Tree Genetics & Genomes (2017) 13: 43
DOI 10.1007/s11295-017-1114-3

ORIGINAL ARTICLE

Genome-wide characterization and evolution analysis of long terminal repeat retroelements in moso bamboo (*Phyllostachys edulis*)

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Received: 14 October 2016 / Revised: 10 January 2017 / Accepted: 23 January 2017 / Published online: 6 March 2017

Abstract The availability of nearly complete moso bamboo genome sequences permits the detailed discovery and cross-species comparison of transposable elements (TEs) between Bambusoideae and other Poaceae species at the whole genome level. Long terminal repeat retroelements (LTR-retroelements) are the single largest components of most plant genomes and can substantially impact the genome in various ways. Through a combination of structure- and homology-based approaches, we initially investigated 982 LTR-retroelement families comprising 2,004,644 LTR-retroelement sequences, which accounted for more than 40% of the moso bamboo genome. Further analysis revealed that the ratio of solo LTRs to intact elements (S/I) in moso bamboo is significantly low (approximately 0.28:1), indicating that bamboo LTR-retroelements might have undergone relatively low frequencies of unequal recombination and illegitimate recombination. Phylogenetic analysis revealed four *Ty1-copia* and five *Ty3-gypsy* evolutionary lineages that were present before the divergence of eudicot and monocot species, but the scales and timeframes within which they proliferated significantly varied across families and lineages. Insertion time estimates showed that LTR-retroelements were amplified for approximately 0~3 million years and had longer periods of activity than those of rice and *Arabidopsis*. These findings suggest that the expansion of LTR-retroelements might be responsible for host large genome size during moso bamboo evolution.

Keywords LTR-retroelement · Moso bamboo [*Phyllostachys edulis* (Carrière) J. Houz.] · Evolution · Genome

Introduction

Transposable elements (TEs) are DNA fragments that can move to different positions within the genome of a single cell. Two broad classes of TEs are recognized based on their mechanism of transposition. Retroelements (class I) utilize an RNA intermediate and thus require reverse transcriptase to produce a DNA copy as well as an integrase for insertion into the host genome, whereas DNA transposons (class II) move directly as DNA and require a transposase or replicase to catalyze the necessary DNA cutting and joining reactions (Feschotte et al. 2002).

Retroelements are ubiquitous in eukaryotes, both in animals and plants (Bennetzen 2000; Eickbush and Jamburuthugoda 2008), and are divided into four main groups: long terminal repeat retroelements (LTR-retroelements), tyrosine recombinase (YR) retroelements, non-LTR-retroelements, and Penelope-like retroelements (Llorens et al. 2009). LTR-retroelements mainly encode two genes (*gag* and *pol*). The *gag* encodes structural proteins that form the virus-like particle (VLP), whereas *pol* encodes enzymatic regions such as protease (PR), reverse transcriptase (RT), RNase H (RH), and integrase (INT). LTR-retroelements are further subdivided into *Ty1-copia*, *Ty3-gypsy*, and *Bel/Pao* superfamilies based on their structure and transposition mechanism (Llorens et al. 2009). *Ty1-copia*

Communicated by C. Chen

Electronic supplementary material The online version of this article (doi:10.1007/s11295-017-1114-3) contains supplementary material, which is available to authorized users.

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and *Ty3-gypsy* retroelements are particularly widely distributed in the plant kingdom (Eickbush and Jamburuthugoda 2008). The former is mainly split into four lineages, which include *Sire*, *Oryco*, *Retrofit*, and *Tork* in plants, and the latter into five lineages, which consist of *Crm*, *Del*, *Reina*, *Athila*, and *Tat* in plants (Llorens et al. 2011). *Sire* elements have an envelope (ENV) domain downstream of the RH domain and are thus considered potential retroviruses (Havecker et al. 2004, 2005). *Sire* elements are widespread among plant species, both in monocots (rice, maize, and sorghum) and dicots (*Arabidopsis*, lotus, medicago, and citrus) (Havecker et al. 2005). *Oryco* (*Ivana*) elements are found in the genome of various plant species such as *Vitis vinifera*, *Arabidopsis thaliana*, *Popolus tricocarpa*, and *Oryza sativa* (Llorens et al. 2009). No ENV domains occur in *Oryco* elements. *Retrofit* lineages, also named *Ale*, together with *Tork* lineages, belong to the *Ty1-copia* superfamily and have been described in the genomes of different plant species, including *Zea mays*, *Solanum lycopersicum*, *V. vinifera*, *Nicotiana tabacum*, and *Vigna radiata* (Llorens et al. 2009). *Crm* elements are plant centromere-specific *Ty3-Gypsy* retroelements (Gorinsek et al. 2004). The *Del* lineage was previously known as the Tekay lineage (Gorinsek et al. 2004) and constitutes a plant *Ty3-Gypsy* superfamily. All *Del* elements encode for the CHR domain. Similar to *Del* elements, *Reina* elements also have a CHR domain that is located proximal to the INT domain (Gorinsek et al. 2004). *Athila* and *Tat* are two ancient lineages (Wright and Voytas 2002): the former encode for an additional putative ENV domain and are considered as potential retroviruses, whereas the latter are considered as giant LTR-retroelements.

LTR-retroelement amplification, along with polyploidization, is largely responsible for genome expansion (Bennetzen et al. 2005). For example, there are more than 26, 55, and 78% of LTR-retroelements in rice (International Rice Genome Sequencing Project 2005), maize (Schnable et al. 2009), and Sorghum (Paterson et al. 2009) genome, respectively. The aggressive proliferation of three families of LTR-retroelements resulted in the doubling of the genome size of *Oryza australiensis*, a wild relative of rice within the last 3 million years (Piegu et al. 2006). On the other hand,LTR-retroelements often undergo severe deletions or fragmentation via unequal homologous recombination and illegitimate recombination (Devos et al. 2002; Ma et al. 2004), which are two major mechanisms that counteract genome expansion (Bennetzen et al. 2005; Wicker and Keller 2007). Elimination through recombination frequently occurs in small genomes such as rice and *Arabidopsis*, but not in larger genomes such as that in maize, barley, and wheat (Bennetzen et al. 2005; Wicker and Keller 2007). In addition to their impact on genome size variations, LTR-retroelements also regulate the expression of adjacent genes in their host genomes as well as encode small RNAs that regulate specific target genes at the transcriptional and post-transcriptional levels (Kashkush et al. 2003; Kashkush and Khasdan 2007).

