Phylogeny and maternal donors of *Elytrigia* Desv. sensu lato (Triticeae; Poaceae) inferred from nuclear internal-transcribed spacer and *trnL*-F sequences

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Abstract

**Background**: *Elytrigia* Desv. is a genus with a varied array of morphology, cytology, ecology, and distribution in Triticeae. Classification and systematic position of *Elytrigia* remain controversial. We used nuclear internal-transcribed spacer (nrITS) sequences and chloroplast *trnL*-F region to study the relationships of phylogenetic and maternal genome donor of *Elytrigia* Desv. sensu lato.

**Results**: (1) E, F, P, St, and W genomes bear close relationship with one another and are distant from H and Ns genomes. E⁰ and E⁵ are homoeologous. (2) In EST genome species, E genome is the origin of diploid *Elytrigia* species with E genome, St genome is the origin of *Pseudoroegneria*. (3) Diploid species *E. elongata* were differentiated. (4) *E. stipifolia* and *E. varnensis* sequences are diverse based on nrITS data. (5) *E. loloides* contains St and H genomes and belongs to *Elymus* s. l. (6) E genome diploid species in *Elytrigia* serve as maternal donor of *E. nodosa* (PI547344), *E. farcta*, *E. pontica*, *E. pycnantha*, *E. scirpea*, and *E. scythica*. At least two species act as maternal donor of allopolyploids (ESt and EStP genomes).

**Conclusions**: Our results suggested that *Elytrigia* s. l. species contain different genomes, which should be divided into different genera. However, the genomes of *Elytrigia* species had close relationships with one another. Diploid species were differentiated, because of introgression and different geographical sources. The results also suggested that the same species and the same genomes of different species have different maternal donor. Further study of molecular biology and cytology could facilitate the evaluation of our results of phylogenetic in a more specific and accurate way.

**Keywords**: *Elytrigia* Desv., Chloroplast *trnL*-F, Nuclear ITS, Phylogeny, Maternal donor

Background

Triticeae in Poaceae includes not only the most economically important cereal crops (wheat, barley, and rye) but also forage grasses and ecological species in grasslands. Approximately 450 Triticeae species exist worldwide [1–3]. Given the wide variety of biological mechanisms and genetic systems, this tribe represents an excellent group for research on plant systematics, genetic diversity, and speciation [4, 5].

As one of the most important perennial genera of Triticeae, *Elytrigia* Desv. includes 40 species, which are distributed in subtropical and warm temperate regions of both hemispheres [6]. *Elytrigia* Desv. was established by Desvaux [7], with *Elytrigia repens* (L.) Nevski as the type species. Morphologically, *Elytrigia* sensu lato is characterized by branched creeping rhizomes and caespitose, long anthers, lanceolate to linear glumes, lanceolate lemma, single spikelet per node, and cross-pollination, and most species were previously categorized under *Agropyron* Gaertner [1, 6, 8]. Cytogenetically, ploidy levels in *Elytrigia* s. l. vary from diploid (2n = 2x = 14) to decaploid (2n = 10x = 70) and...
contain E\textsuperscript{e}, E\textsuperscript{b}, St, ESt, StH, and NsXmStH genomes [1, 3, 8–11]. According to the proposed genomic system of classification, Löve [8] suggested that *Elytrigia* s. l. approximately includes 60 species and varieties and divided them into five genera, namely, *Pseudoroegneria* (Nevski) Á. Löve, *Lophopyrum* Á. Löve (E), *Thinopyrum* Á. Löve (J), *Elytrigia* (ESJ), and *Elymus* (STH). Dewey [1] considered *Elytrigia* s. I. into three independent genera: *Pseudoroegneria* (St), *Thinopyrum* (E or J), and *Elytrigia* (StX). Studies showed similarity of the E and J genomes [12–19]. Wang et al. [19] suggested that E and J should be considered as identical genomes and be distinguished from E\textsuperscript{e} and E\textsuperscript{b}. With the genomic system of classification in Triticeae taxonomy and systematics and genomic constitutions of increasing species identified, the definition of *Elytrigia* becomes narrower than that of traditional *Elytrigia* s. l. and only includes all polyploidy taxa with combination of E\textsuperscript{e}, E\textsuperscript{b}, and St genomes [3, 9, 11]. E\textsuperscript{e} genome originated from *Lophopyrum elongatum* (Host) Á. Löve, E\textsuperscript{b} genome from *Thinopyrum bessarabicum* (Savul. and Rayss) Á. Löve, and St genome from diploid species in *Pseudoroegneria* (Nevski) Á. Löve. However, some studies reported that *E. repens*, a type of *Elytrigia*, is a hexaploid species with StStStStHH genomes and was renamed as *Elymus repens* [3, 11, 20, 21]. Therefore, definition, precise taxonomic ranks, and number of *Elytrigia* species remain controversial.

