

RepeatExplorer

**Classification of repetitive elements based on the analyzes of
protein domains**

**Pavel Neumann
May 2015**

Classification of TEs based on protein domain search

- Repetitive elements are highly diverse – their classification based on DNA sequences requires comprehensive databases which are available for a limited number of species
- Although structural features (LTR, TIR, poly-A etc.) of repetitive sequences are suitable for classification into major repeat types (e.g. LTR retrotransposons, DNA transposons, LINEs), they are usually difficult to recognize in sequence clusters produced by RepeatExplorer
- Coding regions of transposable elements make up a large part of whole elements and encode for proteins which are relatively conserved among TEs of the same type/lineage. Therefore, they are ideal for unambiguous assignment of analyzed sequences to individual types

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- Coding regions of transposable elements make up a large part of whole elements and encode for proteins which are relatively conserved among TEs of the same type/lineage. Therefore, they are ideal for unambiguous assignment of analyzed sequences to individual types
- **but comprehensive database of protein domains of TE does not exist**

Protein domain search: our database

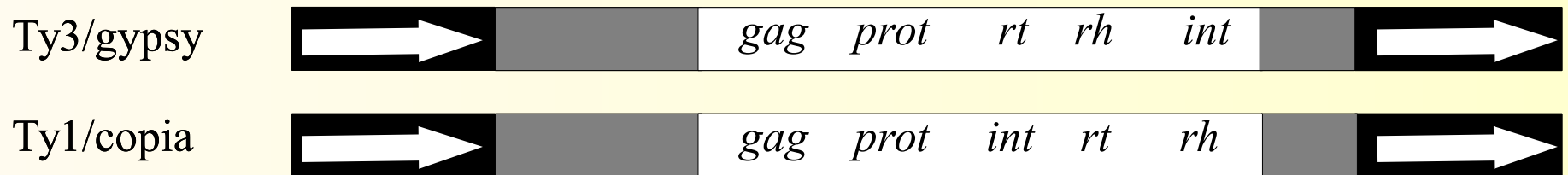
- Protein sequences are more conserved than DNA
- **2942 domains from a total of 1045 elements**
- 398 LTR retrotransposon elements (255 Ty1/copia and 143 Ty3/gypsy)
 - 386 GAG, 394 PROT, 378 RT, 383 RNaseH, 391 INT, 31 ChDII, and 45 CHDCR domains (totally 2317domains)
- 15 pararetroviruses
 - 15 PROT, 15 RT, and 15 RNaseH domains (totally 45 domains)
- 228 nonLTR retrotransposon elements
 - 213 RT, 51 RNaseH, and 190 ENDO domains (totally 454 domains)
- 404 DNA transposons
 - totally 435domains extracted from the following transposon families: DTH (Harbinger), DTM (Mutator/MuDR), DTC (EnSpm/CACTA), DTN (Novosib), DTA (hAT/AC), DTT (Mariner), DHH (Helitron)

Protein domain search: database for blast

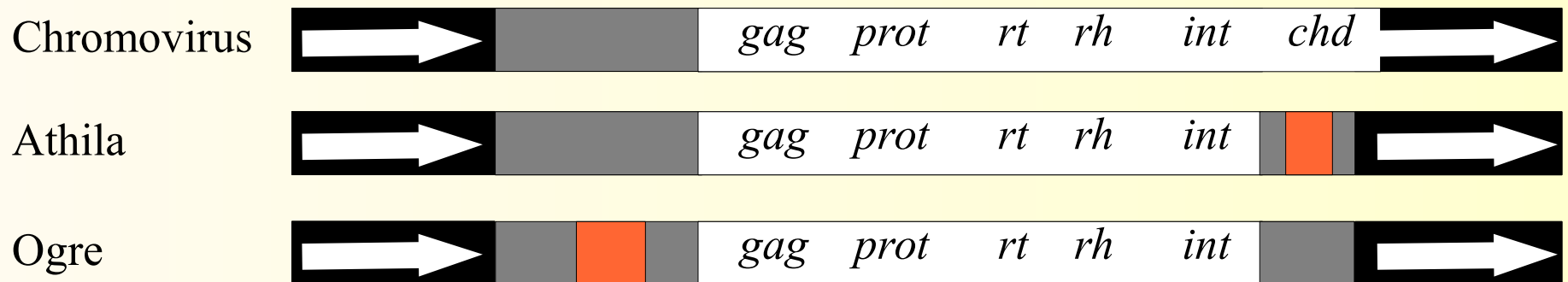
- The database includes only plant elements, therefore, it is highly sensitive **only** for **plant** TE domains!
- Sources:
 - public databases of TEs:
 - RepBase (<http://www.girinst.org/replib/>)
 - GyDB (http://gydb.org/index.php/Main_Page)
 - research articles:
 - Llorens et al., Nucleic Acids Research 2011 (mostly Ty3/gypsy)
 - Wicker et al., Genome Res. 2007 (Ty1/copia)
 - our published research:
 - Macas et al., Gene 2007 (Ogres)
 - Neumann et al., Mobile DNA 2011 (chromoviruses, CRs)
 - our unpublished research
- Protein domain sequences were extracted only from elements that seemed to have their CDS either intact or with only a few mutations

Reliable classification of LTR retrotransposons based on the comparison of polyprotein domains

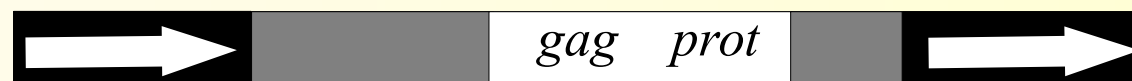
Minimal autonomous elements



Autonomous elements with extra domains or ORFs



nonautonomous elements / deletion derivatives

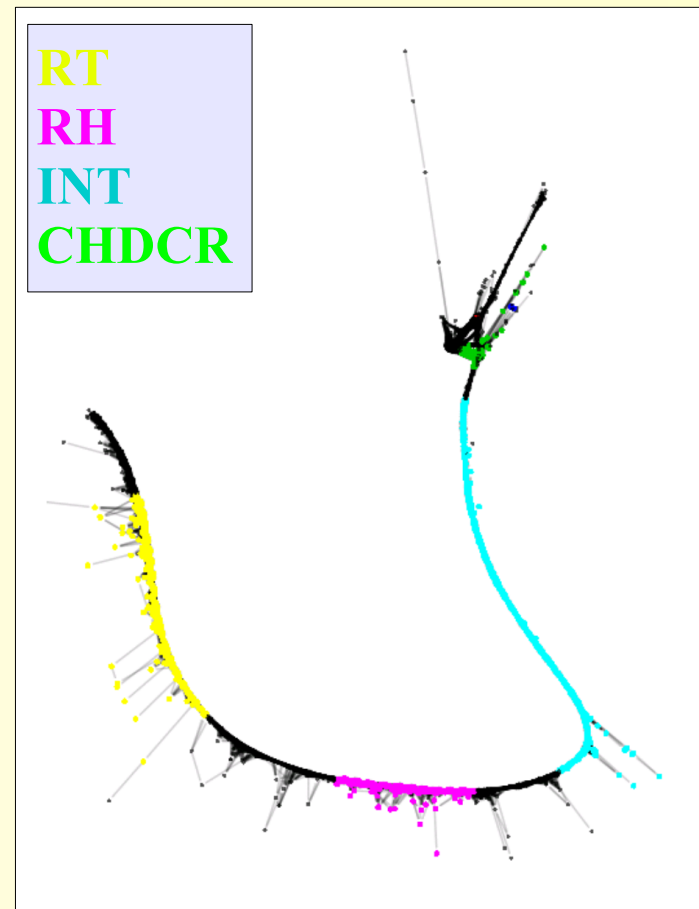


Preliminary classification of TEs based on protein domain search (blast using NGS reads)