Moso bamboo [*Phyllostachys edulis* (Carrière) J. Houz.] is a large woody bamboo that has ecological, economic, and cultural value in Asia and accounts for ~70% of the total bamboo growth area (Fu 2001). The moso bamboo genome contains 24 pairs of chromosomes (2n = 48), with an approximate size of 2.075 Gb, and is diploid (Peng et al. 2013). The published high-quality draft genome sequence of moso bamboo covers 95% of the genomic region, and a total of 31,987 genes have been characterized by gene prediction modeling along with cDNA and deep RNA sequencing data. There are a few reports on TEs in moso bamboo sequences (Gui et al. 2010; Peng et al. 2013), but no detailed study on moso bamboo LTR-retroelements has been conducted to date. In the present study, LTR-retroelements in the moso bamboo genome (BambooGDB, http://www.bamboogdb.org/index.jsp, Zhao et al. 2014) were characterized. We systematically examined the structure, genomic distribution, phylogenetic diversity, and expansion pattern of moso bamboo LTR-retroelements.

Materials and methods

Identification and classification of LTR-retroelements

Genomic sequences and gene annotation information on moso bamboo were downloaded from the bamboo genome database (BambooGDB, http://www.bamboogdb.org/index.jsp, Zhao et al. 2014). A combination of structural analysis and sequence homology comparisons were performed to identify LTR-retroelements in the assembled moso bamboo genome. Initially, the LTR_STRUC program was employed for the identification of LTR-retroelements (McCarthy and McDonald 2003). All of the identified LTR-retroelements were confirmed by manual search against RepBase (version 21.02) and GenBank. The sequences that were identified as LTR-retroelements in the two databases were used in subsequent analyses. The LTR-retroelements were classified into *Ty1-Copia* (INT-RT-RH) and *Ty3-Gypsy* (RT-RH-INT) superfamilies (Llorens et al. 2011). Both superfamilies were further classified into four lineages (*Sire*, *Oryco*, *Retrofit*, and *Tork*) for the *Ty1-Copia* superfamily and five lineages (*CRM*, *Del*, *Reina*, *Athila*, and *Tat*) for the *Ty3-Gypsy* superfamily using BLASTX, with an e-value of <10 by submitting the identified LTR-retroelements against the cores in the Gypsy Database (GyDB) (Llorens et al. 2011).

The identified bamboo LTR-retroelements were assigned to families within the enumerated lineages on the basis of 80% identity, over at least 80 bp in at least 80% of their sequence lengths (80–80–80 rule, Wicker et al. 2007). Each

LTR-retroelement family identified in the present was designated as PhXYZ#, where Ph are the two letters representing Moso bamboo (*P. edulis* (Carrière) J. Houz.), XYZ are the first three letters of the name of LTR-retroelement lineage, and # is the number representing the family.

To mine all the remnant LTR-retroelement sequences, the representative retroelements of families were used as the query sequence to scan the genome sequence by using RepeatMasker with Cross_Match as search engine and a cut-off of >250 (http://repeatmasker.org). The unmatched results were filtered out based on the criteria of a nucleotide identity rate of <80% and a query coverage of <80%. The workflow of identification of bamboo LTR-retroelements is summarized in Supplementary File S1.

Analysis of the structure of bamboo LTR-retroelements

The coding domains of full-length bamboo LTR- retroelements were identified using Pfam and confirmed by alignment with MUSCLE (Edgar 2004) against the domain alignment from GyDb (Llorens et al. 2011). Full-length sequences that contained the GAG, PR, RT, RH, and INT domains were designated as intact bamboo LTR-retroelements. The intact bamboo LTR-retroelements were aligned and analyzed using BioEdit (Hall 1999), and all the coding domains as well as the LTRs and target side duplications (TSDs) could been aligned. The lengths of all domains as well as the LTRs and spacer regions of each element were recorded in an Excel table, and the schematic representation of bamboo LTR-retroelement structures were drawn in Excel.

Estimation of insertion time

Using an approach described by Ma and Bennetzen (2004), the age of the elements with two available LTR sequences were determined by comparing their 5′ and 3′ LTRs. Two LTRs were aligned using the MUSCLE program (Edgar 2004). When necessary, the alignments were manually inspected and corrected using the MEGA6 program (Tamura et al. 2013). The distance (K) between two LTRs was calculated by using the Jukes–Cantor method (Kimura and Ota 1972). An average substitution rate (r) of 1.3×10^{-8} substitutions per synonymous site per year (Ma and Jackson 2006) was used in the calculations. The time (T) since initial insertion was estimated using the formula $T = k/2r$.

Phylogenetic analysis

To assess the evolutionary pattern of moso bamboo LTR-retroelements, representative plant elements of four lineages of *Ty1-Copia* (*Sire*, *Oryco*, *Retrofit*, and *Tork*) and five lineages of *Ty3-Gypsy* (*Crm*, *Del*, *Reina*, *Athila*, and *Tat*) were downloaded from the Gypsy Database (GyDB) (http://www.gydb.org/index.php/Main_Page, Llorens et al. 2011). We also downloaded all full-length sequences of *Ty1-Copia* and Ty3-*gypsy* retroelements of *A. thaliana* and *O. sativa* from the *Arabidopsis* Information Resource (TAIR) (https://www.arabidopsis.org/index.jsp) and Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/home_contacts.shtml), respectively. The *A. thaliana* and *O. sativa* LTR-retroelements were assigned to families by using the 80–80–80 rule (Wicker et al. 2007). The representative intact elements were selected from every family to construct the LTR-retroelement phylogenetic trees. The moso bamboo representative intact elements from each family were also selected for phylogenetic analysis.

All RT protein domain sequences were aligned using MUSCLE with default settings (Edgar 2004), and rare distinct shifts were manually adjusted. The optimal model of amino acid substitution was estimated using MEGA6 (Tamura et al. 2013) with default settings. Bootstrap neighbor-joining trees were built using the Kimura two-parameter method that was integrated in the MEGA6 program (Tamura et al. 2013).