Polyploidization and hybridization are the two main mechanisms in plant speciation and evolution [22, 23]. The changes in the cell size, genome size, gene expression, genomic repatterning, epigenetic effects and retrotransposon activation are caused by the polyploidization and chromosome doubling [5, 22–26]. As a result of these changes, stabilization of hybrid condition and full fertility may occur. And the establishment of phenotypes in nature could be enhanced. Therefore, polyploids could adjust to match with the new ecological niches or become more competitive than parental donors [5, 23, 26, 27]. The evolution of polyploidization alone and/or the combined effects of hybridization and polyploidization may lead to complex lineages, requiring an explanation of the phylogenetic relationship [27]. Molecular genetic analysis bears significance in elucidating phylogenetic relationships and genome evolution patterns in taxa for these kinds of plant groups [27, 28]. The analysis of Molecular phylogenetic exploits DNA sequences elucidated the history of revolution and origins of species in Triticeae. This illustrates their hybridization events and parental lineages contains their formation, and identifies the polyploidization mode [29–38]. Reproducibility and simplicity represent the main qualities that make DNA sequencing a suitable choice for identification of phylogenetic relationships among taxa and genomes [28, 39]. nrITS sequences were widely applied to explain genomic and phylogenetic relationship at a low taxonomic levels [40–42] and Triticeae species containing E, H, Ns, P, St and Xm genomes in *Elymus*, *Hordeum*, *Psathyrostachys*, *Agropyron* *Pseudoroegneria* and *Leymus* [5, 27, 33, 40–43]. Chloroplast DNA (cpDNA) sequences, including intron of trn-L and intergenic spacer of trnH-psbA, trnL-trnF, and trnS-trnG, are also widely used to identify maternal donors of polyploids with extra ability to analyze phylogenetic relationships among relevant species [41, 44–47].

The present study analyzed sequence data of one ITS region of nuclear DNA and one chloroplast gene (the intergenic region of trnL-trnF) from 18 species (subspecies) in *Elytrigia* s. l. with 21 species of related genera in Triticeae. The objectives are as follows: (1) to investigate phylogenetic relationships among species in *Elytrigia* s. l. and related genera; (2) to elucidate interspecific relationships among *Elytrigia* s. l. species; (3) to study phylogenetic relationships among species with different genomes and genome combinations; and (4) to discuss putative maternal donors for ESt genome species.

**Methods**

**Plant materials**

This study included 18 species (subspecies) of *Elytrigia* Desv. sensu lato and 21 species (subspecies) of related genera in Triticeae. Table 1 lists names, accession numbers, genomes, origins, and GenBank accession numbers. *Bromus cartharticus* Vahl. and *Bromus tectorum* L. were used as outgroup [5, 41, 48]. Seed materials with W\textsubscript{e} and PI accession numbers were carefully offered by the American National Plant Germplasm System (Pullman, Washington, USA). We gathered seed materials with Y and ZY numbers. Voucher specimens and plants were deposited at the perennial nursery and herbarium of the Triticeae Research Institute, Sichuan Agricultural University, China.