HTML summary of graph based clustering

Summary of TE domain hits

	Domain	ID	Type	Lineage	Hits	MeanScore
1	Ty3-INT	PiSat1_ID200	Ty3/gypsy	chromovirus	878	62
2	Ty3-RT	PiSat1_ID200	Ty3/gypsy	chromovirus	810	64
3	Ty3-CHDCR	PiSat1_ID200	Ty3/gypsy	chromovirus	629	55
4	Ty3-RH	PiSat1_ID200	Ty3/gypsy	chromovirus	357	60
5	Ty3-INT	MedT1_ID93	Ty3/gypsy	chromovirus	318	64
6	Ty3-INT	MedT2_ID89	Ty3/gypsy	chromovirus	93	67
7	Ty3-RH	LotJ2_ID65	Ty3/gypsy	chromovirus	67	63
8	Ty3-RH	MedT2_ID89	Ty3/gypsy	chromovirus	66	71
9	Ty3-RT	MedT2_ID89	Ty3/gypsy	chromovirus	54	70
10	Ty3-RT	MedT1_ID93	Ty3/gypsy	chromovirus	43	64
11	Ty3-RH	MedT1_ID93	Ty3/gypsy	chromovirus	11	51
12	Ty3-INT	LotJ2_ID65	Ty3/gypsy	chromovirus	9	64
13	Ty3-RT	CarP1_ID79	Ty3/gypsy	chromovirus	6	43
14	Ty3-RT	BraR5_ID117	Ty3/gypsy	chromovirus	4	71
15	Ty3-INT	CRR2_ID16	Ty3/gypsy	chromovirus	4	66
16	Ty3-RT	PopT1_ID21	Ty3/gypsy	chromovirus	4	67
17	Ty3-INT	CRA3a_ID1	Ty3/gypsy	chromovirus	4	60
18	Ty3-CHDCR	MedT1_ID93	Ty3/gypsy	chromovirus	3	38
19	Ty3-INT	SorB1_ID109	Ty3/gypsy	chromovirus	3	60

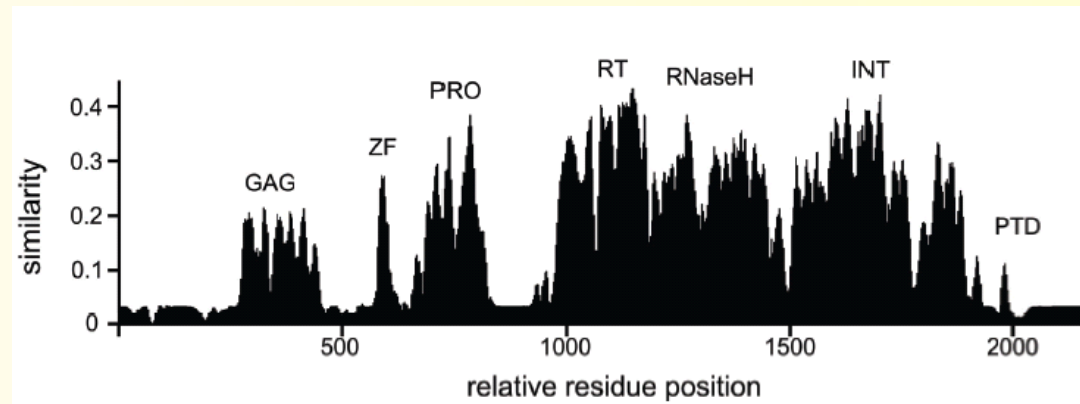


Blast is suitable for a rough preliminary classification of TEs but it is not always sufficient to reliably assign novel sequences into established lineages/clades of LTR retrotransposons

- Blast based classification is based on the best hit but **the best hit is not always a strong hit**
- **the weaker the hit the less reliable classification!**

Protein domain search: database for fasty

- 398 LTR retrotransposon elements (255 Ty1/copia and 143 Ty3/gypsy)
 - 386 GAG, 394 PROT, 378 RT, 383 RNaseH, 391 INT, 31 ChDII, and 45ChDCR domains (totally 2317domains)
- different domains have different level of divergence!

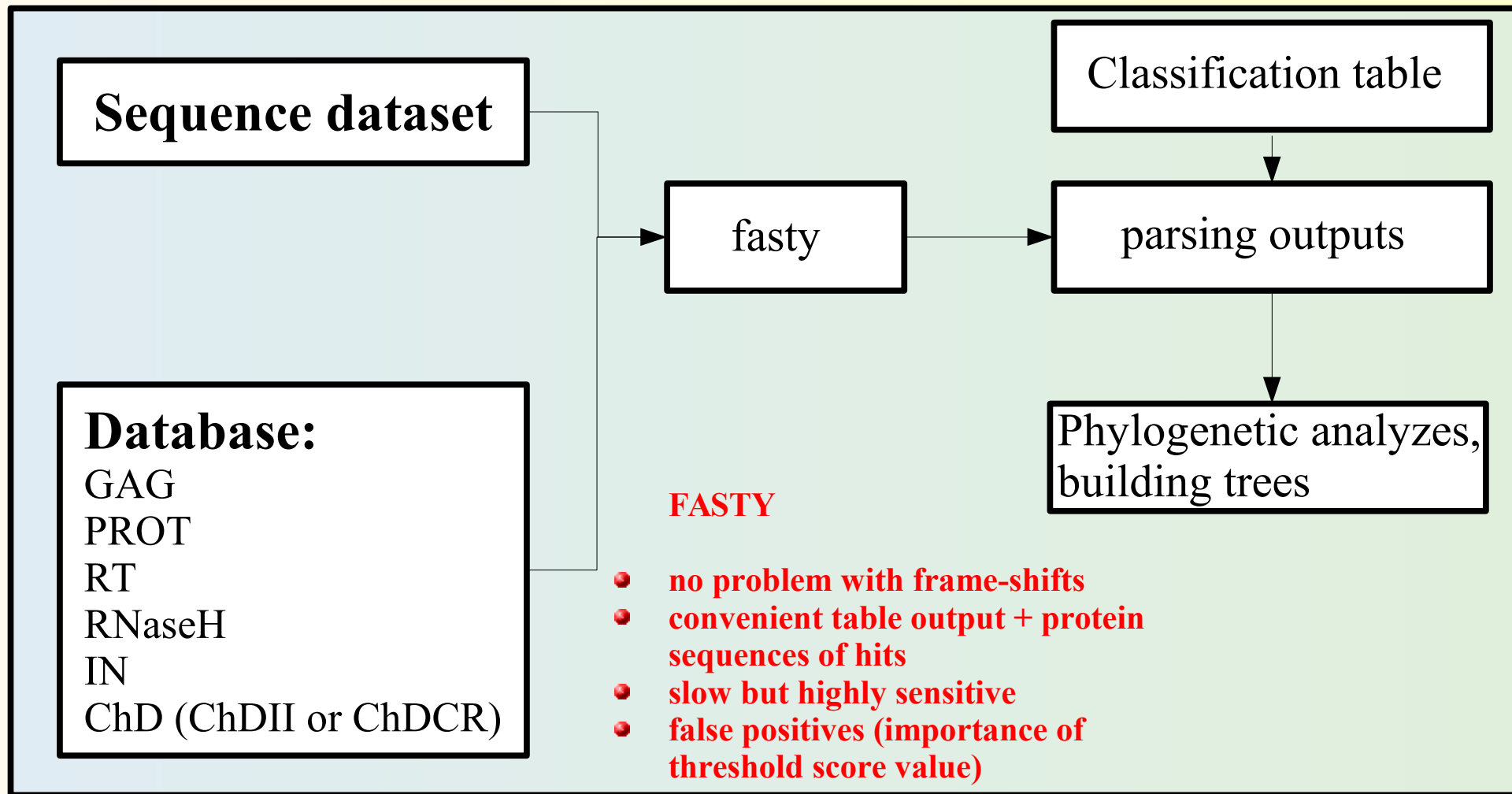


RepeatExplorer: Protein domains tools

1. Protein domain search (it scans DNA sequences with protein domains database using fasty)
2. Filter output (it filters raw fasty output and extracts sequences giving hits above given threshold)
3. Create tree (it calculates and draws phylogenetic tree from a set of sequences)