Results

Characterization of LTR-retroelements in the moso bamboo genome

Using the LTR_STRUC program, we mined 1311 LTR-retroelement candidates in the moso bamboo genome. The 1311 LTR-retroelements were confirmed by manual search against the RepBase version 21.02 (Jurka et al. 2005) and GenBank database. The 1259 of the 1311 sequences were confirmed. Then, by using all-vs-all blastn, 982 LTR-retroelement families were classified based on the 80–80–80 rule (Wicker et al. 2007). Among the 982 LTR-retroelement families, 458 families belonged to the *Ty1-copia* superfamilies, of which 48 families were classified as *Tork* lineages, 249 families as *Retrofit* lineages, 60 families as *Sire* lineages, and 101 families as *Oryco* lineages. There were 501 families that belonged to *Ty3-gypsy* superfamilies, of which 63 families were determined to be *Del* lineages, 181 families belonged to *Reina* lineages, 71 families belonged to *Crm* lineages, and 186 families belonged to *Tat* lineages. Additionally, there were 23 families that were not homologous to the known LTR-retroelement family and were thus designated as Unknown-LTR. The general features of the representative sequence of each moso bamboo LTR-retroelement family such as element size, LTR size, and TSDs are presented in Supplementary Table S2. To retrieve partial or intact LTR-retroelements, the 982 representative sequences were used as reference queries to search the moso bamboo genome using RepeatMasker. A total of 2,004,644 LTR-retroelement sequences were detected in the moso bamboo genome, which accounted for more than 40% of the entire genome (Table 1).

Of the 2,004,644 LTR-retroelement sequences, 829,598 elements belonged to the *Ty1-copia* superfamily (up to 337,053,290 bp) and 1,125,018 elements were classified under the *Ty3-gypsy* superfamily (up to 480,215,478 bp), thereby accounting for approximately 16.43 and 23.41% of the moso bamboo genome, respectively. Among four lineages of the *Ty1-copia* superfamily, the content of the *Sire* lineage was highest (up to 6.11%) and closely followed by the *Retrofit* lineage (5.29%). Previous reports also showed that the *Sire* and *Retrofit* lineages were widely distributed in monocot plants such as *O. sativa* and *Z. mays* (Havecker et al. 2005; Llorens et al. 2009). Among the four lineages of the *Ty3-gypsy* superfamily, the content of the *Tat* lineage was highest (up to 9.67%) and closely followed by the *Del* lineage (9.53%) (Table 1). The *Tat* and *Del* lineages are plant-specific and are ubiquitous in high plant genomes (Havecker et al. 2004; Llorens et al. 2009).

The ratio of the number of *Ty3-gypsy* elements to the number of *Ty1-copia* elements in the moso bamboo was 0.8:1, which was lower than that in maize (1.6:1) (Baucom et al. 2009; Schnable et al. 2009) and much lower than that in rice (4.9:1) (International Rice Genome Sequencing Project 2005; Tian et al. 2009) and sorghum (3.7:1) (Paterson et al. 2009), but considerably higher than that in the eudicot plant, *Medicago* (0.3:1) (Wang and Liu 2008).

The 2,004,644 LTR-retroelement sequences could be classified into three types, including full-length elements, solo LTRs, and truncated elements. The ratio of the number of solo LTRs to the number of full-length elements (S/I) in moso bamboo is approximately 0.28:1 (Table 2). Among the eight lineages, the highest S/I ratio was observed in the *Retrofit* lineage (0.55), whereas the lowest S/I ratio was detected in the *Reina* lineage (0.034) (Table 2).

Structural features of LTR-retroelements in moso bamboo

We identified all the internal coding domains (GAG, PR, RT, INT, RH, ENV, and CHR) of the representative full-length sequences of LTR-retroelements. The ENV domain was identified in all *sire* elements and the CHR domain in both the *Del* and *Reina* elements (Table 1). There were 124 sequences of 458 representative *Ty1-copia* retroelement sequences and 286 sequences of 501 representative *Ty3-Gypsy* retroelement sequences that contain all domains (GAG, PR, RT, RH, INT, /ENV, /CHR). The 410 intact retroelement sequences were further analyzed in terms of structural features.

There was a general pattern in the overall size of the elements from various families. In both superfamilies, two families were determined to be very long (*Sire* and *Del*, 7.2–17.5 kb) and two families were relatively short (*Oryco* and *Reina*, 4.7–8.9 kb) (Fig. 1a, b). Differences in the total length were mainly due to variations in LTR size and the presence and size of spacer regions between the internal coding domains, rather than the gag/pol coding regions (Fig. 1a, b).

The proliferation spectrum of LTR-retroelements in moso bamboo

Although the eight evolutionary lineages were identified in moso bamboo, the proliferation spectrum of LTR-retroelements was highly variable among lineages. The number of families within lineages and the copy numbers of intact and solo elements within lineages identified in the species are listed in Table 2 (truncated elements were not considered here). Among the four *Ty1-copia*

Table 1 The distribution of LTR-retroelements in the moso bamboo genome

Superfamily	Lineage	Family[a]	Structure	Number	Total length (bp)	Percentage of sequences (100%)[b]
Ty1-copia	*Tork*	48	Gag-PR-INT-RT-RH	132,836	57,946,850	2.82
	Retrofit	249	Gag-PR-INT-RT-RH	330,726	108,542,148	5.29
	Sire	60	Gag-PR-INT-RT-RH-env	235,066	125,428,928	6.11
	Oryco	101	Gag-PR-INT-RT-RH	130,970	45,135,364	2.20
	Total	458		829,598	337,053,290	16.43
Ty3-gypsy	*Del*	63	Gag-PR-RT-RH-INT-CHR	443,394	195,466,907	9.53
	Reina	181	Gag-PR-RT-RH-INT-CHR	117,128	36,722,443	1.79
	Crm	71	Gag-PR-RT-RH-INT	158,181	49,677,331	2.42
	Tat	186	Gag-PR-RT-RH-INT	406,315	198,348,797	9.67
	Total	501		1,125,018	480,215,478	23.41
Unknown		23		50,028	10,618,889	0.52
Sum		982		2,004,644	827,887,657	40.35

[a] The number of families within the lineage

[b] The content in percentage of LTR retro-elements in the moso bamboo genome (2,051,719,643 bp)

Table 2 Number of families in each lineage and number of full-length elements and solo elements in each lineage

