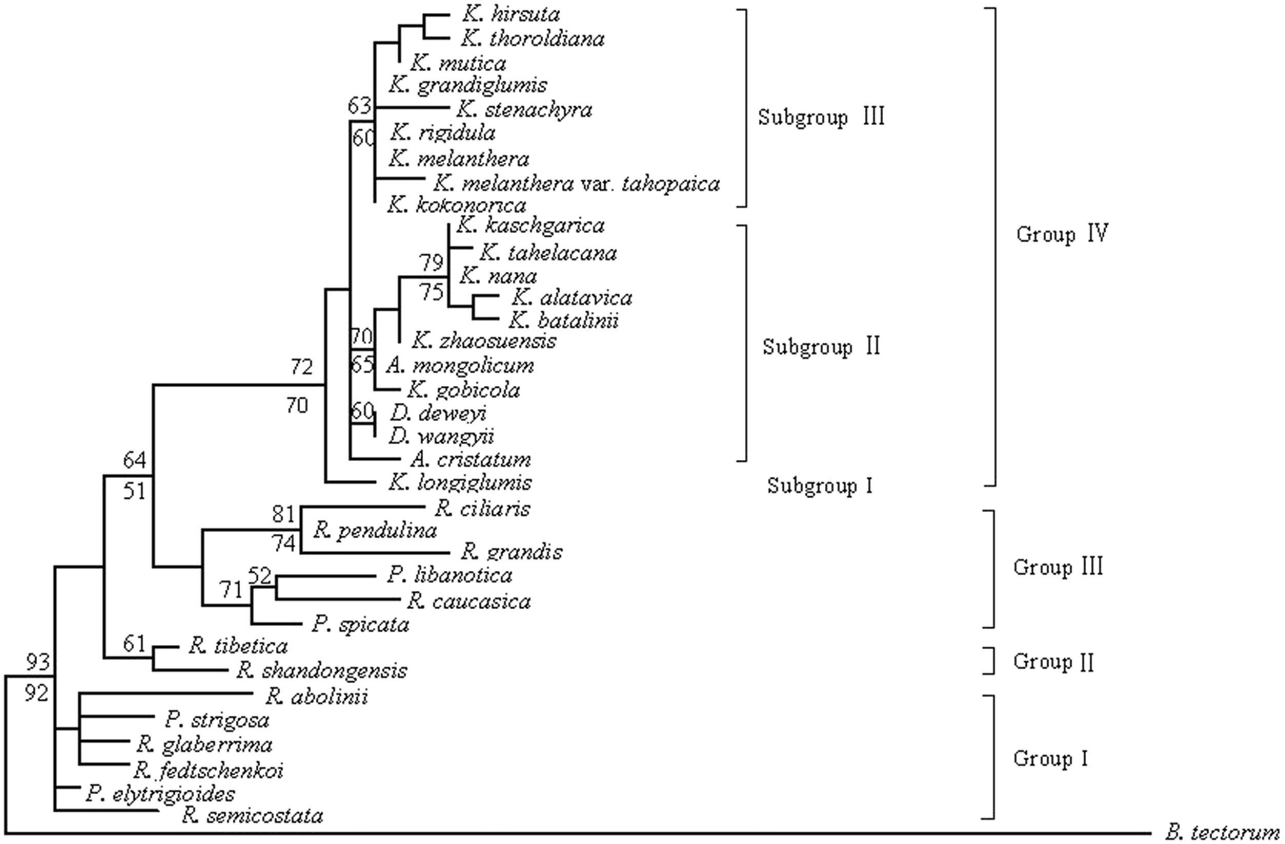

Fig. 1. The strict consensus tree of 486 parsimonious trees inferred from the internal transcribed spacer sequences of all the accessions used in the study (tree length = 146, CI = 0.8151, RI = 0.8412). The topology obtained by maximum likelihood method is the same for some node having different bootstrap values. Number above and below the branches indicate bootstrap values > 50 % by MP and ML analysis, respectively. The different groups are given on the right.