RepeatExplorer: Protein domains tools

What it does:



RepeatExplorer: database and classification table

Database:

```
>Ty1-PROT__Copia-26_SB-I
WYMDTGATDHITSELEKLTTRDKYHGGDQVHTASGSGMEIQHIGHGVLRSPNTNNLHLRNILHVPAANKDLLS
>Ty3-RT__CRM3_ID37
VRESLSPCSVPVLLVPKKDGSRMVCVDCRAINNTIRYRHPIPRLLDDMLDELSGSMIFSKIDLRSGYHQIRMKLGDEWKTSFKTKFGL
YEWLVMPFGLTNAPSTFMRLMNEVLRAFIGRFVVVYFDDILIYSKCLVDHLDHLRAVFNALREARLFGNLDKCTFCTDRVSFLGY
>LINE-RH__LINE1-6_OS
DGSFQESDGKGGIGAVLRNCTGEVIFAACGHVDCSSALETELLACRDGLALALQWTLLPIVIETDCLAMIHLEFRDATGAKSELAFLI
REIDSLLVGNRDISFSKCLRSQNHISH
>DTT-CD1__Mariner-3_SBi
IDEKWFYLLHQKNERYLLPEEDVPHRTCRNKNYIPRLMFLCACARPRFRDGVCTFDGKIGCFPLVHFEPAIRSNERTGRVRGDVVMKP
ITSITGDVIRDFMINKVLPAIREKWPREDLGRPIFIQQDNA
```

Classification table:

Copia-26_SB-I	Ty1/copia	AleI/Retrofit
CRM3_ID37	Ty3/gypsy	chromovirus

- **Classification table was made to classify LTR retrotransposons into lineages**
- **Types of all TEs are encoded in the name line text**

Fasty using RT domain sequences

- RT is one of the most conserved domains of the polyprotein
- several conserved motifs are intermingled with less conserved regions and the length is highly conserved among distinct families (about 260 and 170 aa in Ty1/copia and Ty3/gypsy, respectively) - RT domain sequences are relatively easy to align
- RT domain sequences are sufficiently diverse, allowing to distinguish among distinct lineages/clades
- RT domains have already been used to infer phylogenetic relationships of retrotransposon families in plants

Parsing and filtering output of fasty

```
fasty36 -m 3 -m 9 -b 1 -H -q -O output_file - database_file
```

FASTY compares a DNA sequence to a protein sequence data bank version 35.04 Aug. 25, 2009

Please cite:

Pearson et al, Genomics (1997) 46:24-36

Query: CL46Contig6_(825-2.7-2302), 825 aa

828>>>CL46Contig6_(825-2.7-2302) - 825 aa - 825 aa

Library: all_RT_domains_for_search_extended 181897 residues in 961 sequences

181897 residues in 961 sequences

Statistics: Expectation_n fit: $\rho(\ln(x)) = -93.9197 \pm 0.0186$; $\mu = 591.6836 \pm 0.967$

mean_var=2038.9986 $\pm$ 1740.653, 0's: 0 Z-trim: 325 B-trim: 0 in 0/9

Lambda= 0.028403

Algorithm: FASTY (3.5 Aug 2009) [optimized]

Parameters: BL50 matrix (15:-5) ktup: 2

join: 44, opt: 40, open/ext: -12/-2 shift: -20, subs: -24 width: 16

The best scores are:

				opt	bits	E(961)	%_id	%_sim	sy	alen	an0	ax0	pn0	px0	an1	ax1	pn1
px1	gapq	gapl	fs														
Ty3_454OCU_CL46Contig61_(1860-14.3-26665)				(173)	[r]	1086	58.0	4.6e-10	0.931	0.971	1086	173	604	85	826	1650	1
173	0	0	1														

>>>CL46Contig6_(825-2.7-2302), 825 aa vs all_RT_domains_for_search_extended library

trans. Smith-Waterman score: 1086; 93.1% identity (97.1% similar) in 173 aa overlap (604-85:1-173)

>CL46Co ..

IRPSVSPWGAPVLFVKKKDGSLRMCIDYRELNQLMVKNKYPLPRIEDLFD
QLRGAGTF\SKIDLRSGYHQLKIKECDIPKMAFRTRYGHYEFVVMPPFGLS
NAPAVFMDLMNRVVFHPYSDKFVVIIFIDDILVYSKDKE*HQEHLRITLETL
HKEKLYAKFKKYEIWLDKVGFLGH

>Ty3_45 ..

VRPSVSPWGAPVLFVKKKDGSLRMCIDYRELNRLTVKNKYPLPRIEDLFD
QLRGAGTF-SKIDLRSGYHQLKIKECDIPKTAFRTRYGHYEFVVMPPFGLS
NAPAVFMDLMNRVVFHPYLDKFVIVFIDDILVYSKDKEHQEHLRITLETL
RKEKLYAKFKKCEFWLDRVGFLGQ

