PLANT & ANIMAL GENOME

Clfinder-Orthnet: creating comparative genomics frameworks for closely-related genomes using co-linearity networks

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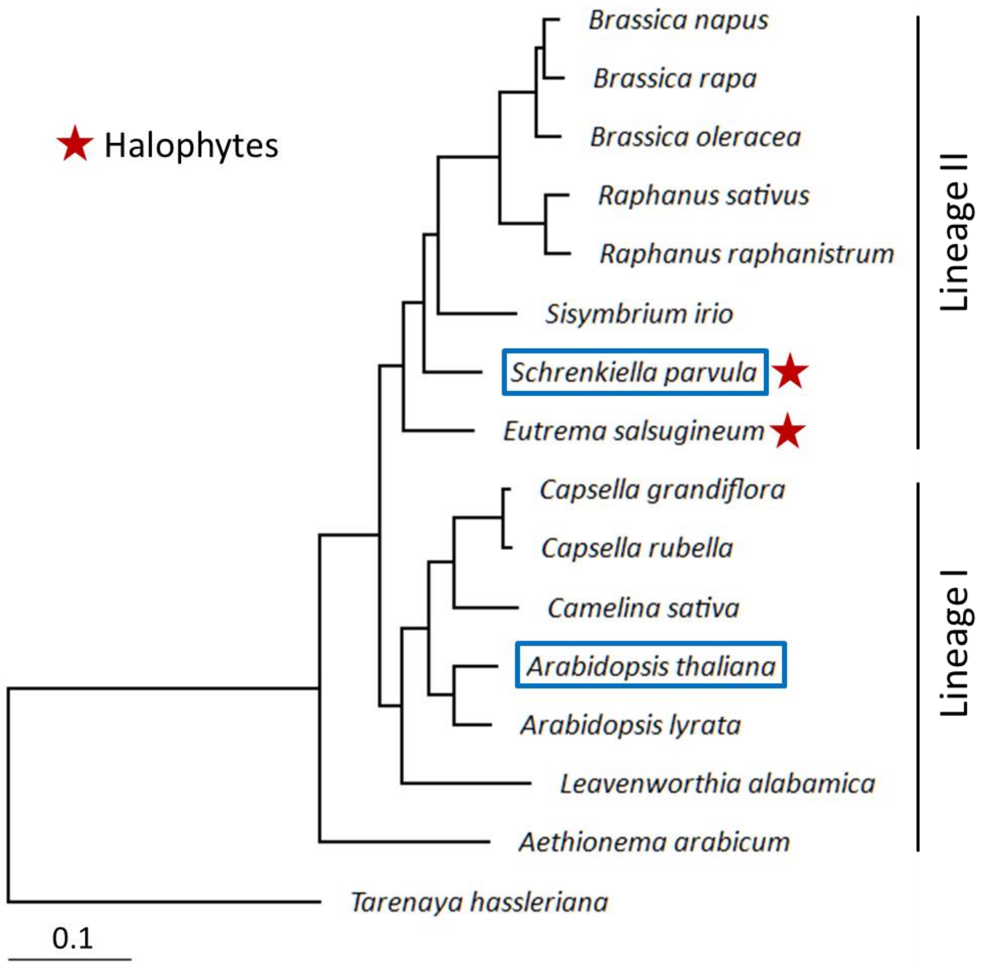
*Presenter

Brassicaceae: one of the most sequenced and annotated plant family

Publicly available Brassicaceae genomes, 2017 Spring



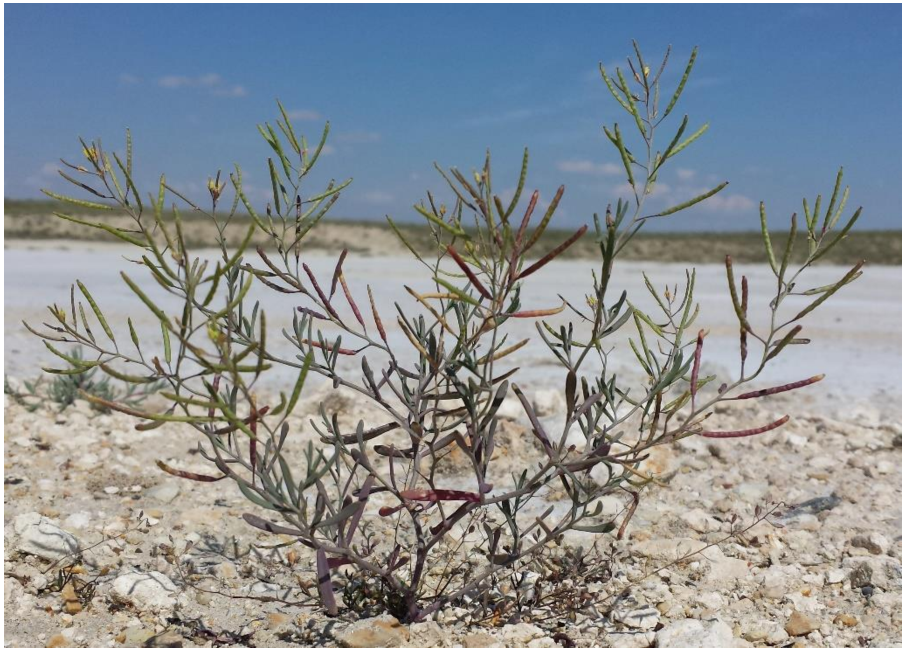
A comparative genomics framework including the two extremophyte/halophyte crucifers, *S. parvula* and *E. salsugineum*. The tree was based on 14,614 alignment of homologous gene clusters.

Schrenkiella parvula from Lake Tuz, Turkey

(NASA, 1994)

(Sun Jeon, c. 2010)

S. parvula in situ (Ozfidan-Konakci et al., Funct Plant Biol 2016)

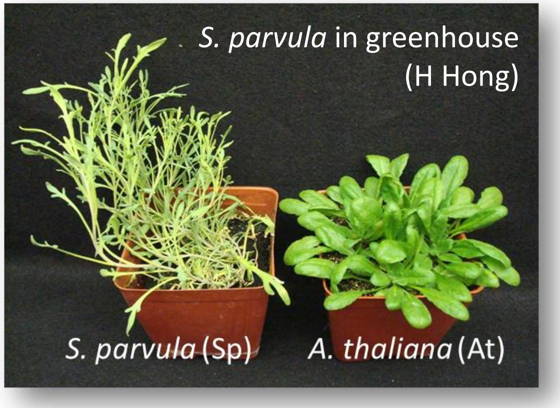


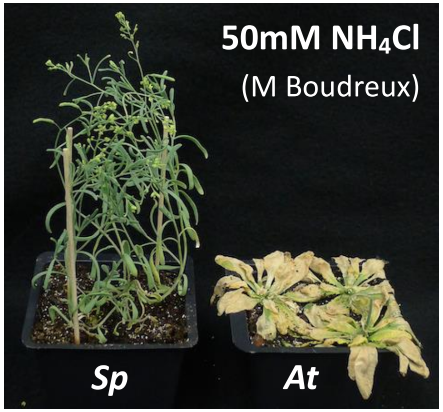
(Helvaci *et al.*, Int Geol Rev 2004)

	pH	Li^+	Na^+	K^+	Mg^{2+} (mg/L)
Burdur Lake	8.8	3	5,500	45	960
Tuz Lake	**6.0**	**325**	**61,000**	**12,000**	**37,500**
Seawater	7.2-8.4	0	10,800	392	1,290

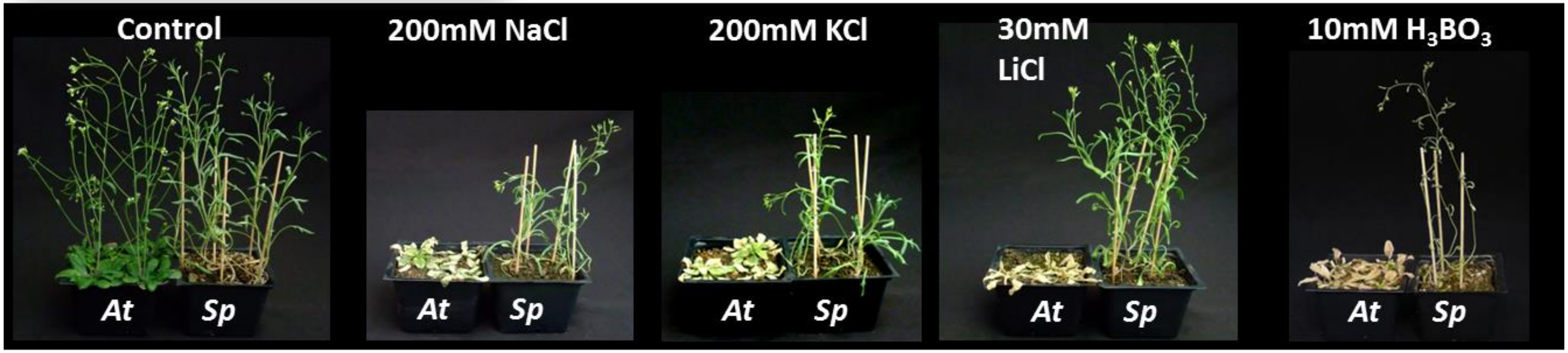
Schrenkiella parvula from Lake Tuz, Turkey

Adaptation to multi-ion salt stresses



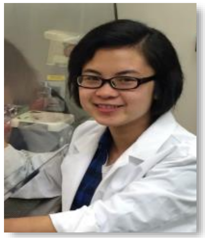


(Oh *et al.*, Plant Physiol 2014)



W489

P495

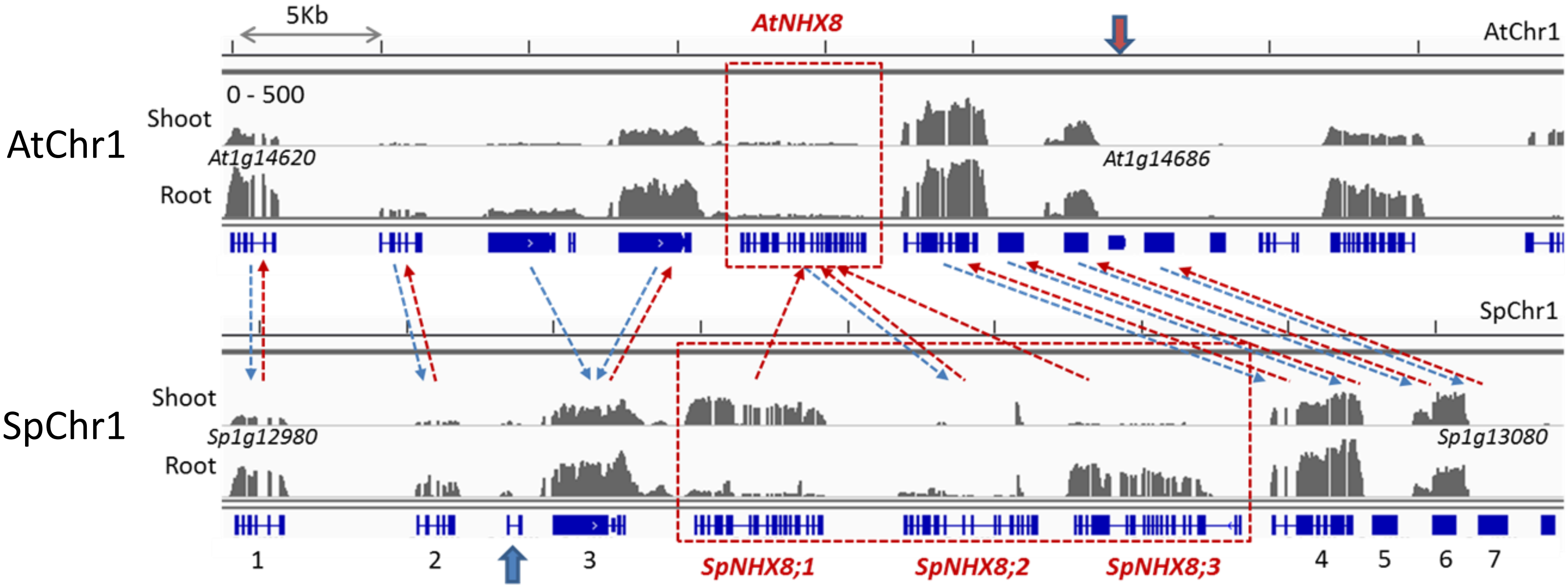


W562

W457

Comparison with a model species (i.e. *S. parvula* vs *A. thaliana*)