**DNA extraction, amplification, and sequencing**

Total genomic DNA was extracted from leaves of single plants by slight modification of Cetyltrimethyl Ammonium Bromide (CTAB) procedure [49]. nrITS sequence and chloroplast trnL-F sequence were amplified with primers described in Table 2. A final volume of 20 μL of mixed reagents was obtained for each polymerase chain reaction (PCR); reagents included 2× Taq PCR Master-Mix (10× ExTaq polymerase buffer, 3 mmol/L MgCl\textsubscript{2}, 500 μmol/L deoxynucleotide, 100 mmol/L KCl, and 20 mmol/L Tris–HCl), 1 μmol/L of each primer, 20–40 ng of template DNA, and distilled deionized water. PCR reactions were performed in GeneAmp T100 Thermal Cycler (Bio-Rad Inc., USA) employing protocols listed in Table 3. PCR products were
| Species | Genome | Accession No. | Origin         | GenBank No. | Abbr. |
|---------|--------|---------------|----------------|-------------|-------|
| Elytrigia Desv. |        |               |                |             |       |
| Elytrigia bessarabica (Savul & Rayss) Dubov. | E<sup>b</sup> | PI531711 | Ukraine        | MF893171 | EBES  |
|          |        | PI531712 | Russian Federation | L36506<sup>a</sup> |       |
| Elytrigia caespitosa (C. Koch) Nevski | E<sup>St</sup> | PI547311 | Russian Federation | EU139480<sup>a</sup> |       |
|          |        |           |                | MF893146 | ECAE  |
|          |        |           |                | MF893147 | ECA2  |
| Elytrigia elongata (Host) Nevski | E<sup>E</sup><sup>o</sup> | W6 21,859 | Iran            | MF893172 | EEO   |
|          |        |           |                | MF893148 | EEL1  |
|          |        |           |                | EF014249<sup>a</sup> | EEL2  |
| Elytrigia farcta (Viv.) Holub | E<sup>E</sup><sup>Pa</sup><sup>e</sup> | PI51655 | Morocco         | MF893175 | EFAR  |
| Elytrigia geniculata (Trin.) Nevski | St<sup>St</sup> | PI565009 | Russian Federation | MF893176 | EGEN  |
| Elytrigia geniculata ssp. pruinifera (Nevski) Tzvel. | St<sup>St</sup> | PI547374 | Russian Federation | MF893177 | EPR   |
|          |        |           |                | EF014229 | EPR1  |
|          |        |           |                | MF893150 | EPR2  |
| Elytrigia intermedia (Host) Nevski | E<sup>E</sup><sup>St</sup> | PI401228 | Iran            | MF893179 | EINT  |
|          |        | PI229917  | Iran            | MF893152 | EIN1  |
|          |        | PI531725  | Germany         | MF893153 | EIN2  |
| Elytrigia folioides (Kar. et Kir.) Nevski | E<sup>b</sup> | PI440059 | Former Soviet Union | MF893180 | ELOL  |
|          |        |           |                | MF893154 | ELO1  |
|          |        |           |                | MF893155 | ELO2  |
| Elytrigia nodosa (Steven) Nevski | E<sup>St</sup> | PI547344 | Turkey          | MF893173 | ENO1  |
|          |        | PI547345  | Ukraine         | MF893174 | ENO2  |
|          |        |           |                | EF014248 | EN11  |
|          |        |           |                | JX624139<sup>a</sup> | EN12  |
| Elytrigia podperae (Nábělek) Holub | E<sup>E</sup><sup>Pa</sup><sup>e</sup> | PI401299 | Iran            | MF893181 | EPOD  |
| Elytrigia pontica (Podp.) Holub | E<sup>b</sup> | PI383583 | Turkey          | MF893183 | EPO   |
|          |        |           |                | MF893157 | EP11  |
|          |        |           |                | AU000768<sup>a</sup> | EP12  |
| Elytrigia pungens (Pers.) Tutin | E<sup>StStP</sup> | PI547268 | Russian Federation | MF893189 | EPO2  |
| Elytrigia pycnantha (Godr.) Á. Löve | E<sup>StP</sup> | PI618742 | Jonufer, Albania | MF893190 | EPYC  |
|          |        |           |                | MF893182 |       |
| Elytrigia rechingeri (Runemark) Hulub | E<sup>E</sup><sup>Pa</sup> | PI531745 | Greece          | MF893184 | EREC  |
| Elytrigia repens (L.) Nevski | St<sup>StH</sup> | Y0814  | China           | MF893185 | EREP  |
|          |        |           |                | MF893160 | ERE1  |
|          |        |           |                | MF893161 | ERE2  |
| Elytrigia scirpea (K. Presl) Holub | E<sup>E</sup><sup>Pa</sup> | PI531749 | Italy           | MF893162 | ESC1  |
|          |        | PI531750  | Greece          | MF893186 | ESC2  |
| Elytrigia scythica (Nevski) Nevski | E<sup>St</sup> | PI502271 | Russian Federation | MF893187 | ESCY  |
|          |        | PI283272  | Former Soviet Union | MF893164 | ES11  |
|          |        |           |                | MF893165 | ES12  |
| Species | Genome | Accession No. | Origin     | GenBank No. | Abbr. |
|---------|--------|---------------|------------|-------------|-------|
| Elytrigia varnensis (Velen.) Holub | Trn | PI281863 | Germany | MF893188 | EVAR |
| | | | | MF893169 | EVA1 |
| | | | | MF893170 | EVA2 |
| Agropyron Gaertn. | P | H10066 | Xinjiang, China | AF519116a | ACRI |
| | | | | AY740891a |
| Australopyrum (Tsvelev) A. Löve | W | M. Pinar 4412b | Turkey | KP723656a | APEC |
| | | D3438 | Australia | L36483a |
| | W | Pis47363 | Australia | EU617319a | ARET |
| | | | | EU617249a |
| Australopyrum velutinum (Nees) B. K | W | D2873–2878 | Australia | AF519119a | AVEL |
| | | | | AF519119a |
| Elymus L. | StH | PI499412 | China | KJ526334a | EC11 |
| | | | | KJ526335a | EC12 |
| Elymus canadensis L. | StH | PI564910 | Russian | AF519119a | E111 |
| | | | | AF519119a |
| Eremopyrum Jaub. Et Spach. | F | HS5552 | Iran | AF519150a | EDIS |
| | | TA2229 | Afghanistan | JQ360120a |
| Eremopyrum distans(C. Koch) Nevski | F | HS5555 | Iran | AF519151a | EORI |
| Eremopyrum orientale(L.) Jaub. Et Spach | F | Y206 | China | JQ360124a | ETRI |
| Hordeum L. | H | PI531761 | China | AY740879a | HBG |
| | | | | AY740876a |
| Hordeum chilense Roem & Schult. | H | Y2054 | Chile | AJ607873a |
| Psathyrostachys Nevski | F | P53192 | Iran | AF519169a | PFRA |
| Psathyrostachys juncea (Fischer) Nevski | F | PI001163 | China | EF591911a | PJUN |
| Psathyrostachys huashanica Keng ex P. C. Kuo | F | Y2054 | China | KT184655a |
| Pseudoroegneria (Nevski) A. Löve | St- | PI420842 | Russian Federation | MF893178 | MF893151 |
| Pseudoroegneria gracillima (Nevski) A. Löve | St | PI228391 | Iran | AF519156a | PLIB |
| | | | | MF893151 |
| Pseudoroegneria libanotica (Hackel) D. R. Dewey | St | P1610986 | United States | MF893166 | MF893166 |
| | | | | MF893166 |
| Pseudoroegneria spicata (Pursh) A. Löve | St | P1566259 | United States | MF893166 | MF893166 |
| | | | | MF893166 |
| Pseudoroegneria stipifolia (Czern. ex Nevski) A. Löve | St | PI635870 | United States | MF893167 | MF893167 |
| | | | | MF893167 |
| Pseudoroegneria strigosa (M. Bieb.) A. Löve | St | PI640095 | Russian Federation | EF396089a | PSTI |
| | | | | EU617052a |
| | | | | MF893168 |