Parsing and filtering output of fasty: outputs

output1: table of fasty hits that passed the filter criteria

Type	lineage	query_name	query_length	hit_name	hit_length	strand	opts	bits	e_val	perc_id	perc_sim	sw	alen	an0	ax0	pn0	px0	an1	ax1	pn1	px1	gapq	gapl	fs
Ty3/gypsy	Ogre/Tat	CL1Contig12	4659	Ty3-RT_RIRE2_J	174	f	878	243.6	2.20E-065	0.678	0.874	878	174	3035	3556	1	4659	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig13	4492	Ty3-RT_RIRE2_J	174	f	894	248	1.10E-066	0.69	0.885	894	174	3060	3581	1	4492	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig23	5275	Ty3-RT_RIRE2_J	174	f	878	243.7	2.50E-065	0.678	0.874	878	174	3301	3822	1	5275	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig26	3906	Ty3-RT_RIRE2_J	174	f	897	248.8	5.30E-067	0.695	0.885	897	174	2891	3412	1	3906	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig27	663	Ty3-RT_RIRE2_J	174	r	840	233.3	4.10E-063	0.678	0.879	840	174	584	61	663	1	1	174	1	174	0	0	2
Ty3/gypsy	Ogre/Tat	CL1Contig30	3399	Ty3-RT_RIRE2_J	174	f	872	242	5.10E-065	0.672	0.868	872	174	1298	1819	1	3399	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig31	4756	Ty3-RT_RIRE2_J	174	f	887	246.1	4.20E-066	0.684	0.879	887	174	2494	3015	1	4756	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig33	2778	Ty3-RT_RIRE2_J	174	f	857	237.9	7.00E-064	0.695	0.885	857	174	2030	2551	1	2778	1	174	1	174	0	0	2
Ty3/gypsy	Ogre/Tat	CL1Contig38	2294	Ty3-RT_RIRE2_J	174	f	870	241.4	5.00E-065	0.678	0.862	870	174	1592	2113	1	2294	1	174	1	174	0	0	0
Ty1/copia	SIRE	CL27Contig4	1238	Ty1-RT_Wicker_Maximus_cons	258	f	1314	361.7	2.60E-101	0.888	0.963	1314	215	592	1236	1	1238	1	215	1	258	0	0	0
Ty1/copia	SIRE	CL27Contig15	13784	Ty1-RT_Wicker_Maximus_cons	258	r	1584	435	2.50E-122	0.884	0.969	1584	258	5359	4586	13784	1	1	258	1	258	0	0	0
Ty1/copia	SIRE	CL27Contig36	1114	Ty1-RT_Wicker_Maximus_cons	258	r	1549	425.4	1.50E-120	0.872	0.957	1549	258	967	194	1114	1	1	258	1	258	0	0	0
Ty1/copia	Angela	CL55Contig6	3043	Ty1-RT_Wicker_BARE1_B_cons	258	f	1586	435.5	3.90E-123	0.933	0.992	1586	255	2	766	1	3043	4	258	1	258	0	0	0
Ty1/copia	TAR	CL89Contig4	5745	Ty1-RT_Wicker_Osr1_Os_cons	257	r	1582	434.4	1.50E-122	0.891	0.973	1582	257	4461	3691	5745	1	1	257	1	257	0	0	0

```
>CL27Contig4_592-1236
WIQAMQEELQQFELNNVWELVKRPDPKRKHNIIGTKWIYRNKQDEHGQVVR
NKARLVAQGYTQVEGIDFDETFAPVARLEAIRILLAYANHHNIFLYQMDV
KSAFLNGKIEEEVYVAQPPGFEDPKRPDMVYKLNKALYGLKQAPRAWYDT
LKDFLKSFGKPGSLDPTLFTKTYDGELFVCQIYVDDIIFGCTVKRYSYE
FGYMMQVQYQMSMMG
>CL27Contig15_5359-4586
WIQAMQEELQQFELNNVWELVKRPDPKRKHNIIGTKWIYRNKQDEHGQVVR
NKARLVAQGYTQVEGIDFDETFAPVARLEAIRILLAYANHHNILLYQMDV
KSAFLNGRIEEVYVAQPPGFEDPKRPDMVYKLNKALYGLKQAPRAWYDT
LKDFLKSFGKPGSLDPTLFTKTYDDELFCVQIYVDDIIFGCTDKRYSDE
FGHMMQEYQMSMMGELKFLLGLQIRQQSNGIFISQEKYLDCLKKFGMQ
DCKGYSTP
>CL27Contig36_967-194
WIQAMHEELQQFELNNVWELVKRPDPKRKHNIIGTKWIYRNKQDENGQVVI
NKARLVAQGYTQVEGIDFDETFALVARLEAIRILLAYANHHNILLYQMDV
KSAFLNGKIEEEVYVAQPPGFEDPKRPDMVYKLNKALYGLKQAPRAWYDT
LKDFLKSFGKPGSLDPTLFTKTYDDELFCVQIYVDDIIFGCTDKRYSDE
FGHMMQEYQMSMMGELKFLLGLQIRQQSNDIFISQEKYLDCLKKFGMQ
DCKGYTTP
```

```
>CL1Contig12_3035-3556
VRGVIHPTWLANPVVVRKANGKWRLCIDFTDVNKAACPKDPFFPLPRIDQIV
DSTAGCELLSFLDAYSGYHQIFMAKEDEEKTAFITPCGTTCYCFIRMPFGLK
NAGSTFARVVYTAFAEPQIHRNVEAYMDDIVVKSksKETLIQDLEETFQNL
RRIQLKLNPEKCVFVPSGKLLGF
>CL1Contig13_3060-3581
VRGVIHPTWLANPVVVRKANGKWRLCIDFTDVNKAACPKDPFFPLPRIDQIV
DSTAGCELLSFLDAYSGYHQIFMAEEDDEEKTAFITPCGTTCYCFIRMPFGLK
NAGSTFARVVHTAFAEPQIHRNVEAYMDDIVVKSksKDKDTLIQDLDETFANL
RKIQLKLNPEKCVFVPSGKLLGF
>CL1Contig23_3301-3822
VRGVLHPTWLANPVVVRKANGKWRLCIDFTDVNKAACPKDPFFPLPRIDQIV
DSTAGCDLLSFLDAYSGYHQIFMAEEDDEEKTAFITPCGTTCYCFIRMPFGLK
NAGSTFARVVHTAFAEPQIHRNVEAYMDDIVVKSksKDRATLIQDLDETFANL
RKINLKLNPEKCVFVPSGKLLGF
>CL1Contig26_2891-3412
VRGVLHPTWLANPVVVRKANGKWRLCIDFTDVNKAACPKDPFFPLPRIDQIV
DSTAGCELLSFLDAYSGYHQIFMAEEDDEEKTAFITPCGTTCYCFIRMPFGLK
NAGSTFARVVHTAFAEPQIHRNVEAYMDDIVVKSksKDKDTLIQDLDETFANL
RKIQLKLNPEKCVFVPSGKLLGF
```

output2: Ty1 domains

output3: Ty3 domains

Parsing and filtering output of fasty: outputs

- Number of identified full-length domains depends on the length of NGS reads (the longer the reads the higher number of full-length domains)
- Fasty is run with default settings - the stringency is very relaxed which leads to many false positives
- False positives can be easily removed at the filtering step but keep in mind that different domains require different values of key parameters
 - minimum similarity [%]: **60**
 - minimum identity [%]: **40**
 - proportion of the hit length from the length of the database sequence [0-1]: **0.8**

Classification of LTR retrotransposons: table or tree?

output1: table of fasty hits that passed the filter criteria

Type	lineage	query_name	query_length	hit_name	hit_length	strand	opts	bits	e_val	perc_id	perc_sim	sw	alen	an0	ax0	pn0	px0	an1	ax1	pn1	px1	gapq	gapl	fs
Ty3/gypsy	Ogre/Tat	CL1Contig12	4659	Ty3-RT_RIRE2_J	174	f	878	243.6	2.20E-065	0.678	0.874	878	174	3035	3556	1	4659	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig13	4492	Ty3-RT_RIRE2_J	174	f	894	248	1.10E-066	0.69	0.885	894	174	3060	3581	1	4492	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig23	5275	Ty3-RT_RIRE2_J	174	f	878	243.7	2.50E-065	0.678	0.874	878	174	3301	3822	1	5275	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig26	3906	Ty3-RT_RIRE2_J	174	f	897	248.8	5.30E-067	0.695	0.885	897	174	2891	3412	1	3906	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig27	663	Ty3-RT_RIRE2_J	174	r	840	233.3	4.10E-063	0.678	0.879	840	174	584	61	663	1	1	174	1	174	0	0	2
Ty3/gypsy	Ogre/Tat	CL1Contig30	3399	Ty3-RT_RIRE2_J	174	f	872	242	5.10E-065	0.672	0.868	872	174	1298	1819	1	3399	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig31	4756	Ty3-RT_RIRE2_J	174	f	887	246.1	4.20E-066	0.684	0.879	887	174	2494	3015	1	4756	1	174	1	174	0	0	0
Ty3/gypsy	Ogre/Tat	CL1Contig33	2778	Ty3-RT_RIRE2_J	174	f	857	237.9	7.00E-064	0.695	0.885	857	174	2030	2551	1	2778	1	174	1	174	0	0	2
Ty3/gypsy	Ogre/Tat	CL1Contig38	2294	Ty3-RT_RIRE2_J	174	f	870	241.4	5.00E-065	0.678	0.862	870	174	1592	2113	1	2294	1	174	1	174	0	0	0
Ty1/copia	SIRE	CL27Contig4	1238	Ty1-RT_Wicker_Maximus_cons	258	f	1314	361.7	2.60E-101	0.888	0.963	1314	215	592	1236	1	1238	1	215	1	258	0	0	0
Ty1/copia	SIRE	CL27Contig15	13784	Ty1-RT_Wicker_Maximus_cons	258	r	1584	435	2.50E-122	0.884	0.969	1584	258	5359	4586	13784	1	1	258	1	258	0	0	0
Ty1/copia	SIRE	CL27Contig36	1114	Ty1-RT_Wicker_Maximus_cons	258	r	1549	425.4	1.50E-120	0.872	0.957	1549	258	967	194	1114	1	1	258	1	258	0	0	0
Ty1/copia	Angela	CL55Contig6	3043	Ty1-RT_Wicker_BARE1_B_cons	258	f	1586	435.5	3.90E-123	0.933	0.992	1586	255	2	766	1	3043	4	258	1	258	0	0	0
Ty1/copia	TAR	CL89Contig4	5745	Ty1-RT_Wicker_Osr1_Os_cons	257	r	1582	434.4	1.50E-122	0.891	0.973	1582	257	4461	3691	5745	1	1	257	1	257	0	0	0