Lineages	Family[a]	Ratio (%)[b]	Full-length elements	Solo LTR	Solo LTR/full-length elements[c]	Full-length elements + solo LTR	Ratio (%)[d]
Tork	48	10.48	403	72	0.18	475	14.43
Retrofit	249	54.37	399	222	0.56	621	18.86
Sire	60	13.10	1022	273	0.27	1295	39.34
Oryco	101	22.05	769	132	0.17	901	27.37
Ty1-copia	458	100.00	2593	699	0.27	3292	100.00
Del	63	12.57	708	333	0.47	1041	23.97
Reina	181	36.13	899	31	0.03	930	21.41
Crm	71	14.17	1219	289	0.24	1508	34.72
Tat	186	37.13	725	139	0.19	864	19.89
Ty3-copia	501	100.00	3551	792	0.22	4343	100.00
Unknown	23	100.00	3	207	69.00	210	100.00
Total	982	100.00	6147	1698	0.28	7845	100.00

[a] Numbers of families

[b] The ratio of numbers of families of every lineage to numbers of families in the corresponding superfamily

[c] The ratio of numbers of solo LTR to numbers of full-length elements in every lineage

[d] The ratio of numbers of full-length elements and solo LTR of every lineage to numbers of full-length elements and solo LTR in the corresponding superfamily

lineages in moso bamboo, *Retrofit* has the largest number of LTR-retroelement families (249), accounting for 54.37% of the *Ty1-copia* families (458) analyzed. However, the 249 families only contained 18.86% (621) of the 3292 *Ty1-copia* elements. In contrast, *Sire* consisted of the highest number of *Ty1-copia* elements (1295, 39.34%); however, these elements belonged to only 60 families. More dramatic differences were observed among the four *Ty3-gypsy* lineages in moso bamboo. For example, the *Tat* lineage contained 186 (37.13%) of the 501 *Ty3-gypsy* families analyzed, but the lineage comprised only 864 elements (19.89% of the 4343 *Ty3-gypsy* elements). The *Crm* lineage showed the highest number of elements, accounting for 34.72% of the *Ty3-gypsy* elements that were identified in the moso bamboo genome. The number of elements of LTR-retroelements within individual lineages reflects their recent amplification, whereas the number of families within individual lineages represents ancient divergent activities. Hence, the above observations suggest that different lineages and families of LTR-retroelements have distinct activities for division and expansion over evolutionary time.

Estimation of insertion date

We estimated the insertion time of 7845 intact and solo LTR-retroelements in moso bamboo. Figure 2 shows that most of the elements (91%) were amplified around 0~3 Mya. The distribution of insertion times of LTR-retroelements in every lineage was represented by a single-peak parabolic curve. These results indicated that the eight lineages were active and amplified within the same evolutionary timeframes.

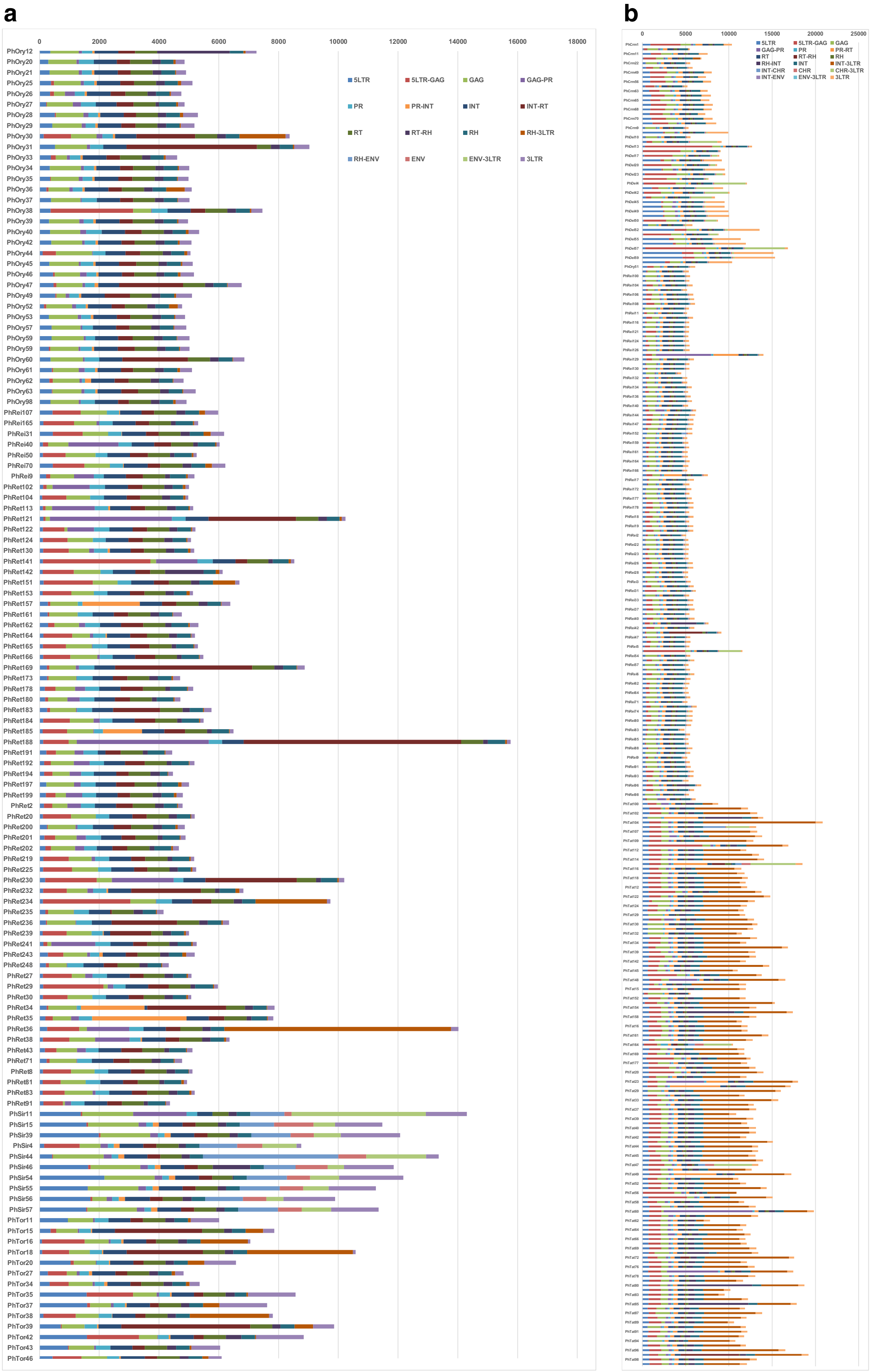
Evolutionary dynamics of moso bamboo LTR-retroelements

To understand the evolutionary dynamics, history, and fates of bamboo LTR-retroelements in moso bamboo over evolutionary time, we used the relatively conserved RT domains from individual elements for phylogenetic analysis. Of the 982 families identified in the moso bamboo genome, 124 *Ty1-copia* and 286 *Ty3-gypsy* family representative sequences that consisted of a complete RT domain were used to construct phylogenetic trees. The other family representative sequences contained deletions of the RT domains.