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electrophoresed on 1.0% agarose gels, and purified using EZNA™ gel extraction kit (Omega, GA, USA), and were cloned into pMD-19 T vector (TaKaRa) following the instructions of manufacturer. All sequences were derived from at least 3 independent clones for diploid species, and 5–8 independent clones for allopolyploid species. Sequencing was performed from both directions by Sunbiotech Company (Beijing, China) [36].

Phylogenetic analysis
Alignment of nrITS and trnL-F sequences were conducted by using Clustal W algorithm [50] with additional manual adjustment. Two data matrices with included nrITS were performed using Maximum likelihood (ML) in PAUP*4.0a (Swofford, D.L., Sinauer Associates, http://www.sinauer.com) and Bayesian inference (BI) in MrBayes version 3.1.2 [51]. Phylogenetic analyses based on trnL-F sequences were performed with MrBayes version 3.1.2. Evolutionary model employed for phylogenetic study was performed using Modeltest v3.7 with Akaike information criterion (AIC) [52]. Best-fit model was GTR + G for nrITS data. ML heuristic studies were carried out with 1000 random addition sequence replications and reconnection branch swapping algorithm and tree bisection [Dong 2013].

Similar to ML analysis, BI analyses of nrITS were performed with the alike evolutionary model. TVM + G was the optimal model for trnL-F data based on AIC in Modeltest v3.7. Observation of consistency and examined log likelihoods among all independent runs showed that burn-in periods very long enough for chains to become stationary [37]. Figures included nonsignificant bootstrap support (BS) of more than 50% and posterior probabilities of more than 70%.

Median-joining (MJ) network method was effectively employed to study detailed progenitor–descendant relationship among polyploidy species within tribe Triticeae [27, 35, 37, 53]. MJ network analysis was conducted by the Network 4.6.1.3 program (Fluxus Technology Ltd., Clare, Suffolk, UK). For the purpose of preventing single insertion/deletion events from being counted as multiple mutational stages in MJ network study, gaps in aligned nrITS and trnL-F sequences were not included in the calculation [37].

Results
nrITS data
Comparison of all species analysis suggested that DNA sequences for nrITS ranged from 596 bp to 605 bp in length. A TTTT insert at positions 58–61 in the nrITS sequence was detected for Et. caespitosa, Et. elongata (W6 21,859), Et. geniculata ssp. pruinifera, Et. intermedia (PI229917), Et. nodosa, Et. pontica, Et. rechingeri, Et. scirpea (PI 531750), Et. scythica, and Et. varnensis (Fig. 1).

Table 1 Species of Elytrigia sensu lato and the related species used in this study (Continued)

| Species | Genome | Accession No. | Origin | GenBank No. | Abbr. |
|---------|--------|---------------|--------|-------------|-------|
| Pseudoroegneria strigosa ssp. aegilopoides (Drobov) A. Löve | 1.0 | P595164 | China | EF396990 | PAEG |
| | | W6 13089 | China | EU617075 | |
| Pseudoroegneria tauri (Boiss. & Bal.) Á. Löve | St | PI401323 | Iran | EF396991 | PTAU |
| | | PI380646 | Iran | EsU617239 | |
| Bromus catharticus Vahl. | 1.0 | | | |
| | | | | |
| Bromus tectorum L. | 1.0 | | | |

Table 2 Names, sequences, and references of primers used in this study

| Gene | Name of primers | Sequence of primer (5′- 3′) | Reference |
|------|-----------------|-----------------------------|-----------|
| nrITS | ITS4 | TCCTCCGCTTATGTGATGC | Hsiao et al. (1995) [59] |
| | ITS-L | TCCTCCGCTTATGTGATGC | |
| trnL-F | C | CGAAATCGTGAGCCTACG | Mason-Gamer et al. (2002) [44] |
| | F | ATTTGACCTGGTGACGAG | |

Table 3 Thermocycling conditions for amplification of genes using the PCR

| Gene | Protocol |
|------|----------|
| nrITS | 1 cycle: 3 min 94 °C; 35 cycles: 1 min 94 °C, 1 min 52 °C, 1 min 72 °C; 1 cycle: 8 min 72 °C |
| trnL-F | 1 cycle: 4 min 94 °C; 25 cycles: 40 s 94 °C, 50 s 60 °C, 2 min 72 °C; 1 cycle: 8 min 72 °C |
Fig. 1 Partial alignment of the amplified sequences of nrITS gene from the ten species of *Elytrigia* sensu lato. A TTTT insert at position 58–61.