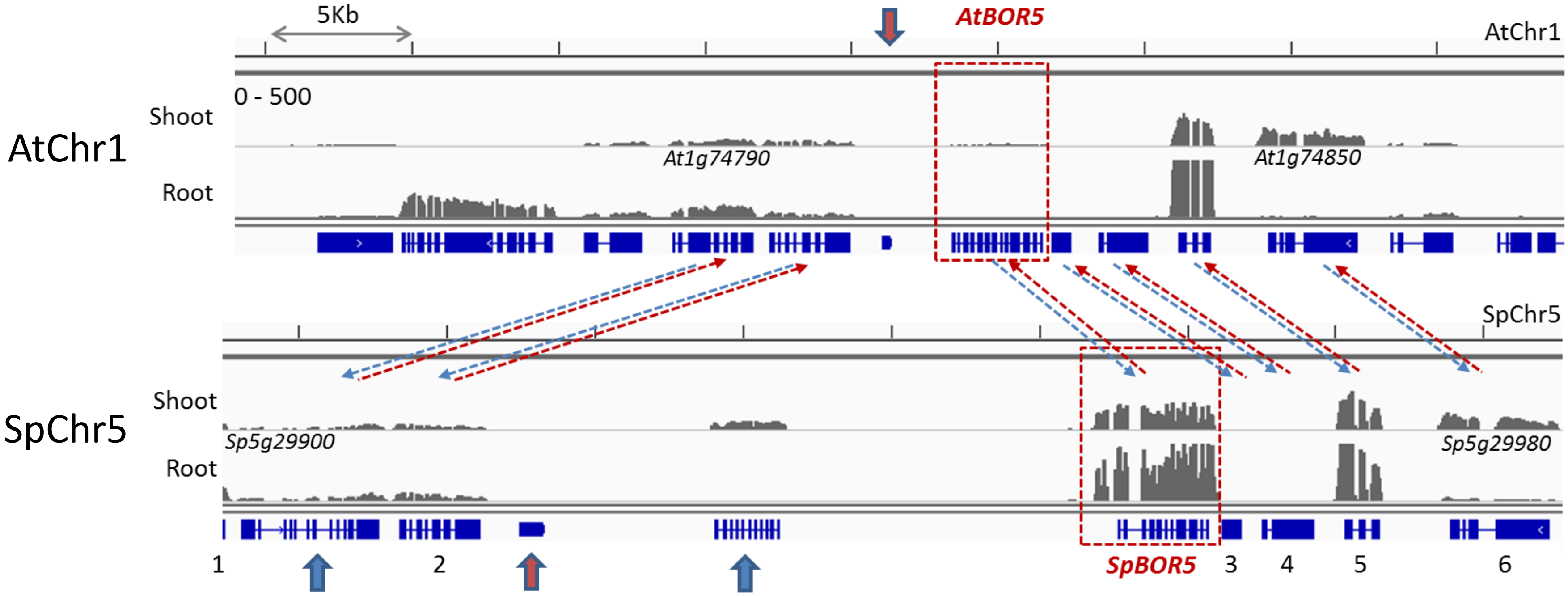
S. parvula-specific tandem duplication of *Na⁺/H⁺ EXCHANGER 8* (*NHX8*)?



(Oh *et al*., Plant Physiol 2014)

Comparison with a model species (i.e. *S. parvula* vs *A. thaliana*)

S. parvula-specific transposition near *BORON TRANSPORTER 5* (*BOR5*)?



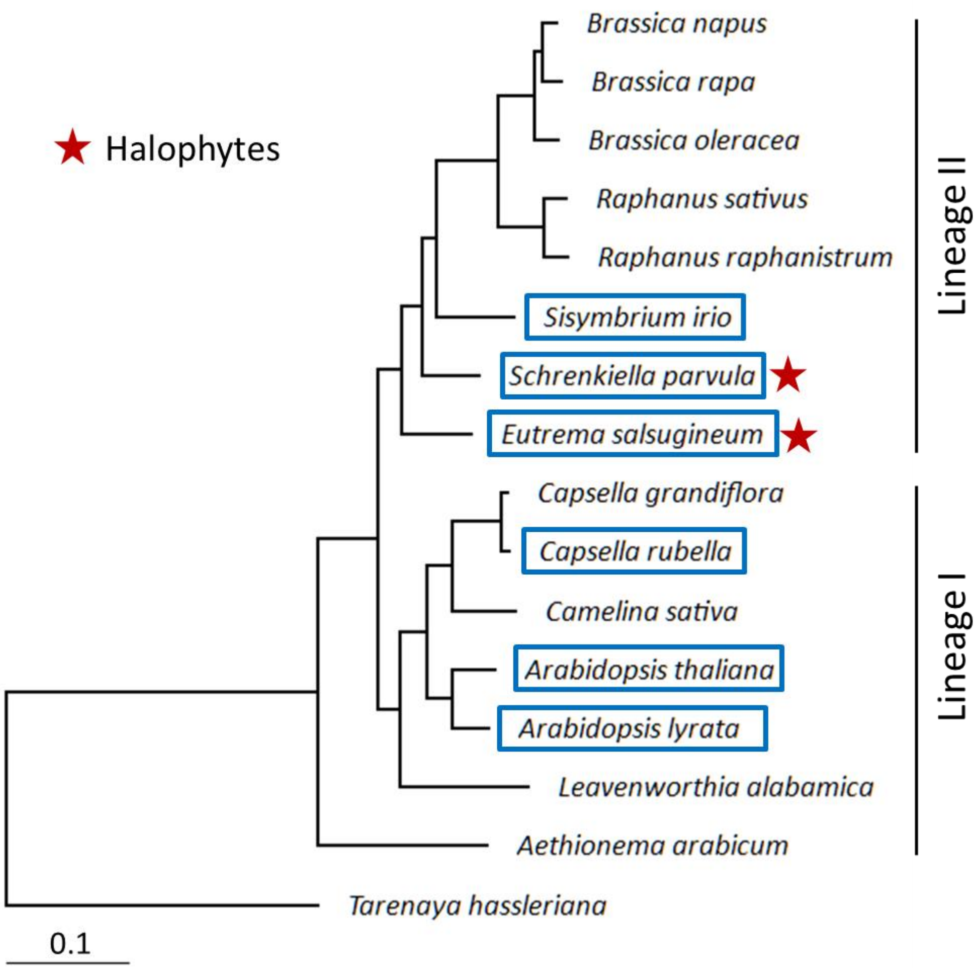
(Oh *et al.*, Plant Physiol 2014)

Criticisms for two-species comparison with a model plant:

Are these events really unique to *S. parvula*?

Expanding genome samples for comparative analyses

Publicly available Brassicaceae genomes, 2017 Spring



(Oh and Dassanayake, *DNA Res, 2018*)

Lineage I

🟦 Aly = *A. lyrata*

🟦 Ath = *A. thaliana*

🟩 Cru = *C. rubella*

Lineage II

🟨 Esa = *E. salsugineum*

🟧 Sir = *S. irio*

🟧 Spa = *S. parvula*

CLfinder-OrthNet

a pipeline to systematically compare multiple closely-related genomes

1. CLfinder (Co-Linearity finder)

   - summarizes annotation and detects tandem duplication (*td*) for each genome
   - detects co-linearity in gene orders in all pairs of genomes

Pair-wise comparisons by CLfinder – but this was not enough...

Summary of CLfinder results showing pairwise comparisons among 6 crucifer species

Query species	# protein-coding genes	CL type[a]	Target species						# TD[b] events (# TD genes)
			Aly	Ath	Cru	Esa	Sir	Spa	
Aly	32,657	*cl*		24,296	23,055	21,416	19,988	21,032	
		tr		4,881	5,375	6,668	8,104	6,478	2,163
		ls		2,876	3,611	3,954	3,902	4,530	(5,733)
		nd		604	616	619	663	617	
Ath	27,206	*cl*	23,436		22,683	21,187	19,821	20,851	
		tr	2,431		2,804	4,032	5,355	4,064	1,747
		ls	1,339		1,719	1,987	2,030	2,291	(4,770)
		nd	0		0	0	0	0	
Cru	26,521	*cl*	22,371	22,836		20,906	19,350	20,436	
		tr	3,036	2,836		4,338	5,817	4,267	1,752
		ls	950	666		1,112	1,154	1,646	(4,996)
		nd	164	183		165	200	172	
Esa	26,351	*cl*	20,384	20,884	20,460		19,699	20,612	
		tr	4,465	4,137	4,460		5,431	4,046	1,646
		ls	1,452	1,274	1,377		1,146	1,631	(4,461)
		nd	50	56	54		75	62	
Sir	32,524	*cl*	19,015	19,538	19,068	19,728		19,766	
		tr	3,062	2,860	2,998	2,722		2,697	1,795
		ls	5,520	5,148	5,496	5,054		4,225	(4,586)
		nd	4,927	4,978	4,962	5,020		5,836	
Spa	26,847	*cl*	19,849	20,358	19,934	20,380	19,546		
		tr	2,830	2,452	2,718	2,534	4,097		1,242
		ls	3,649	3,541	3,688	3,432	2,526		(3,049)
		nd	519	496	507	501	678		

[a] Co-linear (*cl*), transposed (*tr*), lineage-specific (*ls*), or not determined due to too small genome scaffold (*nd*)

[a] Using CLfinder parameters {window_size, num_CL_trshld, gap_CL_trshld} = { 20, 3, 20 }

[b] Using TDfinder parameter max_TD_loci_dist = 4

(Oh and Dassanayake, *DNA Res, 2018*)

CLfinder-OrthNet

a pipeline to systematically compare multiple closely-related genomes

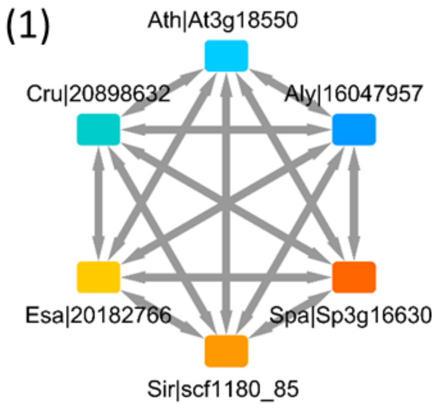
1. CLfinder (Co-Linearity finder)