Agropyron and *Roegneria* in morphology and contain the StYP genomes (Jensen 1990, 1996, Zhou 1994, Zhang *et al*. 2000). The StY genomes of *Kengyilia* come from *Roegneria*, while the P genome is derived from *Agropyron* and the St genome might be derived from *Pseudoroegneria libanotica* and *P. cognata* in Asia (Yen and Yang 1990, Yang *et al*. 1992). Chen *et al*. (1991) reported that *Kengyilia* species are derived from *Roegneria* and evolved forward to *Agropyron* based on leaf epidermis. Yen *et al*. (2006) reported that the species of *Kengyilia* have most probably originated from natural hybridization of a maternal diploid *Agropyron* with a paternal tetraploid *Roegneria* based on cytological studies. In the present study, the species of *Kengyilia* were clustered with *Agropyron* firstly, and then clustered with species of *Roegneria* and *Pseudoroegneria*. It suggested that there are closely related relationships between *Kengyilia* and *Agropyron*, *Roegneria* and *Pseudo-roegneria*.

Species containing the StP genomes were involved in a new genus *Douglasdeweya* (Yen *et al*. 2005). Although species in *Douglasdeweya* was morphologically similar to *Kengyilia* species, there were a lot of distinct morphological differences between them, *i.e*., rhizomes, axillary branches, spikelets and glumes (Yen *et al*. 2005). In this study, two *Douglasdeweya* and two *Agropyron* species were distinctly clustered with *Kengyilia*. The result suggests that there is a close affinity between species of *Douglasdeweya*, *Agropyron* and *Kengyilia*. In addition, it is worth pointing out that species containing the P genome, such as *Kengyilia*, *Agropyron* and *Douglasdeweya*, consistently have 4-bp deletion in their ITS sequences and clustered distinctly into one group. It is most likely that the 4-bp deletion have already occurred in *Agropyron* species before being passed on to the *Kengyilia* and *Douglasdeweya* species during the polyploidization process.

Based on morphological investigation, species in *Kengyilia* were divided into three sections: sect. *Kengyilia*, sect. *Stenachyra* and sect. *Hyalolepis* (Cai and

Zhi 1999). The phylogenetic analysis of *Kengyilia* suggested that sect. *Stenachyra* and sect. *Hyaloepis* was a sister group and had a close affinity, while sect. *Kengyilia* had a farside affinity in relation to sect. *Stenachyra* and sect. *Hyalolepis* (Cai 1999). Zhou *et al*. (2000) and Zhang *et al*. (2005) analyzed relationships among *Kengyilia* species in China by RAPD and RAMP markers respectively, and indicated that there were great genetic differences between the taxa from Qinghai-Tibet plateau and those from Xinjiang province. In the present study, the species mainly distributed in Xinjiang province of China such as *K. gobicola*, *K. kaschgarica*, *K. nana*, *K. tahelacana*, *K. zhaosuensis K. batalinii* and *K. alatavica* formed one subgroup. The relationships among *K. batalinii*, *K. alatavica*, *K. nana*, *K. tahelacana* and *K. kaschgarica* were closer than those of *K. zhaosuensis* and *K. gobicola*. Morphologically, most species in this subgroup had dense spikes except *K. gobicola* and *K. zhaosuensis*. The species from Qinghai-Tibet plateau such as *K. kokonorica*, *K. hirsuta*, *K. mutica*, *K. thoroldiana*, *K. melanthera* var. *tahopaica*, *K. melanthera*, *K. stenachyra*, *K. rigidula* and *K. grandiglumis* formed another subgroup. Morphologically, they had dense spikes except *K. stenachyra* and *K. rigidula*, which come from Gansu province. Cai and Zhi (1999) treated morphologically *K. melanthera* and *K. melanthera* var. *tahopaica* as *K. thoroldiana* var. *melanthera* and *K. hirsuta* var. *tahopaica*, respectively. In this study, *K. melanthera* var. *tahopaica* was closely related to *K. melanthera*, while *K. thoroldiana* was closer to *K. hirsuta* than to the other *Kengyilia* species. Therefore, *K. melanthera* and *K. melanthera* var. *tahopaica* are valid. *Kengyilia longiglumis* alone was clustered in a group and was close to *Roegenria* species. Cai (1998) indicated that the *Kengyilia* was derived from *Roegneria* through *K. longiglumis*.