Fig. 2 Maximum-likelihood tree (~Lnlikelihood = 2553.6868, base frequencies A: 0.2286, C: 0.2966, G: 0.2794, T: 0.1954, pinvar = none, shape = 0.4121) inferred from the nrITS sequences of *Elytrigia* sensu lato and its affinitive species, under GTR+ G model. Numbers above and below branches indicate posterior probabilities (PP) ≥ 70% by BI analysis and bootstrap support (BS) ≥ 50% by ML, respectively.
same topology. The tree demonstrated in Fig. 2 corresponds to the ML tree with posterior probabilities (PP) above and BS below branches [48].

nrITS region from species were divided into five clades (Clades I–V). Clade I was divided into three groups, namely, A, B, and C. Group A (BS < 50 and PP < 70%) consisted of the St-genome sequence and included two Pseudoroegneria species (Pse. spicata PI 506259 and Pse. gracillima), two Elymus species (El. canadensis and El. caninus), and Elytrigia species, such as Et. caespitosa, Et. nodosa, Et. podperae, Et. pontica, and Et. scythica. Group B (PP = 81%) consisted of Et. bessarabica, Et. elongata (PI 531719), Et. farcata (PI 516555), Et. intermedia (PI 531725), and Et. scirpea (PI 531749); this group possesses an E-genome sequence. Group C (BS = 99% and PP = 100%) included 10 species with a TTTT insert at positions 58–61; this insert is a possible variation of E-genome sequence. This group comprised Et. caespitosa, Et. elongata (W6 21,859), Et. geniculata ssp. pruinifera, Et. intermedia (PI 229917), Et. nodosa, Et. pontica, Et. rechingeri, Et. scirpea (PI 531750), Et. scythea, and Et. varnensis. Clade II included St-genome sequences of Pse. spicata (PI 563870), Pse. tauri, and EstP genome species (Et. pungens and Et. pycnantha) and unknown genome sequences of Et. varnensis, P-genome sequences of Agropyron cristatum, W-genome sequences of Australopyrum pectinatum, Au. retrofractum, and F-genome sequences of Eremopyrum distans and E. triticeum. Clade III consisted of St-genome sequences of Pse. libanotica, Pse. stipfolia, Pse. strigosa, Pse. strigosa ssp. aegilopoides, and three Elytrigia s. l. species (Et. geniculata ssp. pruinifera, Et. lolioides, and Et. repens). Clade IV comprised two Elytrigia s. l. species (Et. lolioides and Et. repens), two Elymus s. l. species (El. canadensis and El. caninus), and two Hordeum s. l. (H. bogdanii and H. chilense) (BS = 83%; PP = 100%). Clade V comprised Psathyrostachys juncea and Psa. huashanica (BS = 91% and PP = 100%).

In MJ analysis, each circular network node indicated a single sequence haplotype, and the node size is proportional to the number of isolates with that of haplotype [37]. Median vectors (standing for missing intermediates) present nodes that haven’t sampled deduced by MJ network study, and the numbers along branches illustrate the mutation positions. Distinguishing colors indicated various species that share similar haplotype circular network node. Either alternative genealogies or true reticulation events are represented by network loops in closely related lineages [37]. The MJ network depicted genealogical relationships among 45 nrITS haplotypes from 49 taxa (Fig. 3) [48]. We found that MJ network represented a consistent phylogenetic reconstruction with ML tree. Then, we determined the names and group names of similar clusters to synchronize the MJ network. In nrITS MJ network analysis, five clusters (Clusters N-I to N-V) formed one star-like radiation. Three clusters (Clusters N-III to N-V) represented three different types of haplotypes (St, P, and Ns types) of Elytrigia s. l. and its related genera. Cluster N-I was divided into subclusters N-A, N-B,
and N-C with E and St types, and *Pse. spicata* PI 506259 (PSP1) was placed at the central branching point. Cluster N-II included St type of *Pse. spicata* PI 563870 (PSP2) and *Pse. tauri* (PTAU), P type of *Ag. cristatum* (ACRI), *Et. pungens* (EPUN), and *Et. pycnantha* (EPYC), F type of *Er. distans* (EDIS) and *Er. triticeum* (ETRI), W type of *Au. pectinatum* (APEC) and *Au. retrofractum* (ARET), and unknown type of *Et. varnensis* (EVA1).

**trnL-F data**

Comparison of all species studies showed that the length of *trnL-F* sequences ranged from 809 bp to 882 bp. Likelihood settings from optimal model (TVM + G) were chose by AIC in Modeltest v3.7. Fig. 4 illustrates the BI tree with PP above branches. All *trnL-F* sequences from *Elytrigia* and its related genera species were similar.

![Diagram of Bayesian inference tree](image)

*Fig. 4* Bayesian inference tree inferred from the *trnL-F* sequences of *Elytrigia* sensu lato and its affinitive species. Numbers above branches indicate posterior probabilities (PP) $\geq 70\%$ by BI.
clusters’ names following the name of groups shown in the ML tree. The trnL-F MJ network was divided into four clusters (Clusters N-One to N-Four). All species containing E or St genome were clustered together with E or St diploid species in Cluster N-One. Cluster N-Two included F, P, and W types of haplotypes. Ns type of Psathyrostachys haplotype species and H type of Hordeum haplotype species were grouped, respectively, in Clusters N-Three and N-Four (Fig. 5).