2. ONfinder (OrthNet finder)

   - combines CLfinder results into networks of orthologs (OrthNet),
   - connects “best ortholog” and tandem duplicated (*td*) paralog nodes with the presence (*cl*) or absence (*tr*) of co-linearity as edge property,
   - *iterative Markov clustering (MCL) to “prune” and finalize OrthNets,
   - *searches and clusters OrthNets based on topology

* More on these: W1069 (Systems Genomics)

Encoding the evolutionary history of a locus as a network (OrthNet)

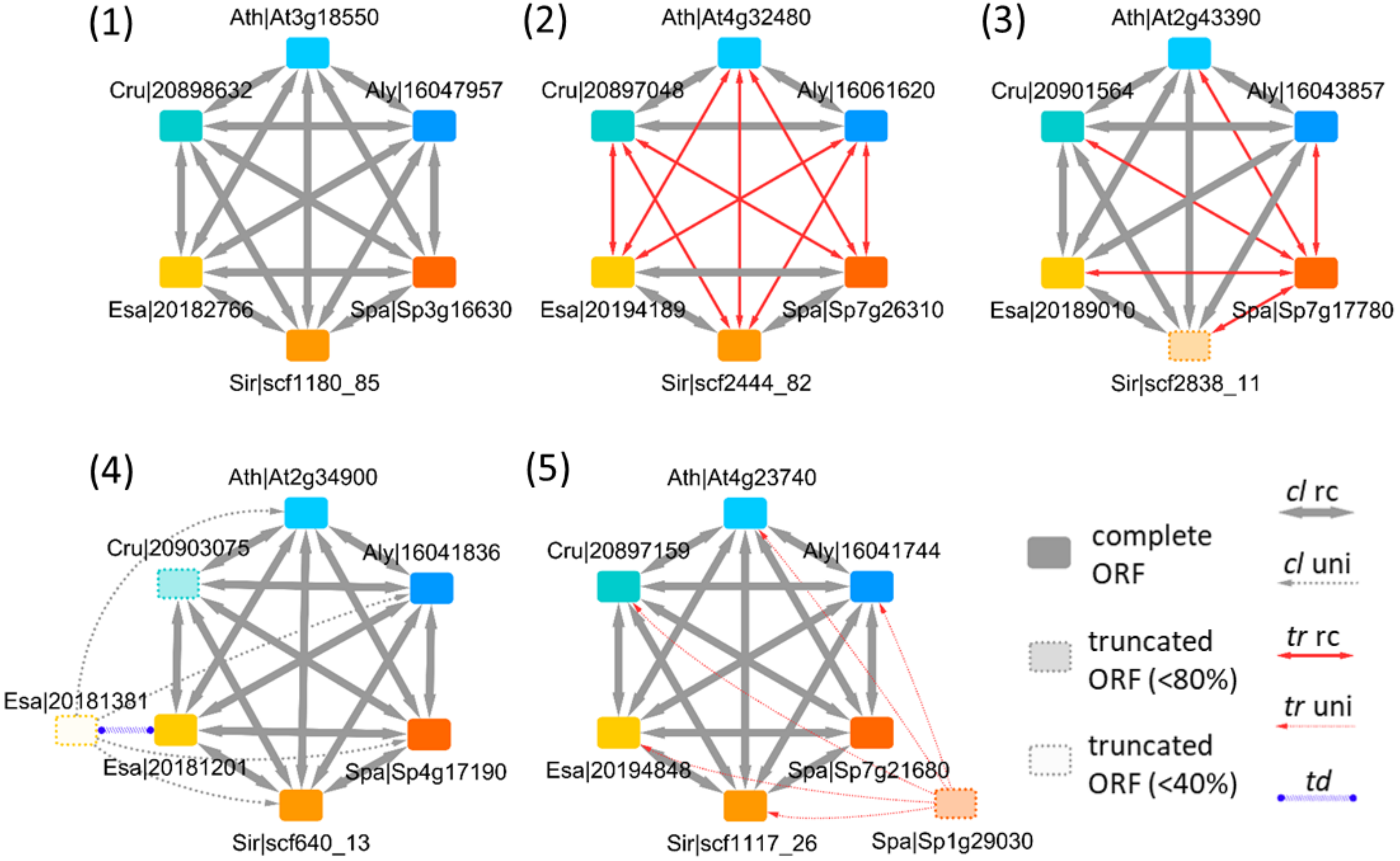


- ■ complete ORF
- ▨ truncated ORF (<80%)
- □ truncated ORF (<40%)

- *cl* rc ↔
- *cl* uni ←
- *tr* rc ↔
- *tr* uni ←
- *td* ●—●

(Oh and Dassanayake,
DNA Res, 2018)

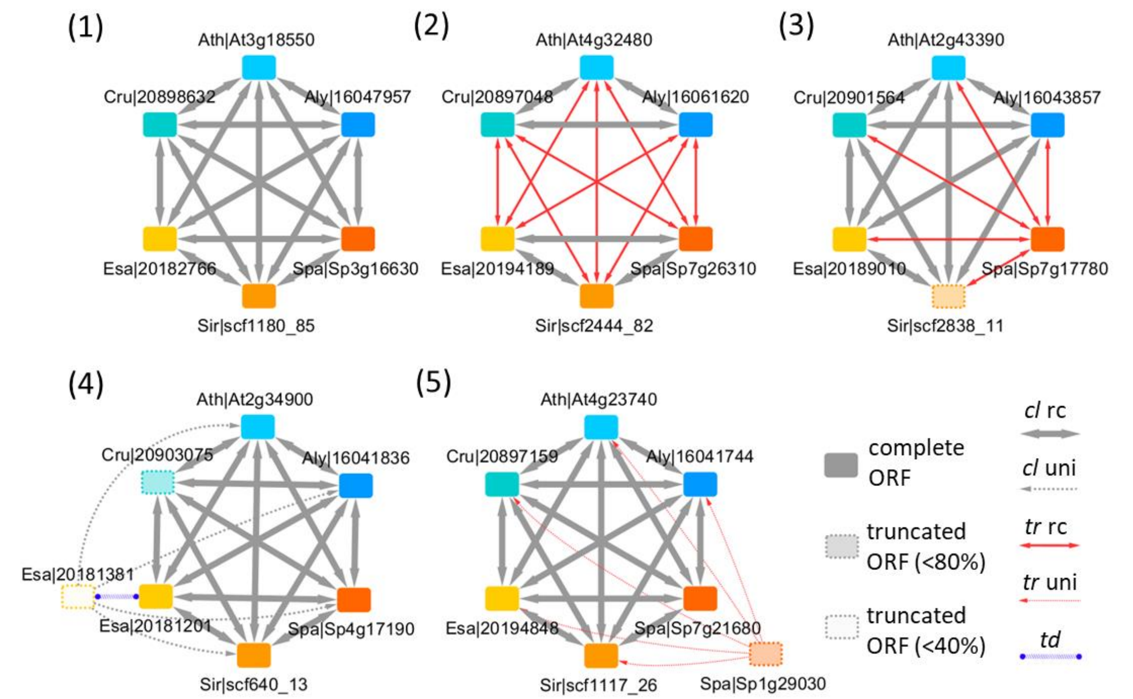
Encoding the evolutionary history of a locus as a network (OrthNet)



(Oh and Dassanayake, *DNA Res, 2018*)

Encoding the evolutionary history of a locus as a network (OrthNet)

enables detection of all orthologs showing a shared "evolutionary context/history"

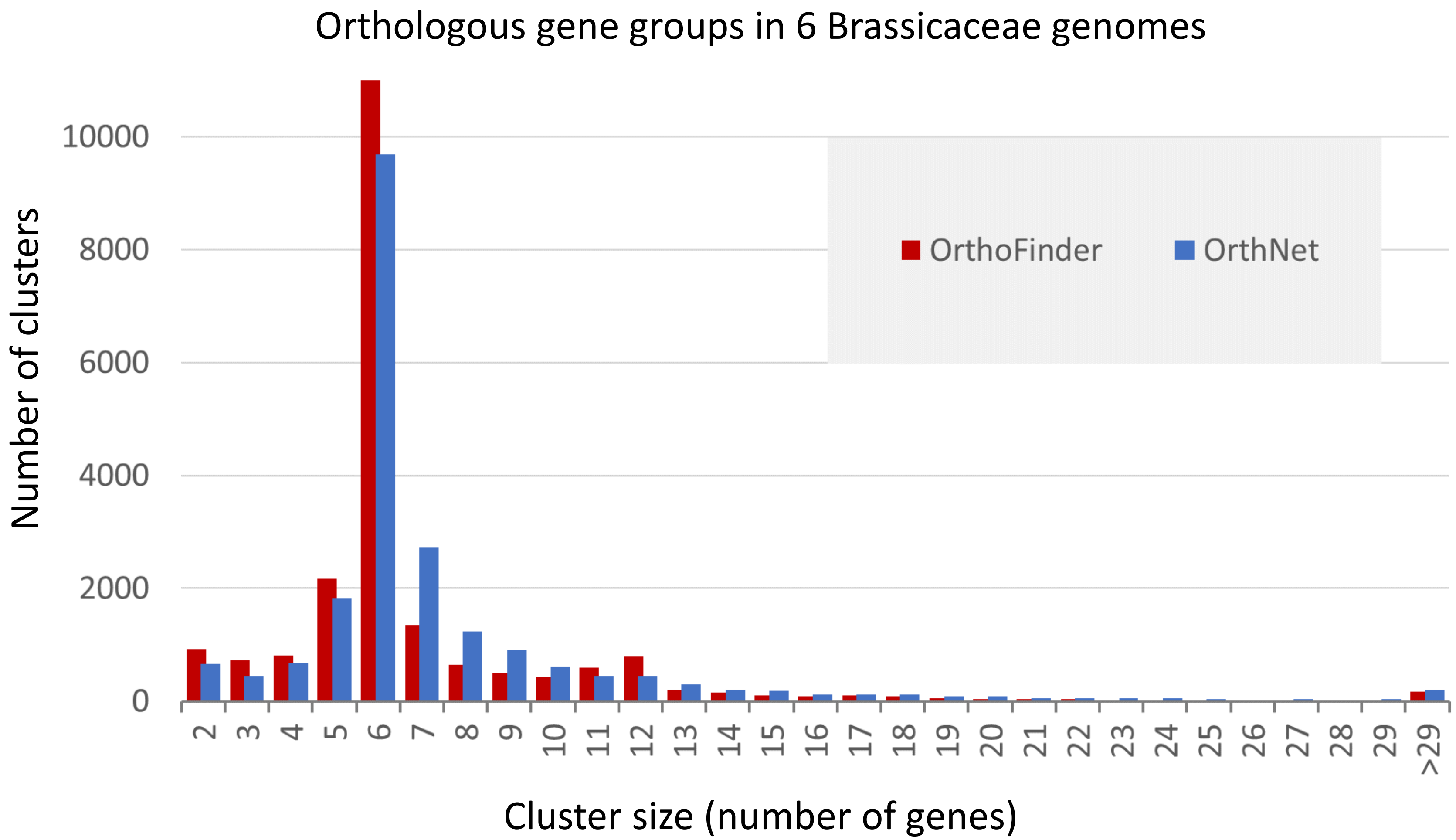


Evolutionary history	Number of OrthNets	Example OrthNet ID (Annotation)
(1) All co-linear, single copy	7,034	On_6361 (*BRANCHED 1*)
(2) Transposition (*tr*) between Lineage I and II	50	On_8867 (unknown function)
(3) Spa-specific transposition	12	On_12904 (unknown function)
(4) Esa-specific tandem duplication (*td*)	44	On_4974 (*IMBIBITION-INDUCIBLE 1*)
(5) Spa-specific transposition-duplication (*tr-d*)	70	On_4629 (LRR kinase family)