For the *Ty1-copia* phylogenetic tree, we grouped 94 *Ty1-copia* retroelements in *A. thaliana*, 85 retroelements in *O. sativa*, 124 retroelements in moso bamboo, and 23 other plant typical *Ty1-copia* elements into four distinct lineages, namely, *Sire*, *Oryco*, *Retrofit*, and *Tork* (Fig. 3a). All families were shared by these three species, which showed that these four lineages all existed before the divergence of eudicots and monocots (Fig. 3a).

For the *Ty3-gypsy* phylogenetic tree, there were 59 *Ty3-gypsy* retroelements in *A. thaliana*, 108 retroelements in *O. sativa*, 286 retroelements in moso bamboo, and 25 other plant typical *Ty3-gypsy* elements, which grouped into five lineages, including *Crm*, *Del*, *Reina*, *Athila*, and *Tat* (Fig. 3b). These five lineages all existed before the divergence of eudicots and monocots, but the *Athila* family was not identified in the moso bamboo (Fig. 3b).

There were two distinctive phenomena in two phylogenetic trees. One is that the bamboo LTR-retroelements and rice LTR-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2000
4000
6000
8000
10000
12000
14000
16000
18000
5LTR
5LTR-GAG
GAG
GAG-PR
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INT-RT
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5LTR
5LTR-GAG
GAG
GAG-PR
PR
PR-RT
RT
RT-RH
RH
RH-INT
INT
INT-3LTR
INT-CHR
CHR
CHR-3LTR
INT-ENV
ENV-3LTR
3LTR

◀ **Fig. 1** The scales and keys for the domains represented in the schematic representations of *Ty1-copia* (**a**) and *Ty3-gypsy* (**b**) are shown. Abbreviation for domains: *LTR* long terminal repeat; *gag* gag; *PR* protease; *RT* reverse transcriptase; *RH* ribonuclease H; *INT* integrase; *ENV* envelope; *CHR* chromodomain

retroelements frequently clustered together, whereas *Arabidopsis* LTR-retroelements clustered together themselves. The other is that there was an abundance of very identical LTR-retroelement sequences among the three species (e.g., *Oscopia44*, *PhOry93*, and *Atcopia205* with the similarity of more than 95% each other) and the presence of relative diverse LTR-retroelement sequences in individual species (e.g., *PhRet191*, *PhSir30*, and *PhTor40* with the similarity of less than 75% each other).

Discussion

LTR-retroelements predominate in the moso bamboo genome

Most genome projects identify and annotate TEs using homology and de novo methods (Hoen et al. 2015), but the most accurate assessment of TE landscapes is currently only possible through a combination of de novo and homology-based repeat identification in conjunction with an additional manual curation step (Platt et al. 2016). One reason for this is that longer elements are often recovered as multiple smaller fragments by both types of searches. Peng et al. (2013) annotated moso bamboo LTR-retroelements with LTRharvest and LTR_FINDER soft. In the study, a combination of structure-based and homology-based approaches was also employed to identify LTR-retroelements in the moso bamboo genome sequence. Initially, the representative sequences of LTR-retroelements with intact LTR sequences were identified by the structure-based approach, LTR_STRUC program (McCarthy and McDonald 2003). After filtering out the false-positive sequences, the rest of the sequences were characterized for LTR, TSD sequences, and GAG, PR, RT, RH, and INT domains. Then, the complete RT domains of individual elements from moso bamboo, *A. thaliana*, and *O. sativa* were used to construct phylogenetic trees to display the evolutionary dynamics of LTR-retroelements. After that, the identified moso bamboo LTR-retroelements were classed into individual superfamilies, lineages, and families of LTR-retroelements. To display the proliferation spectrum of bamboo LTR-retroelements, the full-length elements, solo LTRs, and truncated elements of each superfamily, lineage, and family of LTR-retroelements in the moso bamboo genome were identified using RepeatMasker. This is the first study to perform a systematical survey of the structure, genomic distribution, phylogenetic diversity, and expansion pattern of moso bamboo LTR-retroelements Supplementary File 1).

By using a combination of structure-based and homology-based approaches, a total of 2,004,644 LTR-retroelement sequences were identifiNed in the moso bamboo genome. The content of LTR-retroelement sequences in moso bamboo was up to 40.35%, which is slightly higher than that reported by Peng et al. (37.3%, 2013). The content of all LTR-retroelements in the moso bamboo genome is far lower than that in *Z. mays* (79%) (Schnable et al. 2009) and *S. bicolor* (55%, Paterson et al. 2009) and significantly higher than that in *O. sativa* (26%, International Rice Genome Sequencing Project 2005).

Some LTR-retroelements might be missed due to the incompleteness of the moso bamboo genome. The moso bamboo genome assembly is highly fragmented (>9000 scaffolds). Although we combined the structure-based and homology-based approaches in the identification of LTR-retroelements, the fragmentation of the moso bamboo genome could still cause an underestimation of LTR-retroelement contents. In addition to full-length elements and solo LTRs, massive truncated elements were detected. These truncated elements might be due to the difficulty in assembling full-length TEs, which might have resulted in the inclusion of a high number of truncated elements in the original assembly. Therefore, the 40.35% content of LTR-retroelement sequences identified in this study may be a conservative figure and more LTR-retroelements may be detected in the future with the availability of a higher-quality moso bamboo genome.

Eight evolutionary lineages were identified in moso bamboo (*Sire*, *Oryco*, *Retrofit*, *Tork* of *Ty1-copia* and *Crm*, *Del*, *Reina*, *Tat* of *Ty3-gypsy*). Although the Athila family is widespread in the plant kingdom and also occurs in monocot plants such as sorghum and rice (Steinbauerova et al. 2011), it was not identified in the moso bamboo genome. One possible reason is that *Athila* elements are not suitable for identification using the LTR_STRUC program. The *Athila* elements are usually 8.5–12 kb in size and have relatively long LTR sequences (1.5–2.5 kb, Wright and Voytas 2002), which might be difficult to be recognized by the LTR_STRUC program. Other software with more adjustable parameters may be employed for the identification of Athila elements. On the other hand, Athila elements might be missed due to the incompleteness and fragmentation of the moso bamboo genome. Additionally, there are 23 families and more than 50,000 sequences (0.52%) which were homologous to unknown LTR-retroelements of other species and were named Unknown-LTR. Unknown-LTR sequences in moso bamboo are less than those in maize (181 families, 31.31%) (Schnable et al. 2009).