Discussion
Phylogenetic relationships among species in Elytrigia s. l.
Elytrigia s. l. is distributed in subtropical and warm temperate regions of both hemispheres [6]. Classification and systematic position of Elytrigia remain controversial [7, 8, 54–56]. Traditionally, the classification based on morphology and Elytrigia species contains E^e, E^b, E^E^e, E^F^E^St, E^bE^St, E^St, StSt, StH, and EStP genomes. However, Dewey [1] and Löve [8] showed that taxonomic treatment of Triticeae species should be depended on genomic constitution. Therefore, Elytrigia species must be reclassified. Several current studies used molecular biology to study phylogenetic relationships of Elytrigia s. l. species and its related genera [43, 57, 58]. Hsiao et al. [59] estimated phylogenetic relationships of 30 diploid species of Triticeae (Poaceae) from the nrITS region of nuclear ribosomal DNA. Results illustrated that each genome group of species is monophyletic and consisten with cytogenetic evidence, and Australopyrum (Tzvelev) A. Löve (W) is closely concerned with Agropyron Gaertn. (P) [59]. Kim et al. [43] analyzed nrITS haplotypes, revealing close relationship of E, P, and St genomes. Cytologically, St and Y genomes and St, P, and W genomes are closely related [2, 60–62]. This finding indicates close relationship of E, P, St, and W genomes.

In this study, based on nrITS data, all Elytrigia s. l. species were classified in four groups (E, H, P, and St types) in the ML tree and MJ network. These results indicated that Elytrigia s. l. species contain different genomes. These findings also strongly support previous results. Genome species are not highly supported in Clades I-B, E^e, and E^b (BS < 50% and BI = 81%) (Fig. 2). This phenomenon provides evidence of close affinity between E^e and E^b genome species. Thus, these species are not homologous but homoeologous [63]. Our phylogenetic results also support previous cytological investigations reported by Löve [8, 64, 65], Yen and Yang [3], and Zhou [11].

Phylogenetic relationships between Elytrigia s. l. and related genera
In the present study, in the ML tree and MJ network based on nrITS data, seven types of nrITS region (E, F, H, P, W, Ns, and St types) were obtained from the Elytrigia s. l. species and its related genera. In polyploidy species Et. repens (StStH), H type was clustered with Hordeum species in Clade IV (83% BS, 100% PP), and St type was clustered with Pseudoroegneria (Fig. 2). In this study, we failed to obtain E and St type nrITS sequences from Et. pycnantha (EStP) and Et. pungens (EStP),

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*Fig. 5* Median-joining networks based on trnL-F locus haplotype of Elytrigia sensu lato and its affinitive species. Haplotypes are represented by circles. The numbers along the branches indicate the frequency of mutations. Abbreviations of species names are listed in Table 1.
whereas P type was clustered with *Agropyron* in Clade II (86% BS and 97% PP). In the phylogenetic tree, Clades II and IV formed a monophyletic group, and results support the distant relationship between H genome and other genomes (E, P, St, and W) (Fig. 2). This finding also indicated that St genome is the origin of *Pseudoroegneria*, whereas P genome is the origin of *Agropyron*.

*Eremopyrum* (Ledebr.) Jaub. et Spach and *Agropyron* Gaertn. are highly similar based on one-keeled glumes and Caryopsis morphology [66, 67]. According to molecular phylogenetic analysis, *E. triticeum* and *Er. distans* were clustered with *Agropyron* based on rpoA, cpDNA, DMC1, and β-amylase data [44, 68–70]. Fan et al. [26] showed that *Eremopyrum* and *Agropyron* are closely related based on the presence of Acc1, Pgk1, and Acc1 + Pgk1. In the present nrITS gene data, allopolyploid species of *E. pycnantha* and *Et. pungens* (EStP) were clustered with *E. triticeum*, *Er. distans*, and *A. cristatum* with high statistical support (85% BS and 100% PP). *Et. pycnantha*, *Et. pungens*, *Eremopyrum*, and *Agropyron* species were grouped with St genome diploid species (*Pse. spicata* PI563870 and *Pse. tauri* (92% PP)). MJ network analysis showed that diploid species *Et. bessarabica* (E), *Et. pontica* (PI 547313), *Et. pycnantha* (EStP), *Et. scirpea* (EStP), and *Et. scythica* (92% PP) exhibited the same haplotype in Cluster N-One (Fig. 5). Combined with BI and MJ analyses, we can conclude that E genome-diploid species in *Elytrigia* served as maternal donor of E genome for *Et. farcta*, *Et. nodosa* (PI 547344), *Et. pontica*, *E. pycnantha*, and *Et. scythica*. This conclusion agrees with results of Liao et al. [37]. *Et. nodosa* (P 1547345 Ukraine) was not clustered with *Et. bessarabica*, and its haplotype differs from that of *Et. nodosa* (PI 547344 Turkey). Results showed that (1) at no less than two species served as maternal donor, indicating that formation of *Et. nodosa* occur multiple times. A similar conclusion was observed based on *E. caespitosa*, *Et. intermedia*, *Et. varnensis*, and *Kengyilia* species [45, 58, 79]. (2) Different maternal donors in *Et. nodosa* are affected by altitude and climate conditions [80]. In the BI tree based on trnL-F sequence, we can conclude that *Pseudoroegenaria* species (St genome donor) acted as maternal donor of *Et. repens* (StStH), whereas species of *Agropyron* Gaertn. (P genome donor) acted as maternal donor of *Et. pungens* (EStStP). However, E genome acted as maternal donor of *Et. pycnantha* (EStP). This result indicated that different species served as maternal donors that contributed to species containing the same genomes. Previous findings on *Et. intermedia* were similar to our results [58]. Other polyploidy species in *Elytrigia* and diploid species containing E or St genome formed zero-length branches in Clade One because of the close relation of E and St genomes (Fig. 4). Sources of maternal donor of these genomes remain to be identified.