- Classification included in the table is reliable only in case of **high similarity** to the database sequences
- Sequences that have low similarity to those in the database should be classified using phylogenetic analysis

Classification of LTR retrotransposons using phylogenetic analysis of their domains

- it helps to assign retrotransposon sequences into phylogenetic lineages which were **established previously**
- trees are calculated using **NJ** algorithm because its fast and provides reasonably accurate results
- inferring detailed phylogenetic relationships among identified elements should be done using **more sophisticated** algorithms and models

Need for unified classification of TEs including LTR retrotransposons

- Nomenclature, and classification of TEs into major types is well established but there are no rules for more detailed classification into subclasses/lineages/families
- The classification should be hierarchical, following principals similar to those used for taxonomy of species.
 - Results of different studies on LTR retrotransposons are difficult to compare because relationships among described sequences is often not clear (there are a number of papers describing similar TEs under different names)
- The deeper the level of classification the bigger the problem of category definition (continuum of sequence similarity)
 - e.g. 80-80-80 rule for family definition
- Some attempts have been made but it is still far from perfect
 - Wicker et al. 2007: Class – subclass – order -superfamily -family – subfamily -insertion

GUIDELINES

A unified classification system for eukaryotic transposable elements

Thomas Wicker, François Sabot, Aurélie Hua-Van, Jeffrey L. Bennetzen, Pierre Capy, Boulos Chalhoub, Andrew Flavell, Philippe Leroy, Michele Morgante, Olivier Panaud, Etienne Paux, Phillip SanMiguel and Alan H. Schulman

Abstract | Our knowledge of the structure and composition of genomes is rapidly progressing in pace with their sequencing. The emerging data show that a significant portion of eukaryotic genomes is composed of transposable elements (TEs). Given the abundance and diversity of TEs and the speed at which large quantities of sequence data are emerging, identification and annotation of TEs presents a significant challenge. Here we propose the first unified hierarchical classification system, designed on the basis of the transposition mechanism, sequence similarities and structural relationships, that can be easily applied by non-experts. The system and nomenclature is kept up to date at the WikiPoson web site.

Our knowledge of the structure and composition of genomes has progressed in pace with their sequencing. As was expected early on¹, TEs have been found in virtually all eukaryotic species investigated so far^{2–4}. The only known exceptions are *Plasmodium falciparum* and probably several closely related species. The TEs affect the genome by their ability to move and replicate, thereby generating plasticity. Although the main TE groups are ancient and are present in all kingdoms, TEs display extreme diversity: there are thousands or even tens of thousands of different TE families in plants^{5–8}, constituting 80% or more of the total genomic DNA. Although they are less abundant than those in plants, TEs in fungi and metazoans also represent a substantial part of their genomes (3–20% in fungi and 3–45% in metazoans), and include members of most superfamilies^{9,10}.

More and more large and complex eukaryotic genomes are being selected for sequencing, and they too are expected to be rich in TEs. Given the abundance and diversity of TEs, these genomes will present an enormous TE identification and annotation problem. Hence, there is an increasing

demand for tools to handle these rapidly emerging sequences effectively. This requires straightforward and efficient nomenclature keys and classification strategies that can help non-specialists to easily annotate TEs.

The sequencing of the genomes of multiple related species will facilitate comparative studies and provide insights into genome evolution. However, comparisons will be compromised unless TE annotation is consistent. Until now, TE analysis and classification have generally been carried out on a species-by-species basis, and comparative studies of TEs between taxa have been rare. Thus, no unified system has been applied across species and, therefore, across kingdoms. Accordingly, TE databases are generally restricted to individual or closely related species and tend to lack systematic structure.

Here we propose a common TE classification system that can be easily handled by non-specialists during annotation. It provides a consensus between the various conflicting classification and naming systems that are currently in use. A key component of this system is a naming convention: a three-letter code with each letter respectively denoting

class, order and superfamily; the family (or subfamily) name; the sequence (database accession) on which the element was found; and the 'running number', which defines the individual insertion in the accession. The unified system is also intended to facilitate comparative and evolutionary studies on TEs from different species.

Outline of the classification system

In 1989, Finnegan proposed the first TE classification system, which distinguished two classes by their transposition intermediate: RNA (class I or retrotransposons) or DNA (class II or DNA transposons). The transposition mechanism of class I is commonly called 'copy-and-paste', and that of class II, 'cut-and-paste'¹¹. The discovery of bacterial¹² and eukaryotic^{13,14} TEs that copy and paste but without RNA intermediates, and of highly reduced non-autonomous TEs called miniature inverted repeat transposable elements (MITEs), has challenged the two-class system. These findings led to schemes that either invoke a third class or jettison the two-class system in favour of enzymological classes¹⁵.

Our hierarchical TE classification system (FIG. 1) reconciles both approaches, maintaining two classes while applying mechanistic and enzymatic criteria. It includes (in hierarchical order) the levels of class, subclass, order, superfamily, family and subfamily. These terms were chosen to mirror those used in organismal phylogenetics and to recognize the fact that some terms (such as superfamily) are already in widespread use for TE groups. The highest level (class) divides TEs by the presence or absence of an RNA transposition intermediate, as before¹¹. Subclass, previously used to separate long terminal repeat (LTR) from non-LTR (long and short interspersed nuclear element (LINE and SINE)) class I TEs, here is used to distinguish elements that copy themselves for insertion from those that leave the donor site to reintegrate elsewhere. It concomitantly reflects the number of DNA strands that are cut at the TE donor site. A new taxon — order — marks major differences in the insertion mechanism and, consequently, overall organization and enzymology, thereby replacing subclass for type I TEs.

CORRESPONDENCE

A unified classification system for eukaryotic transposable elements should reflect their phylogeny

Ole Seberg and Gitte Petersen

To assist genome annotators in naming transposable elements (TEs), Wicker *et al.* propose in their Guidelines article (A unified classification system for eukaryotic transposable elements. *Nature Rev. Genet.* 8, 973–982 (2007))¹ a classification and nomenclatural system aimed at simplifying annotation to assist future detailed structural, functional and evolutionary analyses². However, even though the proposed classification system mimics organismal phylogenetic classification, it fails to do so consistently.