(Oh and Dassanayake, *DNA Res, 2018*)

Orthology inference using OrthNets (sequence similarity + co-linearity)

Comparison of OrthNet and OrthoFinder



Orthology inference using OrthNets (sequence similarity + co-linearity)

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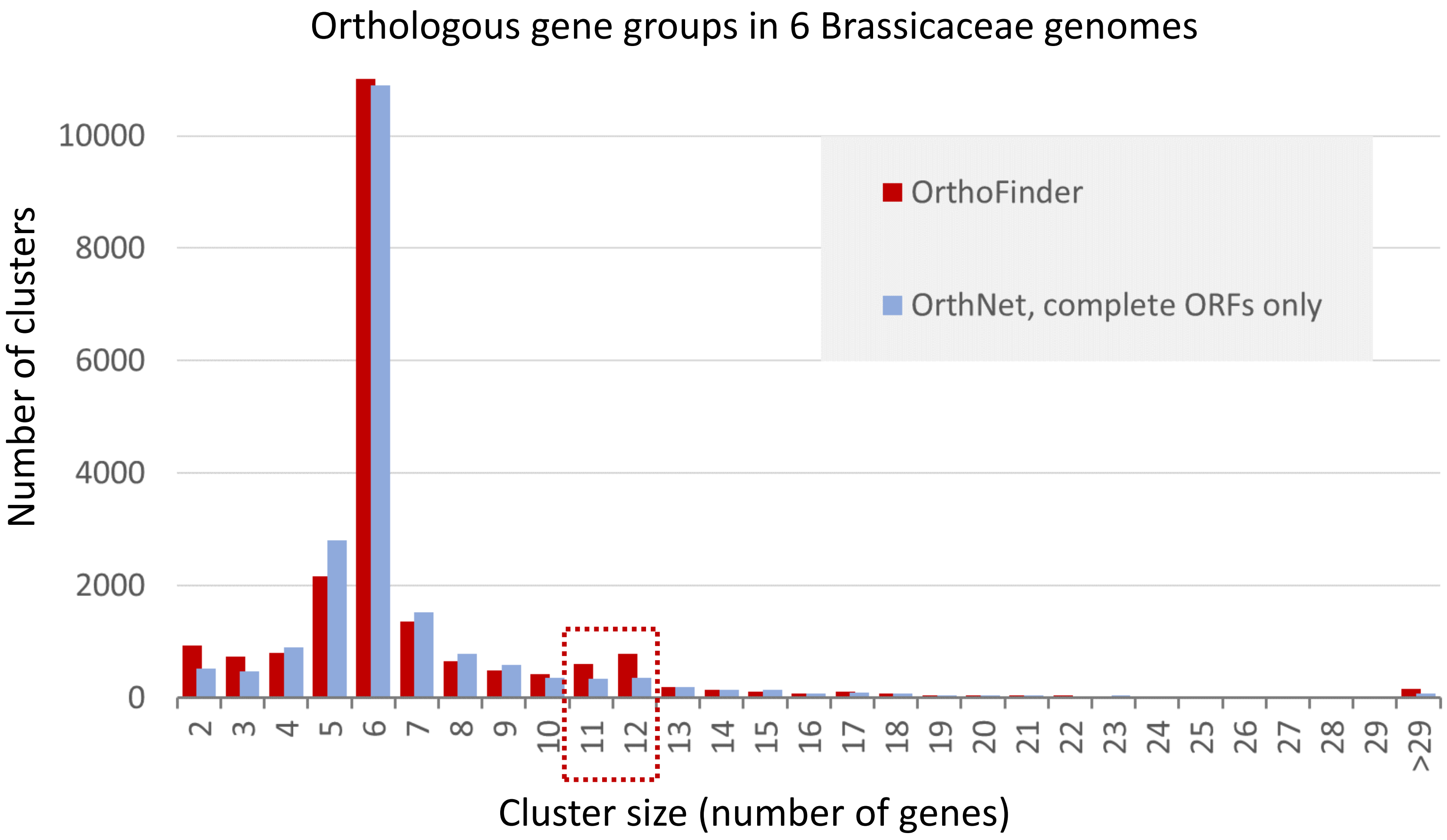
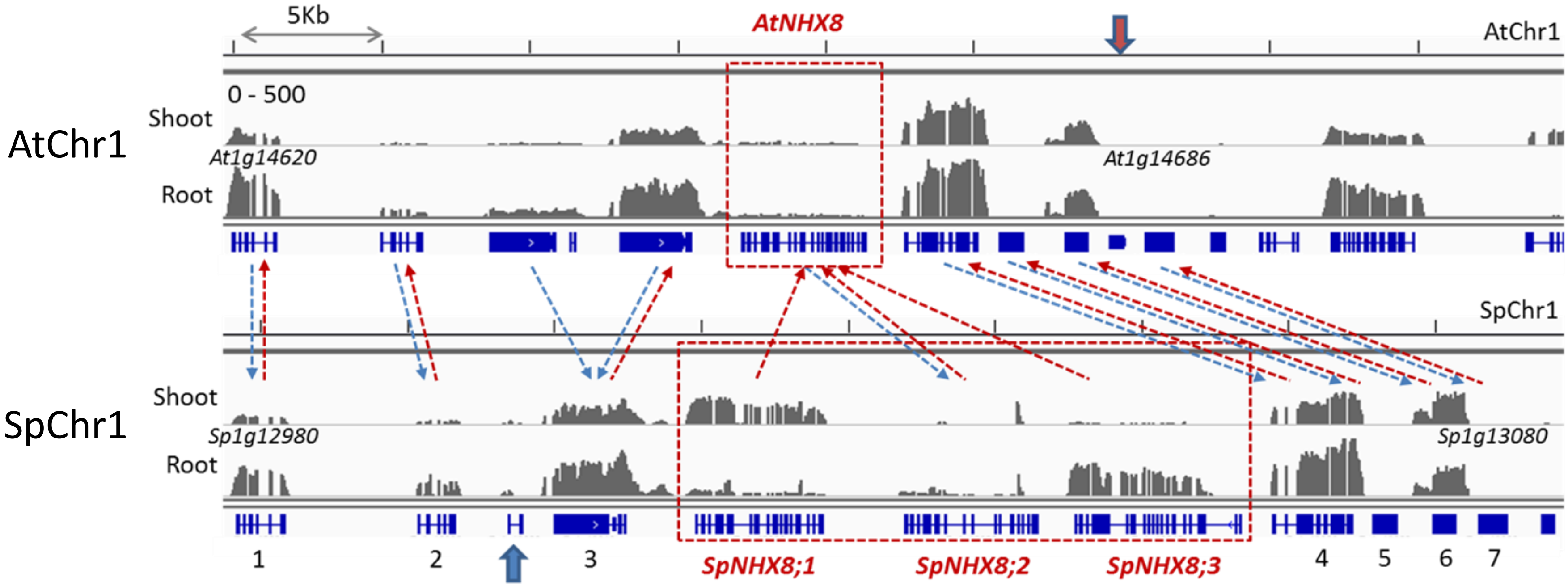


OrthNet reveals the evolutionary history of orthologous gene groups

How many of "*S. parvula*-specific" events really unique to *S. parvula*?

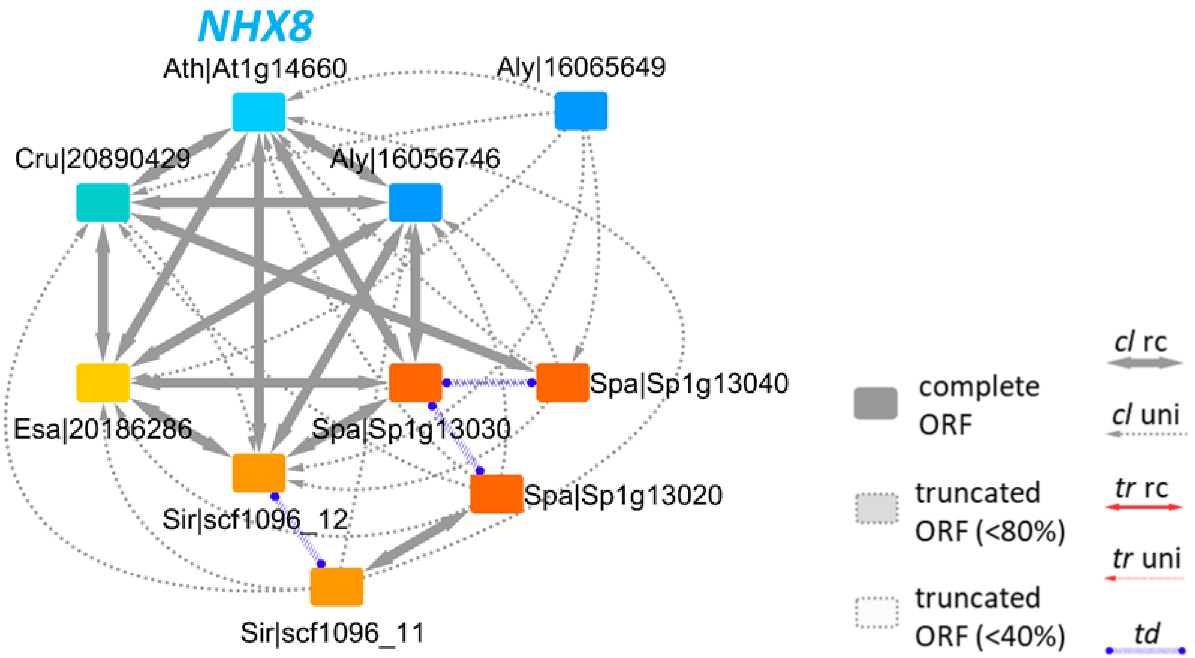


(Oh *et al*., Plant Physiol 2014)

Is this tandem duplication unique to *S. parvula*?

OrthNet reveals the evolutionary history of orthologous gene groups

How many of “*S. parvula*-specific” events really unique to *S. parvula*?



- *NHX8* tandem duplication was found in *S. irio* (Sir), too.
- However, only *S. parvula* contained three copies.