Molecular marker technology such as RAPD, RAMP, AFLP and ISSR has been successfully applied to investigate genetic diversity and phylogenetic relationship among species (Zhou *et al*. 2000, Zhang *et al*. 2005, Rout 2006, Sarker *et al*. 2006). The ITS sequences data presented here provide novel information for taxonomy and systematics of the genus *Kengyilia*. The ITS sequences analysis represents a useful tool, which could help to elucidate phylogenetic relationships within the genus and provide the implications for biogeography and character evolution.

References

Baldwin, B.G., Sanderson, M.J., Porter, M.J., Wojciechowski, M.F., Campbell, C.S., Donoghue, M.J.: The ITS region of nuclear ribosomal DNA: a valuable source of evidence in angiosperm phylogeny. - Ann. Missouri Bot. Garden **82**: 247-277, 1995.

Baum, B.R., Yang, J.L., Yen, C.: Taxonomic separation of *Kengyilia* (*Poaceae*: *Triticeae*) in relation to nearest related *Roegneria*, *Elymus* and *Agropyron* based on some morphological characters. - Plant Syst. Evol. **194**: 123-132, 1995.

Cai, L.B.: On the phylogenetic relationship of *Roegneria* C. Koch based on some morphological characters. - Acta bot. boreal-occident. sin. **18**: 606-612, 1998.

Cai, L.B.: A phylogenetic analysis of *Kengyilia* (*Poaceae*). - Acta bot. boreal-occident. sin. **19**: 707-714, 1999.

Cai, L.B., Zhi, L.: A taxonomical study on the genus *Kengyilia* Yen *et* J. L. Yang. - Acta phytotaxon. sin. **37**: 451-467, 1999.

Chen, S.L., Jin, Y.X., Wu, J.Z.: On the leaf epidermis of genus *Kengyilia* (*Gramineae*) in relation to its taxonomical significance. - Bull. Nanjing bot. Garden Mem. Sun Yat Sen **1**: 1-6, 1991.

Dewey, D.R.: The genome system of classification as a guide to intergeneric hybridization with the perennial *Triticeae*. - In: Gustafson, J.P. (ed.): Gene Manipulation in Plant Improvement. Pp. 209-280. Plenum Press, New York 1984.

Doyle, J.T., Doyle, J.L.: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. - Phytochem. Bull. **19**: 11-15, 1987.

Hsiao, C., Chatterton, N.J., Asay, K.H., Jensen, K.B.: Phylogenetic relationships of the monogenomic species of the wheat tribe, *Triticeae* (*Poaceae*), inferred from nuclear rDNA (internal transcribed spacer) sequences. - Genome **38**: 211-223, 1995.

Jensen, K.B.: Cytology and taxonomy of *Elymus kengii*, *E. grandiglumis,* and *E. batalinii* (*Poaceae*: *Triticeae*). - Genome **33**: 668-673, 1990.

Jensen, K.B.: Genome analysis of Eurasisan *Elymus thoroldianus*, *E. melantherus*, and *E. kokonoricus* (*Poaceae*: *Triticeae*). - Int. J. Plant Sci. **157**: 136-141, 1996.

Jensen, K.B., Chen, S.L.: An overview: systematic relationships of *Elymus* and *Roegneria* (*Poaceae*). - Hereditas **116**: 127-134, 1992.

Keng, Y.L., Chen, S.L.: A revision of the genus *Roegneria* C. Koch of China. - Acta Nanking Univ. **1**: 1-92, 1963.

Liu, Q.L., Ge, S., Tang, H.B., Zhang, X.L., Zhu, G.F., Lu, B.R.: Phylogenetic relationships in *Elymus* (*Poaceae*: *Triticeae*) based on the nuclear ribosomal internal transcribed spacer and chloroplast *trnL-F* sequences. - New Phytol. **170**: 411-420, 2006.