Classifications are a necessary prerequisite for human communication, and most biological classifications are hierarchical. The relationships between entities in any hierarchical system are of two kinds only: either one entity is included in another or they are mutually exclusive. The only rule that applies to the ranks in biological nomenclature is their sequence — a class includes one or more orders, an order one or more families and so on. Most biologists agree that classifications should reflect phylogeny (see REF. 2 for one exception), although how phylogeny is reconstructed is controversial. This aside, the majority of organismal phylogenies are inherently hierarchical, and thus either a clade is included in another clade or two clades are mutually exclusive. Hence, as long as the groups remain monophyletic³ (for an alternative point of view see REFS 4, 5), a

hierarchical classification is ideally suited to reflect phylogeny⁴.

Some groups with formal rank in the TE classification proposed by Wicker *et al.*¹ seem to be well justified (for example, classes). However, the limits of groups become fuzzier as one descends through the hierarchy and, to an increasing degree, loses connection with phylogeny. Some groups are surely not monophyletic, for example, autonomous and non-autonomous partners of the same family are classified as different subfamilies. In addition, although recognizing non-monophyly of miniature inverted repeat elements (MITEs), Wicker *et al.*¹ repeat the classification error by recognition of paraphyletic subfamilies that include only autonomous members, even though they claim that "subfamilies are defined on the basis of phylogenetic data"². Furthermore, the 80–80–80 similarity rule that is used to create families and subfamilies leads to an unpredictable mix of monophyletic, paraphyletic and polyphyletic groups. Naming paraphyletic groups may be misleading⁵, but naming polyphyletic groups has no connection to reality.

Additionally, it must be remembered that, despite their mobility, TEs over time 'travel' within their host genome. Hence, although a TE phylogeny corresponds to a gene tree and need not reflect species phylogeny, the evolutionary histories of the TEs and of their

hosts are tightly linked, and will need to be integrated to achieve a full understanding of both⁷.

It is particularly discouraging that Wicker *et al.*¹, despite being aware of the fact that homology is explained by common descent, misuse^{8,9} the concept by reference to "a continuum of sequence homology"¹. Homology (as with pregnancy, for example) is not measured in degrees: either two sequences are homologous or not. However, in contrast to pregnancy, homology is only a hypothesis^{10,12}.

We absolutely accept the need for a classification system of TEs, but equally urge that such a system must be based on the consistent use of principles. Anything else may retard, rather than improve, our understanding of TE evolution.

Ole Seberg and Gitte Petersen are at the Laboratory of Molecular Systematics, Natural History Museum of Denmark, Solvgade 83, 0pg. 5, DK-1307 Copenhagen K, Denmark.

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CORRESPONDENCE

Reply: A unified classification system for eukaryotic transposable elements should reflect their phylogeny

Thomas Wicker, François Sabot, Aurélie Hua-Van, Jeffrey L. Bennetzen, Pierre Capy, Boulos Chalhoub, Andrew Flavell, Philippe Leroy, Michele Morgante, Olivier Panaud, Etienne Paux, Phillip SanMiguel and Alan H. Schulman

A unified classification system for eukaryotic transposable elements

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Table 2 | Examples of the application of the proposed classification system

	Barbara	Sukkula	Thalos	Xithos
Class	Retrotransposon	Retrotransposon	DNA transposons	Unknown
Subclass	N/A	N/A	1	Unknown
Order	LTR retrotransposon	LTR retrotransposon	TIR	Unknown
Superfamily	Copia	Unknown	Mariner	Unknown
Family	Barbara	Unknown	Stowaway	Xithos
Subfamily	Not defined	Sukkula	Thalos	Not defined
Insertion	RLC_Barbara_AF1234-2	RLx_Sukkula_AF1234-1	DTT_Thalos_AF1234-8	xxx_Xithos_AF1234-1
Structural description	Autonomous retrotransposon	LARD	MITE	-

A unified classification system for eukaryotic transposable elements should reflect their phylogeny

Ole Seberg and Gitte Petersen

We absolutely accept the need for a classification system of TEs, but equally urge that such a system must be based on the consistent use of principles. Anything else may retard, rather than improve, our understanding of TE evolution.