OrthNet reveals the evolutionary history of orthologous gene groups

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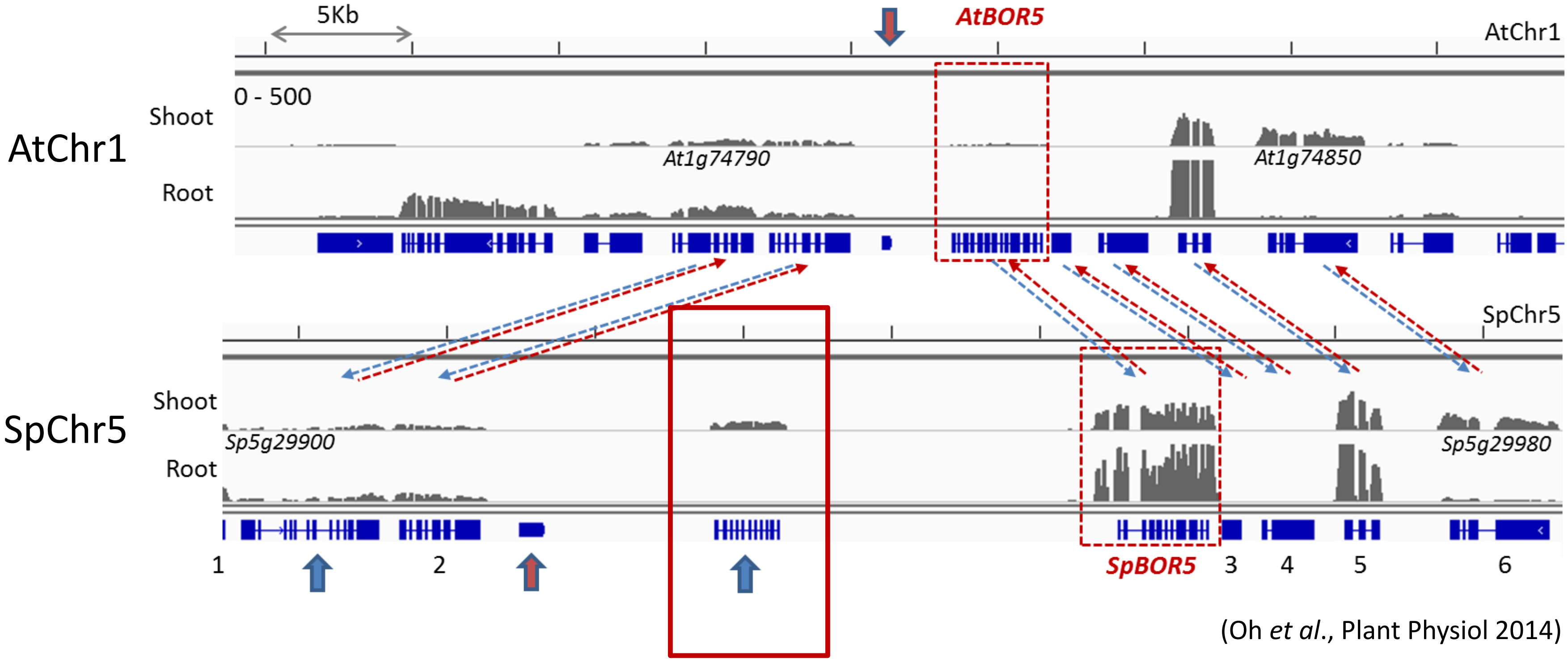


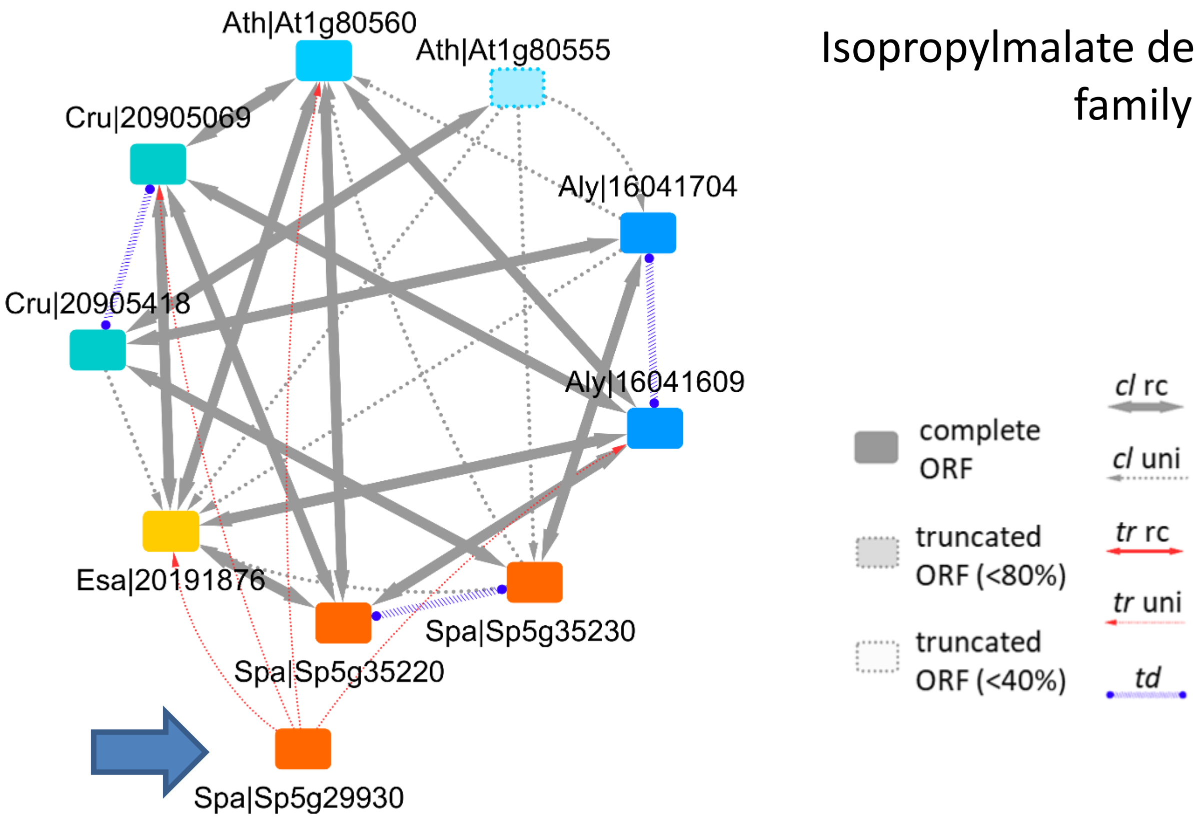
(Oh *et al.*, Plant Physiol 2014)

Is this transposition unique to *S. parvula*?

OrthNet reveals the evolutionary history of orthologous gene groups

How many of "*S. parvula*-specific" events really unique to *S. parvula*?

Isopropylmalate dehydrogenase family



Yes, the transposition-duplication (*tr-d*) of Sp5g29930 (the gene inserted at the 5' of SpBOR5) was unique to *S. parvula*.

OrthNet reveals the evolutionary history of orthologous gene groups

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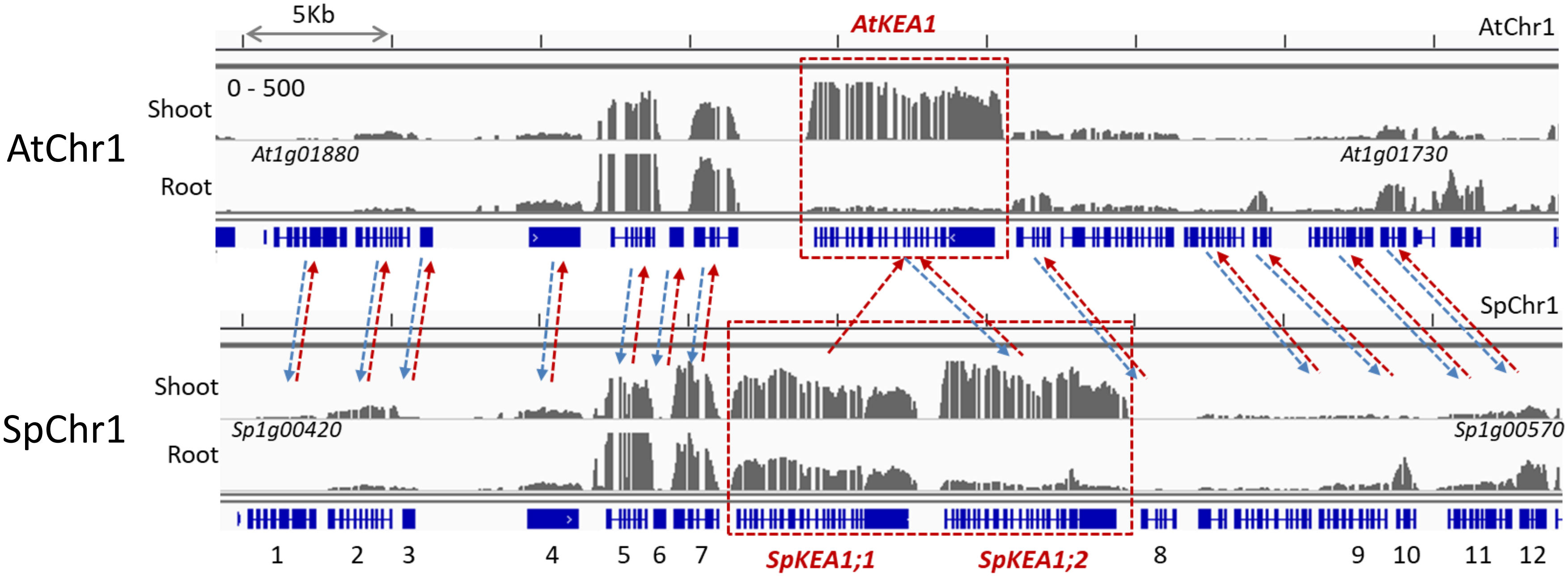

(Oh *et al.*, Plant Physiol 2014)

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OrthNet reveals the evolutionary history of orthologous gene groups