Among the eight evolutionary lineages, the scales and timeframes of activity for proliferation of LTR-retroelements vary tremendously among lineages. A

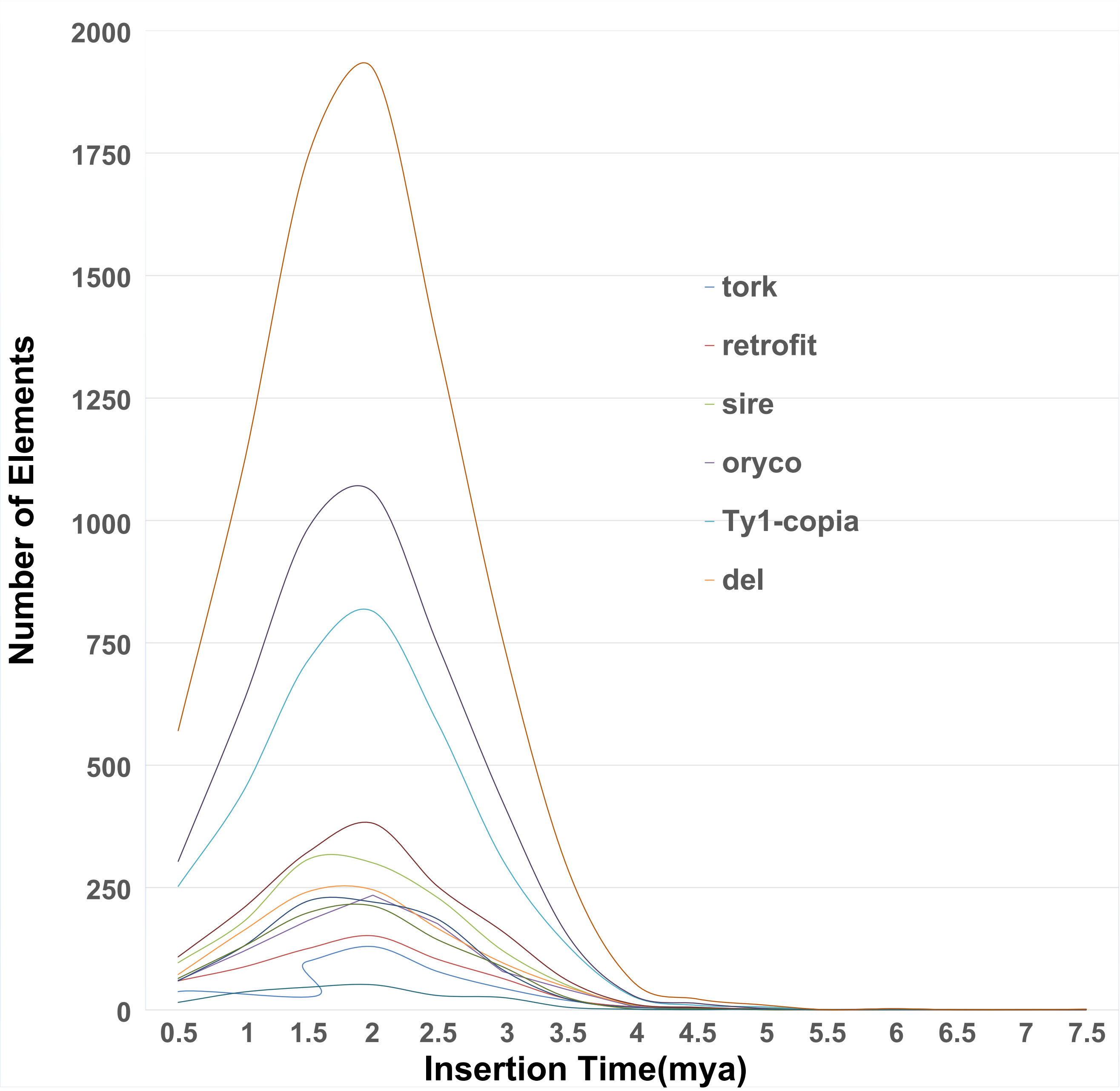

Fig. 2 Distribution pattern of insertion dates of bamboo LTR-retroelements (*Mya*, million years ago)

similar scenario was seen in rice and soybean (Du et al. 2010). There are three lineages (i.e., *Ivana*/*Oryco*, *Ale*/*Retrofit*, and *Reina*) that contain the highest number of families of LTR-retroelements in rice and soybean (Du et al. 2010). In contrast, there are similar three lineages (i.e., *Retrofit*, *Reina*, and *Tat*) that contain the highest number of families of LTR-retroelements in moso bamboo (Table 2). *Arabidopsis* has significantly fewer families in the nine lineages than rice, soybean, and moso bamboo due to overall low activities for LTR-retroelement expansion (Du et al. 2010). The results showed that the moso bamboo genome has the consistently high expansion activities of LTR-retroelements over evolutionary time, which are likely responsible for the current large size of the moso bamboo genome (Du et al. 2010).

Moso bamboo LTR-retroelements might undergo a relative low frequency of unequal recombination and illegitimate recombination

The unequal recombination and illegitimate recombination is thought to be a major process for formation of solo LTRs in plants (Devos et al. 2002; Ma et al. 2004). Our data showed that the ratio of the number of solo LTRs to the number of intact elements (S/I) in moso bamboo is approximately 0.28:1. This estimate is significantly lower than reported in rice (1.62:1) (Tian et al. 2009). These findings thus indicate that the moso bamboo genome might undergo unequal recombination and illegitimate recombination at a relatively low frequency, which is one of the main mechanisms for confrontation of LTR-retroelement expansion activities (Devos et al. 2002; Ma et al. 2004).