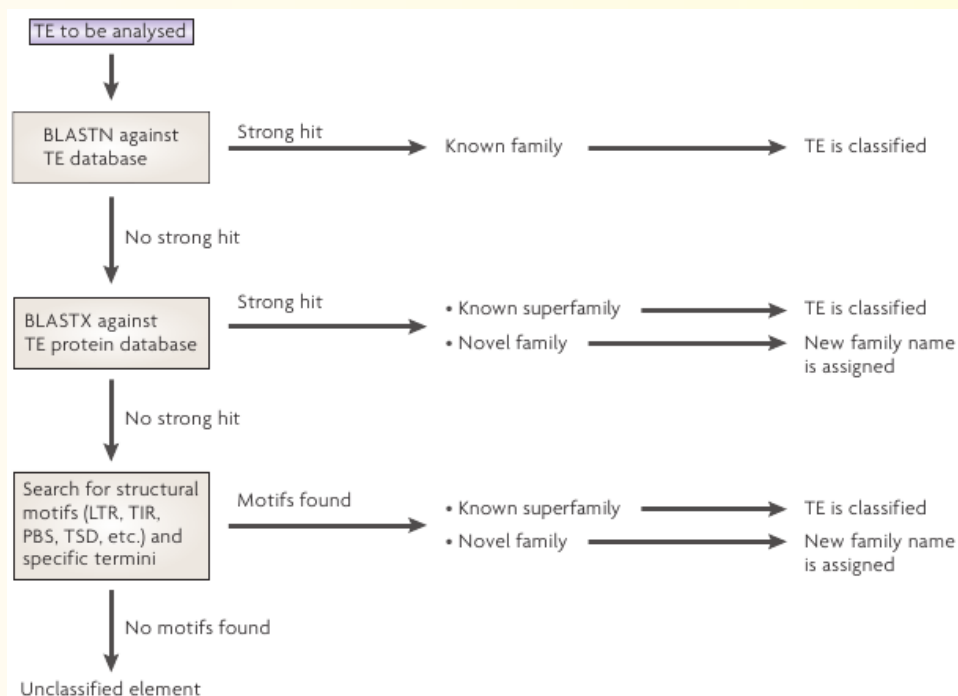
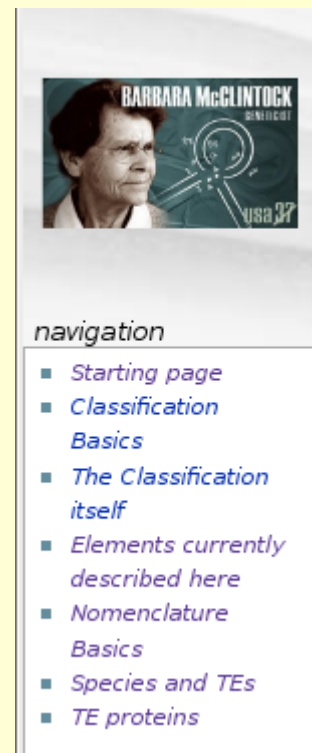
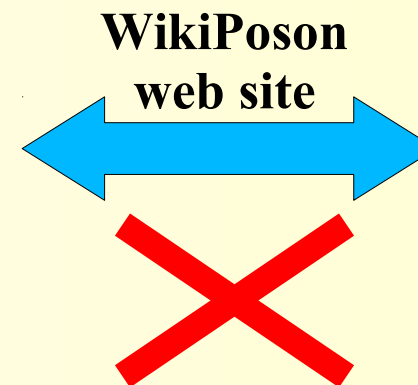
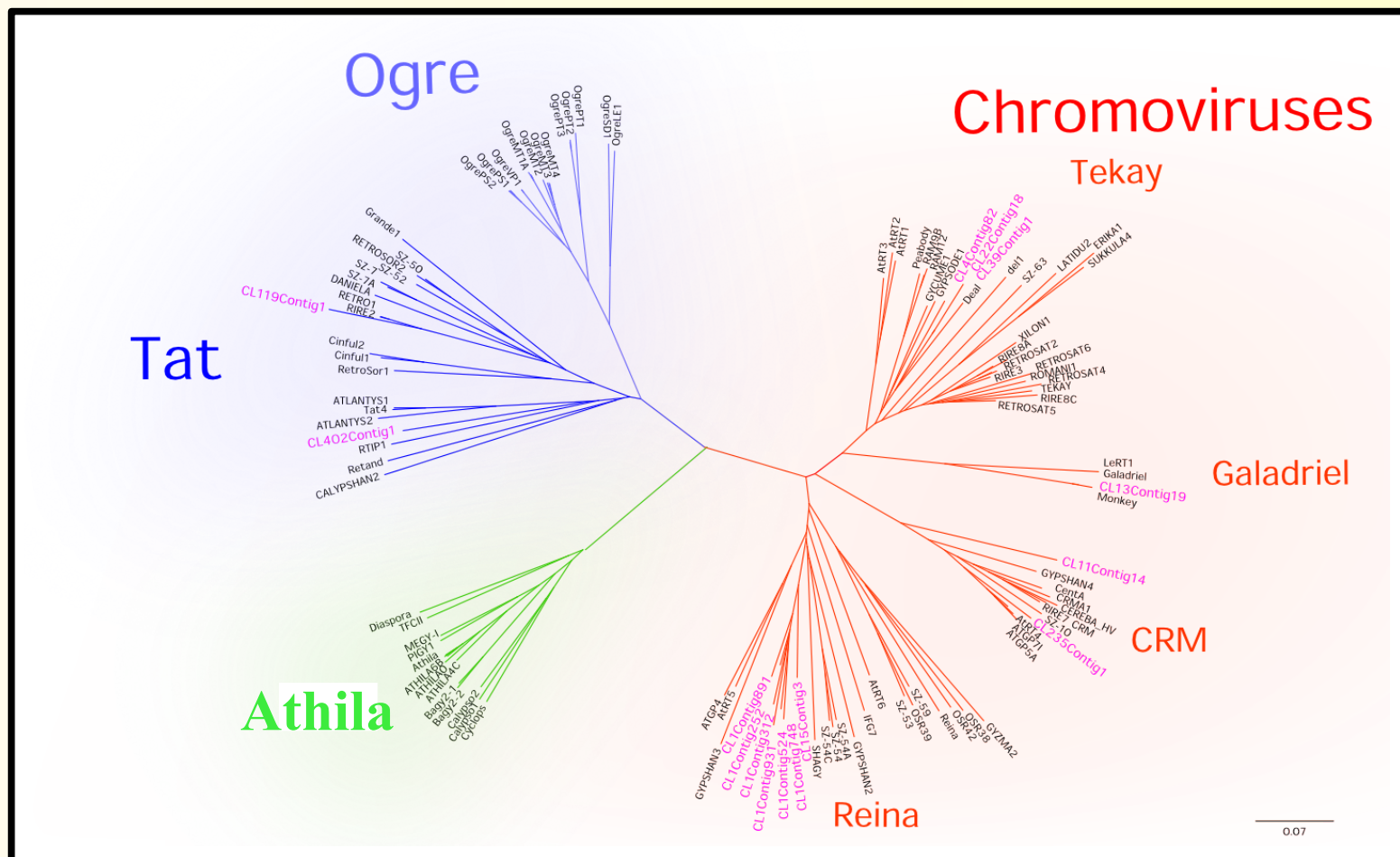


Figure 3 | **Step by step transposable element (TE) classification.** LTR, long terminal repeat; PBS, primer binding site; TIR, terminal inverted repeat; TSD, target site duplication.



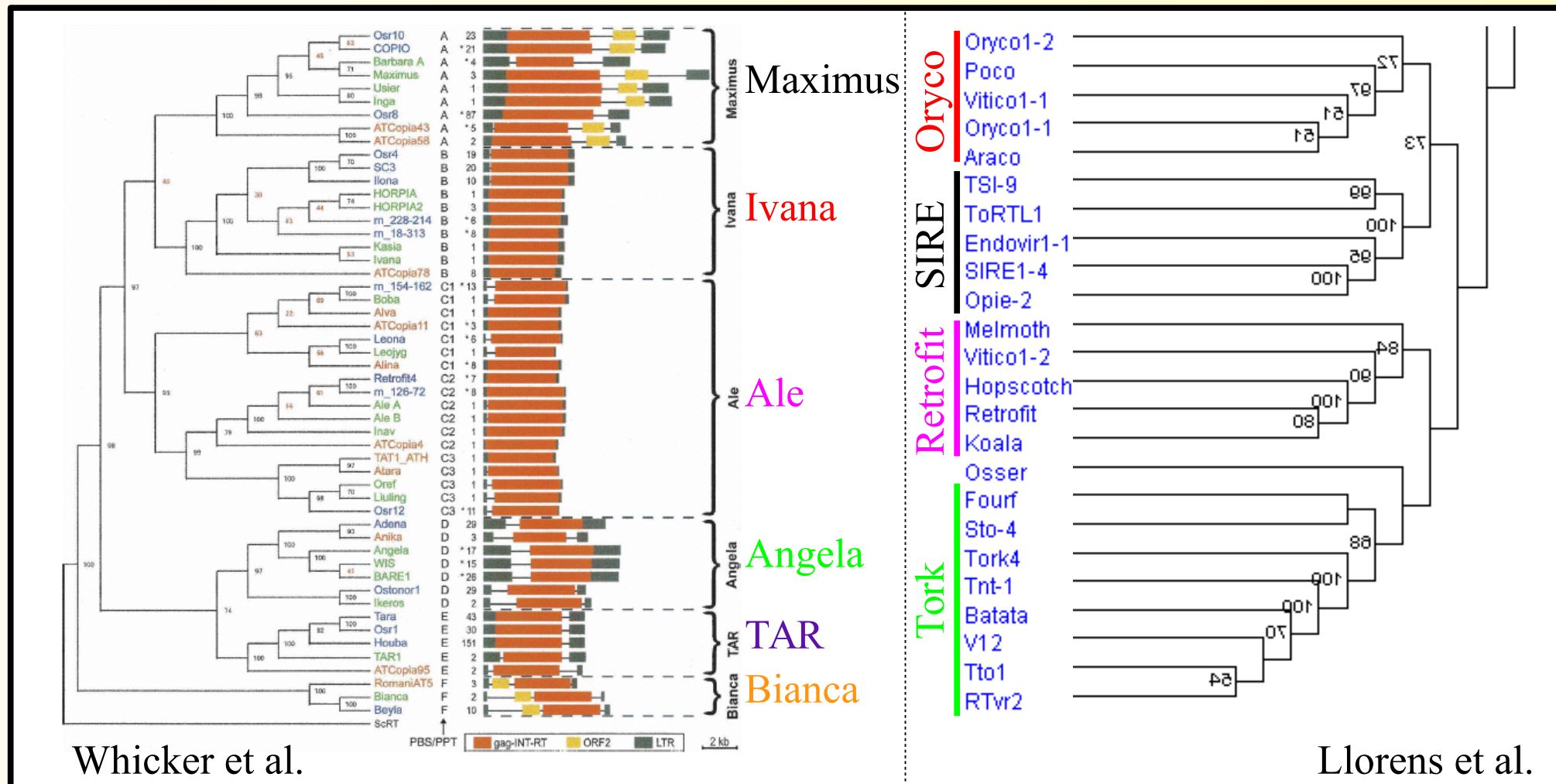
Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
LTR	<i>Copia</i>		4-6	RLC	P, M, F, O
	<i>Gypsy</i>		4-6	RLG	P, M, F, O
	<i>Bel-Pao</i>		4-6	RLB	M
	<i>Retrovirus</i>		4-6	RLR	M
	<i>ERV</i>		4-6	RLE	M
DIRS	<i>DIRS</i>		0	RYD	P, M, F, O
	<i>Ngaro</i>		0	RYN	M, F
	<i>VIPER</i>		0	RYV	O
PLE	<i>Penelope</i>		Variable	RPP	P, M, F, O
LINE	<i>R2</i>		Variable	RIR	M
	<i>RTE</i>		Variable	RIT	M
	<i>Jockey</i>		Variable	RIJ	M
	<i>L1</i>		Variable	RIL	P, M, F, O
	<i>I</i>		Variable	RII	P, M, F
SINE	<i>tRNA</i>		Variable	RST	P, M, F
	<i>7SL</i>		Variable	RSL	P, M, F
	<i>5S</i>		Variable	RSS	M, O
Class II (DNA transposons) - Subclass 1					
TIR	<i>Tc1-Mariner</i>		TA	DTT	P, M, F, O
	<i>hAT</i>		8	DTA	P, M, F, O
	<i>Mutator</i>		9-11	DTM	P, M, F, O
	<i>Merlin</i>		8-9	DTE	M, O
	<i>Transib</i>		5	DTR	M, F
	<i>P</i>		8	DTP	P, M
	<i>PiggyBac</i>		TTAA	DTB	M, O
	<i>PIF-Harbinger</i>		3	DTH	P, M, F, O
	<i>CACTA</i>		2-3	DTC	P, M, F
	<i>Crypton</i>		0	DYC	F
Class II (DNA transposons) - Subclass 2					
Helitron	<i>Helitron</i>		0	DHH	P, M, F
Maverick	<i>Maverick</i>		6	DMM	M, F, O