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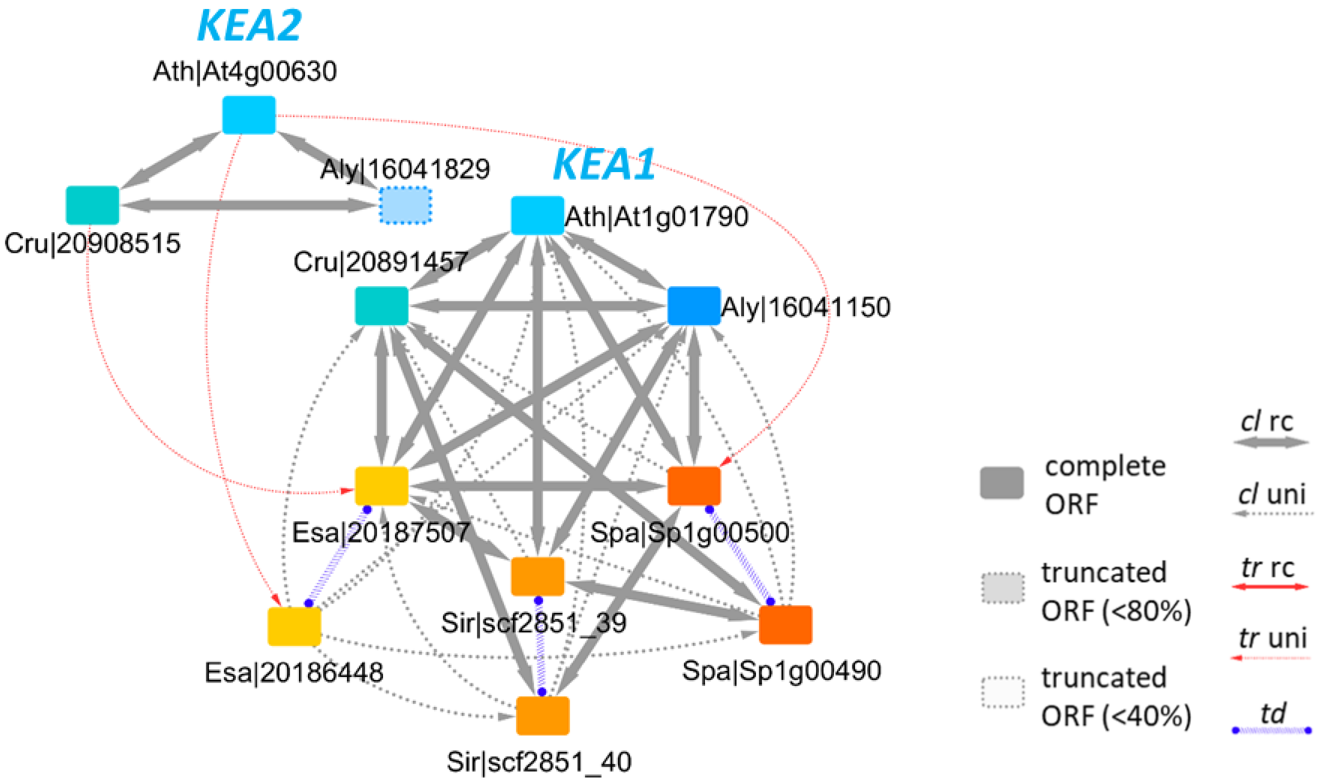
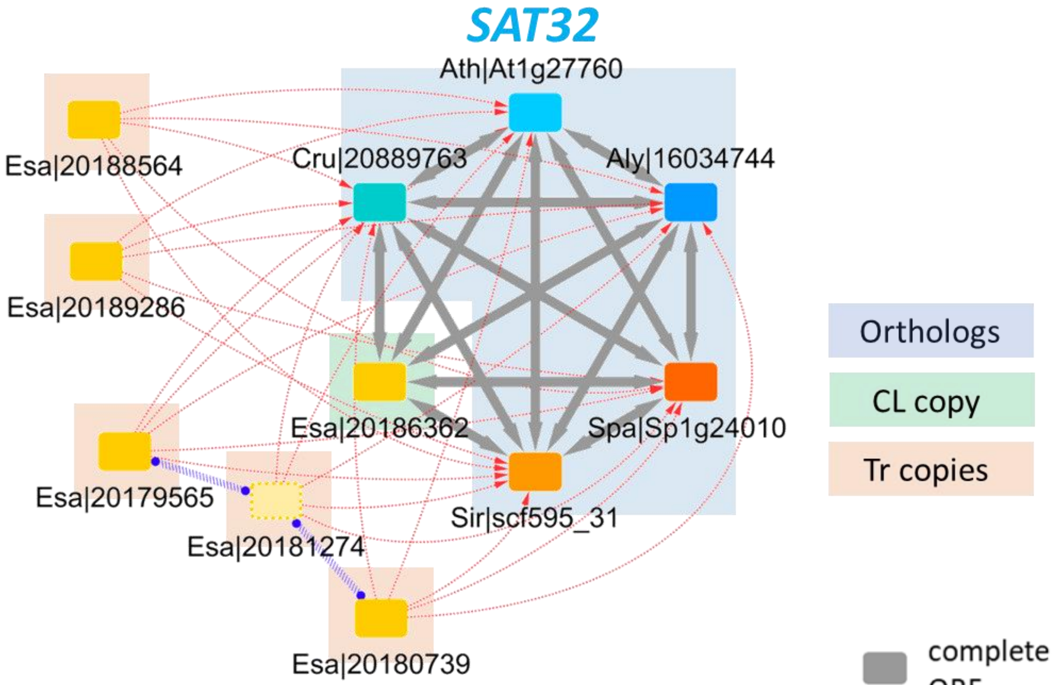
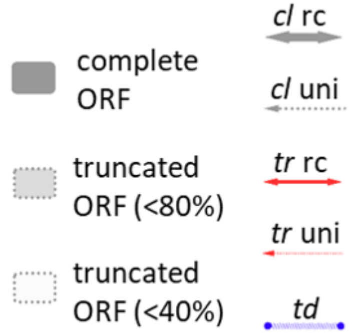


- *SpKEA1* tandem duplication was found in all Lineage II species.
- Lineage I species shared a transposition-duplication (*tr-d*) of *KEA1* (annotated as *KEA2*).

OrthNet reveals the evolutionary history of orthologous gene groups

SALT TOLERANCE 32 (*SAT32*): the largest *E. salsugineum*-specific *tr-d* event





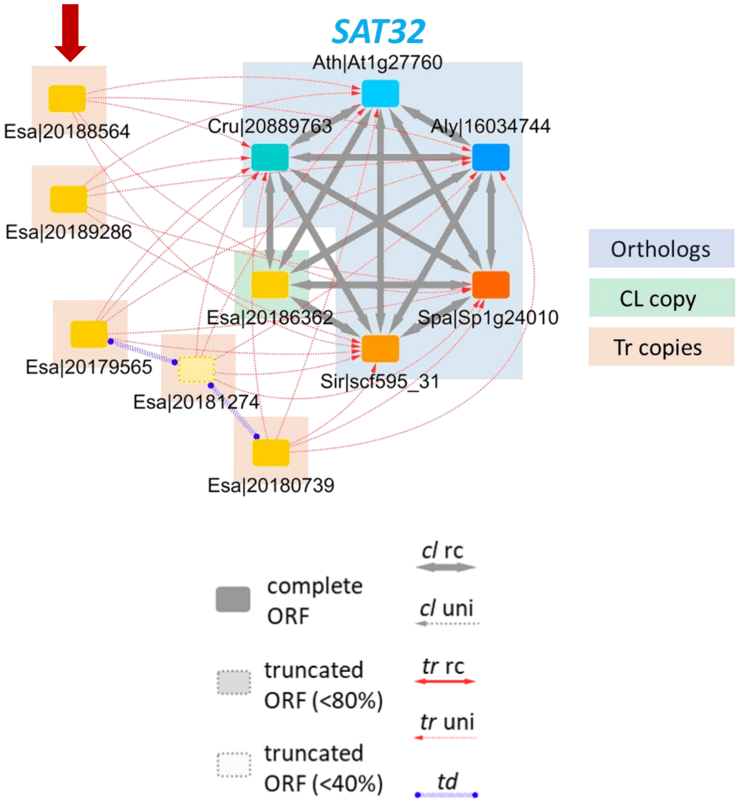
More detail on *tr-d* events in Brassicaceae:

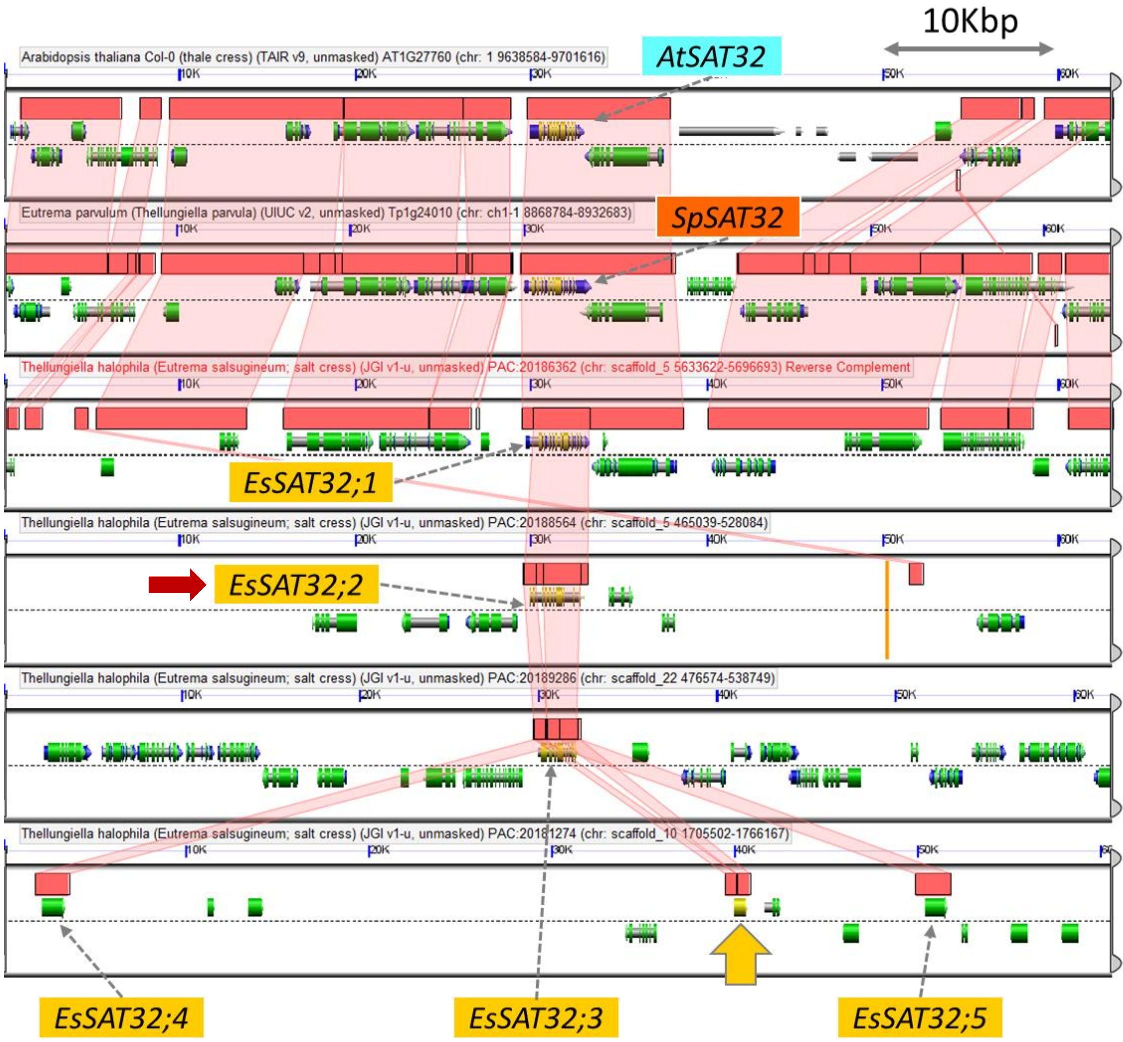
W1069 (Systems Genomics)

(Oh and Dassanayake, *DNA Res, 2018*)

