



Divergence of satellite DNA in the *Cannabaceae* family

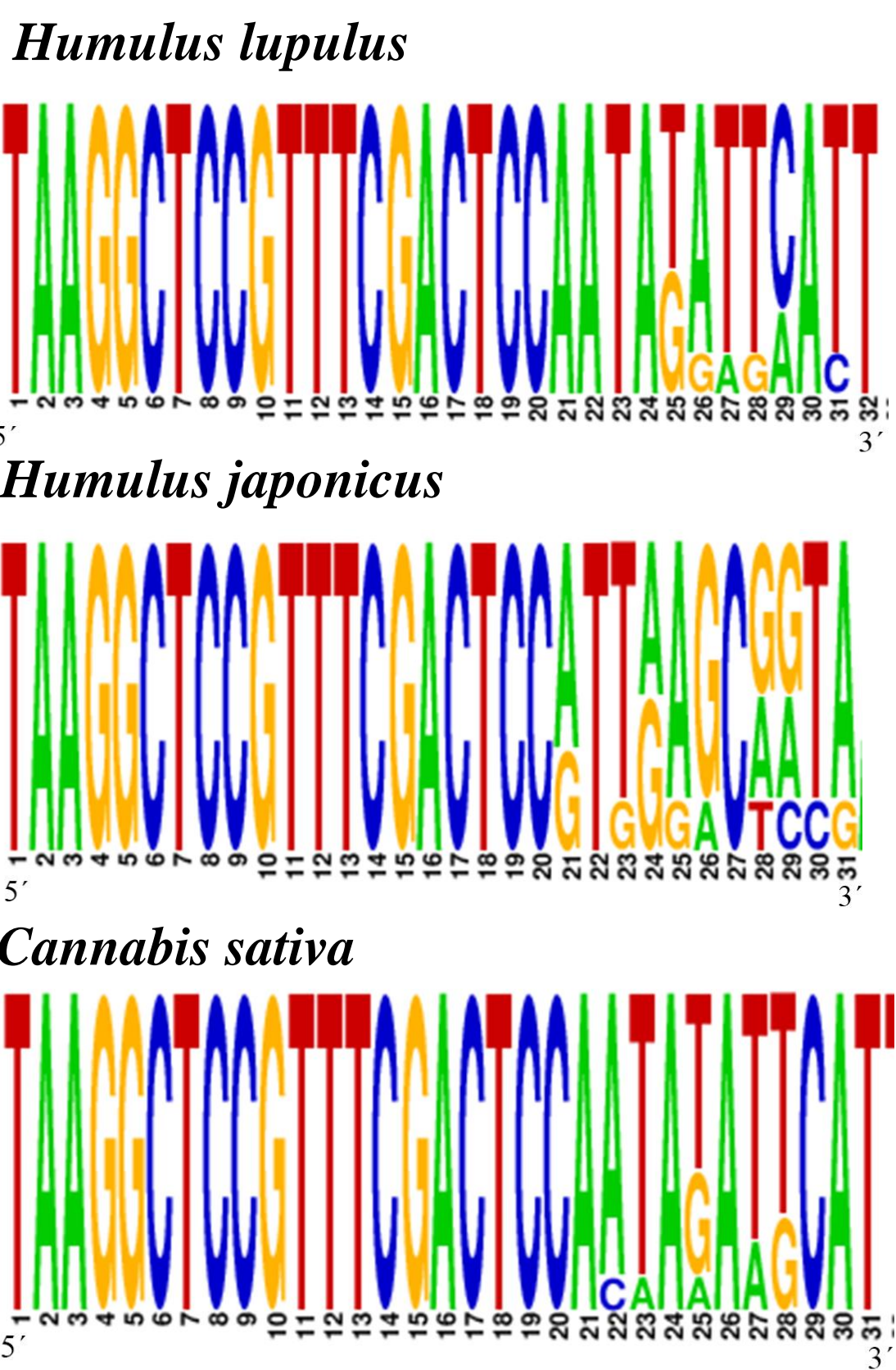
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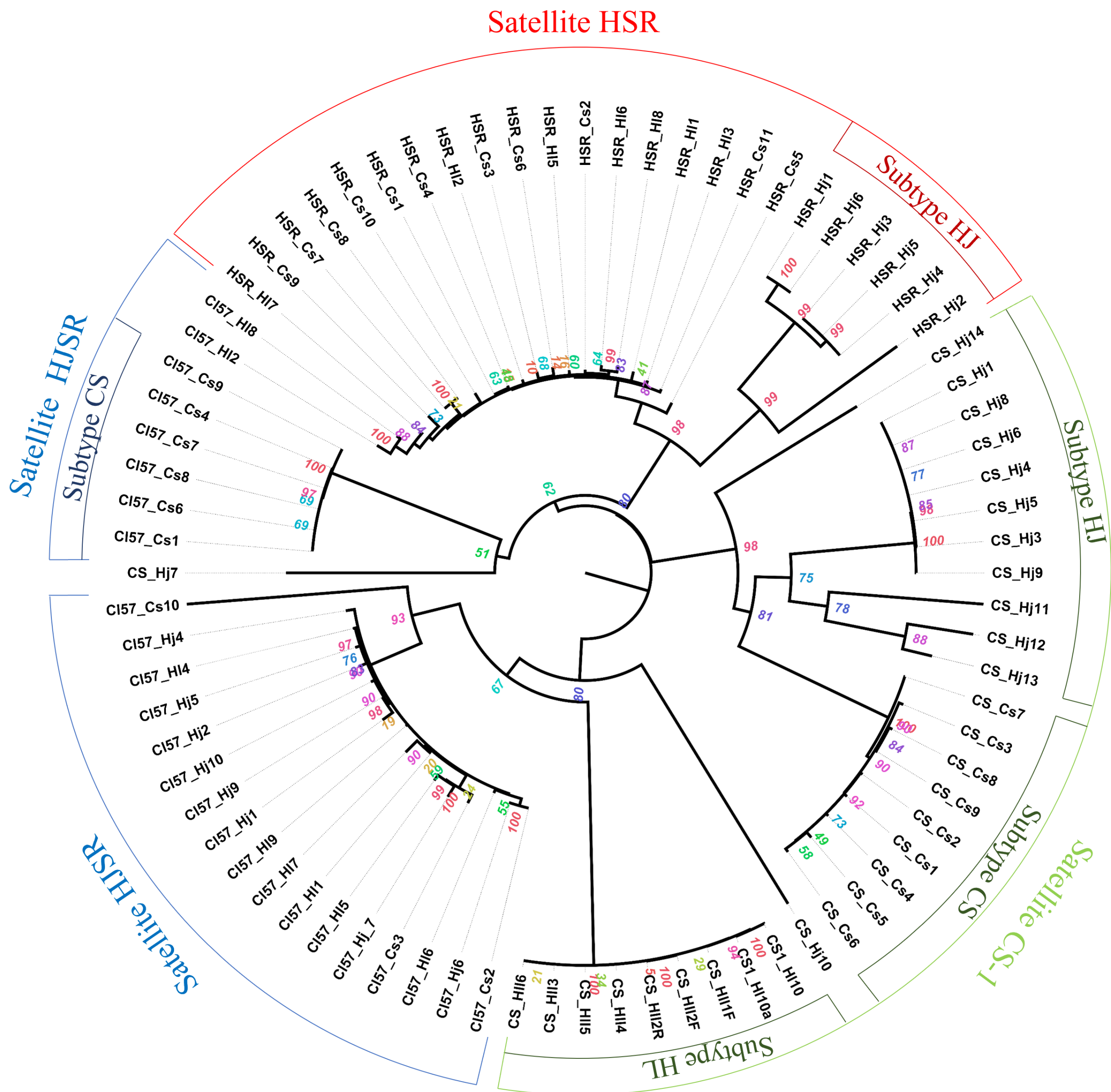
- Humulus lupulus*, *Humulus japonicus*, and *Cannabis sativa* are dioecious species from the *Cannabaceae* family that differ in **genome size**, **chromosome number**, **sex chromosome system**, and life cycle strategy.
- The divergence between the *Humulus* group and *C. sativa* is estimated at **21 - 25.4 Mya**. *H. lupulus* and *H. japonicus* lineages split **6.38 Mya**.
- Subtelomeric satellites **HSR¹** (in *H. lupulus*), **HJSR²** (in *H. japonicus*), and **CS-1³** (in *C. sativa*) are evolutionary conserved sequences and fundamental cytogenetic markers for differentiating autosomes and sex chromosomes.
- Inter-species divergence, evolution, and genomic localization of HSR, HJSR, and CS-1 satellites in genomes of studied species (*H. lupulus*, *H. japonicus*, and *C. sativa*) is unknown.