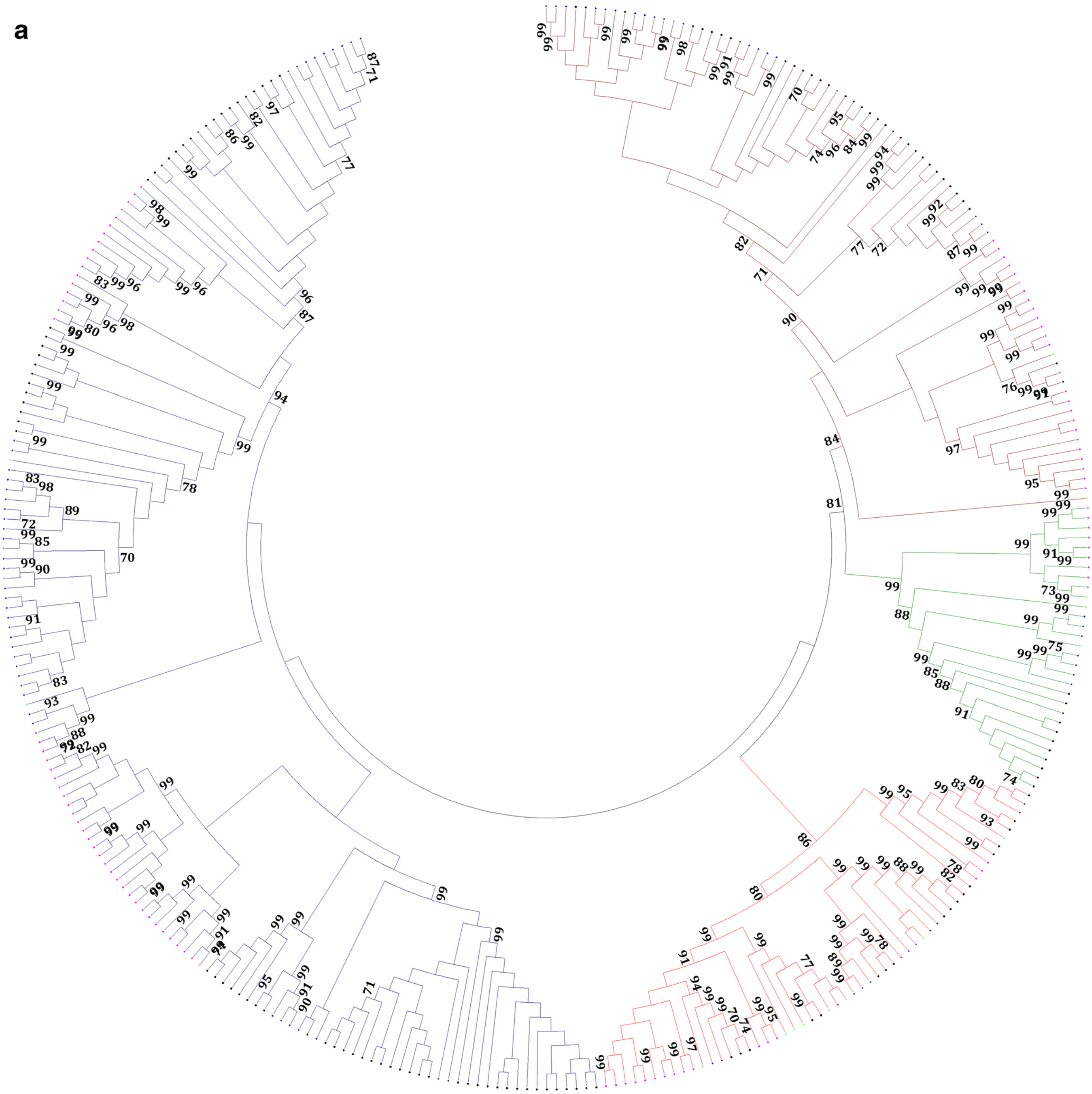

Fig. 3 Phylogenetic relationship of *Ty1-copia* (**a**) and *Ty3-gypsy* elements (**b**) identified in moso bamboo, rice, and *Arabidopsis*. The RT amino acid sequences of individual elements were used to construct the phylogenetic trees. Robustness of the nodes was estimated by 500 bootstrap replications. Individual LTR-retroelements in moso bamboo, rice, and *Arabidopsis* are indicated by *solid black dots*, *blue solid diamonds*, and *pink solid squares*, respectively. **a** The plant typical *Ty1-copia* elements derived from four lineages are represented by *green hollow triangles*. Four lineages, including *Oryco*, *Sire*, *Tork*, and *Retrofit*, were marked by *brown*, *green*, *red*, and *purple branches*, respectively. **b** The plant typical *Ty3-gypsy* elements derived from five lineages are represented by *green hollow triangles*. Five lineages, including *Reina*, *Crm*, *Del*, *Athila*, and *Tat*, are marked by *brown*, *dark green*, *red*, *light green*, and *blue branches*, respectively