Löve, A.: Conspectuse of *Triticeae*. - Feddes Rep. **95**: 425-524, 1984.

Lu, B.R.: The genus *Elymus* L. in Asia. Taxonomy and biosystmatics with special reference to genomic relationships. - In: Wang, R.R.-C., Jensen, K.B., Jsussi, C. (ed.): Proc. 2nd Int. *Triticeae* Symp. Pp. 219-233. Utah State University, Logan 1994.

Nevski, S.A.: Tribe Hordeae Benth. - In: Komarov, V.L., Roshevits, R.Y., Shishkin, B.K. (ed.): Flora USSR. Pp. 590-728. Nauka, Leningrad 1934.

Rout, G.R.: Evaluation of genetic relationship in *Typhonium* species through random amplified polymorphic DNA markers. - Biol. Plant. **50**: 127-130, 2006.

Sarker, M.M., Adamy, S.S., Mohamed, A.A., El-Itriby, H.A.: Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. - Biol. Plant. **50**: 198-204, 2006.

Soltis, D.E., Soltis, P.S., Tate, J.A.: Advanced in the study of

polyploidy since plant speciation. - New Phytol. **161**: 173-191, 2003.
Swofford, D.L.: PAUP*: Phylogenetic analysis using parsimony (*and other method) Version 4.0b10. - Sinauer Associates, Sunderland - Massachusetts 2003.
Thompson, J.D., Plewniak, F., Poch, O.: A comprehensive comparison of multiple sequences alignment programs. - Prog. Nucleic Acid Res. **27**: 2682-2690, 1999.
Tzvelev, N.N.: Tribe 3 Triticeae Dum. - In: Fedorov A.A. (ed.): Poaceae USSR. Pp. 105-206. Nauka, Leningrad 1976.
Wang, C., Shi, S.H., Wang, J.B., Zhong, Y.: Phylogenetic relationship of diploid species in *Aegilops* inferred from the ITS sequences of nuclear ribosomal DNA. - Acta bot. sin. **42**: 507-511, 2000.
Yang, J.L., Yen, C., Baum, B.R.: *Kengyilia*: synopsis and key to species. - Hereditas **116**: 25-28, 1992.
Yang, R.W., Zhou, Y.H., Ding, C.B., Zheng, Y.L., Zhang, L.: Relationships among *Leymus* species assessed by RAPD markers. - Biol. Plant. **52**: 237-241, 2008.
Yen, C., Yang, J.L.: *Kengyilia gobicola*, a new taxon from west China. - Can. J. Bot. **68**: 1894-1897, 1990.
Yen, C., Yang, J.L., Baum, B.R.: *Douglasdeweya*: a new genus, with a new species and a new combination (*Triticeae*: *Poaceae*). - Can. J. Bot. **83**: 413-419, 2005.
Yen, C., Yang, J.L., Baum, B.R.: Biosystematics of *Triticeae* (Vol. 3). - Chinese Agriculture Press, Beijing 2006.
Zhang, X.Q., Yang, J.L., Yen, C., Zheng, Y.L., Zhou, Y.H.: Cytogenetic and systematic analysis of *Kengyilia gobicola, K. zhaosuensis* and *K. batalinii* var. *nana* (*Poaceae*). - Genet. Resour. Crop Evolut. **47**: 451-454, 2000.
Zhang, L., Zheng, Y.L., Wei, Y.M., Liu, S.G., Zhou, Y.H.: The genetic diversity and similarities among *Kengyilia* species based on random amplified microsatellite polymorphism. - Genet. Resour. Crop Evolut. **52**: 1011-1017, 2005.
Zhou, Y.H.: Study on karyotypes of 5 species of *Kengyilia*. - Guihaia **14**: 163-169, 1994.
Zhou, Y.H., Zheng, Y.L., Yang, J.L., Yen C., Jia J.Z.: Relationships among *Kengyilia* species assessed by RAPD markers. - Acta phytotaxon. sin. **38**: 515-521, 2000.