**Putative maternal donor and origin of *Elytrigia* species**

CpDNA is mostly inherited from the female parent in tall plants. Therefore, it can be used to determine maternal donor in polyploids [37, 53, 73]. In the trnL-F ML, the phylogenetic tree showed high numbers of zero-length branches, which are mainly related to multifurcating relationships. Tree-based study methods are unable to represent multifurcating relationships and the coexistence of ancestors with their derivatives [53, 74]. Network approaches were designed to deal with such multifurcations [53, 73–76].

Previous studies based on cpDNA indicated that *Pseudoroegenaria* (St genome donor) species are the maternal donor for species of Triticeae [41, 44, 77]. However, cytologically, Yen et al. [78] considered that rather than the St genome, the maternal donor of *Kengyilia* is the origin of P genome species. In this trnL-F-based BI tree, *Et. bessarabica* (2x) was clustered with polyploidy species *Et. farcta*, *Et. nodosa* (PI 547344), *Et. pontica* (PI 547313), *Et. pycnantha*, *Et. scirpea*, and *Et. scythica* (92% PP). MJ network analysis showed that diploid species *Et. bessarabica* (E), *Et. pontica* (PI 547313), *Et. pycnantha* (EStP), *Et. scirpea* (E), and *Et. scythica* (EST) exhibit the same haplotype in Cluster N-One (Fig. 5). Combined with BI and MJ analyses, we can conclude that E genome-diploid species in *Elytrigia* served as maternal donor of E genome for *Et. farcta*, *Et. nodosa* (PI 547344), *Et. pontica*, and *Et. pycnantha*, and *Et. scythica*. This conclusion agrees with results of Liao et al. [37]. *Et. nodosa* (P 1547345 Ukraine) was not clustered with *Et. bessarabica*, and its haplotype differs from that of *Et. nodosa* (PI 547344 Turkey). Results showed that (1) at no less than two species served as maternal donor, indicating that formation of *Et. nodosa* occur multiple times. A similar conclusion was observed based on *E. caespitosa*, *Et. intermedia*, *Et. varnensis*, and *Kengyilia* species [45, 58, 79]. (2) Different maternal donors in *Et. nodosa* are affected by altitude and climate conditions [80]. In the BI tree based on trnL-F sequence, we can conclude that *Pseudoroegenaria* species (St genome donor) acted as maternal donor of *Et. repens* (StStH), whereas species of *Agropyron* Gaertn. (P genome donor) acted as maternal donor of *Et. pungens* (EStStP). However, E genome acted as maternal donor of *Et. pycnantha* (EStP). This result indicated that different species served as maternal donors that contributed to species containing the same genomes. Previous findings on *Et. intermedia* were similar to our results [58]. Other polyploidy species in *Elytrigia* and diploid species containing E or St genome formed zero-length branches in Clade One because of the close relation of E and St genomes (Fig. 4). Sources of maternal donor of these genomes remain to be identified.

**Differentiation and relationship between E and St genomes**

In Clade I, species containing E, St, and EST genomes and those in Cluster N-I *Pse. spicata* appeared at the central part, indicating close relationship of St and E genome species (Figs. 2 and 3). These findings coincide with previous findings on morphology and molecular biology [44, 72, 81]. In the present study, E and St types were obtained from species containing EST genome grouped with *Elytrigia* or *Pseudoroegenaria* diploid species, respectively. This phenomenon showed that E
genome was the origin of diploid *Elytrigia* species with the E genome. St genome was the origin of *Pseudoroegneria*. Results from morphology, genetics, and molecular biology indicate that species containing E, St, and ESt genomes are closely related with *Elytrigia*.

**Taxonomy of species with ESt and ESTP genomes**

Polyploidization and hybridization are long recognized as prominent forces in evolution of plant species, which feature consequences of genomic changes [22, 23]. Genome relationship and differentiation are often vague in some species because of frequent introgression of alien genes, polyploidization and chromosome segments from wide hybridization [43]. Thus, classification is one of the most important issues that require understanding.