OrthNet reveals the evolutionary history of orthologous gene groups

SALT TOLERANCE 32 (*SAT32*): the largest *E. salsugineum*-specific *tr-d* event

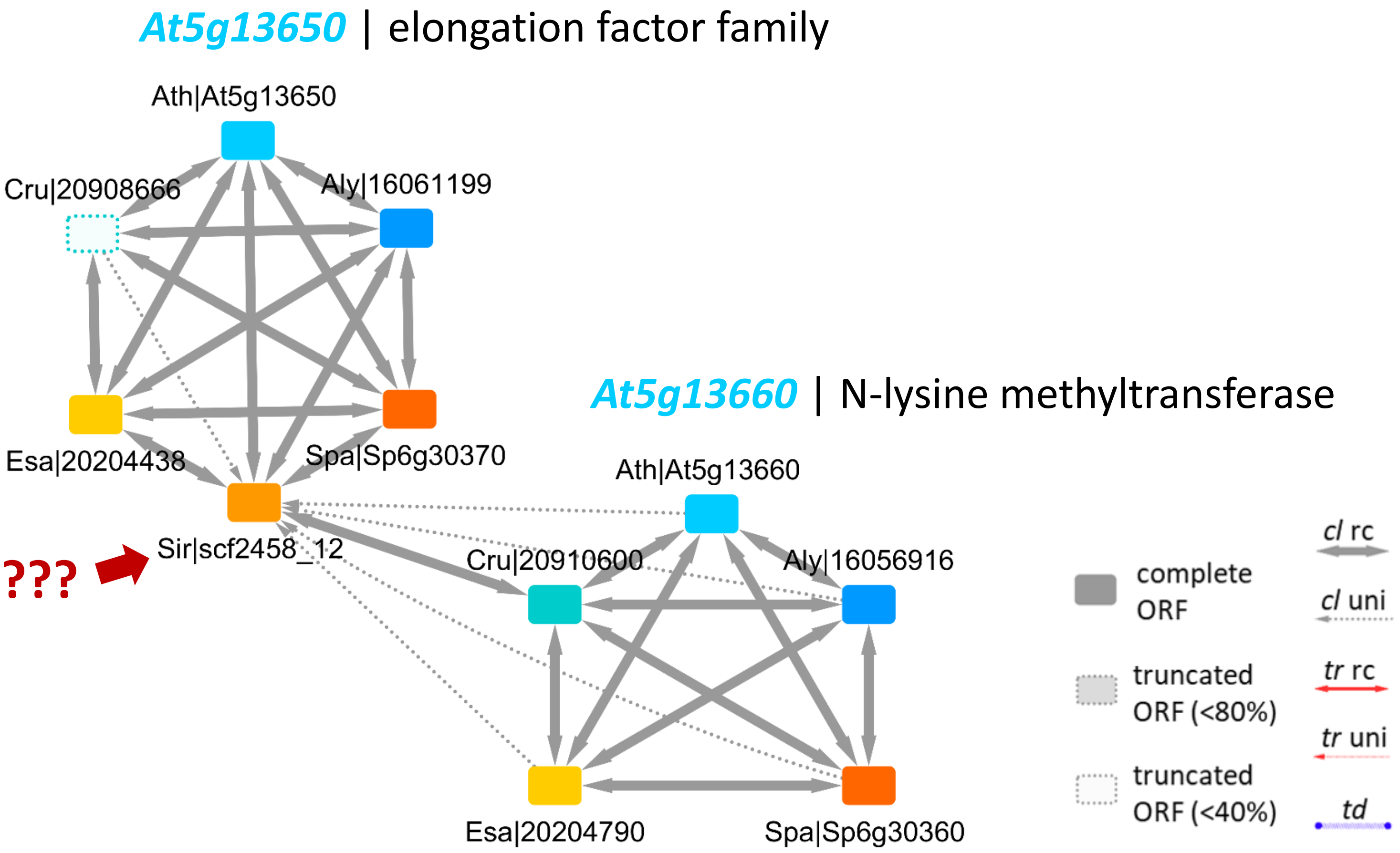



10Kbp
Arabidopsis thaliana Col-0 (thale cress) (TAIR v9, unmasked) AT1G27760 (chr: 1 9638584-9701616)
AtSAT32
Eutrema parvulum (Thellungiella parvula) (UIUC v2, unmasked) Tp1g24010 (chr: ch1-1 8868784-8932683)
SpSAT32
Thellungiella halophila (Eutrema salsugineum; salt cress) (JGI v1-u, unmasked) PAC:20186362 (chr: scaffold_5 5633622-5696693) Reverse Complement
EsSAT32;1
Thellungiella halophila (Eutrema salsugineum; salt cress) (JGI v1-u, unmasked) PAC:20188564 (chr: scaffold_5 465039-528084)
EsSAT32;2
Thellungiella halophila (Eutrema salsugineum; salt cress) (JGI v1-u, unmasked) PAC:20189286 (chr: scaffold_22 476574-538749)
Thellungiella halophila (Eutrema salsugineum; salt cress) (JGI v1-u, unmasked) PAC:20181274 (chr: scaffold_10 1705502-1766167)
10K
20K
30K
40K
50K
60K
EsSAT32;4
EsSAT32;3
EsSAT32;5

(Oh and Dassanayake, *DNA Res, 2018*)

An (unexpected) application: detecting “chimeric” gene models

A characteristic OrthNet topology caused by fused genes

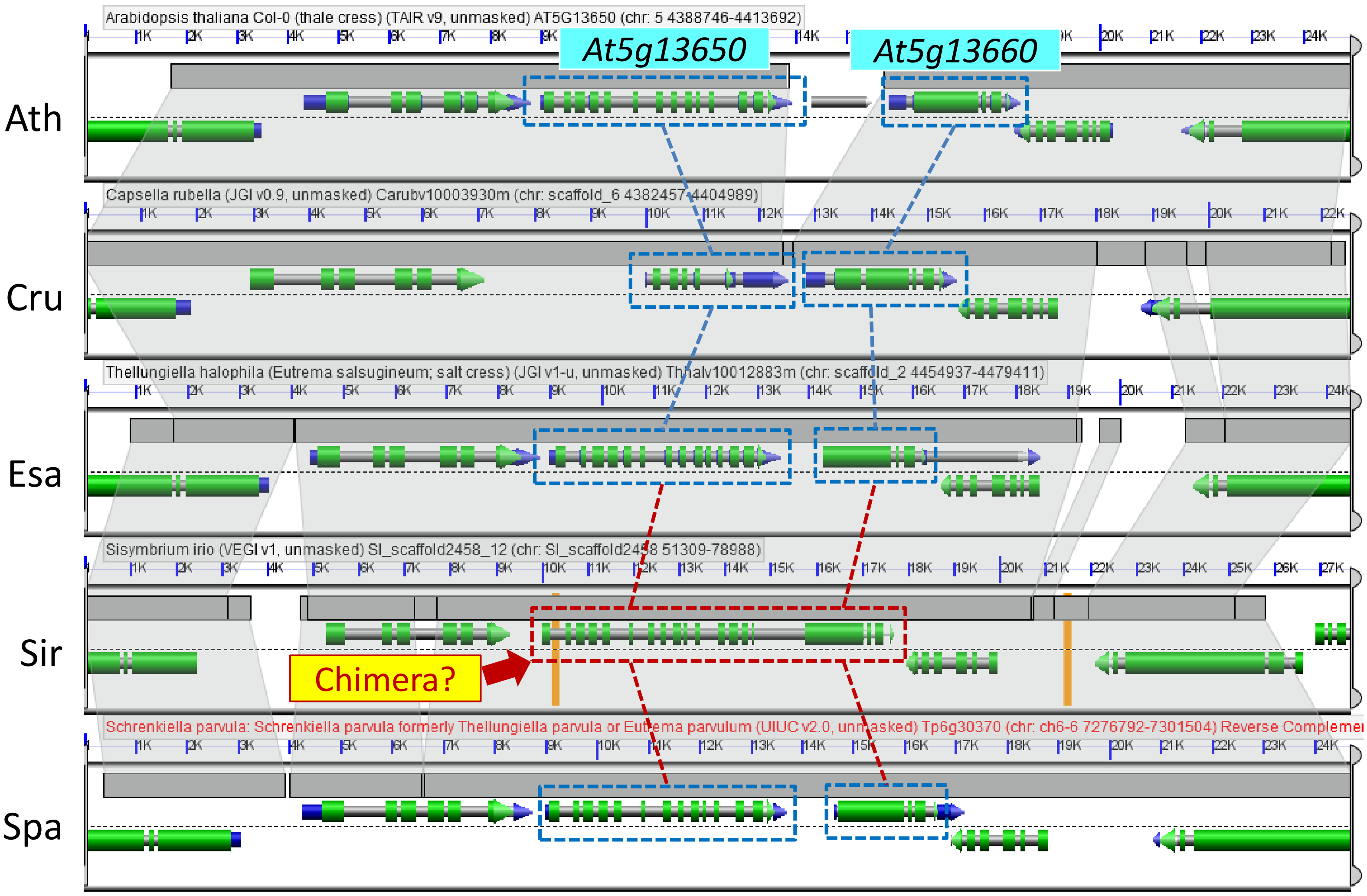


At5g13650 | elongation factor family

At5g13660 | N-lysine methyltransferase

An (unexpected) application: detecting “chimeric” gene models

A characteristic OrthNet topology caused by fused genes



Arabidopsis thaliana Col-0 (thale cress) (TAIR v9, unmasked) AT5G13650 (chr: 5 4388746-4413692)

Capsella rubella (JGI v0.9, unmasked) Carubv10003930m (chr: scaffold_6 4382457-4404989)

Thellungiella halophila (Eutrema salsugineum; salt cress) (JGI v1-u, unmasked) Thhalv10012883m (chr: scaffold_2 4454937-4479411)

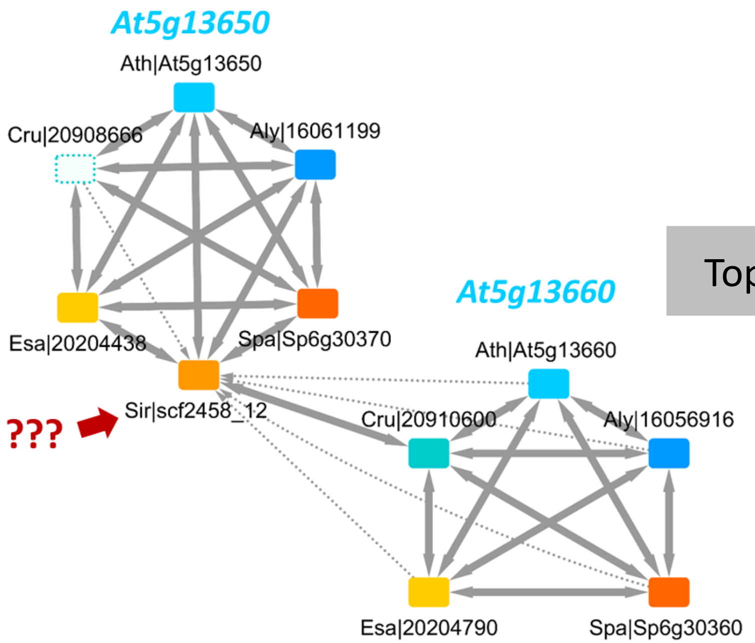
Sisymbrium irio (VEGI v1, unmasked) SI_scaffold2458_12 (chr: SI_scaffold2458 51309-78988)

Schrenkiella parvula: Schrenkiella parvula formerly Thellungiella parvula or Eutrema parvulum (UIUC v2.0, unmasked) Tp6g30370 (chr: ch6-6 7276792-7301504) Reverse Complemen

https://genomevolution.org/r/15k8l

An (unexpected) application: detecting “chimeric” gene models

A characteristic OrthNet topology caused by fused genes



“Chimera” OrthNet topology =

Multiple functionally unrelated, often adjacent, genes captured in the same OrthNet, due to a node in a single (or a subset of) species.