Sequence variability of HSR satellite in studied species



- Analysis of HSR satellite showed conserved regions shared in all three studied species.
- We observed more variable regions of HSR satellite in *H. japonicus*.

Phylogenetic analysis of HSR, HJSR, and CS-1 satellites



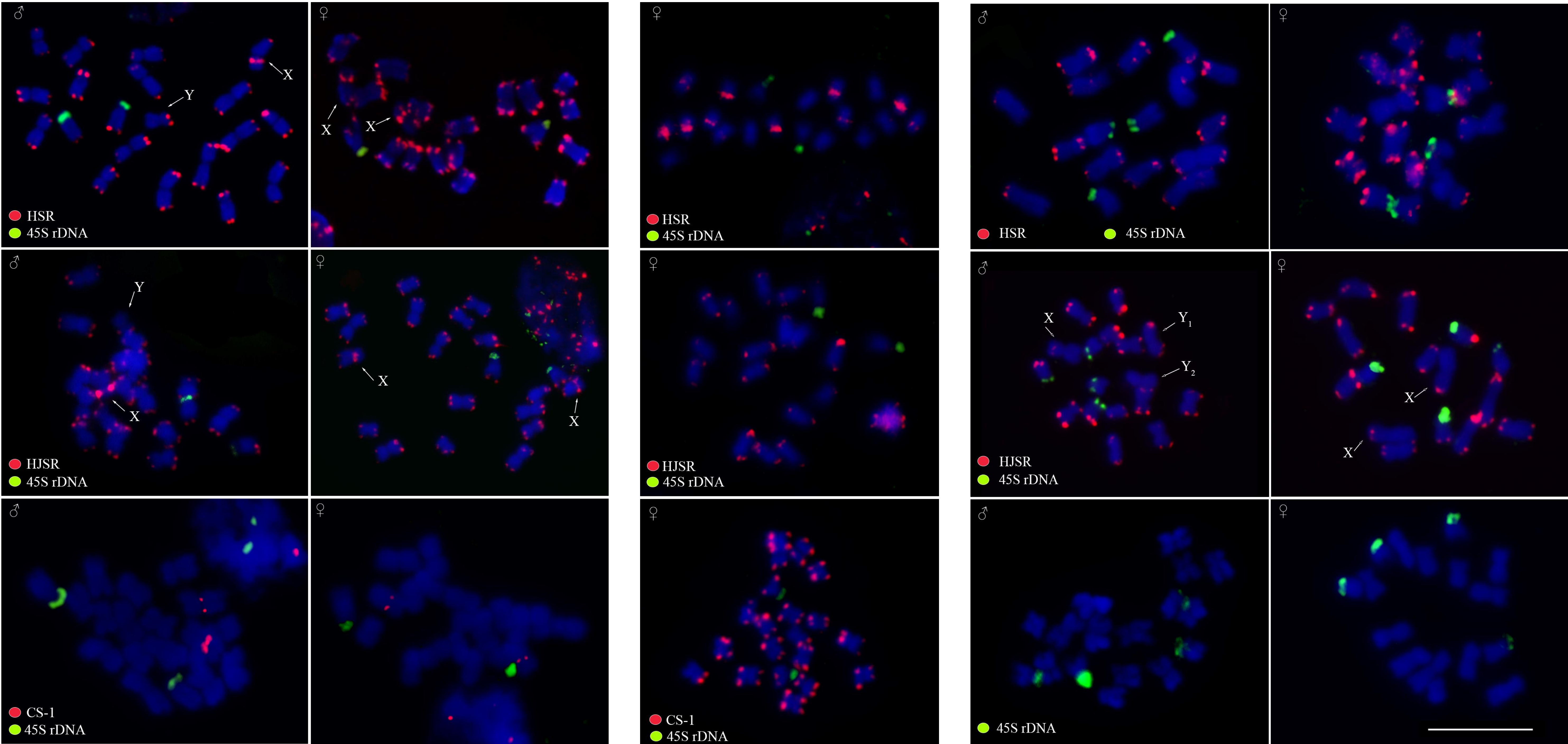
- The most **conserved units** were found in **HSR** satellite between *H. lupulus* and *C. sativa*. Interestingly, in *H. japonicus* shows specific subtype.
- We found **species-specific subtypes** of satellite CS-1 for *H. lupulus*, *H. japonicus* and *C. sativa*.
- Within cluster HJSR, we found a **specific subtype** for *C. sativa*.

Distribution of HSR, HJSR, and CS-1 satellites on metaphase chromosomes

Humulus lupulus (2n = 18 + XX/XY)

Cannabis sativa (2n = 18 + XX/XY)

Humulus japonicus (2n = 14 + XX/XY₁Y₂)



- HSR and HJSR satellites are distributed in **subtelomeric** regions on *H. lupulus* and *H. japonicus* chromosomes. HJSR satellite is localized in subtelomeric regions of *C. sativa* chromosomes.
- HSR satellite is localized in **pericentromeric** regions in *C. sativa* as well as CS-1 satellite in *H. lupulus*.
- CS-1 satellite shows a **subtelomeric** pattern on *C. sativa* chromosomes, this cluster has low abundance in *H. japonicus* genome.

Summary

- We compared DNA sequence subunit similarity for common subtelomeric satellites – HSR, HJSR, and CS-1 in *H. lupulus*, *H. japonicus*, and *C. sativa*. We identified their inter-species localization on metaphase chromosomes using FISH method.
- Our findings show inter-species satellite divergence and suggest chromosomal rearrangements during the evolution of the *Cannabaceae* family.

References
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2. Alexandrov, O. S., Divashok, M. G., Yakovlev, N. A., Karlov, G. I. (2012). Sex chromosome differentiation in *Humulus japonicus* Siebold & Zuccarini, 1846 (*Cannabaceae*) revealed by fluorescence in situ hybridization of subtelomeric repeat. *Comparative cytogenetics*, 6(3), 239.
3. Divashok, M. G., Alexandrov, O. S., Ranzanova, O. V., Kirov, L. V., Karlov, G. I. (2014). Molecular cytogenetic characterization of the dioecious *Cannabis sativa* with an XY chromosome sex determination system. *PlasOne*, 9(1).