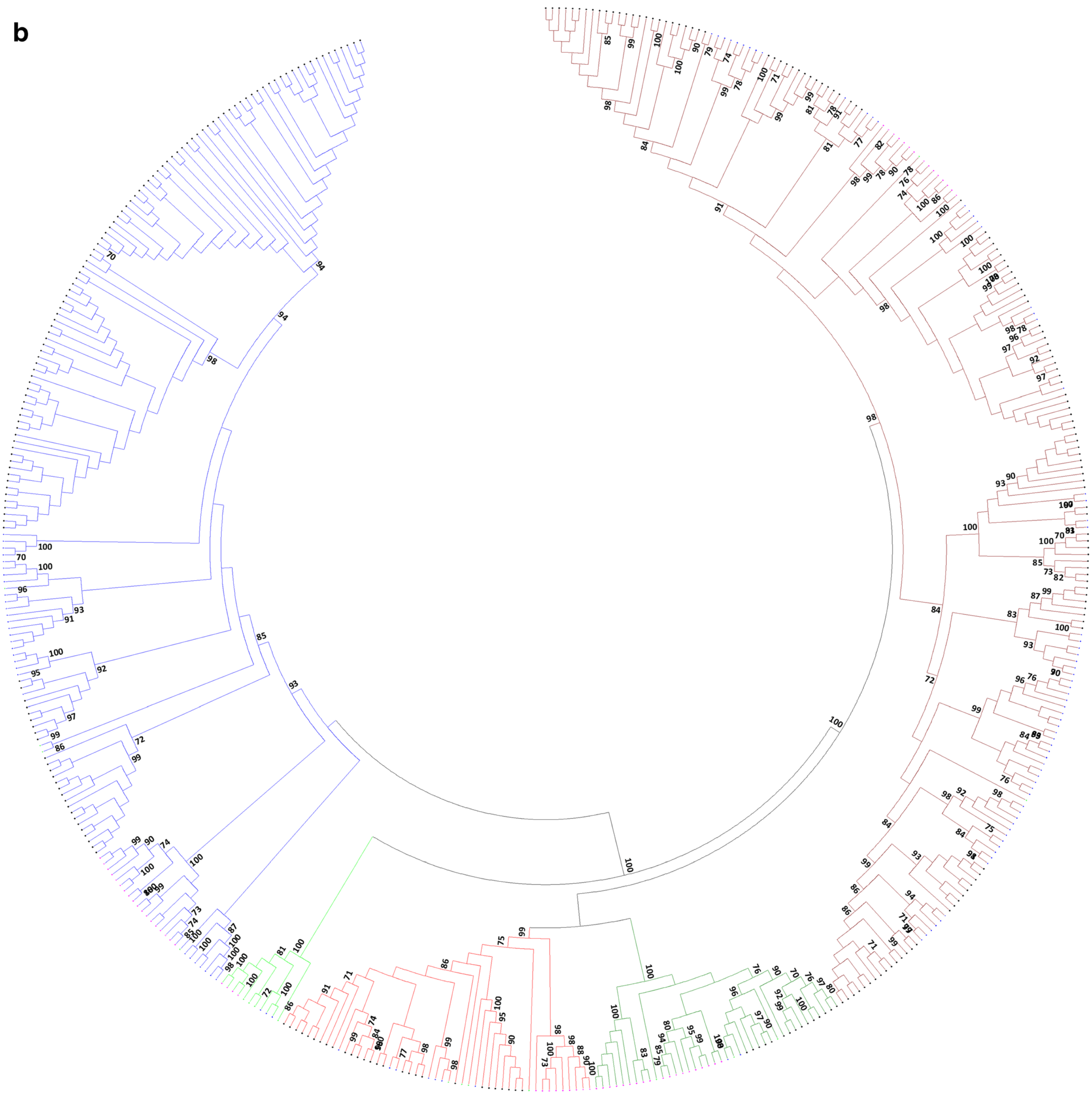

Fig. 3 (Continued)

LTR-retroelements remain relative long existence in moso bamboo genome

We estimated the insertion times of bamboo LTR-retroelements. The largest number of bamboo LTR-retroelements was estimated to be inserted within 0~3 Mya (Fig. 2). The results showed that there might be several bamboo LTR-retroelements that recently transposed.

Additionally, the distribution of insertion times of bamboo LTR-retroelements was represented by a single-peak parabolic curve with a relatively long time span, which was similar to that of barley and wheat (Wicker and Keller 2007). The pattern of insertion times of rice and soybean LTR-retroelements was exponentially distributed (Wicker and Keller 2007; Du et al. 2010). Although the available data set was extremely small to draw definite conclusions, the observed distribution curve of LTR-retroelement insertions may reflect its very long existence in the moso genome (Wicker and Keller 2007), which might be due to the low frequency of unequal recombination and illegitimate recombination for bamboo LTR-retroelements (above discussion).

Bamboo LTR-retroelements are structurally diverse and have evolutionary lineages that are shared between monocots and eudicots

Previous surveys on LTR-retroelements in plants have defined four major common evolutionary *Ty1-copia* lineages and five major common evolutionary *Ty3-gypsy* lineages (Llorens et al. 2011). Four lineages (i.e., *Sire*, *Oryco*, *Retrofit*, and *Tork*) of *Ty1-copia* and four lineages of *Ty3-gypsy* (i.e., *Crm*, *Del*, *Reina*, and *Tat*) were identified in the moso bamboo genome, all of which originated prior to the divergence of monocots and eudicots (Llorens et al. 2009).

Among most families, the bamboo LTR-retroelements and rice LTR-retroelements were frequently clustered together, whereas the *Arabidopsis* LTR-retroelements clustered together with themselves, which indicated that most lineages have diverged after the division between the monocots and eudicots (Fig. 3). However, the *Tork* lineage is an exception. LTR-retroelements of the *Tork* lineage from three species clustered together without discrimination, which indicated that the evolution of the *Tork* lineage was relatively more conserved compared with other lineages (Llorens et al. 2009).

Comparative genome analysis suggested that the moso bamboo diverged from Oryzoideae more than 48 Mya (Peng et al. 2013). Interestingly, there was an abundance of near identical LTR-retroelement sequences between rice and moso bamboo and the presence of relatively diverse LTR-retroelement sequences in individual rice and moso bamboo species (Fig. 3). This might be due to divergent evolutionary rate, stochastic loss, vertical extinction, or horizontal transfer of LTR-retroelements (Du et al. 2010).

Conclusions

Considering the high proportion of LTR-retroelements in the moso bamboo genome, our work contributes to a greater understanding of their impact on genome organization and evolution. We initially investigated 982 LTR-retroelement families comprising 2,004,644 LTR-retroelement sequences, which accounted for more than 40% of the moso bamboo genome. Further analysis revealed that bamboo LTR-retroelements had longer period of activity than those of rice and *Arabidopsis* and might undergo a relatively low frequency of unequal recombination and illegitimate recombination. The knowledge gained from this study constitutes a valuable resource for both the improvement in genome annotation and investigations of the contributions of LTR-retroelements to moso bamboo genome evolution.

Acknowledgements We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Authors' contribution M. B. Zhou—designation of the experiments, identification and classification of LTR-retroelement, and writing of paper; B. J. Hu, Y. H. Zhu—estimation of LTR-retroelement insertion date; all authors read and approved the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Funding Funding the Talents Program of the Natural Science Foundation of Zhejiang Province (Grant No. LR12C16001), and The National Natural Science Foundation of China (Grant Nos. 31470615 and 31270645) supported this study.

Data Archiving Statement Genomic sequences and gene annotation information of moso bamboo presented in this report are available in the bamboo genome database (BambooGDB, http://www.bamboogdb.org/index.jsp). The plant representative elements of four lineages of *Ty1-Copia* (*Sire*, *Oryco*, *Retrofit*, and *Tork*) and five lineages of *Ty3-Gypsy* (*Crm*, *Del*, *Reina*, *Athila*, and *Tat*) are available in the Gypsy Database (GyDB) (http://www.gydb.org/index.php/Main_Page). All full-length sequences of *Ty1-Copia* and *Ty3-gypsy* retroelements of *A. thaliana* and *O. sativa* are available in the *Arabidopsis* Information Resource (TAIR) (https://www.arabidopsis.org/index.jsp) and Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/home_contacts.shtml), respectively.

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