Previous studies indicated that *E. caespitosa*, *E. intermedia*, *E. nodosa*, *E. scytheica*, and *E. varnensis* contain EST genomes, which belong to *Trichopyrum* [3, 17, 82, 83]. Comparison of partial sequences of nrITS gene showed that a TTTT insert at positions 58–61 in nrITS sequence was detected for 11 species (Fig. 1). This finding indicated that introgression of E genome during polyploidization or different independent hybridization events may create the variants in polyploid EST species. In the ML tree and MJ network based on nrITS sequence, one group is formed by EST genome species (*E. caespitosa*, *E. intermedia*, *E. nodosa*, *E. scytheica*, and *E. geniculata* ssp. *pruinifera*) and unknown genome species of *E. varnensis*. This result indicated that these species should be classified under the same genus (*Trichopyrum*). Species containing EST genomes were grouped with diploid species *E. elongata* (E genome), suggesting that E genome may be derived from *E. elongata*. In this study, diploid species of *E. elongata* were differentiated. We selected two *E. elongata* (Iran, France) with different origins, which are divided into Clades I-B and C (Fig. 2). A TTTT insert at positions 58–61 in the sequence was also detected for *E. elongata* (W₆, 21,859) (Fig. 1). This result indicates that EST genome polyploid species and diploid *Elytrigia* species (E genome) displayed hybridization event, resulting in divided E genome.

*E. varnensis* was reported by Löve to contain EST genomes (2n = 12× = 84) [8]. Yang [84] showed that *E. varnensis* is a tetraploid species. Diversity of species ploidy may be caused by chromosome variation under natural conditions. In this study, we discovered that *E. varnensis* clustered with *E. pungens*, *E. pycnantha*, *Ag. cristatum*, *Au. pectinatum*, *Au. retrofractum*, *Er. distans*, and *Er. triticeum* (85% BS and 100% PP) (Fig. 2). We concluded that this species contains P or F genome. Another estimate indicated that St genome allopolyploid species possibly resulted from introgression of *Eremopyrum* or *Agropyron* during polyploidization. Results strongly support those of previous studies in cytogenetics [85].

**Possible genome constitutions and taxonomic treatment of *E. lolioides***

Cytologically, *E. repens* comprised StH genome. *E. lolioides* is a polyploid species, and its genomic constitutions remains unknown [8, 17, 62, 86]. In the present study, *E. lolioides* was clustered with diploid *P. libanotica* (St genome); this result indicated that *E. lolioides* possesses one St genome. *E. lolioides* clustered with *H. bogdanii*, *H. chilense* (H), and *E. repens* (H copy) with high statistical result (98% BS and 100% PP) (Fig. 2). Such finding also indicated that *E. lolioides* contains H genome and is closely related to StH genome species *E. repens*. Thus, we can conclude that genomic constitution of *E. lolioides* includes St and H genomes and belongs to *Elymus* s.l.

**Conclusion**

This study analyzed the phylogenetic relationship among *Elytrigia* s. l. species on the basis of the nrITS sequence data. The results supported the conclusion that *Elytrigia* s. l. consists of various genomes (E, H, P, and St types), which should be classified as different genera. Analyses based on nrITS sequence data and chloroplast trnL-F region show that the E, F, St, P, and W genomes have a close intergroup relationship but are distant with the H and Ns genomes. This finding strongly supports previous studies on morphology, molecular biology, and cytogenetics. nrITS sequence analysis demonstrated that the E genome of species *E. caespitosa*, *E. caespitosa* ssp. *nodosa*, *E. intermedia*, *E. scytheica* and *E. geniculata* ssp. *pruinifera*, which contains EST genomes, originated from *E. elongata* in Lophopyrum. However, differentiation was found in diploid species *E. elongata*; this phenomenon was possibly due to diverse geographical origins or introgression. *E. lolioides*, which is composed of unknown genomes, contains the H and St genomes and has a close genetic relationship with *E. repens* and *E. canadensis*, which contain the St and H genomes. Accordingly, the genome of *E. lolioides* is inferred to contain St and H. In this paper, polyploid species of *Elytrigia* s. l. was deduced based on trnL-F sequence, the female parent of *E. caespitosa* ssp. *nodosa* (P1547344), *E. farcta*, *E. pontica* (P1547313), *E. pycnantha*, *E. scirpea* and *E. scytheica* is the diploid species of *Elytrigia* s. l. containing the E genome; the maternal donor of the polyploidy species *E. caespitosa* ssp. *nodosa* (P1547345), *E. pontica* (P1383583), *E. repens*, *E. geniculata* ssp. *pruinifera* is the St genome. Different maternal donors were also found in allopolyploid species. This result could be attributed to different growth environments, introgression, or incomplete separation of genome E lineage. Thus, different haplotypes were presented.
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Availability of data and materials
The sequencing data from our study was deposited in the National Center for Biotechnology Information (NCBI) under the accession number MF893146-MF893190.

Authors’ contributions
YY designed the study, and wrote the manuscript. YY, FX, ZJ and SLN acquired, analyzed and interpreted the data; YY and WL carried out of nrITS L-F sequences. KHY and ZHQ participated in its design and coordination and helped to draft the manuscript. YY, WL and ZJ participated in the language editing. YY, ZJ and WL gave the good suggestions in the experiments and manuscript. ZHY planned the study, participated in the design of the experiments, and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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