modified classification according to Llorens et al. (2011)

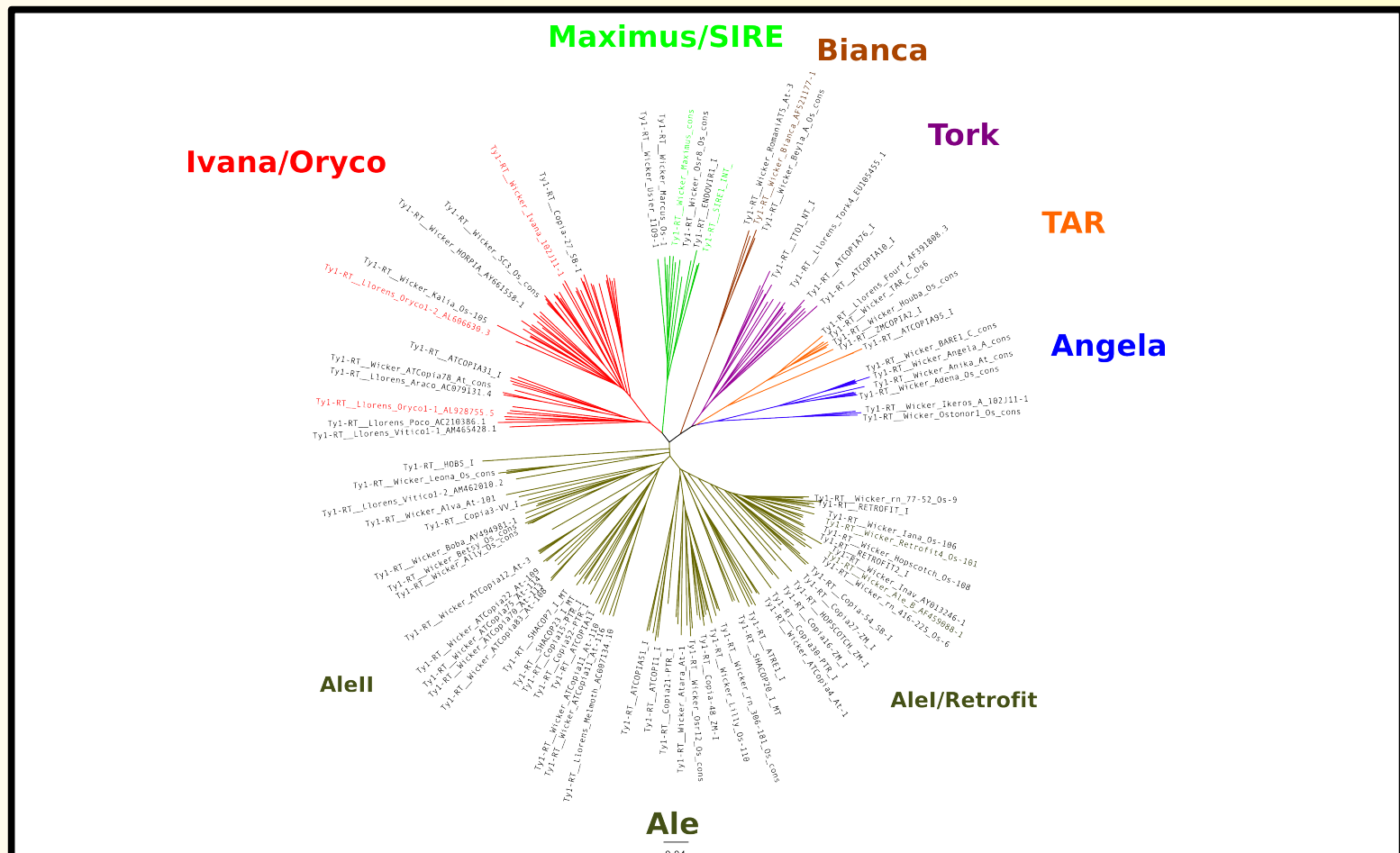


Ty1/copia

classification according to Whicker et al. (2007) and Llorens et al. (2011)



modified classification according to Whicker et al. (2007) and Llorens et al. (2011)



A few tips at the end

- bad alignment leads to a wrong tree
- wrong translation leads to a wrong result
 - continuous translations from multiple frames is convenient but not always correct; consider avoiding old severely mutated sequences - typically but not necessarily those in clusters composed of only a few reads
- analyze Ty3/gypsy and Ty1/copia separately (they are too divergent and difficult to align)
- analyze more domains to get the highest confidence
 - RT, RNaseH, and INT are easy to compare
 - GAG, PROT, CHDII/CHDCR are highly divergent and often difficult to compare but can be used for finer classification of LTR retrotransposons
- if you are not sure take a look at other features (pbs, introns, extra ORF)
- it should be **you** who makes the final decision; do not blindly rely on the automatic outputs

Coming soon ...

- new version of database is being developed to cover elements from a wide range of Viridiplantae species
- DNA transposons: TPase domains will be longer (the short fragments will be removed)
- Classification table will contain all types of TEs (now there are only LTR retrotransposons, other types are recognizable by codes in domains names)
- LINEs will be classified into lineages and clades (L1 – Tal1, BNR, Cin4 .., RTE - ?)
- improved scripts to extract full protein domains
- Other improvements based on your feedback ...