Topology search

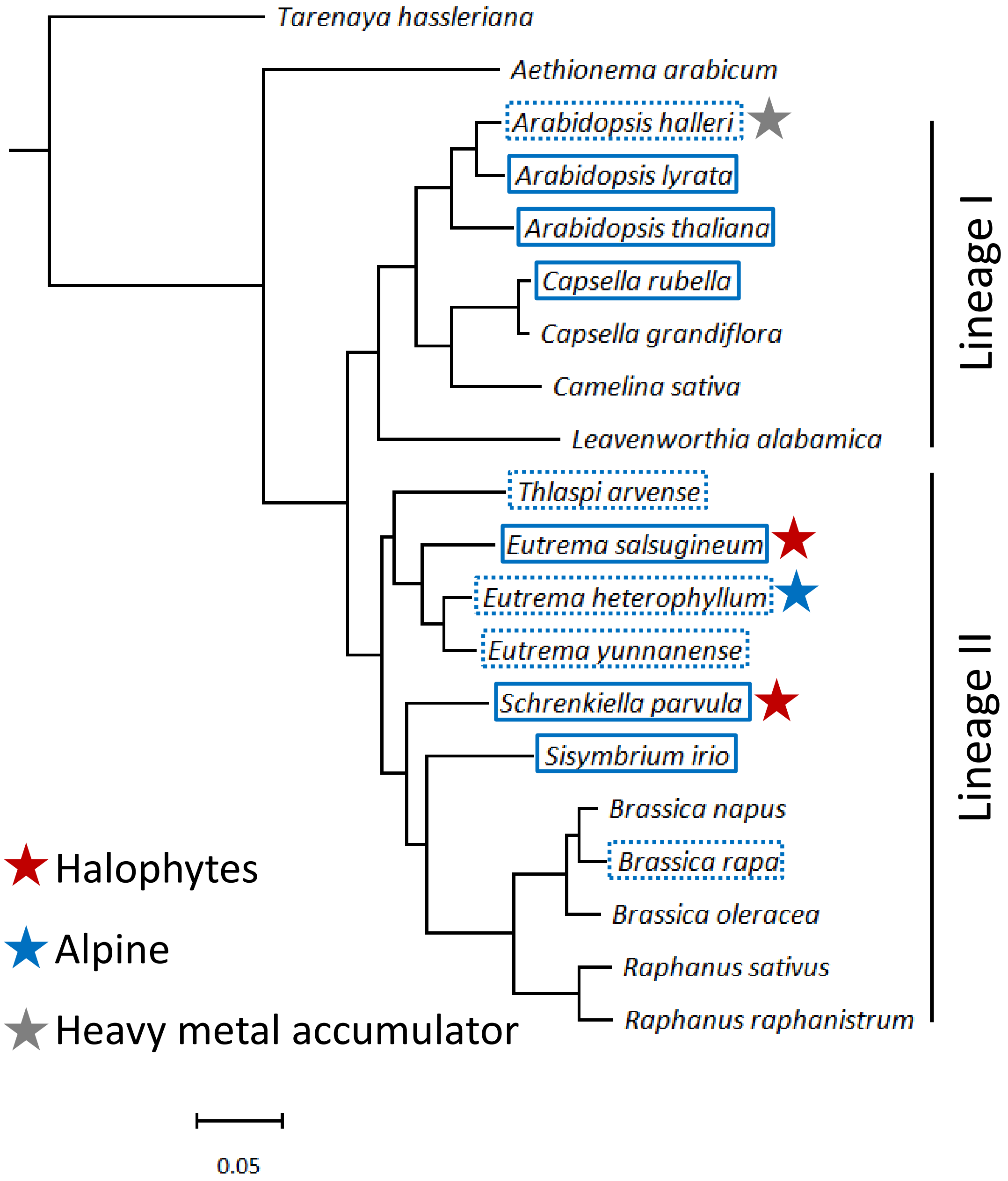
Species	Genome annotation	# of chimeric gene models
Aly	Phytozome 107	47
Ath	Araport11	0
Cru	Phytozome 183	11
Esa	Phytozome 173	8
Sir	ver. 0.2	**285**
Spa	ver. 2.1	10

RNA-Seq or Iso-Seq

Improved gene models

(or confirming a gene fusion event)

Brassicaceae genomes 2018 Fall (part)



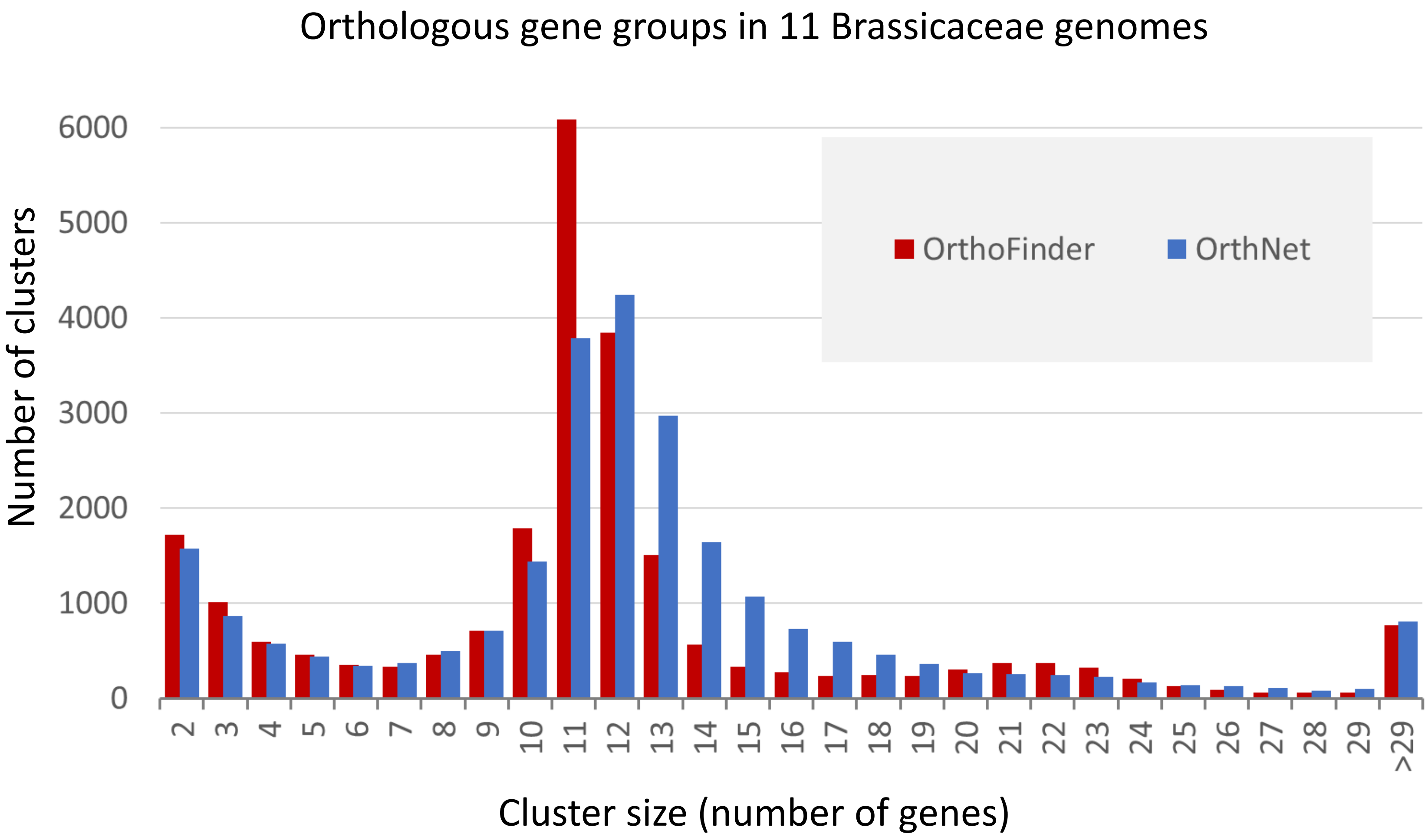
(Guo et al., *DNA Res*, 2018)

Ehe = *E. heterophyllum*

Ehe = *E. yunnanense*

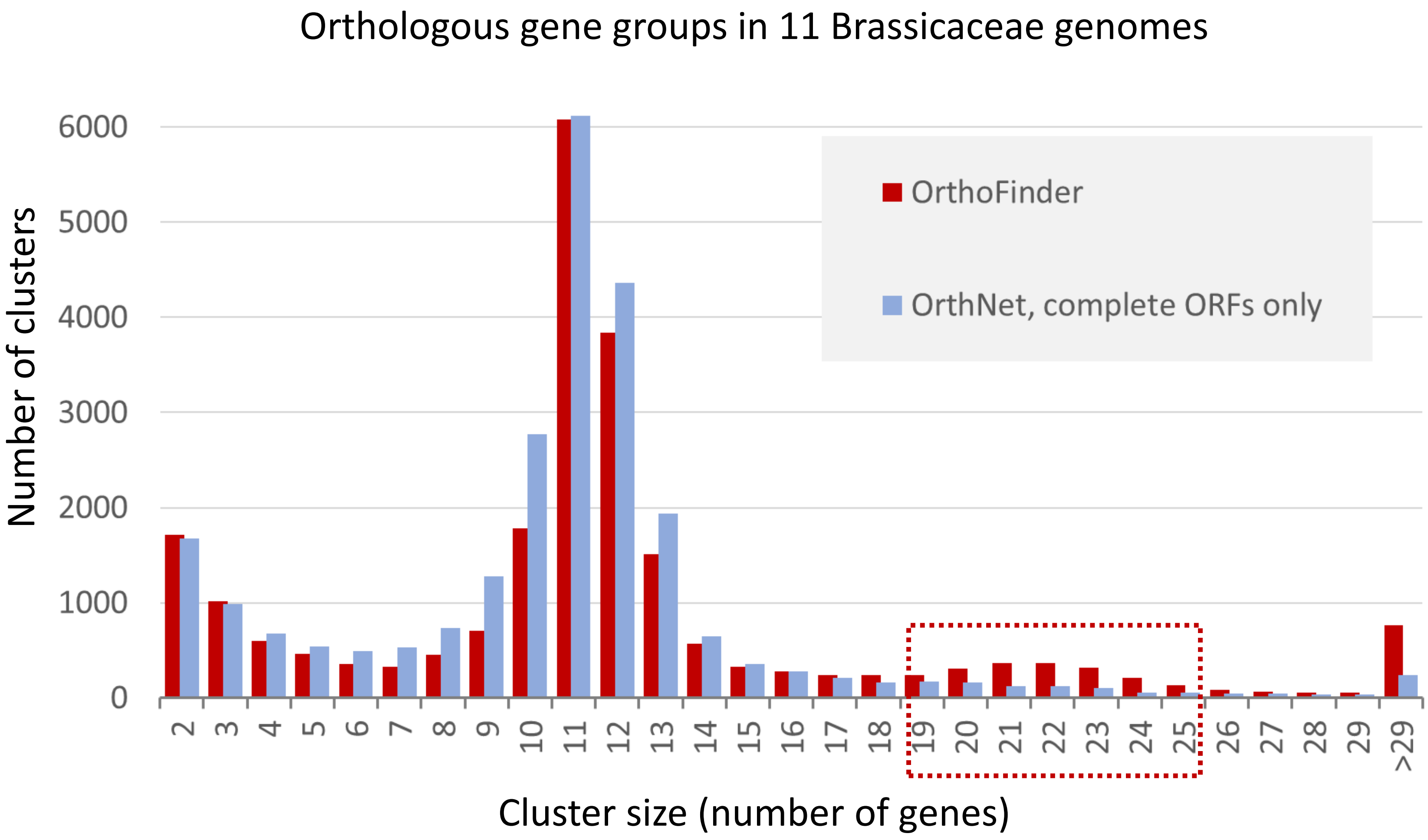
Orthology inference using OrthNets (sequence similarity + co-linearity)

Comparison of OrthNet and OrthoFinder



Orthology inference using OrthNets (sequence similarity + co-linearity)

Comparison of OrthNet and OrthoFinder



Conclusions

- OrthNet encodes evolutionary histories of each gene locus
- OrthNet complements existing tools (e.g. OrthoFinder) in inferring orthology.*
- OrthNet detects truncated and chimeric gene models.
- CLfinder-OrthNet prepares a newly annotated genome (or a set of genomes) into a comparative genomics framework.

*More in W1069 (Systems Genomics)
C20 (Digital Tools and Resources)
Poster #56

Thank you!

Dassanayake Lab.
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